Formal proposal for the conversion to an
Academic Department of Neurological Surgery
within the School of Medicine

April 2020
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EXECUTIVE SUMMARY

The clinical Department of Neurological Surgery provides the full range of contemporary neurosurgical practice. Using a multidisciplinary approach, we provide care to diagnose, treat, and rehabilitate patients with neurological disorders. We also offer residency and fellowship opportunities, and conduct path-defining research that is advancing the field.

While the trajectory of UC San Diego Neurological Surgery has been on the rise over the course of the past five years, the 2018-2019 academic year was a pivotal period of expansion.

Neurological Surgery and Neurosciences maintained its top 50 ranking in *US News & World Report*. Our Quad-Pod of neurosurgical operating rooms in Jacobs Medical Center continued to provide integrated cranial and spinal navigation, intra-operative MRI and CT capabilities, and sophisticated microscopy and imaging tools. In addition to the tremendous opportunities for neuro-oncology and restorative procedures, these suites are home to the stereo-EEG and laser ablation procedures that anchor our Level 4 Epilepsy Center - the only one in the region.

We launched our “Neuro Hub”, an innovative, technology-driven teaching and meeting space designed for surgeons both here and abroad. This space complements and connects our resources of our Quad-Pod, the Altman Clinical and Translational Research Institute, and the Center for the Future of Surgery, as well as our unique institution that is rich in both neurosciences and engineering.

We were the local host of the 2019 American Association of Neurological Surgeons (AANS) conference in April, and were featured in a “History of San Diego Neurosurgery” video that was shown at the opening general plenary session.

We have also broadened our geographic footprint to include a new clinic in Rancho Bernardo, in addition to several other regional locations including Hillcrest, La Jolla, Carlsbad, and Escondido. In addition to our community medical center partnerships of Tri-City and Palomar, we also provide the neurosurgical service needs at Tri-City Medical Center, Palomar Medical Center, the La Jolla VA Medical Center, and Rady Children’s Hospital.

Our two destination neurovascular centers at Hillcrest and Jacobs Medical Center enable us to maintain our status as the third Comprehensive Stroke Center in the country. We receive national-level referrals for complex cases for both adult and pediatric patients.

Our strong tradition of teaching excellence will be further enhanced by the expansion of the Center for the Future Surgery to include a new microsurgical lab and hybrid OR/angiography suite, slated to open in Fall 2019. This visionary, unique facility provides national and international training opportunities as well as a clearinghouse for the research and development of new technologies.

Our world-class Skull Base Surgery program -led by Dr. Marc Schwartz in Neurological Surgery and Dr. Rick Friedman from Otolaryngology- remains one of the largest in the country. Through this program, Drs.
Schwartz and Friedman bring destination integrated care to UC San Diego for the most complex cranial tumors.

The faculty and residency program continue our strong history of academic productivity and national engagement. Faculty were responsible for 96 peer reviewed publications and 4,092 citations in the 2018–2019 academic year. Neurological Surgery continued its strong support of UC San Diego medical students; all four matched successfully to top programs around the country including UC San Francisco and University of Pennsylvania. We were delighted to recruit leading candidates for our own residency program.

We are in a continued period of increased prominence in neurosurgical care and research, with the vision and energy to capitalize on the investments and foundational framework we’ve made. With the upcoming arrival of new clinical and research faculty, we are committed to attracting and retaining the best faculty with a focus on diversity in the recruitment of our faculty, residents, post-docs, and graduate students. Our current faculty are represented at a national level through important academies and societies, including the Council of Neurological Surgeons (CNS), the American Board of Neurological Surgery (ABNS), ACGME Residency Review Committee on Neurosurgery, and the Society of Neurological Surgeons (SNS), to name a few.

Our Neurological Surgery leaders have a strong record of University service:

- Dr. Alksne as founding Division Chief, Dean of the UC San Diego School of Medicine, and Vice Chancellor for Health Sciences
- Dr. Marshall as Dean of Faculty Practice, Chairman of the Executive Committee of Clinical Practice, Associate Dean of Clinical Affairs, CEO of the UC San Diego Medical Group, and Executive Director of UC San Diego’s managed health care plan
- Dr. Khaleesi currently serves on the Council of Chairs and Board of Governors
- Dr. Ciacci currently serves as Academic Community Director for UC San Diego School of Medicine

We remain committed to enhancing existing—and forging new—synergistic clinical and research relationships with many collaborators within our institution and across the Mesa to best achieve our programmatic goals:

- Functional neurosurgery and integration of capabilities of the Jacobs School of Engineering, the Institute of Neural Computation, and Sanford Stem Cell Consortium
- Mesa partnerships with the La Jolla Institute for Allergy & Immunology, the Salk Institute, Sanford Burnham Prebys and the Center for Novel Therapeutics
- Potential Neuro / Ortho Integrated Spine Center with rehab and pain management
- The Neurological Institute with our sister Department of Neurosciences (academic department application in part to facilitate two constituent academic departments organizing as a UC institute)
Devotion to sustaining UC San Diego’s reputation as a technology-forward institution finds us as initial users of a “Second Opinion” telehealth initiative that will inform the existing needs and potential opportunities for such remote clinical expertise.

Our future aspirations include the inception of international courses streamed from our Neuro Hub and strengthening the use of the Center for the Future of Surgery. Our basic and translational research efforts advance imaging, surgical visualization, medical device development, and advance our fundamental understanding of neurologic disease through unique physiologic and histopathologic access opportunities.

We are on a strong trajectory of clinical and financial expansion. Our clinical volume has grown to over 3,000 major cases (2,267 University, 700 Rady Children’s Hospital, 200 La Jolla VA). This is from a historic baseline of under 2,000 major cases. Coupled with a clear-eyed focus on quality and patient experience metrics, we have experienced a 30% relative reduction in cranial mortality and substantial improvement in access and Press Ganey scores.

Neurological Surgery’s operating budget is consistently positive with operating expenses of $12M per year and departmental reserves over $1.1M. This reflects a measured, sustainable expansion since the 2016 resource alignment. On the Health System and Medical Group side, the financial performance of the neurosurgical service line remains strong with $15.4M in profit on a 31% contribution margin. Recent strategic partnerships with Tri-City Medical Center, Palomar Medical Center, Naval Medical Center San Diego (“Balboa Naval Hospital”), and an expansive tele-neurology network further cemented our dominant regional position in neurosurgery.

We have excelled in the five key areas foundational to a successful department: clinical, educational, research, financial, and university service. Our successful transition to an academic department will match the status enjoyed by our national peers, allow our organization into a Neurological Institute, and further the momentum of our growing intra-department research infrastructure. Departmental status will lastly provide further esteem to our nationally-regarded residency and fellowship training programs.
Transition to a Department
We seek to become an academic department for a number of reasons:

1) **Distinct discipline.** Neurological Surgery is an independent and distinct discipline that is separate from General Surgery. Neurological Surgery has separate national meetings with a separate Accreditation Council for Graduate Medical Education (ACGME) and residency review committees. The American Board of Neurological Surgery was founded in 1940 and is one of 24 medical specialty boards that make up the American Board of Medical Specialties (ABMS). We have distinct residency and fellowship programs that go through accreditation channels completely separate from General Surgery.

2) **Enhanced national visibility and reputation.** Academic department status is a natural evolution of our growth and stature. Neurological surgery enjoys department status at our sister UC institutions and our national peers in *U.S. News & World Report (USNWR)* ranking. Our medical students match to top training programs and our own residency program consistently matches our top candidates. We enjoy a 10-year accreditation with no citations from the ACGME. Departmental status will reinforce these strengths and foster stronger collaborative efforts with other entities focused on neurologic disease in the San Diego region and throughout the country.

3) **Faculty retention and recruitment.** School of Medicine department status is important for faculty retention and recruitment. Neurological Surgery department status enhances engagement and faculty satisfaction. Neurological Surgery residents and faculty are recruited by the head of the program and they expect their compensation discussions, offer letters, and promotion letters to come from the Neurological Surgery leader, not the leader of the general surgery department, with whom they have little interaction.

An important additional benefit of academic department status will be the ability to confer adjunct appointments to faculty in allied departments who are working on problems that historically bear on neurosurgical practice. In so doing, we will for the first time be able to present a unified communications platform for the strength and breadth of such work in the broader UC ecosystem. UC San Francisco and UC Los Angeles have undertaken similar strategies to great effect.

Our faculty continues to expand. In the last 18 months, we recruited Drs. Thomas Beaumont, Rick Friedman, Sunil Jeswani, Najla Kfoury-Beaumont, Joseph Osorio, Martin Pham, and Marc Schwartz.

4) **Improved representation of Neurological Surgery at the institutional level.** Improved representation of Neurological Surgery’s specific needs at the institutional level remains a critical issue. While the Chair of Neurological Surgery of UC San Diego Health has enjoyed membership on Council of Health Sciences Chairs, Board of Governors, and Med Staff Executive Committee, there are instances where governance issues arise that the neurosurgical perspective is compromised by a governance distinction on the School of Medicine side. This varies from simple recognition at School of Medicine commencement ceremonies to meaningful input into institutional responses for NIH policies and faculty promotion. While we appreciate the support and partnership of the Department of Surgery, financial operations and faculty promotion / conduct issues have been complicated by our dual division / department status.
In terms of the research and academic mission, the department of Neurological Surgery would continue its ongoing contributions to medical student and resident training. This dynamic manifests itself through surgical conferences, collaborative research opportunities at the Center for the Future of Surgery, NIH-funded clinical trials at ACTRI, and growing funded collaborative ventures in Engineering and Neurobiology. Neurological Surgery stands at the nexus of translational neuroscience research as reflected in the T. Denny Sanford gift and material science applications as demonstrated by first-in-man surgeries performed within the Department.

In summary, the clinical Department of Neurological Surgery of UC San Diego Health seeks the status of academic Department of Neurological Surgery within the UC San Diego School of Medicine. We believe that academic department status is a natural evolution of our growth and independence and will: recognize our distinctiveness as a discipline; enhance faculty, fellow, and resident recruitment and retention; enhance our representation for neurosurgery programmatic issues at the institutional level; and facilitate our growth and excellence as one of the most outstanding Neurological Surgery departments in the country. A strong Department of Neurological Surgery will bring further academic, clinical, and educational recognition and profitability to UC San Diego.
DEPARTMENT OF NEUROLOGICAL SURGERY GOALS

Overall
1. Deliver compassionate, highest quality neurosurgical care to the people of San Diego, Southern California, and around the world.

2. Provide premier educational programs that will train and develop the next generation of exceptional neurological surgeons.

3. Generate innovative and impactful research that will contribute to the advancement of neurosurgical science.

4. Advance the UC San Diego School of Medicine and Health Sciences goals in clinical care, education, and innovation.

5. Be responsible contributors and resource stewards for UC San Diego Health Sciences.

Specific

2. Develop an international profile for UC San Diego Neurological Surgery through an expanded communications platform and curricula that will leverage the Center for the Future of Surgery’s microsurgical lab and hybrid OR/angiography suite, Quad-Pod, and Neuro Hub to feature our faculty expertise and facilitate exchange with engineering and neurosciences colleagues. Adjunct appointments will enhance this effort.

3. The Department will be active partners in the redevelopment of Hillcrest. Complex spine, neurorestoration, rehab, neuro critical care, and interventional psychiatry will all require foundational neurosurgical support. Neurological Surgery already supports our Level 1 Trauma and Comprehensive Stroke Center certifications at Hillcrest.

4. North County agreements with Tri-City Medical Center and Palomar Medical Center complement our expansive tele-neurology network and affirm the regional dominance for our department. At present, no one needs to leave San Diego for world-class neurosurgical care and we already serve as a destination provider in several sub-specialty areas including neurovascular and skull base surgeries. For context, UC San Diego Health System inpatient market share is 16% and our department is responsible for upwards of 65% of the craniotomies performed in the region.

5. Expand, complement basic full-time researchers within the department for a 5-year goal of $5M in NIH funding and the completion of an R25 Training Grant within Neurological Surgery. Lastly, the department will support Moores Cancer Center application for a brain tumor SPORE grant in that same time frame.
HISTORY OF NEUROLOGICAL SURGERY IN SAN DIEGO

In 1971, the relatively new University of California, San Diego School of Medicine appointed Dr. John Alksne as Division Chief of Neurosurgery, launching San Diego’s only academic neurosurgery program. Neurosurgery at UC San Diego has grown tremendously in the past five decades under the guidance of four chiefs: Dr. John Alksne, Dr. Lawrence Marshall, Dr. Bob Carter, and Dr. Alexander Khalessi (now also the chair of the clinical Department of Neurological Surgery). Neurosurgery at UC San Diego has been - and continues to be- home to innovative and pioneering research and is home to many well respected neurosurgeons and neurosurgical trainees. The Division is known for its tradition of service to its patients, and the efforts of past and present faculty to push the frontiers of neurosurgical care.

The history of UC San Diego Neurological Surgery is closely intertwined with the development of UC San Diego as a major public research institution and with the emergence of La Jolla as one of the nation’s leading biotechnology clusters. Simultaneous with the University’s founding, the Salk Institute was gifted 27 acres adjacent to UC San Diego overlooking the Pacific Ocean. The Salk Institute opened its doors in 1963 to become one of the nation’s renowned institutes of research in the neurosciences and neurological disorders, genetics, and cancer biology. In the years ahead, both the Sanford Burnham Prebys Institute (1979) and The Scripps Research Institute (1980) consolidated operations on the Torrey Pines ‘Mesa’ to create an internationally prominent nucleus of biologic research in La Jolla. It was in this scientific ecosystem that the UC San Diego School of Medicine was founded in 1968 and created a nascent Division of Neurosurgery.

The formal beginning of neurosurgery at UC San Diego began with the recruitment of Dr. John Alksne in 1971. Dr. Alksne received his medical degree and neurosurgical training at the University of Washington. Prior to arriving in San Diego, from 1964 to 1967, Dr. Alksne served as Chief of Neurosurgery at Harbor General Hospital at UC Los Angeles (UCLA). His time at UCLA would ultimately pave the way to a rich practice in cranial nerve compression disorders at UC San Diego.

Dr. David Hume, who is well known for his role on the team that performed the first successful kidney transplant, as well as his research on the hypothalamus and neuro-endocrine systems, recruited Dr. Alksne to the Medical College of Virginia in 1967. Dr. Alksne served as Chair of Neurosurgery from 1967 to 1971 and, while in Virginia, helped integrate a racially segregated intensive care unit to ensure that equal care was given regardless of race. Dr. Marshall Orloff, whom Dr. Alksne had originally met and befriended at UCLA but now was Chair of Surgery at UC San Diego, recruited Alksne to become the first Chief of Neurosurgery at UC San Diego.

Dr. Alksne was viewed as an innovator in research and clinical care; some of his earliest work at UC San Diego focused on using iron acrylics for the stereotactic thrombosis of surgically inaccessible intracranial aneurysms. In hindsight, this research was pivotal because it foreshadowed the idea that aneurysms could be treated via alterations in flow dynamics as an alternative to microsurgical reconstruction; in essence, this novel treatment was an early version of modern endovascular coiling techniques.
Dr. Alksne’s early administrative task was to build both a faculty and a residency; he recruited two surgeon scientists as the first faculty: Dr. Hoi Sang ‘Ben’ U from UC San Francisco and Dr. Lawrence Marshall from University of Pennsylvania. In the model of a surgeon-scientist that influences the Division today, Dr. U focused on cerebrovascular disease and basic science; he worked closely with colleagues in neuroanesthesia including Dr. John Drummond to improve the safety of surgical resections of complex arteriovenous malformations. Laboratory work included early papers discussing the role of epidermal growth factor receptor (EGFR) amplification in glioblastoma and the development of gene therapy initiatives.

In order to meet the growing demands of the Division, a formal neurosurgery residency was created. Dr. David Barba, whose father Dr. Manuel Barba was one of the first neurosurgeons in San Diego and an early advocate for establishing a formal training program to meet the neurosurgical needs of the San Diego community, was selected as the first resident of the program in 1980. Drs. Barba and Alksne’s first paper together in 1984 on trigeminal neuralgia highlights the strong tradition of faculty/resident mentorship that is a hallmark of the UC San Diego program today. Dr. Barba spent a year as a research fellow at the National Institute of Neurological Disorders and Stroke (NINDS) working on the use of interleukins and thymidine-kinase mediated killing of brain tumors before returning to UC San Diego as a faculty member to develop the functional neurosurgery program.

After twenty-five years as Division Chief, Dr. Alksne was tapped to lead larger academic initiatives within the institution. He was appointed Dean of the UC San Diego School of Medicine in 1992, and was named Vice Chancellor for Health Sciences in 1994. Due to his new responsibilities as Dean, Dr. Alksne stepped down as Chief of the Division of Neurosurgery in 1994. While Dean of the medical school, Dr. Alksne had a major role in the initial development of the La Jolla campus and the construction of Thornton Hospital. He performed the first operation at Thornton Hospital, paving a way for the future development of the ‘East’ campus as the major hub of medical care for UC San Diego Health. One specific initiative in the Alksne years was the development of an integrated clinical and teaching program with the Kaiser San Diego hospital system. Initially, UC San Diego neurosurgeons provided the neurosurgical coverage to the Kaiser Permanente group. While the Kaiser Permanente group later developed a fully independent Division of Neurosurgery, resident rotations and joint appointments of the Kaiser Neurosurgery staff as voluntary faculty at the University has been an ongoing result of Dr. Alksne’s early work.

In 1995, Dr. Lawrence Marshall succeeded Dr. Alksne as Division Chief. Dr. Marshall had been initially recruited as an adult and pediatric brain tumor specialist from Philadelphia. Dr. Marshall received his medical degree from the University of Michigan. He completed his neurosurgical training at the University of Pennsylvania and spent a year as a research fellow at the Institute of Neurological Sciences in Glasgow, Scotland. He then went on to complete a fellowship in pediatric neurosurgery at the Children’s Hospital of Philadelphia.

Dr. Marshall led a major expansion in the research infrastructure of the Division, much of it focusing on developing a bench to beside laboratory at Hillcrest, where UC San Diego’s Level 1 Trauma Center was situated. Several grants awarded to UC San Diego in the late 1970s paved the way for the designation as a comprehensive head injury program. This designation opened the door for numerous clinical trials on the treatment of traumatic brain injury. Additionally, UC San Diego was recognized as one of four centers
in the country involved in the National Traumatic Coma Data Bank. Information obtained from the data bank led to groundbreaking discoveries in the categorization and treatment of traumatic brain injury. Along the same lines, under the guidance of Dr. Marshall, UC San Diego became one of the centers for the National Acute Spinal Cord Injury study. Although controversial, work looking at methylprednisolone compared to naloxone or tirilazad mesylate has helped a generation of investigators think about spinal cord injury, and its treatment in new and exciting ways.

Many of the breakthroughs in medical and surgical management of traumatic brain injury (TBI) were based on papers published from UC San Diego under Dr. Marshall’s leadership with significant influence in larger literature (Dr. Marshall’s work has been cited over 27,000 times). The Marshall CT classification (Table 1.1) has become a staple of neurosurgical practice, and has been adopted extensively in literature as a means of grading head injury.

Table 1.1

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
<th>Mortality</th>
</tr>
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<tbody>
<tr>
<td>Diffuse Injury I</td>
<td>No visible intracranial pathology seen on CT scan</td>
<td>9.6%</td>
</tr>
<tr>
<td>Diffuse Injury II</td>
<td>Cisterns are present with midline shift of 0-5 mm</td>
<td>13.5%</td>
</tr>
<tr>
<td>Diffuse Injury III (swelling)</td>
<td>Cistern compressed or absent, shift less than 5mm</td>
<td>34%</td>
</tr>
<tr>
<td>Diffuse Injury IV (shift)</td>
<td>Shift greater than 5 mm Any surgically evacuated lesion</td>
<td>56.2%</td>
</tr>
<tr>
<td>Evacuated Mass Lesion V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-evacuated Mass Lesion VI</td>
<td>High or mixed density lesion greater than 25cm³</td>
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Dr. Marshall’s pioneering work in intracranial pressure monitoring and the use of medical therapy for reducing intracranial pressure has been instrumental in our understanding of TBI. Many of the strategies developed at UC San Diego to manage complicated intracranial hypertension are still in use today. For example, work done in the 1970s by Dr. Marshall using barbiturate coma for severe head injury is utilized throughout the world in the management of patients with poorly controlled intracranial hypertension.

In addition to Dr. Marshall’s clinical and research efforts, he also became Dean of Faculty Practice, Chairman of the Executive Committee of Clinical Practice, Associate Dean of Clinical Affairs in 1986, and CEO of the UC San Diego Medical Group in 1986, as well as Executive Director of UC San Diego’s managed health care plan.
The Division expanded substantially during Dr. Marshall’s tenure as Division Chief. One focus was in the domain of spinal neurosurgery. Dr. William Taylor, who trained with Dr. Paul McCormick in spine surgery at Columbia University, led the development of a major initiative in minimally invasive spine surgery with a focus on lateral approaches. Dr. Taylor worked closely with the San Diego biotech environment to bring several innovations related to intra-procedural neurological monitoring to clinical use. In the domain of spinal neurosurgery, Dr. Marshall also recruited Dr. Joseph Ciacci, who had a special interest in complex spinal reconstruction for traumatic and degenerative diseases.

Dr. Ciacci later teamed with scientists at the Sanford Stem Cell Initiative, a collaborative joint venture between Salk, UC San Diego, Sanford Burnham Prebys, and The Scripps Research Institute (TSRI) to develop UC San Diego’s first phase one clinical trial in the country for the direct injection of stem cells into adult patients with chronic spinal cord injury. In 2009, after 15 years of service as Division Chief, Dr. Marshall elected to step down and a national search was undertaken for a new Neurosurgery Division Chief, led by Dr. Mark Talamini, Chair of the Department of Surgery.

Dr. Bob Carter was recruited from Massachusetts General Hospital (MGH) and Harvard Medical School to UC San Diego. Dr. Carter received his MD and PhD degrees from Johns Hopkins School of Medicine and Public Health. His neurosurgical training was completed at MGH and he remained on faculty at Harvard and MGH until coming to San Diego in 2010. Dr. Carter had published extensively in the academic literature on the topics of gene therapy and CAR T-cells as treatment for glioblastoma and outcomes research in cerebrovascular and cranial neurosurgery. His work on exosomes (nano-sized vesicles secreted by glioblastoma cells) fundamentally re-shaped the development of diagnostic biomarkers for brain cancer patients. Dr. Carter’s background as both a scientist and surgeon were uniquely suited to the UC San Diego environment. He doubled the size of the faculty, increased case volume and market share to become a regional leader, supported new research and education programs, and increased philanthropic gifts.

At the start of Dr. Carter’s tenure, neurosurgical oncology, neurovascular clinical care, and neurocritical care were early needs. New faculty -including Drs. Clark Chen, Justin Brown, Alexander Khalessi, and Andrew Nguyen- were recruited with the goal of expanding the faculty. Each new faculty member made major programmatic contributions and proved invaluable to the Division’s growth and success.

In 2012, UC San Diego opened the Center for the Future of Surgery (CFS). This $65M building hosts 45,000 square feet of educational space including 22 operative stations, an endoscopy suite, as well as ICU and ED simulation suites. Neurological Surgery has generated substantial revenue for the CFS through societal and industry courses. Dr. Khalessi raised $20M in in-kind donations to outfit the microsurgical lab and hybrid OR/angiography suite. The neurosurgery residents have used the CFS to learn spinal instrumentation techniques; in addition, a state of the art endovascular simulator has helped resident trainees appreciate the complex nature of endovascular treatment.

The neurosurgery service continued to expand. In 2015, UC San Diego recognized the growing influence of the faculty with formal designation of the Division to a clinical Department of Neurological Surgery.
In 2017, after seven years of service, Dr. Carter was recruited by his alma mater to lead the Department of Neurosurgery at MGH and Harvard Medical School. Dr. Alexander Khalessi was named acting Clinical Chief.

In January 2018, after an extensive national search, Dr. Alexander Khalessi was named Chair of the clinical Department of Neurological Surgery at UC San Diego Health and Chief of the Division of Neurosurgery in the Department of Surgery at UC San Diego School of Medicine. Notably, the search was conducted by the Vice Chancellor for Health Sciences’ office in anticipation of an academic department application. The process involved campus-wide lectures by the finalists. Dr. Khalessi is one of the youngest and most distinguished faculty members to have earned these critical clinical and academic leadership positions, and was previously the Division’s first Vice Chair of Clinical Affairs.

Dr. Khalessi initiated a new skull base surgery program by recruiting the outstanding team of Drs. Marc Schwartz and Rick Friedman, and developed a new alliance with Balboa Naval Hospital for trauma care at Hillcrest - both of which were accomplished within a year. Working closely with UC San Diego Health CEO Patty Maysent, Dr. Khalessi ensured the clinical Department remained on an aggressive growth trajectory for the future.

With established thought leadership in medical device innovation, Dr. Khalessi has chaired several data safety monitoring boards and clinical events committees for FDA neurovascular device assessment. Dr. Khalessi further helped pioneer first-in-man surgical cases for endoscopic evacuation of intracranial hemorrhage and 4K-3D exoscope visualization in neurological surgery.

Dr. Khalessi led efforts at Jacobs Medical Center to expand its neurosurgical capabilities through a combination of key recruitments, new technologies, and process quality improvements. He also led the Hospital’s successful designation as the nation’s third Comprehensive Stroke Center (with sites in Hillcrest and La Jolla). Additionally, Dr. Khalessi led the expansion of the Center for the Future of Surgery to include a microsurgical lab and hybrid OR/angiography suite, slated for opening in Fall 2019. This involved securing $20M of in-kind investments.
CLINICAL HIGHLIGHTS

Neurological surgery constitutes a medical discipline and surgical specialty that provides care for adult and pediatric patients in the treatment of pain or pathological processes that may modify the function or activity of the central nervous system. For example: (brain, hypophysis and spinal cord), the peripheral nervous system (cranial, spinal and peripheral nerves), the autonomic nervous system, the supporting structures of these systems (meninges, skull and skull base and vertebral column) and their vascular supply (intracranial, extracranial and spinal vasculature).

Treatment encompasses both non-operative management (prevention, diagnosis – including image interpretation– and treatments such as, but not limited to neurocritical intensive care and rehabilitation) and operative management with its associated image use and interpretation (endovascular surgery, functional and restorative surgery, stereotactic radiosurgery and spinal fusion) including its instrumentation.

UC San Diego Neurological Surgery has been and continues to be a leader in the complex sub-specialties encompassed within neurological surgery.

*Note: please find current clinical organizational chart below (Table 1.2). Short biographies on all clinical team members can be found in Appendices III and IV. Enlarged formats of all informational tables can be found in Appendix VI.

Table 1.2

<table>
<thead>
<tr>
<th>NEUROTRAUMA</th>
<th>NORTH COUNTY</th>
<th>NEUROVASCULAR</th>
<th>SPINE</th>
<th>EPILEPSY / FUNCTIONAL</th>
<th>PEDIATRIC</th>
<th>NEURO-Oncology</th>
<th>VA</th>
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<tr>
<td>Jeffery Tanis, MD</td>
<td>Andrew Nguyen, MD PhD</td>
<td>Scott Olson, MD</td>
<td>William Taylor, MD</td>
<td>Sharone Ben-Haim, MD</td>
<td>Michael Levy, MD PhD</td>
<td>Michael Schwartz, MD</td>
<td>Marc Gazz, MD</td>
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<tr>
<td>Lawrence Marshall, MD</td>
<td>Suniti Joshi, MD</td>
<td>J. Scott Pannell, MD</td>
<td>Joseph Oxoro, MD PhD</td>
<td>John Alkow, MD</td>
<td>David Gonda, MD</td>
<td>Joseph Ciallo, MD</td>
<td>Anne Ostrer, MD</td>
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<tr>
<td>Elisa DiFranco, PA</td>
<td>Howard Tang, MD</td>
<td>Samantha Wheeler, PA</td>
<td>Martin From, MD</td>
<td>Denis Essle, MD</td>
<td></td>
<td>Rick Friedman, MD PhD**</td>
<td>Hai Liang (Lin) Li, MD</td>
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<td>Gege Lambert, MD PA-C</td>
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<tr>
<td>Alexander A. Khaleesi, MD MBA Chair of Neurological Surgery Professor of Surgery, Radiology and Neurosciences</td>
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<tr>
<td>Eric Daj, RN</td>
<td>Karl O'Connor, NP</td>
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<td></td>
<td></td>
<td>**Adjunct</td>
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</table>
Neurovascular

UC San Diego has a long history of excellence and innovation in the treatment of cerebrovascular disease. Dr. Alksne’s pioneering work with direct stereotactic magnetically controlled embolization of aneurysms with iron filings set the tone and cadence for innovative thought. The work of Drs. U and Kerber built a foundation for arteriovenous malformation (AVM) treatment solidifying the standard approach of staged embolization and resection that continues to be followed today.

The neurovascular service has since evolved into a model of multi-disciplinary care integrating neurocritical care, stroke neurology, and neurosurgery with a collaborative approach between services and unification of open surgical and endovascular approaches to cerebrovascular disorders.

Dr. Alexander Khalessi, trained at the University of Southern California in neurosurgery and SUNY Buffalo in endovascular techniques under Dr. Nick Hopkins, was appointed Chief of Neuroendovascular Surgery and Surgical Director of Neurosurgical Critical Care. Dr. Khalessi built an outstanding multidisciplinary team housed within neurosurgery including Dr. Scott Olson (Interventional Neurology) and Dr. J. Scott Pannell (Interventional Neuroradiology) that led UC San Diego’s effort to establish the nation’s third Comprehensive Stroke Center. Notably, the entire neurovascular program faculty have primary appointments in neurosurgery to maintain a cohesive, multi-disciplinary team.

Dr. Khalessi’s research interest is focused on carrying out clinical trials that test novel endovascular treatments for stroke and aneurysms, as well as, designing quality measures and management algorithms for cerebral ischemia and subarachnoid hemorrhage. Under Dr. Khalessi’s leadership, the neurovascular multi-disciplinary team at UC San Diego is involved with numerous clinical trials including MISTIE3, MR Rescue, WEAVE, THERAPY, and DEFUSE 3. UC San Diego has a U01 grant as a regional coordinating center for StrokeNet and Dr. Khalessi serves on the Interventional Advisory Panel for StrokeNet.

The neurovascular service remains committed to excellence in academic research and clinical medicine. We look forward to a bright future of continued innovation.
Spine

The spinal neurosurgery service is internationally known for its innovation in minimally invasive techniques. Based in La Jolla, the Minimally Invasive Spine Division is currently led by Dr. William Taylor. Dr. Taylor also currently serves as the President of the Society of Lateral Access Surgery and is the Founder and President of the Society for Minimally Invasive Spine Surgery.

The service also has an active trauma and reconstructive practice at the Hillcrest Level I Trauma Center led by Dr. Joseph Ciacci, who has been with the Division for over 15 years. Dr. Ciacci continues to provide strong academic leadership in multiple areas. He is the Residency Director for Neurological Surgery, and is intimately involved in medical student graduate education. In addition, his academic interests are specifically tumor trauma and he currently leads one of the few stem cell treatment programs for spinal cord injury. He is a leader in the field of spinal cord injury and trauma with multiple clinical trials and scientific publications in the stem cell and regenerative medicine field.

Many innovations have been made here at UC San Diego, including the development of the X-LIF procedure which is a lateral minimally invasive technique for accessing lumbar inter body pathology. In the early 2000s, Dr. Taylor teamed up with neurosurgery residents Drs. Burak Ozgur, Henry Aryan, and Luiz Pimenta to develop dedicated devices to support minimally invasive spine surgery. In the first decade after the introduction of this technique, over 100,000 patients were treated.

We recently added Dr. Martin Pham to our Spine team who is an expert in the field of robotics. Dr. Pham recently completed his residency at University of Southern California and a fellowship at the prestigious Columbia University deformity program. Dr. Pham completed some of the first cases with the Mazor X™ spine robotics system at UC San Diego and presented this work at the American Association of Neurological Surgeons (AANS) meeting. UC San Diego will be the national training site for Mazor X™ technology with a footprint at the Jacobs Medical Center and the Center for the Future of Surgery.

Dr. Joseph Osorio joins the faculty to develop a reconstructive and deformity spine practice. He is an MD/PhD graduate of UC San Francisco and is also a world expert in deformity surgery. He is widely published in the areas of adult lumbar and cervical deformity surgery. He is charged with developing this integrated program and expanding our footprint in spinal clinical trials.

Our program is rounded out by providers all of whom are specialty and fellowship-trained including surgeons providing academic complex care at Palomar Medical Center and Tri-City Medical Center. With Drs. Sunil Jeswani, Andrew Nguyen, and Howard Tung, the Department has an opportunity to expand our residency complement or undertake a Committee on Advanced Subspecialty Training (CAST)-certified spine fellowship in the North County region. At minimum, these respected regional providers expand the footprint of UC San Diego as a destination for complex spinal care.

We currently have approximately 10 clinical trials within the spine specialty and continue to build our academic program, including degenerative disc disease, bone graft infusions, stem cell treatment, and regenerative medicine along with robotic and minimally invasive procedures.
Epilepsy

UC San Diego is one of the most advanced centers for surgical epilepsy care in Southern California, and is the only accredited adult Level 4 Epilepsy Center in San Diego County.

There are currently 6 epileptologists within UC San Diego’s Department of Neurosciences. Dr. Jerry Shih is the current Director of the Epilepsy Center and was recruited in 2015 from the Mayo Clinic in Jacksonville, Florida, where he served as the Director of the Mayo Clinic Comprehensive Epilepsy Center. Dr. Shih is well known nationally, and has authored numerous publications in the field of epilepsy, in addition to his research program centered on brain-machine interfaces. Since his arrival, Dr. Shih has recruited an additional epileptologist from UC San Francisco, Dr. June Yoshii-Contreras, who specializes in utilizing technology for 3D source localization of seizures from EEG monitoring. Drs. David Lee and Leena Kansal are also fellowship-trained epileptologists who have been recruited to UC San Diego over the past five years.

Additionally, the epilepsy faculty continue to benefit from the expertise of Drs. Vicente Iragui and Evelyn Tecoma, the former Directors of the UC San Diego epilepsy program. Dr. Tecoma is a world-renowned epileptologist who has authored numerous articles and continues to lecture widely on areas of her specialty including: clinical trials; pre-surgical evaluation; vagal nerve stimulation therapy; reproductive hormones and epilepsy; and the diagnosis and management of epilepsy. Dr. Iragui is similarly a renowned epileptologist who has authored numerous publications in the field and continues to be active both clinically and in his research profile.

We have two members of our neurosurgical team who are actively involved in our surgical epilepsy center: Drs. Sharona Ben-Haim and David Barba.

Dr. Ben-Haim was recruited to the faculty in 2015 to help foster the growth and inclusion of new technology in our center. She trained as a neurosurgeon at the Mount Sinai Hospital in New York, and obtained fellowship training in Epilepsy Surgery at Yale University. She subsequently underwent additional fellowship training in deep-brain stimulation surgery at Oxford University in England before being recruited to UC San Diego. Dr. Ben-Haim specializes in minimally invasive therapy for epilepsy, including the placement of stereotactic EEG electrodes, and laser interstitial thermal therapy, in addition to other open neurosurgical procedures.

Dr. David Barba is a board certified functional neurosurgeon who also specializes in epilepsy surgery. Dr. Barba’s areas of expertise include craniotomy for the placement of grid and strip electrodes, as well as anterior temporal lobectomy.

The UC San Diego surgical epilepsy team benefits from a large and varied team of clinical experts. This team meets twice per month to hold a multidisciplinary surgical epilepsy conference, where patients under consideration for surgery are discussed. In addition to epileptologists and epilepsy neurosurgeons, the team includes neuroradiologists, neuropsychologists, fellows, a nurse coordinator, and several integral EEG technologists.
Our epilepsy center provides state of the art epilepsy surgical care to our patient population. We have undertaken innovative surgical approaches to the management of these patients including being the first and only center in San Diego to implant the NeuroPace® Responsive Neurostimulation System (RNS) and to implant deep brain stimulation electrodes (DBS) for the treatment of epilepsy. Our access to cutting edge technology like our intraoperative MRI suites allows us to utilize progressive techniques like MR guided laser interstitial thermal therapy (MRgLITT) for the minimally invasive treatment of epilepsy. These recently introduced innovative approaches have allowed us to re-invigorate interest amongst referring practitioners to our center as well as to patients themselves.

In a select number of patients, specialized surgery for the placement of intracranial EEG electrodes is undertaken. This may be performed via a craniotomy for the placement of strip, grid, and/or depth electrodes, or may be performed in a minimally invasive fashion using the stereotactic EEG (SEEG) method. The latter procedure was recently innovated to allow for many channels of intracranial recordings in a minimally invasive fashion. Our center performs approximately 10-15 procedures of this type per year, ranking us amongst the top programs in the country.

In addition to being an important clinical tool, intracranial electroencephalography is a unique research tool that has the capacity to provide very unique access into the human brain to ask questions that range from understanding the underlying pathophysiology of epilepsy to understanding the neurophysiology of basic human functions such as speech, language development and sleep, amongst many others.

At UC San Diego, our epilepsy team is closely partnered with our rich basic neuroscience environment allowing for true cross-disciplinary collaboration. Currently, there are multiple laboratories from a range of academic departments working with our epilepsy patients under four separate IRBs. These laboratories are studying a variety of topics from the basics of language to brain-machine interface development to the engineering of new types of electrodes that allow us to understand new information about the human brain.

As our center continues to grow, we anticipate expanding our patient access area continually to the north and to regions east of San Diego through education and the advertisement of our unique team and resources. We concurrently anticipate that as our volume continues to grow, our research profile will also continue to expand and engender further cross-disciplinary collaboration with the basic science resources at UC San Diego as well as neighboring institutions including the Salk Institute and The Scripps Research Institute.
Pediatric Neurosurgery

The Division of Pediatric Neurosurgery at Rady Children’s Specialists of San Diego provides pre-operative evaluation, surgical intervention, and pre-operative and post-operative care for disorders of the brain, spine, calvarial vault, and peripheral nerves. More surgeries are completed at Rady Children’s Hospital each year than at any other facility in the United States. It is additionally one of the top 5 largest children's hospitals in the United States.

More than 600 major surgeries (cerebrospinal fluid diversion excluded) are performed a year, including pediatric brain tumor surgery, interventional and open cerebral vascular surgery, spinal cord tumor surgery, cerebrovascular spinal fluid diversion or shunting procedures, craniofacial procedures, epilepsy surgery, and surgery for congenital abnormalities. Stereotactic adjuvant radiation is also offered via the gamma knife or proton beam therapy.

Whenever possible, minimally invasive procedures are used, with the assistance of highly advanced technology. The Division is among a select group of pediatric programs using Visualase laser ablation technology and the Robotic Stereotactic Assistance (ROSA®) surgical system to perform minimally invasive tumor and epilepsy surgery.

Rady Children’s Hospital is also one of the only children’s hospitals using three-dimensional endoscopy for complex surgical approaches. Infrared and virtual technologies, along with 3D endoscopes, are used in performing minimally invasive procedures to remove brain tumors. Three-dimensional applications of magnetic resonance (MRI) and computed tomography (CT) imaging with stereolithographic models help surgeons to perform complex cerebral vascular surgeries and craniofacial reconstructions.

Rady Children’s Hospital first opened its doors for patients on August 19, 1954. At the time, the city of San Diego was battling a polio epidemic and the children’s hospital was considered a godsend. Since then, Rady Children’s Hospital has grown to become the largest children’s hospital in the state of California. It is ranked nationally in all 10 specialties including neurosurgery in the most recent US News & World Report surveys. Neurological Surgery has long been an important part of the hospital and has been strongly affiliated with UC San Diego and its surgeons.

One of the earliest neurosurgeons to establish a regular practice at Rady Children’s Hospital, then known as the Children’s Hospital of San Diego, was Dr. Thomas Waltz. Dr. Waltz served as a flight surgeon in the Air Force and came to San Diego in 1970 from the University of Baylor where he received his neurosurgical training. Dr. Waltz treated pediatric patients both at the Children’s Hospital and in the community through the Scripps Clinic. He became an esteemed member among the Children’s Hospital staff and among the community physicians. Later in his career, he would move on to become president of the Scripps Clinic Medical Group and chief executive officer of the Scripps Clinic (1990-2000) providing a profound influence on the San Diego medical community as a whole. Upon his death, he was memorialized at Rady Children’s Hospital through the naming of the medical staff lounge in his honor.

When Dr. Lawrence Marshall arrived to UC San Diego neurosurgery in 1975, there was little interaction between the Children’s Hospital and UC San Diego neurosurgery. Dr. Marshall initiated a pediatric practice
at the UC San Diego hospitals. Dr. Marshall developed the earliest pediatric brain tumor experience at UC San Diego and would go on to include the pediatric population in his head trauma studies which would help define the management of head injuries in the dawn of the CT scan era. He multiplied the volume of pediatric neurosurgical cases being done at UC San Diego within his first few years, prompting the hire of another pediatric neurosurgeon to join the group, Dr. Hector James, in 1977. Dr. James was one of the first in San Diego to limit his neurosurgical practice almost predominantly to the pediatric population. Over 140 publications in the field of neurosurgery were made by Dr. James on the topics of hydrocephalus, treatment of cerebral edema in intracranial hypertension, and healthcare delivery models. He worked at Rady Children’s Hospital until 2003 when he was recruited to head the University of Florida’s Division of Pediatric Neurosurgery in Jacksonville at Wolfson Children’s Hospital, where there is now an endowed chair held in his name.

During this same time period, Dr. Thomas Luerssen would begin his pediatric neurosurgical academic career at UC San Diego. Dr. Luerssen worked closely with Dr. Marshall as part of the Congress of Neurological Surgeons (CNS) head injury research group. Among their successes together was the creation of the Camino fiber optic ICP monitor that is among the most used ICP monitors around the country to this day. UC San Diego would be the catapult to send Dr. Luerssen on as a leader in the field of neurosurgery, sitting as Chief of Neurosurgery at Riley Hospital for Children in Indianapolis from 1988 to 2006. He then served as Chief of Neurosurgery at Texas Children’s Hospital from 2006 until his retirement in 2016, and sat on the founding executive committee of the Hydrocephalus Clinical Research Network. With much of the UC San Diego pediatric volume having shifted to Rady Children’s Hospital with Dr. James in 1988, UC San Diego began a loose affiliation with the neurosurgical department, starting to send residents on mandated clinical rotations in 1992. Dr. John Hsiang was the first UC San Diego resident to rotate at Rady Children’s Hospital, soon followed by Dr. Hal Meltzer. They would train under the guidance of Dr. Hector James and the attendings covering pediatrics from Scripps Clinic: Drs. Thomas Waltz, Brian Copeland, and Hertzel Soumekh.

Dr. Meltzer was recruited back to UC San Diego on completion of his residency and fellowship training to continue building the pediatric program. Dr. Meltzer would partner with plastic surgeon Dr. Steve Cohen to build one of the largest craniofacial surgical programs on the west coast. He is known for his work on craniosynostosis and pioneering minimally invasive surgical approaches to craniofacial reconstruction through the use of endoscopy.

This longstanding but loose relationship between physicians at Rady Children’s Hospital and UC San Diego became formalized in 2001 when physicians and leadership of UC San Diego, Rady Children’s Hospital, and Children’s Specialists of San Diego unified pediatric patient care, research, education, and community service programs creating a university-affiliated children’s health system to serve the region.

As part of this consolidation of care, Dr. Michael Levy was recruited from Children’s Hospital of Los Angeles to lead the pediatric neurosurgical efforts at Rady Children’s Hospital in 2002. Dr. Levy is an internationally recognized skull base and cerebral vascular surgeon with a strong laboratory interest in stem cells. Specifically, Dr. Levy’s PhD work evaluated the ability of toxins to allow for the movement of dendritic stem cells into the central nervous system. Dr. Levy has over 390 peer review publications and over 500 presentations at peer review meetings to date. Under his leadership, and along with Dr. Meltzer,
a formal Division of Pediatric Neurosurgery at UC San Diego was created and the surgical case load quadrupled. Neurosurgical practice at Rady Children’s Hospital was limited to the UC San Diego team exclusively. Under their combined efforts, neurosurgery at Rady Children’s Hospital would become the highest volume pediatric neurosurgical center in California. The neurosurgical service was the first in the United States to utilize 3D endoscopy, visualization via head mounted displays, and exoscopy during surgery.

The brain tumor program continues to attract international referrals for Dr. Levy’s operative expertise. In 2016, the UC San Diego pediatric neurosurgery group would bring on a third partner, Dr. David Gonda. Dr. Gonda would direct the development of a surgical epilepsy program at Rady Children’s Hospital, incorporating minimally invasive approaches with the use of laser thermal ablation and robotic assisted procedures.

Lastly, the neurosurgical Residency Review Committee (RRC) requires a pediatric neurosurgery rotation and Rady Children’s Hospital provides this exposure for PGY-6 and PGY-3 residents; it is one of the most well regarding pediatric educational experiences in the country. Rady Children’s Hospital has long been a favorite rotation of residents in the UC San Diego neurosurgery residency. A pediatric neurosurgery fellowship program was started in 2010 and continues to attract some of the brightest graduating residents for training. Nine pediatric fellows have been trained in addition to over 20 international fellows to date. Several residents in recent years have pursued academic careers in Pediatric Neurosurgery including Dr. David Gonda (Fellowship at Texas children's Hospital), Dr. Brandon Gable (Fellowship at Emory University Children's Hospital), and Dr. Lissa Baird (Fellowship at Boston Children's Hospital). Dr. Baird is currently the Chief of Pediatric Neurosurgery at Oregon Health Sciences University. International Fellow Dr. Sebastian Vigueras is currently the Chairman of Neurosurgery at the Hospital De Niños in Viña del Mar, Chile.
Neurosurgical Oncology

In 2010, a formal Division of Neurosurgical Oncology was formed under the leadership of Dr. Bob Carter. Dr. Clark Chen was subsequently recruited as Vice Chair of Research with the specific goal of creating a strong translational environment in which to develop the neurosurgeon-scientists of the future. His clinical work at UC San Diego focused on developing minimally invasive neurosurgical approaches for the treatment of brain tumors. At UC San Diego, Dr. Chen performed the first intracranial injection of viral gene therapy, as well as the first laser thermal ablation as a treatment for glioblastoma. His laboratory work focuses on microRNA and epigenetic regulation of DNA repair processes in glioblastoma as mechanisms of therapeutic resistance.

Neurocritical care, particularly in relation to the Marshall era’s focus on head trauma, was long a discipline exclusively practiced by neurosurgeons. Yet, with the rise in the number of trained specialists whose singular focus was in the critical care unit and not in the operating room, it became apparent that there was value to a model whereby patients had the dedicated attention by a specialist in neurocritical care techniques. Working with Dr. Bill Mobley, former Chair of Neurosciences, a plan was made to create a new neurocritical care service in 2011. In 2012, the neurocritical care service was reinstituted at UC San Diego. With the recruitment of Dr. Navaz Karanjia from Johns Hopkins University, the service was reformed at Hillcrest. A concept of collaborative care was initiated with the neurosurgical service. The service rapidly grew to be a multidisciplinary service including neuroanesthesia, neurology, and neurosurgery providers.

Soon after Dr. Chen’s arrival, the Center for Theoretical and Applied Neuro-Oncology (CTAN) was created to study novel treatment options for patients with malignant brain tumors. This multi-disciplinary group consisting of neurosurgeons, neuro-oncologists, neuro-radiologists, and basic scientists produced numerous peer-reviewed papers in the scientific literature and, for the first time, established a strong translational research presence in the Department. The center served as the central node for multidisciplinary translational work by facilitating fruitful collaborations between clinicians in neuro-oncology and basic scientists from many disciplines.

Presently, this work has been carried forward by the Brain Tumor Epigenomics Laboratory under the direction of Drs. Thomas Beaumont and Najla Kfoury-Beaumont, recently recruited to the Division. Broadly, the laboratory focuses on identifying epigenetic mechanisms associated with tumorigenesis and progression with the goal of developing novel targeted therapeutics. Dr. Kfoury-Beaumont also has a secondary focus investigating epigenetic mechanisms underlying sex-specific differences in outcome in patients with malignant brain tumors. Through collaborations with colleagues in the Sanford Consortium for Regenerative Medicine, the program’s broad reaching goal is to develop a wide array of targeted therapeutics that can be used to create personalized treatment paradigms, not only for malignant brain tumors such as glioblastoma, but also for challenging skull base lesions such as chordoma and craniopharyngioma. This approach represents the foundation of precision neuro-oncology, an exciting initiative to which many centers within the Sanford Consortium are dedicated. There is also an effort to create a brain tumor research consortium throughout the greater UC system and beyond that would facilitate data and tissue sharing while streamlining state-wide clinical trial enrollment.
The clinical arm of the neurosurgical oncology program offers comprehensive care for the most complex brain and skull base tumors including the highest volume acoustic neuroma program in the nation as well as one of few centers in the country offering the expanded endoscopic endonasal approach (EEA). There is a program-wide initiative focused on clinical outcomes associated with modern minimally invasive techniques such as endoscope-assisted craniotomy and laser interstitial thermal therapy (LITT).

Leveraging strong relationships with industry partners, the program has remained on the cutting edge of technology and innovation in neurosurgery. The current suite of neurosurgical operating rooms offers intraoperative MRI (iMRI) and full 3D/4K technology allowing the integration of advanced imaging such as functional MRI (fMRI) and diffusion tensor tractography for advanced surgical planning.

Through campus wide digital integration, the neurosurgical operating rooms, along with our state-of-the-art microsurgical laboratory at the Center for the Future of Surgery (CFS) are connected to our centrally-located Neuro Hub that provide a rich substrate for research, innovation and surgical education. In the coming year, several courses for resident and faculty education will be hosted at CFS, including our first-ever comprehensive skull base course that will include local, national, and international experts.

The neuro-oncology program currently includes three destination neurosurgery faculty. Dr. Khalessi is a recognized expert in the resection of brain metastases and intrinsic tumors. The primary brain tumor program further benefits from well-developed awake craniotomy and intraoperative MRI teams. These service areas are complemented by the posterior and lateral skull base program developed by Dr. Marc Schwartz. Dr. Tom Beaumont is charged with the development of an anterior skull base, integrated pituitary, and extended endoscopic practice. Taken together, these surgeons provide the full breadth and depth of complex cranial surgery to our comprehensive NCI designated Moores Cancer Center.

**Peripheral Nerve Surgery**

A new Division of Peripheral Nerve Surgery was created with the recruitment of Dr. Justin Brown in 2011. Dr. Brown developed a major program in distal nerve transfer for functional reanimation in patients with spinal cord injury. Since Dr. Brown’s departure, Dr. Khalessi is working collaboratively with Orthopedics and Plastic Surgery at the direction of Interim Dean Garfin to develop a multi-disciplinary peripheral nerve program.
NEUROLOGICAL SURGERY TEAM

The Neurological Surgery team currently includes 21 surgical faculty (Table 1.3), 14 residents, 7 Advanced Care Practitioners (ACPs), 1 Triage Nurse (based on a highly successful quality improvement pilot), 1 PhD Researcher (the first of three), 15-17 staff, and 3 ongoing recruitments.

We have two FTEs allocated to Neurological Surgery. One FTE is currently held by Dr. U. Dr. Khalessi split his full FTE as Department Chair to contribute another 0.5FTE for a pending PhD research recruit. Health Sciences and the School of Medicine have also committed to providing another 1.0FTE upon Neurological Surgery’s successful transition to an academic department for a dedicated PhD research recruit.

Our physicians cover UC San Diego Health hospitals in Hillcrest and La Jolla, Veterans Affairs San Diego Healthcare System, Rady Children’s Hospital and are actively involved in extending the reach of UC San Diego Health through the Clinical Integration Network. US News & World Report consistently ranks the neurology and neurosurgery programs at UC San Diego Health among the top 50 in the nation.

*Note: please find listing of all current surgical faculty on the following page (Table 1.3). Short biographies on each faculty member can be found in Appendix III.
### Table 1.3 “Surgical Faculty”

<table>
<thead>
<tr>
<th>Faculty</th>
<th>Title</th>
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</thead>
<tbody>
<tr>
<td>Alksne, John, MD</td>
<td>RTAD - Professor Emeritus of Surgery, Past Chief (Ladder Rank)</td>
</tr>
<tr>
<td>Barba, David, MD</td>
<td>HS Clinical Professor of Surgery, Director of Functional Surgery</td>
</tr>
<tr>
<td>Beaumont, Thomas, MD PhD</td>
<td>Assistant Professor of Clinical Surgery <em>(Clin X in process)</em></td>
</tr>
<tr>
<td>Ben-Haim, Sharona, MD</td>
<td>HS Assistant Clinical Professor of Surgery</td>
</tr>
<tr>
<td>Ciacci, Joseph, MD</td>
<td>Professor of Clinical Surgery <em>(Clin X)</em>, Residency Program Director, Chief of Neurosurgery VASDHS</td>
</tr>
<tr>
<td>Gonda, David, MD</td>
<td>HS Assistant Clinical Professor of Surgery</td>
</tr>
<tr>
<td>Jeswani, Sunil, MD</td>
<td>HS Associate Clinical Professor of Surgery <em>(in process)</em></td>
</tr>
<tr>
<td>Khalessi, Alexander A., MD MBA MS FAANS</td>
<td>Chair of Neurological Surgery; Professor of Surgery, Radiology and Neurosciences (Ladder Rank / In Residence)</td>
</tr>
<tr>
<td>Levy, Michael, MD</td>
<td>Professor of Clinical Surgery <em>(Clin X)</em></td>
</tr>
<tr>
<td>Marshall, Lawrence, MD</td>
<td>RTAD - Professor Emeritus of Surgery, Past Chief (Ladder Rank)</td>
</tr>
<tr>
<td>Nguyen, Andrew, MD PhD</td>
<td>HS Associate Clinical Professor of Surgery</td>
</tr>
<tr>
<td>Olson, Scott, MD</td>
<td>HS Associate Clinical Professor of Surgery</td>
</tr>
<tr>
<td>Osorio, Joseph, MD PhD</td>
<td>Assistant Professor of Clinical Surgery <em>(Clin X in process)</em></td>
</tr>
<tr>
<td>Oygar, Ahmet, MD</td>
<td>HS Clinical Professor of Surgery (non-salaried)</td>
</tr>
<tr>
<td>Pannell, J. Scott, MD</td>
<td>HS Assistant Clinical Professor of Surgery and Radiology</td>
</tr>
<tr>
<td>Pham, Martin, MD</td>
<td>Assistant Professor of Clinical Surgery <em>(Clin X in process)</em></td>
</tr>
<tr>
<td>Schwartz, Marc, MD</td>
<td>HS Clinical Professor of Surgery <em>(in process)</em></td>
</tr>
<tr>
<td>Taylor, William, MD</td>
<td>HS Clinical Professor of Surgery</td>
</tr>
<tr>
<td>Tomlin, Jeffrey, MD</td>
<td>HS Clinical Professor of Surgery (non-salaried; <em>in process</em>)</td>
</tr>
<tr>
<td>Tung, Howard, MD</td>
<td>HS Clinical Professor of Surgery <em>(in process)</em></td>
</tr>
<tr>
<td>U, Hoi S, MD</td>
<td>Professor of Surgery (Ladder Rank)</td>
</tr>
</tbody>
</table>
ADMINISTRATIVE ORGANIZATION

Administratively, the academic Department of Neurological Surgery will be under Eric Wilson, Department Business Officer (Professional School) and Michelle Ziemba, Senior Director of Neurological Institute, Rehabilitation Services, Trauma & Burn (Health System and Physician Group). Mr. Wilson and Ms. Ziemba have been jointly managing the professional and clinical administrative functions of both Neurosciences and Neurological Surgery, the overlap of which is described as the “Neurological Institute”, since June 2016.

Neurological Surgery’s administrative core has expanded to efficiently support substantial growth. Four additional clinical care coordinators are responsible for the scheduling and interaction of patients and faculty. An academic administrative assistant is currently being recruited to preserve the faculty ratio to administrative load with the growth of the faculty contingent. Neurological Surgery essentially enjoys a full separation of all business functions from the Department of Surgery. With the approved and posted replacement of an assistant financial manager, Neurological Surgery is well positioned to become a full-fledged Academic Department without additional cost.

Neurological Surgery recognizes the importance of keeping a lean administrative core, and will strive to share business expertise with HR/Faculty affairs administration with resources in the Department of Neurosciences as appropriate in the formation of the UC San Diego Neurological Institute. Neurological Surgery relies on central shared services for the functions of grant/research management (RSC), human resources/payroll (HHR), faculty administration & academic affairs (ARC). Clearly, UCOP criteria for the development of an institute requires two academic departments. This process is therefore a critical step in the broader strategic effort shared with the Chancellor and senior Health Sciences leadership.

*Note: please find current administrative organizational chart on the following page (Table 1.4). Short biographies on Administrative Leadership can be found in Appendix V.
CLINICAL OVERVIEW

The clinical Department of Neurological Surgery is on the leading edge of treating neurological conditions using the latest innovations and techniques in neurological surgery. The neurosurgeons at UC San Diego Health provide advanced treatment for injuries and disorders of the brain, spine, and spinal cord. They are experts in using innovative technology and therapies to deliver the best possible care, including: minimally invasive surgery; intraoperative MRI / CT scan; computer-assisted brain surgery; stereotactic radiosurgery; awake brain surgery; deep brain stimulation; complex tumors; as well as endovascular disease and conditions.

A significant investment has been made in innovative technology to advance the neurosurgical clinical mission. The state-of-the-art Quad-Pod at UC San Diego Health Jacobs Medical Center is home to two intraoperative MRI suites and two CT suites. This technology is also home to the latest in surgical navigation equipment. Enabling our surgeons to perform cases that cannot be done in this region cements our status as a destination for complex neurosurgery and related research.

The clinical Department of Neurological Surgery’s clinical activity has steadily grown over time and is on a strong trajectory of clinical and financial expansion (Tables 1.5 Inpatient Operative Cases and 1.6 Outpatient Visits). Our clinical volume has grown to over 3,000 major cases (2,267 University, 700 Rady Children’s Hospital, 200 La Jolla VA). This is from a historic baseline of under 2,000 major cases.

Table 1.5

<table>
<thead>
<tr>
<th>Year</th>
<th>Operative Cases (University sites only)</th>
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<tbody>
<tr>
<td>FY19</td>
<td>2500</td>
</tr>
<tr>
<td>FY18</td>
<td>2250</td>
</tr>
<tr>
<td>FY17</td>
<td>2100</td>
</tr>
<tr>
<td>FY16</td>
<td>2000</td>
</tr>
<tr>
<td>FY15</td>
<td>1500</td>
</tr>
</tbody>
</table>

Table 1.5: Operative Cases (University sites only)
Coupled with a clear-eyed focus on quality and patient experience metrics, we have experienced a 30% relative reduction in cranial mortality (Table 1.7), substantial improvement in readmission rate (Table 1.8), access (Table 1.9), and faculty provider Press Ganey scores.

Table 1.6

<table>
<thead>
<tr>
<th>FY19 (ANNUALIZED)</th>
<th>FY18</th>
<th>FY17</th>
<th>FY16</th>
<th>FY15</th>
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<tbody>
<tr>
<td>8000</td>
<td>8000</td>
<td>8000</td>
<td>6500</td>
<td>6500</td>
</tr>
</tbody>
</table>

Outpatient Clinic Visits

Table 1.7

**Mortality Observed / Expected (O/E)**
Table 1.8
30 Day Related Readmission
30-Day Related Readmission per Fiscal Quarter Trend - All Data

Table 1.9
Percentage of New Patients in 7 Days
Percentage of New Patients in 7 Days per Fiscal Quarter Trend - All Data
The clinical Department of Neurological Surgery is foundational in several distinguished programs for UC San Diego Health:

- Level I Trauma program at Hillcrest
- Joint Commission Comprehensive Stroke Program at Hillcrest and Jacobs Medical Center
- Level 4 Epilepsy program, the only one in the region.
- Parkinson’s Center for Excellence
- One of the largest Acoustic Neuroma programs in the country

The clinical Department has, over recent years, expanded its regional presence through strategic partnerships and ventures. Neurological Surgery has broadened its geographic footprint and has clinics in several regional locations including Hillcrest, La Jolla, Carlsbad, Escondido and Rancho Bernardo. There are contracts for call coverage at two community medical centers: Palomar Medical Center and Tri-City Medical Center. Our excellence in clinical care has made these valuable partnerships possible, and allow us to serve an even more diverse and expansive population.

In addition to our community medical center partnerships, we also provide the neurosurgical service needs at the VA Medical Center and Rady Children’s Hospital. There is an existing partnership with Balboa Naval Hospital neurosurgical providers that ensures their team remains “battle field” competent. The Navy neurosurgeons are a partner in meeting the Level I Trauma Neurosurgical call responsibilities at Hillcrest.

Neurological Surgery’s excellence in clinical care is evidenced by top 50 rankings is *US News & World Report* for the past several years. The trends in clinical excellence continue to improve and the rankings are expected to move even higher for future reporting.
REGIONAL FOOTPRINT OF USCD NEUROSURGERY

Outpatient Clinic Locations
- Carlsbad
- Rancho Bernardo
- Escondido
- La Jolla
- Hillcrest

Medical Center / Hospitals
- UCSD Hillcrest
- UCSD Jacobs / La Jolla
- Palomar Medical Center
- Tri City Medical Center
RESEARCH

Neurological Surgery’s total-awarded, research-related funding has varied from year to year. Historically, funding has ranged from $100,000-800,000. Neurological Surgery’s research portfolio traditionally has involved clinical trials and is expanding to other basic and translational areas. A basic research project was awarded by the Morton Cure Paralysis Fund in this current fiscal year (FY19) to Dr. Ciacci. 14 proposal submissions are currently pending, half of which are federal/non-profit/foundation grant funded.

The strategic approach has been around collaborative research dyads. There is substantial collaborative involvement with other departments. As of June 30, 2019, Neurological Surgery faculty are listed as co-investigators on proposals totaling $5.9M in direct costs, across five departments (Table 1.10). Neurological Surgery faculty rarely draw meaningful salary support from these grants but are engaged partners in these broad institutional research efforts. As previously detailed, adjunct appointments offered by a Department of Neurological Surgery will only strengthen these collaborative research relationships.

Table 1.10

<table>
<thead>
<tr>
<th>Discipline</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical And Computer Engineering</td>
<td>$321,571</td>
</tr>
<tr>
<td>Mechanical And Aerospace Engineering</td>
<td>$186,104</td>
</tr>
<tr>
<td>Orthopedic Surgery</td>
<td>$1,601,110</td>
</tr>
<tr>
<td>Neurosciences</td>
<td>$300,000</td>
</tr>
<tr>
<td>Radiology</td>
<td>$3,472,028</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>$5,880,813</strong></td>
</tr>
</tbody>
</table>

Neurological Surgery has engaged the services of the Vice Chancellor for Health Sciences’ Research Service Core (RSC) for pre- and post-award management since FY2015.

Neurological Surgery has recently recruited Thomas Beaumont, MD, PhD, and Najla Kfoury-Beaumont, PhD whose focus is the development of a Brain Tumor Epigenomics Laboratory which will contribute to a stable funding pattern in the coming years. Neurological Surgery is currently engaged in a nationwide search for a senior researcher to further develop and expand their research portfolio. They are part of a broader Moores Cancer Center strategy in conjunction with Drs. Jeremy Rich and Bob Hevner to support the neuro-oncology components of our NCI-designated comprehensive cancer center.

This short term view merely reflects the recent period of transition and we anticipate continued strength in research funding based on pending proposal submissions. Despite this interruption in funding, Neurological Surgery maintained robust academic productivity with over 96 peer reviewed publications and 4,092 citations in the 2018–2019 academic year.
Intermediate Department research objectives include:
- brain tumor SPORE grant
- Agility Center for Engineering
- R25 Training grant with Neurosciences

Neurological Surgery faculty currently support:
- U01 grant for StrokeNet
- U01 grant for Auditory Brain Stem Implants
- lead key trials administered by the Alpha Stem Cell clinic

Neurological Surgery will play a key role in the T. Denny Sanford $100M gift to study the neurobiology of empathy and compassion. Neurological Surgery is also poised to partner with the Department of Neurosciences in dispensing the net of the University of Southern California-Alzheimer's settlement for neurodegenerative disease (of a $50M total) upon Chancellor’s approval.

Eventually, these collaborative research efforts will be housed in a freestanding Neurological Institute. Examples of active research are reflected in the robust bibliographies provided as an appendix to this application. Key departmental research publications include:


“HDAC and PI3K Antagonists Cooperate to Inhibit Growth of MYC-Driven Medulloblastoma”, Cancer Cell

“First-in-Man Clinical Experience Using a High-Definition 3-Dimensional Exoscope System for Microneurosurgery”, Operative Neurosurgery


“PI-3K Inhibitors Preferentially Target CD15+ Cancer Stem Cell Population in SHH Driven Medulloblastoma”, PLOS ONE


SPACE ALLOCATION AND PHYSICAL FOOTPRINT

Primary administrative offices for Neurological Surgery’s faculty and staff are located in the East Campus Office Building near Jacobs Medical Center, with a small ancillary office footprint in Hillcrest. Educational facilities at the Center for the Future of Surgery provide highly technical teaching space.

Neurological Surgery clinics are located in Rancho Bernardo, Carlsbad, Hillcrest, La Jolla, the Koman Family Outpatient Pavilion, and Escondido, with new clinic space currently under renovation in the Perlman Medical Offices. In addition to Jacobs Medical Center and Hillcrest, neurosurgical service needs are provided at Tri-City Medical Center, Palomar Medical Center, the La Jolla VA Medical Center, and Rady Children’s Hospital.

Natural research locations for expansion would be to the Altman Clinical and Translational Research Institute, the Center for Novel Therapeutics, and Moores Cancer Center.
EDUCATION

The UC San Diego Neurological Surgery residency program has experienced a ten year period of outstanding stability and improvements. The program has been recognized for its outstanding academic accomplishments by being granted a rare increase in the number of resident positions in 2011, as well as a current maximum 10-year accreditation from the ACGME with no major citations. Career guidance and academic advice is consistently provided to residents with frequent one-on-one meetings. The educational and mentoring activities extend to all levels of neurological surgery, and range from working with younger faculty, the broad array of residents in the program, and our medical students who are considering careers in neurosurgery.

Several of our faculty and residents have been recognized with teaching awards, and have provided significant mentorship in research. Drs. Hoi Sang U, Jeffrey Steinberg and David Santiago-Dieppa have been awarded the Kaiser Excellence in Teaching Award. Dr. Arvin Wali has been awarded The Gold Humanism Honor Society Resident Teaching Award in Surgery. Drs. William Taylor and Joseph Ciacci have been awarded Resident Teaching Awards. Dr. Khalessi received the Golden Suture award from Neurological Surgery residents.

UC San Diego Neurological Surgery has led in national neurosurgery educational initiatives as part of the Society of Neurological Surgeons Residency Boot Camp Courses since their inception, and continues to serve on the committee for neurosurgery resident education courses at the national level. The West Coast regional Neurological Surgeons Residency Boot Camp Course has been hosted at UC San Diego’s Center for the Future of Surgery for the past 7 years. UC San Diego Neurological Surgery’s role in this course is expanding to include additional residents from throughout the United States, and top faculty from other programs in the country.

Dr. Joseph Ciacci, the Residency Director for Neurosurgery, also serves the UC San Diego School of Medicine as an Academic Community Director, and has done so from the inception of the new curriculum. The Academic Communities form one of the cornerstones of the Integrated Scientific Curriculum (ISC) in our School of Medicine. Dr. Ciacci is the only surgeon who has been selected as an Academic Community Director. This role encompasses mentoring responsibility, advocacy for students from diverse backgrounds, guiding students through academic difficulties, and other significant life events for medical students from all four years of the medical school classes. This connection has allowed UC San Diego Neurological Surgery to be a strong champion for increased diversity in the many surgical specialties, and we have helped students match to outstanding residency programs throughout the United States.
The following lists UC San Diego medical students who have recently matched in Neurological Surgery (Table 1.11).

<table>
<thead>
<tr>
<th>Year</th>
<th>Resident Graduate</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>2019</td>
<td>Altattar, Ali MD</td>
<td>University of Pittsburgh</td>
</tr>
<tr>
<td></td>
<td>Brandel, Michael MD</td>
<td>UC San Diego</td>
</tr>
<tr>
<td></td>
<td>Dalle Ore, Cecilia MD</td>
<td>UC San Francisco</td>
</tr>
<tr>
<td></td>
<td>Lopez Ramos, Christian MD MPH</td>
<td>OHSU</td>
</tr>
<tr>
<td></td>
<td>Schupper, Alex, MD</td>
<td>Mt. Sinai</td>
</tr>
<tr>
<td>2018</td>
<td>Ali, Mir Amaan MD</td>
<td>Vanderbilt</td>
</tr>
<tr>
<td></td>
<td>Carroll, Kate MD</td>
<td>University of Washington</td>
</tr>
<tr>
<td></td>
<td>Wali, Arvin MD MAS</td>
<td>UC San Diego</td>
</tr>
<tr>
<td>2017</td>
<td>Steed, Tyler MD PhD</td>
<td>Emory</td>
</tr>
<tr>
<td></td>
<td>Treiber, Jeffrey MD</td>
<td>Baylor</td>
</tr>
<tr>
<td></td>
<td>Wilson, Bayard MD</td>
<td>UC Los Angeles</td>
</tr>
<tr>
<td>2016</td>
<td>Hirshman, Brian MD</td>
<td>UC San Diego</td>
</tr>
<tr>
<td></td>
<td>Scheer, Justin MD</td>
<td>UC San Francisco</td>
</tr>
<tr>
<td></td>
<td>Zhou, Tianzan MD</td>
<td>Georgetown</td>
</tr>
<tr>
<td>2015</td>
<td>Marcus, Logan MD</td>
<td>UC Los Angeles</td>
</tr>
<tr>
<td></td>
<td>McCutcheon, Brandon MD</td>
<td>Mayo Clinic</td>
</tr>
<tr>
<td></td>
<td>Peck, Ted MD</td>
<td>University of Southern California</td>
</tr>
</tbody>
</table>

Program Structure
The seven year residency (including internship) provides comprehensive training in all aspects of modern neurosurgery, including neurosurgical critical care, neurotrauma, neurovascular surgery, brain tumors, functional neurosurgery, epilepsy, pediatric neurosurgery, peripheral nerve, and spine/spinal instrumentation surgery. The program is academic in its orientation, with input from world-renowned basic science researchers from the UC San Diego School of Medicine, the UC San Diego main campus, The Scripps Research Institute, and the Salk Institute. The program is also designed to be flexible in training and strives to accommodate specialized research and/or clinical interests.

During PGY-1 year, each intern spends 6 months on the neurosurgery service, where they learn to run the service and perform bedside procedures. For the remainder of the year, they spend time on neurocritical care, endovascular, trauma, and ENT services. The PGY-2 and PGY-3 years concentrate on developing skills in basic neurosurgery and in neurosurgical critical care. Introduction to operative techniques commences with rotations on both the Tumor and Spine services, and these years provide dedicated study in fields
within neurosurgery in order to allow the resident to develop mastery in these areas with undivided attention. This includes endovascular neurosurgery exposure, neuroradiology, and neuropathology as well as six months at Rady Children’s Hospital on the pediatric neurosurgery service.

Following this experience, the PGY-4 and PGY-5 years are dedicated to in-depth research and/or subspecialty residency training. The Senior Resident and Chief Resident years hold the maximum opportunity and responsibility as these residents oversee all neurosurgical care at both Hillcrest and Jacobs Medical Center, as well as selected cases at the Veterans Administration Medical Center in La Jolla.

In years past, residents have scored in the eightieth to ninetieth percentile on national neurosurgery board examinations and have far surpassed caseloads required by the American Board of Neurological Surgeons. The 2019 ABNS written exam scores were the highest in the program’s history.

**Medical Student Clerkships**
Dr. Martin Pham, one of our most recent faculty recruits, is currently the neurosurgery course director for medical student clerkships in neurosurgery. He directs the MS3’s and the mentoring of all visiting Fourth Year Students as well as UC San Diego fourth year students (Sub-I’s). There will be 12-16 visiting MS4 students for 2019-2020. We have hosted students from top medical schools across the country, including UC Los Angeles, Vanderbilt, NYMC, University of Texas, and Georgetown. The clerkships include weekly meetings with students that include specific didactic lectures and educational learning objectives. The students are guided through the creation of their own end-of-rotation presentation that they present to the neurosurgical faculty at Grand Rounds. Students are embedded in the neurosurgical service and participate in the care of neurosurgical patients for a highly meaningful experience.

**Grand Rounds and Academic Day Educational Activities**
Dr. Sharona Ben-Haim directs neurosurgery Grand Rounds and has developed a robust educational program. UC San Diego Neurological Surgery dedicates 3-4 hours per week to its educational mission and this time is divided into a 2-hour long Grand Rounds program, followed by a 1-hour long dedicated faculty/resident teaching and mentorship period. Grand Rounds is attended by all Neurological Surgery faculty members and residents, as well as all medical students currently on our third and fourth year rotations. Additionally, members of the Department of Neurosciences are invited to attend as well as faculty in the neuropathology, neuroradiology and orthopedic departments. The pediatric neurosurgery division from Rady Children’s Hospital present their cases at our conferences as well.

A portion of the Grand Rounds schedule includes a consistent monthly rotation of programming. The first Friday of the month is reserved for a 2-hour long Quality Assurance (Morbidity and Mortality) conference. During this time, residents present the details of all neurosurgical inpatients from the preceding month where any Quality Assurance questions were raised during patient admission. Educational content from the residents is often included into the presentation structure, and the cases are then discussed amongst the group. The second Friday of the month includes a one-hour long joint conference with the neuropathology and neuroradiology departments. During this conference, cases deemed to have high-
educational value are presented and discussed by a combination of the Neurological Surgery residents, neuropathology and neuroradiology fellows, allowing contributions from their various areas of expertise.

The remainder of the Grand Rounds programming during the 8am-10am time frame is more variable in order to meet the needs of Neurological Surgery and to capitalize on opportunities based on guest lecturers’ availability. There are 2-4 hours reserved in the program for guest lectures during a one-month period. These include a variety of basic science, translational, and clinical lectures given by a diverse assortment of faculty members both internally from UC San Diego as well as visiting faculty from outside institutions. These are focused broadly on topics relating to neurosurgery and the neurosciences, as well as a wide range of other disciplines from palliative care to cell biology to engineering. There is an emphasis on engaging the broader San Diego neurosurgery and neurosciences community by including lecturers from other local institutions. We also regularly include lectures from internal members of our department, including our chief residents as well as various faculty members.

Approximately twice per month, we set aside time within our program for Case Presentations. These are opportunities for our residents to present recent, interesting cases for the benefit of the education of the department. There is a faculty mentor assigned to each presentation with their goal being to facilitate teaching and discussion amongst the members of the department.

These two-hour long Grand Rounds programs are followed by a dedicated, one-hour long session from 10am-11am reserved for direct faculty-resident education and mentorship. During these sessions, the residents and one faculty member transition from the auditorium to a nearby conference room to facilitate an environment conducive to round-table discussion. During this time, the faculty member will present on topics of their personal expertise in a question-and-answer format. Our residents have found that this less-formal environment enables a lively discussion. Once per month, the 10am-11am hour is reserved for journal club, where several recent articles from the neurosurgical literature are chosen in advance and discussed.

The Chair meets alone with the residents once per month to confidentially discuss issues and suggestions regarding the residency program. Additionally, Neurological Surgery is initiating a Wednesday morning microsurgical video conference in the Neuro Hub for the 2019-20 academic year. This reflects our growing infrastructure to support these novel educational approaches; the team was recently featured in an AAMC tour of academic leaders at UC San Diego for these cutting edge offerings.

Our dedicated, academic teaching is continuously evolving to meet the needs of the department. Regular feedback is solicited from both the faculty and the residents, and adjustments to the programming are made accordingly.

Dr. Ciacci and Neurological Surgery resident Dr. Joel Martin—as well as colleagues from the Department of Radiology and UC San Diego’s Jacobs School of Engineering—were the recipients of a Galvanizing Engineering in Medicine (GEM) award. GEM, an initiative of the Altman Clinical and Translational Research Institute (ACTRI) and the UC San Diego Institute of Engineering in Medicine (IEM), supports projects that
identify clinical challenges for which engineering solutions can be developed and implemented to improve health care. Drs. Ciacci and Martin were awarded this special honor for their project entitled “Next-generation Spinal Cord Neuro-electronic Interface Implant for the Potential Treatment of Paralysis”. Among the different neuromodulation therapies for spinal cord injury, epidural and intraspinal cord stimulation and recording has shown promising results. The project’s goal was to develop a next-generation spinal cord neuro-electronic implant to treat paralysis. The implant is an intradural spinal cord stimulation device that includes novel microelectrodes, uses less power and is “smarter” and minimally invasive. The aims were threefold: to investigate the safety and optimal design parameters and device geometries in acute and chronic recording and stimulating in spinal cord injury pig models, with the goal of developing a chronic implant for a human patient; to use chronically implanted flexible electrodes to test the effect of periodic low-power spinal cord stimulation on recovery of function; and to determine the effect of the combined treatment composed of spinal parenchymal delivery of neural stem cells and periodic low power spinal cord stimulation on recovery of function. The innovation was measured by the flexibility of the surgical implant, and the fidelity and spatial arrangement of the recording and stimulating electrodes. Recorded data from the device aided in a variety of neuroscience research, ranging from pain, spinal cord injury, and brain-machine-interface engineering research.

Neurological Surgery resident Drs. Arvin Wali and Michael Brandel received an NIH TL1 pre-doctoral training grant through the ACTRI. The grant funds medical students to complete a clinical research training program consisting of a mentored clinical research project and a Master’s of Advanced Studies (MAS) in Clinical Research program. Drs. Christian Lopez and Peter Abraham similarly completed this program in neurosurgery as UC San Diego med students.

Resident Research and Publications
UC San Diego Neurological Surgery has a strong tradition of supporting and mentoring resident research and publications. Residents have been successful in collaborating across the spectrum of excellence in research surrounding UC San Diego and the La Jolla campus. The following is a list of selected recent resident publications:


FINANCIAL

Neurological Surgery has experienced consistent year-over-year growth in clinical revenue, despite the transition of four faculty (~20% of total dedicated UC San Diego faculty) in FY17. The new leadership has emphasized growth in faculty, revenue, work RVUs, and market share. Both academic division and Medical Center profitability of the Neurological Surgery program remain strong. Neurological Surgery’s professional fee revenue (Medical Group collections) has grown ~20% from $5.8M in 2016 to $7M (projected) in 2019. The $7M in collections is against $6.5M CARE payment (Table 1.12) to the clinical Department.

Neurological Surgery has nearly fully recovered via recruitment of new faculty and collections, and is on an upward growth trajectory. We model continued 6-8% growth in professional revenue per year in the next three years as recently hired faculty ramp up to full productivity, and new faculty are recruited to enhance continue our growth.

Table 1.12

<table>
<thead>
<tr>
<th></th>
<th>Neurological Surgery CARE Payment Clinical Revenue</th>
</tr>
</thead>
<tbody>
<tr>
<td>FY 2017</td>
<td>$5.5M</td>
</tr>
<tr>
<td>FY 2018</td>
<td>$6.2M</td>
</tr>
<tr>
<td>FY 2019-Annualized</td>
<td>$6.7M</td>
</tr>
</tbody>
</table>

In 2019, Neurological Surgery generated $15.4M on a 31% Contribution Margin.

{remaining financial information on this page redacted}
{page redacted}
{page redacted}
{page redacted}
{page redacted}
{page redacted}
PHILANTHROPY

Under Dr. Bob Carter, Neurological Surgery saw great progress in gaining philanthropic support. From fiscal year 2014 to fiscal year 2016, philanthropy increased by 530%. A key initiative in 2014 was the first Neurological Surgery faculty “giving campaign” which was launched in support of the Intraoperative Suite in Jacobs Medical Center. This initiative raised $336,000, showing true commitment by the faculty. Notably, the first endowed chair in the department was established in 2015: the Sharon B. and Lawrence F. Marshall, MD Presidential Chair in Neurosurgery. The first endowed visiting professorship – the John Alksne, MD Visiting Professorship – was established in 2017.

The Chairmanship search process temporarily interrupted philanthropic momentum. With Dr. Alexander Khaledi being named Chair, philanthropy is on the rise. It is anticipated that Neurological Surgery will raise nearly $5M in philanthropic support in fiscal year 2019. This would be a 253% increase over Neurological Surgery’s best philanthropic year ever.

Due to Neurological Surgery’s long-planned expansion of the Center for the Future of Surgery to include a microsurgical lab and hybrid OR/angiography suite, industry partners have enthusiastically provided educational support. They are further motivated by the broadcast and curriculum opportunities afforded by the Neuro Hub. By the end of FY19 - which coincides with the expansion completion timeline- it is anticipated that Neurological Surgery will be responsible for $4.95M in donated equipment support from both new and existing industry partners. It is important to note that the fair market value of this equipment equates to nearly $20M. Please see Table 1.19 below for a summary of fundraising activity for FY14–FY19.

Table 1.19

<table>
<thead>
<tr>
<th></th>
<th>FY 13/14</th>
<th>FY 14/15</th>
<th>FY 15/16</th>
<th>FY 16/17</th>
<th>FY 17/18</th>
<th>FY 18/19</th>
<th>Anticipated Final - FY19</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0</td>
<td>$369,490</td>
<td>$738,399</td>
<td>$1,959,982</td>
<td>$570,478</td>
<td>$365,270</td>
<td>$520,750</td>
<td>$4,961,554</td>
</tr>
</tbody>
</table>

Please see Table 1.19 below for a summary of fundraising activity for FY14–FY19.
CLOSING SUMMARY

In summary, the UC San Diego clinical Department of Neurological Surgery has expanded its clinical, research, and educational performance significantly over the last five years.

Designation as a School of Medicine academic department represents a natural progression that will:

1. Recognize our position as a top neurosurgical department nationally
2. Allow formal UCOP recognition of planned Neurological Institute
3. Facilitate adjunct academic appointments that will allow greater institutional level collaboration
4. Contribute to the recruitment and retention of leading faculty through independent promotion processes
5. Maintain current positive trajectory in volume, impact, and visibility
6. Improve philanthropic position for dedicated endowed chairs and research funds
7. Mirror neurological surgery standing at sister UC and peer institutions
APPENDIX I

LETTERS OF SUPPORT: LEADERSHIP AND HS FACULTY COUNCIL

(Found on pages 51-54; Alphabetized by Author)
January 28, 2020

Maripat Corr, MD
Chair, Academic Senate
UC San Diego Division
MC 0002

Dear Dr. Corr:

As Vice Chancellor for Health Sciences, I am pleased to write this strong letter of support for the transition of Neurosurgery from a hospital department and division of the Department of Surgery to an independent academic department within the School of Medicine. I believe this represents a natural progression from a series of shared strategic conversations with Dr. Alexander Khaleessi and his predecessor, Dr. Robert Carter. This transition additionally has unanimous support from the Health Sciences Faculty Council and Interim Dean Steven Garfin.

Neurosurgery’s evolution has accelerated over the past several years, and its faculty and residents have been strong partners to our tripartite mission. Its residency program has not only become highly recognized by its peers—in fact, it recently received a maximum accreditation from the Neurological Surgery Residency Review Committee—but has also maintained an exceptional level of quality, as evidenced by numerous School of Medicine teaching awards. Its research portfolio continues to grow and clearly has strong synergies with allied departments in Health Sciences, such as Neurosciences, Radiology, Emergency Medicine, Radiation Medicine and Applied Sciences, and Orthopaedic Surgery. The letters of support from these departments demonstrate the enthusiasm and productivity of these partnerships, which I expect will proliferate as the department grows its research faculty. Clinical productivity continues to expand, with the number of annual cases increasing from 2,200 to 3,400, providing a strong financial foundation for its faculty and leadership to foster novel academic and educational programs.

I remain strongly supportive of Dr. Khaleessi’s vision of the department. Neurosurgery remains one of the few specialties at UC San Diego consistently ranked in the top 50 in US News & World Report. This transition reflects the distinct training and academic culture of Neurosurgery and mirrors the organization of other UC institutions and every other top 25 Neurosurgery program. As Health Sciences actively seeks to broaden its diverse community, all School of Medicine departments, including Neurosurgery, are currently engaged in developing a three-year plan to assess and enhance diversity across several domains, including faculty, staff, and resident representation. Neurosurgery is also hosting the 30th anniversary of Women in Neurosurgery event and providing additional funding for UC San Diego trainees to participate in the event.

More broadly, academic department status will also provide further impetus for the formal organization of Neurosurgery and Neurosciences into a comprehensive Neurological Institute, a long-held vision that will greatly benefit UC San Diego as a whole. (A UC San Diego Neurological Institute would be perennially rank No. 1 or
No. 2 in NIH funding!) This institute has incredible potential to elevate the University’s prominence across multiple disciplines and represents the collaborative approach to healthcare that is a hallmark of UC San Diego Health Sciences.

I am confident that Neurosurgery has the required framework to thrive as an independent department, one that will bring further academic, educational, and clinical recognition to UC San Diego. I recommend departmental status for Neurosurgery with enthusiasm and without reservation and would be happy to discuss this further at your request.

Sincerely,

David A. Brenner, MD
Vice Chancellor for Health Sciences

cc: S. Constable
    S. Garfin
    R. Ross
September 23, 2019

David A. Brenner, MD
Vice Chancellor for Health Sciences
UC San Diego
MC 0602
La Jolla, CA  92093

Dear Dr. Brenner,

I am pleased to endorse the application to establish a Department of Neurological Surgery at UC San Diego. As articulated in the proposal, and its accompanying materials, I believe that Neurological Surgery has demonstrated a strong commitment to the Health Sciences tripartite mission and created the necessary infrastructure to support this transition. Creating an academic Department of Neurological Surgery will also enhance the stature of the School of Medicine among other top-tier peer institutions.

I would like to recommend Alexander Khalessi serve as Interim Chair until a permanent chair is recruited. Dr. Khalessi was selected from a national pool of candidates to serve as Chair of the hospital department, and I am confident he has the leadership experience to incorporate the appropriate elements into an independent academic unit. We will initiate a search for a permanent Chair once the department is approved by the UC San Diego Academic Senate and Chancellor Khosla.

Sincerely,

Steven R. Garfin, MD
Interim Dean
School of Medicine

cc:  R. Ross
December 12, 2019

ALEXANDER A. KHALESSI, M.D.
Department of Neurological Surgery
7893

Subject: New Academic Department of Neurological Surgery

Dear Dr. Khalessi:

Thank you for attending the November 5, 2019 meeting of the Health Sciences Faculty Council (HSFC). The Council was very impressed by your presentation. I am pleased to inform you that the HSFC has voted unanimously to endorse the establishment of a new academic Department of Neurological Surgery.

We wish you success as you continue your efforts in creating this department.

Sincerely,

Elizabeth Winzeler, Ph.D.
Chair, Health Sciences Faculty Council

c: David A. Brenner
Morgan Tupper
APPENDIX II

LETTERS OF SUPPORT: DEPARTMENT CHAIRS AND COUNCIL

(Found on pages 56-66)
June 21st, 2019

Chancellor Pradeep Khosla

Re: Letter in Support of Neurosurgery as an Academic Department

Dear Chancellor Khosla,

As Chair of the Department of Neurosciences and Co-Director of the Neurosciences Institute, I am delighted to provide my strongest support for the establishment of Neurosurgery as an academic department.

As you are aware, the Department of Neurosurgery has clearly distinguished itself in serving the tripartite mission and, under the new visionary leadership of Dr. Alexander Khalessi, has taken a new trajectory toward cementing its status as a highly renowned department that leads research in a broad array of basic and clinical research in science relevant to neurosurgery and neuro-oncology.

The establishment of the Department of Neurosurgery as an academic department is well-deserved and sends a strong signal of our institution's commitment to lead the nation in neurosurgical care, education, and research. With the team that Dr. Khalessi has assembled and continues to build upon, combined with his dedicated and skilled leadership, I have no doubt that the UC San Diego Department of Neurosurgery will meet and exceed all measures of success applied to our university's academic departments.

Thank you for your support of the broad reaching endeavors we are pursuing together in the Neurosciences Institute and for considering this request of our close and critical partner.

Sincerely,

James B. Brewer
Professor and Chair, Department of Neuroscience
Director, Shiley-Marcos Alzheimer’s Disease Research Center (ADRC)
June 5, 2019

To: Steve Garfin, MD
   Dean, School of Medicine
   David Brenner, MD
   Vice-Chancellor for Health Sciences
   University of California, San Diego

Re: Application of Neurosurgery for Academic Department Status

Dear Drs. Garfin and Brenner,

I am pleased to write this letter of strong and enthusiastic support for the transition of Neurosurgery from hospital department to independent academic department in the School of Medicine at the University of California, San Diego. As Chair and longstanding faculty member of the Department of Emergency Medicine which underwent a similar transition a few years ago, I believe that the Division of Neurosurgery is well-deserving and also well-positioned for success as an independent academic department at our University.

As you are aware, under the recent leadership of Dr. Alexander Khalessi, as well as his predecessor, Dr. Bob Carter, Neurosurgery has undergone tremendous growth over the last few years with a robust clinical practice, highly-ranked teaching programs, and a burgeoning research portfolio. Many current and emeriti faculty are world-renowned in the specialty, and their ranks include our former Medical School Dean and Vice Chancellor for Health Sciences. As an Emergency physician I can attest to the outstanding clinical care provided by the Division’s faculty for patients in acute need of emergent neurosurgical attention. Similarly, the education programs including its residency and fellowship trainings programs are highly regarded and attract the very best students from top-tier schools. Given that these programs are accredited separately from general or other surgical specialties, it is clear that Neurosurgery is a distinct academic specialty with an independent body of knowledge and
experience. In addition, the faculty’s research productivity has grown markedly and a number of faculty are recognized at a national level for their innovative clinical programs.

By becoming an academic department, Neurosurgery will continue and indeed accelerate this impressive trajectory. Given that the division is financially self-supporting as an independent hospital department already, the risk of such a transition is minimal. In fact, the greater risk lies in not transitioning Neurosurgery to a full academic department. Our sister institutions at UCSF, UCLA, UC Davis and UC Irvine, have already established academic Neurosurgery Departments. Maintaining the current status will put UCSD at a disadvantage when recruiting faculty and trainees compared with other institutions that recognize Neurosurgery as a distinct specialty in the House of Medicine and in academia.

As Chair of an academic department that made a similar transition just in the past decade (in a specialty whose board certification developed much later than Neurosurgery), I look forward to collaborating with a future academic Department of Neurosurgery at UCSD - continuing to build outstanding educational, research, and academic opportunities for both our Departments. I foresee strong collaborations with my Department not only in acute neurosurgical care in the Emergency Department, but also with our new geriatric and senior initiatives now taking shape.

In summary, I fully support the transition of Neurosurgery from hospital department to full academic department in the School of Medicine. The division’s record of clinical service, education, and research is outstanding and the transition to an academic department will insure continued success into the future. I have no doubt that an academic Department of Neurosurgery will contribute immensely to the missions of the School of Medicine and University.

Should you have any further questions for me, please do not hesitate to contact me at any time.

Sincerely,

Theodore Chan, MD
Professor and Chair
Department of Emergency Medicine
University of California, School of Medicine
July 20, 2019

To: Pradeep K. Khosla, Ph.D.
    Chancellor, UC San Diego

CC: Steve R. Garfin, M.D.
    Interim-Dean, School of Medicine

David A. Brenner, M.D.
    Vice Chancellor, Health Sciences

Re: Application of Neurosurgery for Academic Department Status

Dear Chancellor Khosla,

I am writing to express my thoughts and considerations regarding the proposed transition of the Division of Neurosurgery to full departmental status. By way of context, shortly after my arrival as Chair of the Department of Surgery in 2015, Vice-Chancellor and then Dean, David Brenner set in motion a process to transition the Division of Neurosurgery to full departmental status. In doing so, Dr. Brenner was fulfilling a commitment to then-division chief Dr. Bob Carter who had been recruited from Boston by my predecessor, Dr. Mark Talamini. Drs. Brenner and Carter were confident that this was a natural and necessary transition for Neurosurgery, and one that was in keeping with organizational patterns present in the leading academic medical institutions. Indeed, fewer than 10% of academic neurosurgery programs remain housed in Departments of Surgery nationwide (i.e. most are fully independent departments).

To be transparent it was my recommendation at that time that Neurosurgery remain as a division in the Department of Surgery. While the division possessed very solid educational programs that were and are distinct from other surgical disciplines, my principal objection was that the division was too small and its clinical and research programs were not at a level of maturity that would be consistent with departmental status. While I understood the desire on the part of Drs. Carter (and Khalessi) to be independent, I did not, and still do not fully accept many of the purported advantages of departmental status with respect to the ability to recruit top talent, develop top tier research and educational programs, and successfully advocate within the health system for resources needed to build great clinical programs. In fact, all of these are attainable under a divisional structure with the right departmental
and divisional leadership. (By way of example, we have achieved all of these things in the Division of Otolaryngology over the past four years.). Furthermore, I do believe that there are benefits for surgical specialty programs in maintaining a common bond and infrastructure through the umbrella of a broad surgery department structure. Breaking up a robust and successful Department of Surgery into smaller departments is not always advantageous.

While I will not speak for Dr. Brenner, it was my impression that he was much more convinced of the benefits of a transition than I. Under his directive, Neurosurgery has functioned over the past four years as a “hospital-based department” with a substantial degree of autonomy in administering its clinical and academic programs. While I may have disagreed, I respected Dr. Brenner’s decision and have worked to support his desire to see this transition succeed. Dr. Carter and subsequently Dr. Khalessi have in my opinion, provided excellent leadership of Neurosurgery during this transitional period. It should be noted that in response to the departure of Dr. Carter, a national search process was conducted to identify and recruit a new leader of Neurosurgery. To be fair, I believe that a narrative of departmental transition was incorporated into the discussions with the candidates, including Dr. Khalessi. I will also point out that Dr. Khalessi has put together a thorough application that is on par with the recent and successful application of the Division of Urology which very recently secured Departmental status.

The Division of Neurosurgery in our modest scale academic medical enterprise will like other surgical subspecialty departments be relatively small. I still maintain reservations regarding the level of maturity of its clinical and research programs with respect to the justification for Departmental status. Nonetheless, I am supportive of Dr. Khalessi who I believe has the skills and vision to bring the Division forward. He has secured a number of excellent junior level hires and has articulated a strategic plan for advancing the research programs.

In summary, I am confident in the leadership of Neurosurgery and I strongly suspect that the transition to Departmental status will ultimately prove to be a success. The separation will afford Neurosurgery more freedom to creatively pursue its missions and reach its full potential. These missions include partnership with the Department of Neurosciences in the development of a UCOP-recognized Neurological Institute. I am fully committed to working collaboratively with Dr. Khalessi in facilitating this transition should this be the direction that you choose to pursue.

Sincerely,

Bryan M. Clary, M.D., M.B.A.
Professor and Chair, Department of Surgery
M.J. Orloff Family Endowed Chair in Surgery
Surgeon-in-Chief
UC San Diego Health
Dear Drs. Garfin and Brenner,

I would like to express my strong support for the establishment of a UCSD School of Medicine (SOM) Department of Neurological Surgery. As Chair of the SOM Department of Radiation Medicine and Applied Sciences (RMAS), I am intimately aware of the contributions that Dr. Khalessi and his faculty have made to the institution as a whole across all academic missions and the importance of the existence of an independent Department has for the future of neurological surgery here at UCSD.

Dr. Khalessi and his colleagues are extremely committed to providing cutting-edge neurosurgical care to our patients and have developed multiple novel clinical programs over the years. Many neurosurgical faculty are integral members of the Moores Cancer Center and greatly contribute to the multi-disciplinary care of brain tumor patients, both adults and children. Moreover, department faculty are involved with the care of patients well beyond those with benign and malignant tumors. Their clinical competence and expertise is extremely high and valued by all of us. It is a true honor and pleasure to call them my colleagues.

In addition to their clinical expertise, multiple neurosurgical faculty are involved with research and closely collaborate with several of my faculty on novel research projects including new approaches to delivering radiation therapy sparing eloquent areas in the brain. I am also impressed with the involvement of neurosurgical faculty in the medical school and in the teaching and training of residents. As a longtime Community Director, Joe Ciacci MD is closely involved in the education of medical students and is highly respected for his contributions and commitment. Multiple other neurosurgical faculty take service to heart and participate in a wide variety of hospital and university committees. Neurological surgery is truly a valuable member of our institution and helps raise the reputation of UCSD as a whole.

The time is truly right to establish an independent UCSD SOM Department of Neurological Surgery, a status held by other UC Schools and leading academic institutions throughout the country. Over his tenure as Chair, Dr. Khalessi has demonstrated his ability to lead the faculty and has greatly contributed to the Health System as a whole. Failure to establish an independent department will have an adverse impact on recruitments of future chairs, future clinical and research faculty, residents, post-docs and graduate students and their ability to successfully compete for grant funding.

Please do not hesitate to contact me with any future questions.
Regarding: Neurosurgery academic department status, letter of support.

Vice-Chancellor Brenner;

Please accept this strong letter of support regarding granting the Department of Neurosurgery at UC San Diego academic department status. As a lifelong academician, interventional neuroradiologist, and diagnostic neuroradiologist I have practiced at the intersection of radiology, neurosurgery, and neurology. I have been privileged to hold appointments and to have worked in well-respected and recognized academic departments including Stanford, Massachusetts General Hospital, the Brigham and Women’s Hospital, Boston University, and now UC San Diego.

I was privileged to serve as chair of radiology at Boston University for over eleven years, and part of my body of work was the unsolicited decision to first separate radiation oncology as a clinical department, and then to grant radiation oncology independent academic department status. This empowerment resulted in a stronger and infinitely more academically competitive radiation oncology department that tangibly competed in a more robust and effective manner where recruitment was concerned, and resulted in a department that proudly and more autonomously planned its own course and future.

As would be expected, I have a high degree of familiarity with my current and prior housing departments, and I am also most familiar with a large number of departments and academic medical centers I have visited or collaborated with in my specialty over the past 26 years since my initial academic appointment. I am well acquainted with all radiology chairs at the top twenty departments by dint of my currently serving as president of the society of academic radiology chairs, and I am also professionally and personally acquainted with many of the neurosurgery and neurology chairs in the top twenty neurosurgery and neurology departments. Given this lifelong familiarity, I believe I am suitably qualified to speak candidly about the need for expedient and unencumbered conferral of full academic status to the department of neurosurgery.

Insofar as my perspective is concerned, there are at least two major benefits related to the conferral of departmental status to Neurosurgery at UC San Diego; the first is elevation of the
department to the necessary tier in order to grant it the essential luster to compete in recruitment and retention of world class translational and fundamental scientists and practitioners, and the second is to recognize and empower the department regarding the high degree of unique and internal expertise it possesses regarding its own academic and intellectual domain.

To elaborate, renowned scientists and expert neurosurgeons both internally and externally will look at UC San Diego through the lens of our competing institutions, and without academic departmental status we will lack the level playing field necessary to effectively compete with the top twenty neurosurgical departments in the US. Currently, we are at such a disadvantage when compared with UC San Francisco, UC Los Angeles, UC Davis, and UC Irvine. Academic departmental status demonstrates the earnest commitment of the university to promoting and celebrating the essential translational role that neurosurgery plays in connecting bench neuroscience with translation to bedside, and then from bedside to marketplace delivery of surgical care. To possess the premier neuroscience program in the country such as ours without also empowering an independent and robust academic neurosurgical department places an unnecessary ceiling to growth where the accomplishments of the cumulative enterprise are concerned. The equity established by conferring academic departmental status to neurosurgery will also lower barriers and facilitate forging novel and direct institutional partnerships with other fundamental and empowered UC San Diego innovators, such as with the school of pharmacology, school of engineering, or school of global policy.

With Dr. Alexander Khalessi serving as the chair of neurosurgery, the department benefits uniquely from his established credibility as a thoughtful and responsible strategist who is consistently mindful of the larger institutional needs and opportunities in every instance. I can think of no better leader to ensure the preservation and strengthening of the medical school and university while forging the unique benefits to science and innovation that can only be made possible by conferral of academic departmental status.

Please allow me to restate not only my support of neurosurgery in securing academic departmental status, also my belief that this is an essential strategic need for our medical school and university, and that Dr. Alexander Khalessi will serve as an exceptional guarantor for this necessary step in our development as we seek to realize our full potential.

Most Sincerely,

[Signature]

Alexander Norbash, MD, MS
February 3, 2020

Dear Dr. Ross:

This letter is written at your request, to represent and summarize feedback that I obtained from the Health Sciences Chairs (HSC), regarding the establishment of the Department of Neurosurgery as the 19th Academic Department in the School of Medicine at the University of California, San Diego. While some Chairs wrote very comprehensive letters, others emailed me directly with shorter feedback and others spoke with me without any written comments about establishing such a Department.

In general, there is strong support from the various HSC for establishing the Department of Neurosurgery. The reasons are summarized as follows:

1. The overwhelming schools of medicine across the nation have such Departments, and UCSD is in the very small minority at present for not having such a Department. Furthermore, all the UC campuses and sister schools do have a Department of Neurosurgery, and, if UCSD continues not to have such a Department, this would jeopardize our ability to compete when recruiting faculty and trainees.
2. In general, and through the collective experience of the HSC, establishing an Academic Department of Neurosurgery would foster and facilitate its growth in all three missions. Failure to do so would stunt its growth.
3. Establishing such a Department would be necessary since the discipline in general has grown greatly in the past few decades. In addition, because of the good trajectory of the current hospital Department, the HSC believe that the risk is not in establishing such a Department but in not establishing one.
4. In general, HSC were laudable of the leadership of the current Hospital Chair, Dr. Khalessi, and his achievements so far; hence they believe that we have the right leader for such a transition.

There were, however, some HS Department chairs who felt that:

a) It might be premature to transition into such a Department because the current Hospital Department is rather small; and that they have reservations about the maturity of its programs.
b) Some chairs expressed reservations about whether such a Department can grow academically since the current primary faculty are mostly clinical and that it does not appear that the academic mission at present is being fostered.

In summary, although there were some reservations, most of the HSC support the establishment of an Academic Department of Neurosurgery. I am happy to discuss this further with you in case you have any questions.

All the best,

Gaby
Alexander A. Khalessi, MD, MBA, MS, is a board-certified neurosurgeon who specializes in cranial and endovascular surgery. He is Chair of the clinical Department of Neurological Surgery, and Professor of Surgery, Radiology and Neurosciences. He provides both open surgical and catheter-based approaches to complex neurosurgical problems, including brain tumors, aneurysms, arteriovenous malformations (AVM), and carotid disease. His vascular training informs his surgical approach to complex tumors in the brain. He is a fellow of the American Association of Neurological Surgeons (FAANS), American College of Surgeons (FACS), American Heart Association Stroke Council (FAHA), and senior member of the Society of NeuroInterventional Surgery (SNIS).

Dr. Khalessi is an active clinical leader in the field of neurosurgery and the treatment of cerebrovascular disease. He serves on the Interventional Advisory Panel for the StrokeNet Steering Committee. Developed by National Institutes of Health (NIH), StrokeNet is a stroke trials network that conducts clinical trials and research studies to advance acute stroke treatment and prevention, and recovery and rehabilitation following a stroke.

Dr. Khalessi serves on the Executive Committee of the Congress of Neurological Surgeons (CNS) and represents the CNS and American Association of Neurological Surgeons to the American College of Radiology. Dr. Khalessi has further served as Scientific Program Chair and Annual Meeting Chair for CNS. Regionally, Dr. Khalessi served two terms as president of the San Diego Academy of Neurological Surgery.

His research interests include open surgical and endovascular therapies for intracranial aneurysms, extracranial carotid disease, large-vessel ischemic stroke, and intensive care management and prognosis in head injury. Dr. Khalessi has been a contributing author to the national guidelines efforts that define clinical practice standards, and was a writing group member the American Heart Association/American Stroke Association’s guidelines regarding both the extended use of IV-tPA and mechanical thrombectomy for ischemic stroke.

Dr. Khalessi is a section editor for Cerebrovascular and Endovascular Neurosurgery for World Neurosurgery, the official journal of the World Federation of Neurological Societies (WFNS). He further
chairs the Guidelines Committee for the CNS/AANS Joint Cerebrovascular Section and has authored Multi-
Society Consensus Training Guidelines for *Neurovascular Surgery*.

As a Professor of Surgery, Radiology and Neurosciences, he directs national and international courses,
including the intermediate resident course for the Society of Neurological Surgeons (SNS). Dr. Khalessi is a
full member of the Senior Society of Neurological Surgeons (SNS). Dr. Khalessi served on the
Accreditation Council for Graduate Medical Education (ACGME) Residency Review Committee (RRC) for
Neurological Surgery and the editorial board for the *Journal of Graduate Medical Education*. He was also
named as an ACGME working group member for Neurosurgery and Endovascular Surgical Neuroradiology
Milestone Projects.

He completed his endovascular neurosurgery fellowship at SUNY Buffalo and neurosurgical residency at
University of Southern California. Dr. Khalessi earned his medical degree at Johns Hopkins School of
Medicine and his Master of Business Administration from Massachusetts Institute of Technology (MIT)
Sloan School of Management.

John F. Alksne, MD, is a board-certified neurosurgeon who focuses on the care of patients with
neurological conditions such as trigeminal neuralgia (tic doloreux/facial pain), facial tic, glossopharyngeal
neuralgia, and acoustic neuroma.

Dr. Alksne has pioneered new surgical techniques and technologies to advance the treatment of diseases
and disorders of the central nervous system. He established the Epilepsy Surgery Program at UC San Diego
Health, and was honored with a special commendation by the Epilepsy Society of San Diego County in
1992. He was instrumental in establishing the UC San Diego Program in Gene Therapy, which includes
research, training and clinical trials.

As Professor Emeritus of Surgery at UC San Diego School of Medicine, Dr. Alksne trains residents and
fellows. He joined UC San Diego in 1971 as a professor of neurological surgery and served as Chief of the
Division of Neurosurgery until 1995. He was appointed Dean of the School of Medicine in 1992, Vice
Chancellor for Health Sciences in 1994, and continued in both positions until 1999.

Dr. Alksne was Chief of Neurosurgery at Harbor General Hospital at UC Los Angeles from 1964-67 and
Professor and Chair of Neurological Surgery at the Medical College of Virginia from 1967-1971.
He completed his residency training at the University of Washington in Seattle, where he also earned his medical degree.

Dr. Alksne is a member of many scientific and professional societies, including the American Association of Neurological Surgeons, the Latin American Federation of Neurosurgeons, the World Society of Stereotactic and Functional Neurosurgery, the Association of American Medical Colleges, and the Association of Academic Health Centers.

David Barba, MD, is a board-certified neurosurgeon focusing on surgical treatment of people with epilepsy and movement disorders.

While often presumed a last resort, surgery can be performed as a highly effective therapy for epilepsy, movement disorders (such as Parkinson’s disease and essential tremor), and certain pain syndromes (trigeminal neuralgia). This type of surgery is called functional neurosurgery. Dr. Barba has extensive experience and training in functional neurosurgical techniques including microsurgery, deep brain stimulation, vagal nerve stimulation, awake brain surgery and stereotactic radiosurgery. He also performs surgery to remove spinal and brain malignancies, pituitary tumors, and to relieve spinal conditions including sciatica.

Dr. Barba joined the neurosurgical faculty at UC San Diego Health in 1988. He has been invited as a distinguished lecturer or panelist at over 50 conferences. He is also a member of many scientific and professional societies, including the American Association for the Advancement of Science, the San Diego Academy of Neurological Surgeons, and the Research Society of Neurological Surgeons.

As a professor in the Department of Surgery, Dr. Barba trains surgeons during their fellowship as well as established surgeons from medical centers across the country on functional neurosurgical techniques. He is active in clinical research including studies of brain activity related to language and as a participant and consultant in studies of gene therapy for neurodegenerative diseases including Alzheimer’s and Parkinson’s diseases.

Dr. Barba did a research fellowship in microsurgical techniques for cerebral aneurysms and the posterior fossa at the Shinshu University in Matsumoto, Japan. He was also the EC-IC bypass fellow at the University of Western Ontario in London, Ontario, Canada. In addition, Dr. Barba spent two years as a
senior research fellow at the National Institute of Neurological and Communicative Disorders and Stroke at the National Institutes of Health in Bethesda, Maryland. He completed a residency in neurological surgery at UC San Diego School of Medicine.

Dr. Barba earned his medical degree at University of Southern California Keck School of Medicine. He is board-certified in neurological surgery and is a fellow of the American Association of Neurological Surgeons (FAANS).

Thomas Beaumont, MD, PhD, is a neurosurgeon who specializes in skull base and minimally invasive cranial surgery. He performs both open microsurgery and endoscopic approaches for brain, skull base and pituitary tumors where he tailors the surgical approach to provide maximally effective yet minimally invasive neurosurgical care. Dr. Beaumont has a passion for the treatment of meningiomas, pituitary tumors, craniopharyngiomas and clival chordomas, and is an expert in the endoscopic endonasal approach (EEA). Additionally, he treats cavernous malformations, cranial nerve compression syndromes and craniocervical junction disease.

Also trained as a scientist, Dr. Beaumont has extensive research experience in genomics and epigenetics and has received funding from the National Institutes of Health (NIH). Dr. Beaumont currently studies the role of chromatin remodeling in tumorigenesis with the goal of developing novel targeted therapeutics for aggressive skull base tumors. He also conducts clinical outcomes studies and has developed risk prediction models for rare clinical diseases.

As an assistant professor of clinical surgery, Dr. Beaumont instructs medical students, residents and fellows at UC San Diego School of Medicine. He is widely involved in scholarly activities and is an active reviewer for several neurosurgical journals and grant agencies.

Dr. Beaumont completed a fellowship in skull base and minimally invasive cranial surgery at The Ohio State University. He completed a residency in neurological surgery at Washington University School of Medicine in St. Louis. Dr. Beaumont earned both his medical and doctoral degrees in a combined program at Wayne State University School of Medicine.
He is a member of many professional organizations, including the American Association of Neurological Surgeons, the Congress of Neurological Surgeons, the North American Skull Base Society and the Society for Neuro-Oncology.

Sharona Ben-Haim, MD, is a neurosurgeon who specializes in caring for people with treatment-resistant epilepsy, chronic pain including facial pain and trigeminal neuralgia, spasticity, and movement disorders such as Parkinson’s disease, dystonia and essential tremor. She performs deep brain stimulation (DBS) surgeries, including "awake" microelectrode-guided DBS and "asleep" MRI- and CT-guided DBS.

She is an expert in leading-edge surgical epilepsy treatments, including selective temporal lobectomies, responsive neurostimulation, and MRI-guided laser induced interstitial thermotherapy (laser ablation) for treatment-resistant epilepsy as well as gamma knife radiosurgery.

Dr. Ben-Haim’s research is focused on novel tools that will help localize and treat epileptic seizures more selectively using minimally invasive techniques. Dr. Ben-Haim’s work in movement disorders and improvements in technique for deep-brain stimulation insertion has been published in numerous peer-reviewed journals, including *European Spine Journal, Neurosurgery, Journal of Craniofacial Surgery, World Neurosurgery, Pediatric Neurosurgery, Neuropathology, and Stereotactic and Functional Neurosurgery*. In 2014, she authored a book chapter for *Textbook of Cortical Brain Stimulation*.

Dr. Ben-Haim has been invited to present nationally on topics including risk factors for hemorrhage during deep brain stimulation and occupational hazards in neurosurgical training. She also serves as a neurotrauma consultant to the National Football League.

She completed a fellowship in epilepsy and functional surgery at Yale School of Medicine in New Haven, Connecticut, and was a visiting fellow in functional neurosurgery at University of Oxford in Oxford, England. Dr. Ben-Haim completed residency training in neurosurgery at Mount Sinai Medical Center in New York. She earned her medical degree from UC San Diego School of Medicine.

Dr. Ben-Haim has received several awards over the years, including the Robert E. Hertzka Student Leadership Award from the San Diego County Medical Society, and the Grace Fimognari Memorial Award from the Department of Molecular and Cell Biology, Honors Research Committee at University of California, Berkeley. She serves as neurosurgeon liaison to the executive committees for the American
Joseph Ciacci, MD, is a board-certified neurosurgeon with extensive experience in neuro-oncology of the spine and brain. Dr. Ciacci’s primary interests are tumors of the spine and brain, complex spinal reconstruction, and stereotactic radiosurgery, a form of radiation therapy that focuses high-power energy on a small area of the body.

As a professor of clinical surgery, Dr. Ciacci instructs medical students, residents and fellows at UC San Diego School of Medicine. He also directs the Neurosurgery Residency Program.

Dr. Ciacci was on the faculty at the Johns Hopkins School of Medicine for nine years prior to joining the faculty at UC San Diego. He also has been a visiting professor and delivered lectures throughout the U.S. and internationally.

He completed neuro-oncology fellowships at UC Los Angeles and the National Institutes of Health, completed his residency at Northwestern Memorial Hospital in Chicago, and interned at Mount Sinai School of Medicine in New York. He earned his medical degree from University of Buffalo, SUNY School of Medicine and Biomedical Sciences. He is board certified in neurological surgery.

Rick Friedman, MD, PhD is a board-certified neurotologist, a specialist who treats neurological disorders of the ear, and is a world-renowned expert in the treatment of acoustic neuroma. In addition to leading the acoustic neuroma program with Dr. Marc Schwartz, he also treats meningioma, neurofibromatosis
type 2 (NF2), glomus tumors, hearing impairment, otosclerosis, Meniere’s disease, cholesteatoma, superior canal dehiscence and chronic ear infections.

As a professor in the Department of Surgery, Dr. Friedman trains medical students, residents and fellows at UC San Diego School of Medicine. He receives funding from the National Institutes of Health (NIH) for his research on the genetics of common forms of hearing loss.

Prior to joining UC San Diego Health, Dr. Friedman served as the division director of otology, neurotology and skull base surgery at the Keck School of Medicine of USC. He was also the director of the USC Acoustic Neuroma Center and has experience treating over 1,000 acoustic neuromas. In addition, he served as medical director of the Cranial Base Surgical Center at Cedars Sinai Medical Center in Los Angeles. Dr. Friedman completed his fellowship training in neurotology at House Ear Clinic in Los Angeles. He did his residency training in otolaryngology at UC San Diego School of Medicine, where he also earned his medical degree. He holds a doctorate in molecular pathology, also from UC San Diego. He is board certified in neurotology.

A popular speaker, Dr. Friedman has presented his work at more than 30 medical conferences around the world. He has published some 100 articles and book chapters in peer-reviewed publications such as Human Molecular Genetics, American Journal of Medical Genetics, Otology & Neurotology and Laryngoscope, to name a few. Dr. Friedman belongs to most of the societies in his area of specialization, including the American Academy of Otolaryngology - Head and Neck Surgery, the American Otological Society, the Acoustic Neuroma Association-Medical Advisory Board (co-chair), the American Neurotology Society, the North American Skull Base Society, the Triological Society, and the NIH/National Institute of Deafness and other Communication Disorders (NIDCD), where he serves as an ad hoc reviewer.

David Gonda, MD, is a pediatric neurosurgeon at Rady Children's Hospital San Diego. Dr. Gonda's clinical areas of expertise are epilepsy surgery, MRI laser thermal ablation surgery, pediatric spine abnormalities and craniovertebral junction abnormalities. His research area of expertise is minimally invasive epilepsy surgery.

He comes to Rady Children's from Texas Children's Hospital, where he was a pediatric neurosurgery fellow, and Baylor College of Medicine, where he was a clinical instructor. Dr. Gonda completed his residency training at UC San Diego and earned his medical degree at The Ohio State University.
Dr. Gonda has specialized training in utilizing a new robotic assist device called ROSA, which Rady Children’s has obtained. He utilizes the robot for epilepsy surgery and other neurological procedures.

Sunil Jeswani, MD, is a neurosurgeon who treats people with cranial and spinal problems. He performs surgery for brain tumors, pituitary tumors, skull base tumors, stereotactic radiosurgery, hydrocephalus, and trigeminal neuralgia. He also does minimally invasive spinal surgery and treats spinal conditions such as degenerative disease, tumors, scoliosis and spinal trauma.

Dr. Jeswani completed fellowships in minimally invasive and complex spine surgery at University of Southern California and at Cedars-Sinai Medical Center in Los Angeles, where he also did his residency in neurosurgery. He earned his medical degree with high distinction from Wayne State University School of Medicine in Detroit.

He is a member of several professional organizations, including Alpha Omega Alpha Honor Medical Society, the Congress of Neurological Surgeons and the American Association of Neurological Surgeons. Dr. Jeswani’s research has been published in several leading academic journals.

Najla Kfoury-Beaumont, MS, PhD, received her Bachelor of Science in Medical Laboratory Technology from the American University of Beirut and her Master of Science and Ph.D. in Molecular Biology and Genetics from Wayne State University in Michigan.
Her doctoral research focused on the implications of C/EBPs in the control of biological processes critical to neuronal development and survival. For her postdoctoral training, she focused on pursuing translational research where she joined the laboratory of Dr. Marc Diamond at Washington University in St. Louis to investigate Tau aggregate transfer and propagation as well as potential antibody-based therapies to target tau trans-cellular propagation in Alzheimer’s disease.

Subsequently, Dr. Kfoury-Beaumont returned to her initial research passion of genetics and epigenetics, particularly their role in development and cancer. She joined the laboratory of Dr. Joshua Rubin where her focus was on how the normal epigenetics of sexual differentiation interact with the abnormal epigenetics of brain tumors to produce cell intrinsic sex differences in brain tumor biology.

Her current research interests extend across cancer biology, development and epigenetics with special emphasis on sex differences in glioblastoma. Her long-term research interests include the study of epigenetics and transcriptional mediators of sex differences in brain tumors and developmental disorders. By using sex differences in glioblastoma phenotype as a tool to better understand cancer risk and progression, she hopes to ultimately develop sex-specific therapies to improve survival outcomes for all patients.

Michael L. Levy, MD, PhD is a board-certified neurosurgeon at UC San Diego Health and the Chief of Pediatric Neurosurgery at Rady Children’s Hospital San Diego. Dr. Levy's primary surgical interests are in Pediatric Brain Tumors, Pediatric Skull Base Tumors, and Cerebral Vascular Neurosurgery.

Dr. Levy’s research includes the evaluation of Surgery, Genomics, and multi-modality treatment methodologies in children with brain tumors. He also provides management (surgical and otherwise) of complex cerebral vascular malformations in children. He has evaluated the longevity and outcome in children with brain tumors and the relationship of certain variables and treatments to their survival. He has also evaluated survival and predicted models of outcome including surgical management in pediatric and adolescent head trauma and community-based efforts at prevention and communication.

Dr. Levy has been significantly involved with surgical missions and establishing sister programs for the past 20 years throughout the world. Current sister programs exist in Nicaragua, Bolivia, Chile, Peru, Mexico, Samoa, Uganda, and Ukraine.
Dr. Levy also serves as a Professor of Clinical Surgery, inspiring the next generation of adult and pediatric neurosurgeons.

His vascular research includes the relationships of cerebral blood flow to cardiac output in patients with aneurysmal induced subarachnoid hemorrhage and vasospasm. His research has led to treatments with both hyperdynamic protocols and the use of inotropes throughout the country. Further research involves the use of surgery and/or rheological agents to further increase cerebral blood flow and perfusion to ischemic regions of the brain during cerebral vasospasm or in children with Moya disease.

Dr. Levy’s other interests include technical methodologies within neurosurgery. He has developed novel techniques of intervention for children and adults using modified endoscopes for catheter placement within the ventricular system of the brain, and using endoscopes as adjuvants during microsurgery. He has further developed picture-in-picture image graphics for the operating microscope and the use of head mounted display systems for both endoscopic and microscopic neurosurgical procedures. More recently he has been involved in the development of exoscopes to be utilized during surgery, contemporary head mounted displays, and robotics. He also studies the 3-dimensional representation of the central nervous system and the relationship of neoplastic and vascular abnormalities to functional structures to maximize both neurosurgical approaches and patient outcome.

He completed his neurosurgical residency at the University of Southern California (USC). He also obtained a PhD degree in biophysics from USC. He then completed a two-year fellowship in pediatric neurological surgery at Children's Hospital Los Angeles. He earned his medical degree from the University of California, San Francisco (UCSF) and is board-certified in both adult and Pediatric Neurosurgery. Dr. Levy joined the USC faculty as a pediatric and vascular neurosurgeon in 1993 and left to develop the pediatric neurosurgical program at Rady Children's Hospital San Diego in August 2002.

Lawrence Marshall, MD, is a board-certified neurosurgeon and an internationally renowned expert in traumatic head injury. Dr. Marshall’s surgical interests cover all of neurosurgery but especially diseases of the spine and spinal cord, brain tumors, and peripheral nerves.

Operations he performs include craniotomy, anterior cervical diskectomy and laminectomy, thoracic diskectomy, lumbar diskectomy and laminectomy and fusion, and exploration of the brachial plexus, among others.
Dr. Marshall served as Chief of the Division of Neurosurgery from 1995-2010. He has served in many key roles at the UC San Diego, including associate dean for the Faculty Practice Plan and executive director of managed care.

As Professor Emeritus of Surgery at the UC San Diego School of Medicine, he has conducted research studies in a wide range of areas related to central nervous system trauma and severe head injury.

Dr. Marshall completed a fellowship at the Institute of Neurological Sciences in Glasgow, Scotland after his residency training at the University of Pennsylvania. He earned his medical degree at the University of Michigan in Ann Arbor. Dr. Marshall is board certified in neurological surgery.

He has served on over 75 scientific and professional committees. He has held over 60 positions for various scientific and professional organizations. Dr. Marshall has been invited as distinguished lecturer or panelist at over 500 meetings and conferences.

Dr. Marshall is also a member of over 50 scientific and professional societies, including the American Association of Neurological Surgeons, San Diego Academy of Neurological Surgeons, Society of Critical Care Medicine, Society of Neurological Surgeons, Society of Neurotrauma, and the Western Federation of Neurological Surgeons.

Andrew Nguyen, MD, PhD, is a Board-certified and Fellowship-trained neurosurgeon who specializes in endovascular treatments for neurovascular diseases and minimally invasive spine surgery.

As an HS Associate Clinical Professor of Surgery, Dr. Nguyen trains neurosurgical residents and fellows at UC San Diego School of Medicine.

He completed his neurosurgical residency at UC San Diego School of Medicine, where he also earned his medical degree and a PhD through the Medical Scientist Training Program in conjunction with the Salk Institute for Biological Studies. Dr. Nguyen joined UC San Diego Health System and the School of Medicine in 2010 following a research fellowship at The Scripps Research Institute in La Jolla.

During his clinical fellowship training, Dr. Nguyen worked with pioneers in the field of endovascular neurosurgery at UC San Diego and became one of the first neurosurgeons trained in endovascular
treatments in France at the Hospital Lariboisiere in Paris. He enjoys treating a wide range of neurosurgical diseases from complex neurovascular diseases, traumatic brain and spinal injuries, metastatic and primary tumors, to degenerative and complex spinal disorders.

Scott E. Olson, MD, is a board-certified vascular neurologist with additional fellowship training in interventional neuroradiology/endovascular neurosurgery. He specializes in evaluating and treating disorders of the blood vessels of the brain and spinal cord, which can lead to ischemic or hemorrhagic stroke. His expertise includes both medical and endovascular care for individuals with extra and intracranial stenoses, brain aneurysms, and vascular malformations, whether congenital or acquired.

Dr. Olson is part of the neurovascular team at UC San Diego Health’s Stroke Center. He enjoys working with a multidisciplinary team of physicians and nurses who share his passion for treating cerebrovascular disease and providing the highest level of care to patients and their families.

As an HS Associate Clinical Professor of Surgery, he also instructs medical students, residents and fellows. He has given numerous lectures and co-authored many publications on cerebrovascular disease. He is currently involved in National Institute of Health (NIH) and industry funded research involving ischemic and hemorrhagic cerebrovascular disease. Dr. Olson is a senior member of the Society for NeuroInterventional Surgery.

Dr. Olson completed his stroke and interventional neuroradiology/endovascular neurosurgery fellowships at UC San Diego School of Medicine. His focus was on new, image-guided, minimally invasive therapies and he was one of the first neurologists in the country trained in this emerging field. He completed a residency in neurology at The University of Arizona College of Medicine where he was selected for his clinical and leadership abilities to serve as chief resident. Dr. Olson earned his medical degree from the University of Cincinnati College of Medicine. He is board certified in neurology and vascular neurology.
Joseph Osorio, MD, PhD is a native of San Diego who completed his undergraduate education at the University of California, Irvine in Civil Engineering. He then earned a Ph.D. in Bioengineering at University of California, Berkeley/University of California, San Francisco (UCSF), with a focus on translational research in brain tumor imaging.

Dr. Osorio went on to complete both medical school and Neurosurgery residency at UCSF, where he was recognized and awarded for his clinical performance (Krevan’s Award for Surgical Intern of the Year, 2013), research (Top Senior Research Paper Award, 2018), and teaching (Rosegay Resident Teaching Award, 2018). He was mentored during his residency by Dr. Christopher Ames who trained at UC San Diego.

After his Neurosurgery training, he completed a post-graduate spine fellowship in the Department of Orthopedics under the guidance of Dr. Lawrence Lenke at Columbia University in New York City. Dr. Osorio’s dual-training provides an interdisciplinary perspective for the most complex spine pathologies including adult spinal deformity, cervical deformity, and spinal tumors.

Ahmet Oygar, MD, graduated from the Harvard Medical School in 1975 and specializes in Neurological Surgery. Dr. Oygar is affiliated with VA San Diego Healthcare System and has 44 years of experience.
J. Scott Pannell, MD, is a neurointerventional radiologist who specializes in endovascular neurosurgery and neuroradiology. Dr. Pannell focuses on the catheter-based treatment of ischemic stroke, cerebral aneurysms, arteriovenous malformations, carotid stenosis, vascular tumors, and interventional management of pain associated with disorders of the spine.

His research interests include ischemic stroke, endothelial injury, and simulator-based angiography training.

Dr. Pannell completed fellowships in endovascular neurosurgery and neuroradiology at UC San Diego Health. He completed his radiology residency at the University of Alabama at Birmingham and his internship at Emory University. Dr. Pannell earned his medical degree from the Medical College of Georgia. He is board certified in diagnostic radiology.

During his medical school training and residency, Dr. Pannell developed interest in cerebrovascular imaging and minimally invasive treatment of cerebrovascular disorders.

Following his endovascular neurosurgery fellowship in 2015, Dr. Pannell joined the multidisciplinary neurovascular team at UC San Diego Health as a crucial member of one of the nation’s first comprehensive stroke centers.
Martin Pham, MD, is a neurosurgeon who focuses on the neurosurgical evaluation and treatment of spinal disorders. This includes adult scoliosis and spinal deformity, complex spinal reconstruction, robotic and minimally invasive spine surgery, motion preservation of the spine, spine tumors, and spine trauma.

Having completed spine fellowship training with both neurosurgeons and orthopedic surgeons, Dr. Pham has a comprehensive understanding of the spine that allows him to tailor his treatment to each individual using a wide range of available surgical and nonsurgical options.

Dr. Pham is published extensively within the field of spinal conditions and has an active interest in the development of evidence-based spine care. His work has appeared in *Journal of Neurosurgery: Spine*, *The Spine Journal*, *Neurosurgery*, and *JAMA Surgery*, among others.

Dr. Pham completed a fellowship in robotic, spinal deformity, and motion preservation surgery at Columbia University’s NewYork-Presbyterian Daniel and Jane Och Spine Hospital. He also completed a fellowship in minimally invasive and complex spinal surgery at Keck School of Medicine of USC, where he completed his residency in neurological surgery. Dr. Pham earned his medical degree from Northwestern University Feinberg School of Medicine in Chicago.

He is a member of the American Association of Neurological Surgeons, the Congress of Neurological Surgeons, the North American Spine Society, and AOSpine North America. He speaks Spanish in addition to English.
Marc Schwartz, MD, is a board-certified neurosurgeon who is an internationally renowned expert on the treatment of patients with acoustic neuromas, skull base tumors, and neurofibromatosis type 2 (NF2). He is also the recognized neurosurgical leader in the field of auditory brainstem implants. In addition, Dr. Schwartz treats complex cranial problems, meningiomas, brain tumors, pituitary tumors, cranial nerve disorders, and intradural spine tumors.

Dr. Schwartz conducts research in tumor biology and genetics, especially in relation to schwannomas and neurofibromatosis type 2. He is a national leader in the development and refinement of the auditory brainstem implant, which is used for hearing restoration in deaf adults and children who are unable to benefit from hearing aids or cochlear implants.

Dr. Schwartz has published numerous articles and book chapters in his areas of interest. These articles focus not only on basic and clinical science but also on the psychological and social aspects of clinical decision-making. He is a frequent speaker at national and international conferences and has been a visiting professor at medical schools on three continents.

Prior to joining UC San Diego Health, Dr. Schwartz was the senior neurosurgeon at the House Ear Clinic in Los Angeles, where he treated over 2,000 patients with acoustic neuromas and associated disorders. Prior to this, he was the director of the Skull Base Surgery Program at the Albany Medical College.

Dr. Schwartz completed fellowship training in skull base surgery at Oregon Health and Sciences University. He did his neurosurgical residency at the Brigham and Women’s Hospital, Harvard Medical School in Boston. He earned his medical degree from Harvard Medical School. He is board certified in neurosurgery.

Dr. Schwartz is a member of many professional societies, including the American Association of Neurological Surgeons, the Congress of Neurological Surgeons, the California Association of Neurological Surgeons, the Western Neurosurgical Society, and the North American Skull Base Society. He is on the editorial board of *Skull Base: Operative Videos* and regularly reviews manuscripts for a number of other journals. He is a member of the medical advisory board of the Acoustic Neuroma Association.
William Taylor, MD, is a board-certified neurological surgeon. His practice focuses on the neurosurgical evaluation and treatment of spinal disorders. He is also dedicated to further developing and refining minimally invasive surgical procedures used to treat spinal conditions and diseases.

Dr. Taylor is renowned in the medical community for his expertise in minimally invasive spine surgery and receives patient referrals from around the world. As a patients’ rights advocate, he has spoken before the U.S. Food and Drug Administration (FDA) and created several position statements to help ensure that patients with spinal conditions have access to adequate care.

He is the executive director of the Society for Minimally Invasive Spine Surgery (SMISS), which develops educational training for surgeons. Dr. Taylor is also editor of The Scientific World Journal and a member of many professional scientific organizations, including the American Medical Association and the American Association of Neurological Surgeons.

He is an HS Clinical Professor of Surgery and has been a member of the faculty at UC San Diego School of Medicine since 1994. He serves on the Admissions Committee and the Education Committee for the Division of Neurosurgery, and has received several teaching awards during his career.

Dr. Taylor completed his residency training at Albert Einstein College of Medicine in the Bronx in New York City, and completed his specialty training in spinal surgery at New York-Presbyterian/Columbia University Medical Center. He earned his medical degree from UCLA School of Medicine.
CAPT Jeffrey M. Tomlin, MD, MBA, served as the Neurosurgical Specialty Leader to the Navy Surgeon General from June 2014 to February 2018, and is on Active Duty in practice at Naval Medical Center San Diego where he was the Chief of the Medical Staff from July 2016 through December 2017.

CAPT Tomlin graduated from the US Naval Academy in 1989 with a BS in Aerospace Engineering, and then completed training in nuclear propulsion training to initially serve in the submarine force, completing tours on USS Annapolis (SSN-760) as a plankowner for its commissioning, and also at FACS FAC San Diego. He then attended medical school at Uniformed Services University in Bethesda, MD, graduating in 1999. After completing residency in neurosurgery at the University of Rochester in 2006, he served in Bethesda, MD and was deployed to the combat support hospital in Landstuhl, Germany as well as the NATO Multinational facility in Kandahar, Afghanistan. Most recently, CAPT Tomlin completed his Executive MBA through the Naval Postgraduate School in September 2017.

CAPT Tomlin is an active staff member at various trauma centers, including UC San Diego, Albert Einstein Medical Network in Philadelphia, PA, and Iowa Methodist Hospital where he takes call for emergency neurosurgical care. He is a fellow in both the American Academy of Neurological Surgeons, the Congress of Neurosurgeons, the American College of Surgeons, and a member of the Neurosurgical Society of America, serving as the Academic Program Chair for their annual meeting in 2018. His interest in clinical practice and research has been focused in neurotrauma care with special interests in cranial trauma, skull reconstruction, and traumatic brain injury. His volunteer work has been as a member of the Board of Directors for the Vail Veterans Program, fortifying the strategic partnership to provide healing for wounded service members through leading clinics for injured active duty service members and veterans since 2012. He is also the lead instructor for their pilot program, Veterans Path to Success, in partnership with the Human Performance Institute division of Johnson & Johnson.

He is a physician member of the National Ski Patrol as well as a previous national registry of emergency medical technicians (NREMT) member. Within his educational interests, he has led topic-focused neurotrauma training for the Jackson Hole Ski Patrol, the Teton Gravity Research International Pro Rider’s Workshop, and as a guest lecturer for Outdoor Emergency Care Refresher courses across a spectrum of outdoor athletes, professionals, and first responders.
Howard Tung, MD, has been practicing medicine since 1992 and became board certified in Neurosurgery in 1995. Dr. Tung received his medical degree from Dartmouth Medical School in 1985. He served his internship specializing in General Surgery at the University of Southern California (USC) in Los Angeles and served his residency specializing in Neurosurgery at USC.

Hoi Sang U, MD, is a board-certified neurosurgeon who is an internationally recognized specialist in the treatment of vascular anomalies known as AVMs. Prior to 1979, AVMs in the basal nuclei had been deemed inoperable. With the assistance of Drs. Michael Todd, John Drummond and Charles Kerber, an anesthesia technique entailing the elective induction of barbiturate coma was developed. In addition, the staged elimination of large lesions through embolization and staged operations evolved. This has allowed for the approach to these deep structures where giant AVMs were excised with acceptable excellent outcomes. The neuropsychiatric performance of these patients is especially shown to improve and benefit from AVM excision. Patients have traveled to UC San Diego from as far away as Australia, Hong Kong, the Sultanate of Brunei, Africa and the Kingdom of Saudi Arabia to take advantage of his expertise.

His neurovascular practice also includes treatment of aneurysms and cavernous hemangiomas. Dr. U's other surgical interests include the treatment of brain masses, including meningiomas, astrocytomas, craniopharyngiomas, and pituitary adenomas.

As a Professor of Surgery at UC San Diego School of Medicine, Dr. U is a renowned expert in brain tumor and stem cell research. He has conducted studies on diseases ranging from human pituitary adenomas to glioblastomas being one of the first to describe EGF-R gene amplification in these tumors in 1986. In addition, Dr. U collaborated with Dr. “Rusty” Gage (the Salk Institute) in performing pioneering work in
gene therapy and brain tissue transplantation especially in the treatment of Alzheimer’s disease (AD). This led to the first trial using ex-vivo gene therapy in AD in 2001 by Drs. U and Tuszynski.

Dr. U joined UC San Diego in 1978. He obtained his medical degree from Tufts University School of Medicine in Boston and obtained an internship at Stanford followed by residency at UC San Francisco. While at UCSF, he invented the detachable balloon catheter for the endovascular treatment of vascular diseases. He is board-certified in neurological surgery. Dr. U has served on many scientific and professional committees. He has been invited as a distinguished lecturer or panelist at over 100 meetings and conferences. He is also a member of the Society of Neurologic Surgeons, the American Association of Neurological Surgeons, the San Diego Academy of Neurological Surgeons, Congress of Neurological Surgeons, the North American Skull Base Society and the Royal Society of Medicine, London.
Elina DeFranceschi is a physician assistant who received her Master of Science degree in Physician Assistance from Jefferson College of Health Sciences in December 2014.

She began her career working as a neurosurgery physician assistant subspecializing in vascular neurosurgery at Johns Hopkins Bayview Medical Center in February 2015. During this time, she also served as the neurosurgical representative for the Stroke Oversight Committee and contributed to the successful accreditation of the hospital as a Comprehensive Stroke Center in July 2016. In January 2017, she moved to North Carolina where she continued her career in neurosurgery at Vidant Medical Center. She subspecialized in functional neurosurgery and assisted with the foundation of the deep brain stimulation program.

Elina joined UC San Diego Health in September 2018 as a neurotrauma physician assistant. She provides nocturnist coverage and performs consultations for emergency and traumatic neurosurgical patients in addition to assisting in emergency operations and procedures.

Eric Do, BSN, RN started his nursing career with a new graduate program at Emory University Hospital. After his initial years of critical care experience, he pursued his nursing career to California. Since then, he has trained and worked in various cases of pre- and post-op cares and OR procedures. He joins the
Neurological Surgery team as the program’s first Triage Nurse, a position that was created due to a highly successful quality improvement pilot.

Homira Feely is a native of Iran. She has been licensed as an Acute Care Nurse Practitioner since 2009. She obtained a Doctorate of Nursing Practice (DNP) from the University of San Diego in 2018.

She has been a neurosurgery nurse practitioner at UC San Diego since 2010. Her principal focus is brain tumors and she works on the Acoustic Neuroma Team with Drs. Schwartz and Friedman. She also works on brain tumor patients with Drs. U and Beaumont. She works closely with the neuro-oncology team.

She is a member of American Academy of Nurse Practitioners.

Gage Lambert, MS, PA-C is a board-certified PA with extensive experience in Neurosurgery. He was born in San Diego, California.

He obtained his undergraduate degree from USF with a bachelors in Biology/Neuroscience, and Masters of Sciences from Western University of Health Sciences.

Mr. Lambert joined UC San Diego as clinical research associate, Division of Neuro-oncology in 2012, with emphasis in primary gliomas. His current role as neurosurgical PA includes diagnosis and treatment of acute and chronic neurosurgical conditions and 1st assist in surgery.
His clinical interest include management of Traumatic brain injuries and primary brain tumors. Mr. Lambert is a member of AANS, AAPA, CAPA, and SDPAS.

Paul McNally was born in Dallas, TX and studied psychology at Boston University. While working in inpatient psychiatric care in Dallas he developed an appreciation of the ways that non-physician providers could play a vital role in the management of chronic health conditions.

Paul moved to San Diego to attend University of San Diego's Nurse Practitioner program. While earning his Masters in Nursing he worked as an inpatient RN at Scripps La Jolla on the Ortho/Neuro/Trauma unit. In doing that work he was introduced to the world of neurosurgery.

After finishing his Masters of Science in Nursing and completing national board certification as a Nurse Practitioner in 2009 he began working in Neurosurgery in San Diego.

Joining UC San Diego’s clinical Department of Neurological Surgery in 2015, he now works with Drs. Jeswani, Nguyen, and Pham specializing in neurotrauma, brain/spinal tumors, and spinal reconstructive surgery.

Keri O’Connor graduated with honors in 2012 from California State University, Channel Islands with a bachelor’s degree in nursing and a minor in psychology. Upon graduation, she obtained her registered nurse and public health nurse licensure.
Keri worked as a registered nurse in surgical services, including medical/surgical floor, post anesthesia care unit (PACU), and pre-operative unit, for nearly 5 years.

Ms. O’Connor went on to attend The University of Cincinnati, where she graduated in 2016 with honors and a master’s degree in nursing, specializing as a family nurse practitioner. She is certified as a family nurse practitioner by the American Association of Nurse Practitioners (AANP).

Keri is a member of the American Association of Nurse Practitioners (AANP), California Association of Nurse Practitioners (CANP), Sigma Theta Tau honor society of nursing, and San Diego Neurosciences Society.

Samantha Wheeler is a native of Boston, Massachusetts. She is certified by the National Commission on Certification of Physician Assistants and has been a licensed Physician Assistant since 2012. She graduated from Simmons College in Boston, MA with a Bachelors in Biology and a Minor in Chemistry in 2010.

She went on to attend the University of Pittsburgh where she received her Masters of Science in Physician Assistant Studies in 2012. Prior to coming to UC San Diego, Samantha worked as a Physician Assistant in Pediatric Neurosurgery at the Children’s Hospital of UPMC in Pittsburgh, Pennsylvania.

She currently works as a Senior Physician Assistant with the clinical Department of Neurological Surgery at UC San Diego. She has worked very closely with our Chair, Dr. Alexander Khalessi, in Neurovascular and Endovascular Neurosurgery and is attributed for their high patient satisfaction scores. She has transitioned to the inpatient setting where she has launched the inpatient neurosurgery ACP service to improve quality of care, readmission rates, and resident education. She currently supports all of the neurosurgery faculty.

She is a member of the Surgical Quality board and partners with Dr. Barba and Dr. Khalessi in the departmental quality improvement project. She has helped to develop comprehensive stroke center at JMC and HC (currently the 3rd in the country). She precepts medical students, residents and fellows in clinic and provides shadowing for undergraduate and high school students who are interested in the physician assistant career.

She was nominated for Physician Assistant of the Year in 2018.
Maureen Parsons graduated in 2001 with a Bachelor of Science degree in Kinesiology followed by a Bachelor of Science degree in Nursing in 2003. Maureen started her career in healthcare as a critical care nurse in the Surgical Intensive Care Unit at UC San Diego, specializing in trauma, neurosurgery, transplant, cardiothoracic, and pulmonary medicine. From 2007 – 2011 she worked in the Thornton Intensive Care Unit at UC San Diego, specializing in medical, surgical, oncology, neurosurgery and cardiothoracic surgery.

In 2011, Maureen participated in the opening of the Sulpizio Cardiovascular Center and was one of the founding intensive care nurses, providing team care to advanced cardiovascular and thoracic surgery and medicine patients, including heart and lung transplants and pulmonary endarterectomy patients from around the world. Ms. Parsons then went on to attend California State University, San Marcos where she graduated Magna Cum Laude and received a Master of Science Degree in Nursing, specializing as a Family Nurse Practitioner in 2014.

Maureen was involved with the Pulmonary Division at UC San Diego from 2014-2017 where she worked with patients with advanced lung disease. While there, she conducted a clinical research trial for Esbriet (Pirfenidone) for patients with Idiopathic Pulmonary Fibrosis. In June of 2017, she joined the Neurological Surgery team where she currently works closely with Dr. William Taylor, Dr. Joseph Ciacci, and Dr. Howard Tung specializing in neurosurgical evaluation and treatment of spinal disorders as well as tumors of the brain.

Maureen is certified as a Family Nurse Practitioner by the American Association of Nurse Practitioners (AANP) as well as a member of the American Association of Nurse Practitioners (AANP), California Association of Nurse Practitioners and Sigma Theta Tau Honor Society of Nursing.
APPENDIX V

ADMINISTRATIVE LEADERSHIP BIOGRAPHIES

In her role as Executive Director in the Office of the Chair of Neurological Surgery, Morgan Tupper works directly with Dr. Alexander Khalessi and manages departmental programs, projects, and initiatives. She provides strategic insight and guidance for philanthropic endeavors, communications, business development, and short- and long-term operational planning. Morgan also oversees both the clinical coordinator and administrative assistant teams.

Prior to her current role, Morgan was the Executive Director of Donor Engagement, Strategy, and Operations for UC San Diego Health Sciences Advancement (HSA), as well as Chief of Staff to the Associate Vice Chancellor for HSA. She played a senior role in raising $250M+ over 2.5 years and oversaw seven distinct areas: donor engagement and experience, operations, stewardship, communications, Board of Advisors strategy and operations, Compass (patient navigation program), and special events.

She was recruited to UC San Diego from New York City where she held management positions with First Republic Bank, Columbia University Medical Center, and Christie’s Auction House in New York City.

Morgan has over 16 years of leadership experience in project management, business development, donor and client relations, marketing, and event production. She graduated from the University of Southern California with a Bachelor of Arts in Communications and now resides in La Jolla, CA.
Eric Wilson is the Department Business Officer for the Departments of Neurological Surgery and Neurosciences in the School of Medicine at UC San Diego Health Sciences. Eric has worked with UC San Diego Health Sciences since 2001, holding various administrative finance positions including: Department of Medicine Director of Finance (2001-2008); Department of Ophthalmology Ast. Business Officer and Interim Business Officer (2008-2010); and Vice Chancellor for Health Sciences Affiliations Business Manager (2010-2015).

Eric holds a bachelor degree in Business Administration. He also serves as a board member for the Veteran’s Medical Research Foundation (VMRF).

In her role as Senior Director for Neuro, Trauma Burn and Rehab service lines, Michelle Ziemba is charged with the development and operations of the Neurological Institute and to the Neuro service line oversight, strategic program development, and operations for Trauma, Burn, and Rehab Services.

Michelle also works with the Departments of Neurological Surgery and Neurosciences to improve clinical care, patient access, and clinical operations for both ambulatory and inpatient services and is responsible for Trauma and Burn Program Verification, program operations and strategy. Additional responsibilities include budget planning, financial sustainability, faculty recruitment, departmental program development, and marketing as well as providing oversight of rehabilitation services, physical therapy, occupational therapy, and speech therapy operations for both inpatient and outpatient services.
Prior to her current role, Michelle was the Associate Vice President of Trauma, Emergency, and Perioperative Services where she provided oversight over two hospitals and an ambulatory surgery center for the University of Arizona. There she oversaw all departmental operations, strategic planning, budget and organizational alignment. She was appointed by the executive leadership team to serve as an operational leader/liaison to clinical and revenue cycle operational leaders to facilitate a successful transition to the new electronic medical record system.

Michelle has 20 years of experience as a passionate healthcare executive and enjoys being a change agent and leading operational initiatives to improve patient care and organizational excellence.
### Table 1.1

#### The Marshall Classification

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
<th>Mortality</th>
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<tbody>
<tr>
<td>Diffuse Injury I</td>
<td>No visible intracranial pathology seen on CT scan</td>
<td>9.6%</td>
</tr>
<tr>
<td>Diffuse Injury II</td>
<td>Cisterns are present with midline shift of 0-5 mm</td>
<td>13.5%</td>
</tr>
<tr>
<td>Diffuse Injury III (swelling)</td>
<td>Cistern compressed or absent, shift less than 5mm</td>
<td>34%</td>
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<tr>
<td>Diffuse Injury IV (shift) Evacuated Mass Lesion V</td>
<td>Shift greater than 5 mm Any surgically evacuated lesion</td>
<td>56.2%</td>
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<tr>
<td>Non-evacuated Mass Lesion VI</td>
<td>High or mixed density lesion greater than 25cm³</td>
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</tr>
<tr>
<td>Faculty</td>
<td>Title</td>
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<tr>
<td>Alksne, John, MD</td>
<td>RTAD - Professor Emeritus of Surgery, Past Chief (Ladder Rank)</td>
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<td>Barba, David, MD</td>
<td>HS Clinical Professor of Surgery, Director of Functional Surgery</td>
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<td>Beaumont, Thomas, MD PhD</td>
<td>Assistant Professor of Clinical Surgery (Clin X in process)</td>
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<td>Ben-Haim, Sharona, MD</td>
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<td>Ciacci, Joseph, MD</td>
<td>Professor of Clinical Surgery (Clin X), Residency Program Director,</td>
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<td>Chief of Neurosurgery VASDHS</td>
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<td>Jeswani, Sunil, MD</td>
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<td>Khalessi, Alexander A., MD MBA</td>
<td>Chair of Neurological Surgery; Professor of Surgery, Radiology and</td>
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<td>MS FAANS</td>
<td>Neurosciences (Ladder Rank / In Residence)</td>
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<td>Marshall, Lawrence, MD</td>
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<td>Tomlin, Jeffrey, MD</td>
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<tr>
<td>U, Hoi S, MD</td>
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Table 1.5

Operative Cases *(University sites only)*

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<th>Year</th>
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<td>FY19 (ANNUALIZED)</td>
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</tr>
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<td>FY18</td>
<td>2300</td>
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<tr>
<td>FY17</td>
<td>2200</td>
</tr>
<tr>
<td>FY16</td>
<td>2100</td>
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<td>FY15</td>
<td>1900</td>
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Table 1.6

Outpatient Clinic Visits

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<td>8800</td>
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<tr>
<td>FY17</td>
<td>8700</td>
</tr>
<tr>
<td>FY16</td>
<td>8300</td>
</tr>
<tr>
<td>FY15</td>
<td>6700</td>
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### Table 1.7

Mortality Observed / Expected (O/E)

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Table 1.8
30 Day Related Readmission
### Table 1.10

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### Table 1.11

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<td>2019</td>
<td>Altattar, Ali MD</td>
<td>University of Pittsburgh</td>
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<td>Brandel, Michael MD</td>
<td>UC San Diego</td>
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<td>Dalle Ore, Cecilia MD</td>
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<td>Lopez Ramos, Christian MD MPH</td>
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<td>Schupper, Alex, MD</td>
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<td>Ali, Mir Amaan MD</td>
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<td>Carroll, Kate MD</td>
<td>University of Washington</td>
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<td>Wali, Arvin MD MAS</td>
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<td>Treiber, Jeffrey MD</td>
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<td>Zhou, Tianzan MD</td>
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Table 1.12

![Bar chart showing Neurological Surgery CARE Payment Clinical Revenue for FY 2017, FY 2018, and FY 2019-Annualized.]

Table 1.13

{redacted}
Table 1.14

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Table 1.15

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APPENDIX VII

SELECT DEPARTMENT PUBLICATIONS
[Median amount of 84 publications per year from January 1, 2014 – May 22, 2019]

2019 (January - May)


Gonda DD, Huang M, Briceno V, Lam SK, Luerssen TG, Jea A. “Protecting Against Post-Operative Dyspnea and Dysphagia After Occipitocervical Fusion.” Operative Neurosurgery. ACCEPTED, 2019


Bakhsheshian J, Wheeler S, Strickland BA, Pham MH, Rennert RC, Carmichael J, Weiss M, Zada G. Surgical Outcomes Following Repeat Transsphenoidal Surgery for Nonfunctional Pituitary Adenomas: A
Retrospective Comparative Study. Oper Neurosurg (Hagerstown). 2019 Feb 01; 16(2):127-135. PMID: 29767762


2018


From the American Association of Neurological Surgeons (AANS), American Society of Neuroradiology (ASNR), Cardiovascular and Interventional Radiology Society of Europe (CIRSE), Canadian Interventional Radiology Association (CIRA), Congress of Neurological Surgeons (CNS), European Society of Minimally Invasive Neurological Therapy (ESMINT), European Society of Neuroradiology (ESNR), European Stroke Organization (ESO), Society for Cardiovascular Angiography and Interventions (SCAI), Society of Interventional Radiology (SIR), Society of NeuroInterventional Surgery (SNIS), and World Stroke Organization (WSO), Sacks D, Baxter B, Campbell BCV, Carpenter JS, Cognard C, Dippel D, Eesa M, Fischer U, Hausegger K, Hirsch JA, Shazam Hussain M, Jansen O, Jayaraman MV, Khalessi AA, et al. Multisociety Consensus Quality Improvement Revised Consensus Statement for Endovascular Therapy of Acute

Rafii MS, Tuszynski MH, Thomas RG, Barba D, Brewer JB, Rissman RA, Siffert J, Aisen PS; AAV2-NGF Study Team. Adeno-Associated Viral Vector (Serotype-2) -Nerve Growth Factor for Patients With Alzheimer Disease: A Randomized Clinical Trial. JAMA Neurol. 2018 Jul 1;75(7):834-841

John Hermiz, a Nicholas Rogers, b Erik Kaestner, c Mehran Ganji, a Daniel R Cleary, d Bob S. Carter, d David Barba, d Shadi A. Dayeh, e f Eric Halgren, g h Vikash Gilja. Sub-millimeter ECoG Pitch in Human Enables Higher Fidelity Cognitive Neural State Estimation. Volume 176, 1 August 2018, Pages 454-464


Lopez Ramos C, Brandel MG, Steinberg JA, Wali AR, Rennert RC, Santiago-Dieppa DR, Sarkar RR, Pannell JS, Murphy JD, Khalessi AA. The impact of traveling distance and hospital volume on post-surgical


Robert C. Rennert, MD, Arvin R. Wali, MD, Jeffrey A. Steinberg, MD, David R. Santiago-Dieppa, MD, Scott E. Olson, MD, J. Scott Pannell, MD and Alexander A. Khalessi, MD MS. Epidemiology, Natural History, and Clinical Presentation of Large Vessel Ischemic Stroke. Neurosurgery NEU-D-18-01293R1. December 2018

Clemens M. Schirmer, MD, PhD, Adnan H. Siddiqui, MD, PhD, Ilya Frid, BS, Alexander A. Khalessi, MD, MS, J Mocco, MD, MS, Christoph J. Griessenauer, MD, Oded Goren, MD, Shamsher Dalal, MD, Gregory Weiner, MD, Adam S. Arthur, MD MPH. Modern Training and Credentialing in Neuro-Endovascular Acture Ischemic Stroke Therapy. Neurosurgery NEU-D-18-01386R1. December 2018


2017


Hoshide R, Meltzer H, Dalle-Ore C, Gonda D, Guillaume D, Chen CC. “Impact of Ventricular-peritoneal Shunt Valve Design on Clinical Outcome of Pediatric Patients with Hydrocephalus: Lessons Learned from Randomized Controlled Trials.” Surg Neur Int 8:49; Apr 5, 2017


Unusual and Rare Locations for Craniopharyngiomas: Clinical Significance and Review of the Literature
Brandon C. Gabel, Daniel R. Cleary, Joel R. Martin, Usman Khan, Vivian Snyder and Hoi Sang U World Neurosurgery 98:381-387, 2017


2016


Calayag M, Gabel BC, Hong DS, Hatefi D, Gonda DD, Meltzer HS, Levy ML. Considerations in Relationship to the Approach for the Treatment of Lateralized Posterior Fossa Tumors in Children. Neurosurgery. 63:177-178, August 2016. (Abstract) PMID 27399475


Rennert RC, Scott Pannell J, Levy ML, Khalessi A. Sphenoid Wing Dysplasia and Plexiform Neurofibroma in Neurofibromatosis type 1. ANZ J Surg. 2016 Apr 08. PMID 27061833


Sun Choe Daly, Hoi Sang U, and John Drummond "Neuroanesthesia in the Treatment of Pituitary Tumors" - the proofs have been approved for publication in 2016

Intracranial Rhabdomyoma: Case Report and Review of the Literature David R. Santiago-Dieppa, Tianzan Zhou, Karra A Muller, James Y Chen, Lawrence A Hansen, and Hoi Sang U Cureus Accepted for publication 2016


2015


Page 131 of 136


Brandon Gabel, Mary Goolsby, Lawrence Hansen, and Hoi Sang U "Inflammatory Myofibroblastic Tumor of the Left Sphenoid and Cavernous Sinus Successfully Treated with Partial Resection and High Dose Radiotherapy: Case Report and Review of the Literature." Cureus 7(9): e328, 2015


2014


Drummond J, Ciacci JD, Lee R: Direct Pressure on a Pseudomeningocele Resulting in Intraoperative Cerebral Ischaemia. Canadian Journal of Anesthesia/Journal canadien d’anesthésie, 2014; 61(7), 656-659


APPENDIX VIII

REPRESENTATIVE PUBLICATION REPRINTS
(Commencing on page 137)
Clinical Progress

Cell Stem Cell

A First-in-Human, Phase I Study of Neural Stem Cell Transplantation for Chronic Spinal Cord Injury

Graphical Abstract

Preclinical Studies
- Differentiation of grafted NSI-566:
  - neurons
  - oligodendrocytes
  - astrocytes
- Migration of grafted NSI-566 cells

Intraspinal Injections
- Phase 1 clinical trial
- n=4 adult subjects
- Thoracic ASIA A spinal cord injury
- 1-2 years after injury
- 6 bilateral injections
- 2 x 10^8 cells/injection via stereotactic floating cannula

Clinical Follow-up
- Presence of antibodies
- ISNCS1 Exam
- Neurophysiology
- Imaging
- Quality of Life
- Pain

Highlights
- NSI-566 grafted injured spines in rats with near complete cavity-filling
- The differentiation profile of grafted cells showed all three neural lineage cells
- High-density human axonal sprouting was seen throughout the NSI-566 grafted region
- NSI-566 transplanted in the spinal injury site of patients can be performed safely

Authors
Erik Curtis, Joel R. Martin, Brandon Gabel, ..., Catriona Jamieson, Martin Marsala, Joseph D. Ciacci

Correspondence
jciacci@ucsd.edu

In Brief
After promising results were seen in a pre-clinical human-spinal-cord-derived neural stem cell NSI-566 transplantation study for spinal cord injury in rats, a phase I clinical trial for NSI-566 transplantation was initiated in patients with complete thoracic SCI.
A First-in-Human, Phase I Study of Neural Stem Cell Transplantation for Chronic Spinal Cord Injury

Erik Curtis, Joel R. Martin, Brandon Gabel, Nikki Sidhu, Teresa K. Rzesiewicz, Ross Mandeville, Sebastiaan Van Gorp, Marjolein Leerink, Takahiro Tadokoro, Silvia Marsala, Catriona Jamieson, Martin Marsala, and Joseph D. Ciacci

1Department of Neurological Surgery, University of California, San Diego, La Jolla, CA 92037, USA
2Department of Medicine, Division of Regenerative Medicine and CIRM Alpha Stem Cell Clinic, University of California, San Diego, La Jolla, CA 92037, USA
3Department of Neurosciences, University of California, San Diego, La Jolla, CA 92037, USA
4Department of Anesthesiology, University of California, San Diego, La Jolla, CA 92037, USA
5These authors contributed equally
6Lead Contact
*Correspondence: jciacci@ucsd.edu
https://doi.org/10.1016/j.stem.2018.05.014

SUMMARY

We tested the feasibility and safety of human-spinal-cord-derived neural stem cell (NSI-566) transplantation for the treatment of chronic spinal cord injury (SCI). In this clinical trial, four subjects with T2–T12 SCI received treatment consisting of removal of spinal instrumentation, laminectomy, and durotomy, followed by six midline bilateral stereotactic injections of NSI-566 cells. All subjects tolerated the procedure well and there have been no serious adverse events to date (18–27 months post-grafting). In two subjects, one to two levels of neurological improvement were detected using ISNCSCI motor and sensory scores. Our results support the safety of NSI-566 transplantation into the SCI site and early signs of potential efficacy in three of the subjects warrant further exploration of NSI-566 cells in dose escalation studies. Despite these encouraging secondary data, we emphasize that this safety trial lacks statistical power or a control group needed to evaluate functional changes resulting from cell grafting.

INTRODUCTION

Current pharmacological- or physical-rehabilitation-based therapies for chronic spinal cord injury (SCI) are limited and primarily focused on modulating symptoms associated with chronic SCI such as pain (Gwak et al., 2016; Saulino and Averna, 2016) and/or muscle spasticity (Koulousakis and Kuchta, 2007; McIntyre et al., 2014). With the exception of nerve transfer procedures aimed at optimizing motor control in dermatomes just below the injury (Emamhadi et al., 2016; Medina et al., 2017; Ray et al., 2016; Simcock et al., 2017), no therapy exists that would lead to a clinically relevant improvement of motor or sensory function in chronic spinal trauma patients. Over the past 3 decades, extensive pre-clinical data have suggested a beneficial-functional effect of cell-replacement-based therapy for treatment of a variety of spinal neurodegenerative disorders. In the majority of experimental models, multipotent neural precursors (NPCs) derived from animal or human fetal CNS (FT) (Cizkova et al., 2007; Hefferan et al., 2012; Kakinohana et al., 2012; Lu et al., 2012; Rosenzweig et al., 2018), embryonic stem cells (ESCs) (Brustle et al., 1999; Keirstead et al., 2005; Nistor et al., 2005), or induced pluripotent stem cells (iPSCs) (Fujimoto et al., 2012; Kobayashi et al., 2012; Nori et al., 2011) have been used for in vivo spinal grafting, resulting in a population of post-mitotic neurons, astrocytes, and oligodendrocytes that differed in frequency depending on the NPC source. Because of the established lineage commitment potential of FT-derived NPCs and lack of teratoma formation, they have the most favorable safety profile. The NSI-566 line is a human neural stem cell line authorized by the FDA for clinical testing (Johe et al., 1996; Lu et al., 2012; Usvald et al., 2010; van Gorp et al., 2013). While the NSI-566 neural stem cell line showed a favorable safety profile in completed phase I and II clinical trials for patients with ALS (Glass et al., 2016), its safety, tolerability, and proof-of-concept data supporting further use in patients with SCI had not been evaluated. Historical clinical data show that patients with complete SCI (ASIA-A) that is stable at 1 week after injury have less than a 2% chance of spontaneous sensory improvement one dermatome below the level of injury (Burns et al., 2003; Harrop et al., 2009). Here we report the results of the first cohort of patients enrolled in a phase I first-in-human clinical trial of implantation of a neural stem cell product, NSI-566, into the injury site of patients with chronic ASIA-A grade thoracic SCI.

RESULTS

Pre-clinical Efficacy and Safety Studies with a Spinally Grafted NSI-566 Cell Line

Extensive pre-clinical efficacy and safety studies were conducted using a spinally grafted NSI-566 cell line in small and large animal models of spinal injury or in naive immunodeficient or...
continuously immunosuppressed animals. A statistically significant improvement in motor and sensory neurological function, amelioration of muscle spasticity, and evidence of functional synaptic coupling between grafted NSI-566 cells with the host spinal neuronal circuitry was demonstrated in rat models of L3 spinal compression (van Gorp et al., 2013), complete spinal cord transection (Lu et al., 2012), or irreversible spinal ischemic injury (Cizkova et al., 2007). A comparable functional efficacy signal was recently reported using a non-human primate model of cervical hemisection (Rosenzweig et al., 2018). In addition, a cell dose escalation study to define the equivalent human cell dose was completed in adult pigs that have similar spinal cord anatomical dimensions to those of adult humans (Usvald et al., 2010).

To define the pre-clinical safety of spinally grafted NSI-566 cells, a long-term (9–10 months) study was conducted in adult spinally injured immunodeficient rats. A total of 90 athymic nude rats (nu/nu, NCI) were randomly assigned to injections of vehicle or NSI-566 (450,000 cells/45 μL of the vehicle) into the injury epicenter 7 days after T10 spinal segment impact injury (MASCIS 12.5 mm) (Figure 1A). After cell grafting, animals survived for an average of 9.7 months and were assessed for neurological function deterioration. Qualitative confocal immunofluorescence microscopic analysis and quantitative analysis of graft survival, cell proliferation, and axonal outgrowth at 9 months post-grafting revealed

Figure 1. Survival and Long-Term Safety of Spinally Grafted NSI-566 in Th10 Spinal Segment-Injured Immunodeficient Rat
(A) Schematic diagram of the experimental design.
(B) Near complete injury-induced cavity-filling effect by injected NSI-566 cells (hNUMA+ cells) and continuing extensive syringomyelia in vehicle-injected animals (white asterisks).
(C–E) Differentiation of grafted NSI-566 to neurons (C) (hNSE), oligodendrocytes (D) (OLIG2), and astrocytes (E) (GFAP).
(F) Extensive migration of grafted NSI-566 cells (hNUMA+ cells) from cell-injected injury epicenter into host tissue.
Scale bars: (B), 500 μm; (C), 20 μm; (D), 100 μm; (E), 50 μm; (F), 200 μm.
Figure 2. Extensive Axonal Sprouting from Grafted NSI-566 and Innervation of Human Grafts by Descending Motor Axons of the Host in Immunodeficient Rat

(A) High density of human axons (rostral to the lesion site) (HO14; red) derived from grafted NSI-566 cells in lateral funiculi. Numerous GFP-tagged (green) descending motor axons of the host can also be seen.

(B–D) High density of descending GFP-tagged corticospinal and/or rubrospinal axons innervating hNSE + grafts.

(E) Human-specific synaptophysin terminals (hSYN; green) derived from grafted NSI-566 neurons. Numerous hSYN+ puncta residing on membrane of the host α-motoneuron (NeuN) distal to the injury epicenter can be identified.

Scale bars: (A), 100 μm; (B), 200 μm; (C), 50 μm; (D), 40 μm; (E), 10 μm.
increased axonal outgrowth in NSI-566 grafted animals (Figures 1 and 2; Table S6). Confocal images obtained following staining of sections with human-specific nuclear antibody showed near complete cavity-filling by grafted cells. This was in contrast to the extensive syringomyelia seen in vehicle-injected animals (Figure 1B). Examination of the differentiation profile of grafted cells showed all three neural lineage derivatives, including hNSE+ (human-specific neuronal enolase) neurons, OLIG2+ (oligodendrocyte transcription factor) oligodendrocytes, and hGFAP+ (human-specific glial fibrillary acidic protein) astrocytes (Figures 1C–1E). Analysis of rostrocaudal migration of hNUMA+ cells showed a robust migration of injected cells through the thoracic spinal cord (Figure 1F). Analysis of axonal sprouting from grafted neurons showed a high density of human axons throughout the grafted region (rostral to the lesion site) and in lateral funiculi between GFP-tagged descending motor tracts of the host (Figure 2A). Endogenous GFP-tagged motor axons showed extensive innervation of human hNSE+ grafts (Figures 2B–2D). Moreover, human-specific synaptoysin+ punctae derived from grafted human neurons, on the membranes of the host interneurons and α-motoneurons above and below the level of injury, were seen (Figure 2E). In summary, previously published pre-clinical proof-of-concept and safety studies provided a scientific rationale and a large animal translational platform for designing the human clinical protocol for treatment of chronic SCI by spinal NSI-566 grafting.

**A Phase I Neural Stem Cell Implantation Clinical Trial for Chronic Thoracic SCI**

A total of four subjects have received NSI-566 spinal cord implantation (Video S1) with a post-procedure follow-up ranging from 18 to 27 months (Table 1). All subjects tolerated the procedure well with no serious adverse events in the post-procedure period. Prospective data have been collected including ISNCSCI scores, functional and pain surveys, SCIM scores, electromyography (EMG), Brain Motor Control Assessment (BMCA), and serial MRI. The presence of donor-specific HLA antibodies was also monitored periodically.

The first patient enrolled in the study is a 26-year-old female (subject 001) with a T8 neurological level of injury after a motor vehicle accident. After neural stem cell implantation, ISNCSCI neurological assessment showed two-level sensory and motor improvements (from T8 through T10) bilaterally at 6, 12, and 18 months, and one-level sensory and motor improvement at 27 months, compared to pre-treatment baseline. Moreover, BMCA showed newly developed muscle responsiveness in lower limbs to reinforcement maneuvers. This improvement developed between 18 to 27 months post-cell grafting visits with no evidence of circulating anti-donor HLA class I or class I antibodies at 3, 12, 18, or 27 months (Figures 3A–3D; Tables S1 and S5).

The second patient in the cohort is a 33-year-old male (subject 008) with a T7 neurological level of injury after a motorcycle accident. While no change in overall ISNCSCI neurological score was noted initially, the 12-month EMG analysis showed new voluntary activity in the right rectus abdominus and left T6 to T8 paraspinal muscles (Figures 3E and 3F) as well as the development of sensation during EMG needle insertion between T6 and T9 bilaterally at 18 months after grafting (Tables S2 and S5).

The third patient in the cohort of this phase I trial is a 35-year-old male (subject 009) who suffered a T2 neurological level of injury after a motor vehicle accident. No change in ISNCSCI neurological score (motor and sensory) was noted for up to 12 months after cell grafting (Tables S3 and S5).

Finally, the fourth patient in the cohort is a 24-year-old male (subject 010) with a T5 neurological level of injury after a motor vehicle accident. ISNCSCI neurological assessment showed one level of sensory and motor improvement (from T5 to T6) on the right side and two levels of sensory and motor improvement (from T5 to T7) on the left side at 6, 12, and 18 months visits compared to baseline. The functional response included gain in voluntary bilateral motor control of T6 to T7 abdominal wall musculature. Moreover, EMG analysis showed previously unrecoerred activity in the right superficial paraspinal muscle at T7, one level lower at the 12 months visit than at the 6 months visit. This voluntary activity was reconfirmed at the 18 months visit (Figures 3G and 3H). Subject 010 was noted to have anti-HLA antibodies on post-implantation day 98. Measured anti-HLA antibodies of CW1, CW8, DRB1*04:04, and DR16 were not antibodies with specificity against the HLA alleles of the donor cells. The subject denied additional transfusions or blood products post-implantation. A bystander immune response was ruled out when months 12 and 18 revealed similar anti-HLA antibody results. Upon analysis of these assays and patient report, it was concluded that the immunoreactivity present in the patient was not related to the stem cell treatment (Tables S4 and S5).

For all patients, no significant change in quality of life scores were observed. MR imaging for all patients demonstrated varying degrees of focal spinal cord malacia, with one patient having bony fragments within the spinal canal at T11 level (subject 001). No patient demonstrated immediate or delayed complications after the stem cell injections. There were no new areas of cord or soft-tissue edema, enhancement, development of swelling, or fluid collections on immediate post-procedural or follow-up imaging. Evaluation of the area of cord malacia and cell injection site was limited in all patients due to susceptibility artifact either from metallic or bony fragments from the previous injury or from the sequelae of the previous fusion hardware. No visible morphologic change was observed in the area of spinal cord malacia on either the pure anatomic or diffusion tensor sequences. In all four patients, diffusion tensor imaging (DTI) revealed a stable appearance of spinal cord tracts both at the injury site and rostral/caudal to the injury site but did not show extensive evidence of remodeling or improvement of tractography (Figure 4).

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<td>Number of injections (mean)</td>
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<td>6</td>
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<td>Hospital length of stay (mean/range)</td>
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<td>mean: 6.75 days; range: 6–8 days</td>
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<td>Time since implantation (mean)</td>
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<td>mean: 372 days; range: 175–553 days</td>
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DISCUSSION

SCI represents a devastating neurological condition. Depending on the segmental level and completeness of spinal injury, the neurological deficit can clinically be presented as paraparesis or quadriparepsis or fully developed paraplegia or quadriplegia. Current pharmacological or physical rehabilitation-based therapies for SCI are limited and primarily focused on modulating symptoms associated with chronic SCI such as pain (Gwak et al., 2016; Saulino and Averna, 2016) and/or muscle spasticity (Koulousakis and Kuchta, 2007; McIntyre et al., 2014). With the exception of nerve transfer procedures aimed at optimizing motor control in dermatomes just below the injury (Emamhadi et al., 2016; Medina et al., 2017; Ray et al., 2016; Simcock et al., 2017), at present no therapy exists that would lead to a clinically relevant improvement.

Figure 3. Brain Motor Control Assessment (BMCA) and EMG Assessments to Identify Voluntary or Reinforcement Maneuver-Initiated EMG Activity

(A and B) Recording of voluntary or reinforcement maneuver-triggered BMCA activity showed no detectable BMCA responses at 18 months after cell transplantation in subject 001 (4.4.2016 tracers).

(C and D) Subsequent recording performed at 27 months after transplantation showed the presence of EMG response in triceps surae after a reinforcement maneuver (D). No activity is noted with volitional attempts at plantar flexion or dorsiflexion of the left ankle in either study (C). Red arrowheads show new activity in triceps surae with a reinforcement maneuver (neck flexion and deep breath) compared to prior recording; horizontal black bar indicates onset marker.

(E and F) EMG recording from right T6 paraspinal muscle in subject 006. In comparison to baseline recordings, multiple new motor units (red arrowheads) were recorded at 12 months after cell transplantation.

(G and H) EMG recording from T6 and T7 paraspinal muscles in subject 010. In comparison to baseline (12 weeks) recording, a new motor unit (read arrowheads) was recorded at 6 and 18 months after cell grafting from T7 paraspinal muscle.
improvement of motor or sensory function in chronic spinal trauma patients.

Over the past 3 decades, a substantial number of experimental studies have tested the treatment potency of spinally grafted cells with the aim of restoring trauma-induced loss of function. Based on known pathophysiology of SCI characterized by (1) loss of segmental neurons at the site of injury, (2) demyelination of descending axons and associated loss of signal conductivity, and (3) decreased synthesis of neurotrophic factors and increased and long-lasting inflammation, the cell replacement therapies and a specific cell source used for treatment can be considered in three principal categories. First are studies that aim to restore local axonal myelin by implanting oligodendrocyte-type cells (Brustle et al., 1999; Keirstead et al., 2005; Nistor et al., 2005). Second, the implantation of neurotrophic factor-producing cells was employed. In these studies, for example, implantation of BDNF or NGF-producing fibroblasts has been shown to promote axonal sprouting and facilitate functional recovery in rat and primate models of SCI (Grill et al., 1997; Liu et al., 1999; Tuszynski et al., 2002). Third, grafting of neuron-producing neural precursors (such as the NSI-566 line used in our current phase I study) was extensively used. The primary goals in these studies aim at reconstituting regional neuronal pools at the spinal injury site in order to (1) restore local segmental neuronal circuitry by newly established synapses between grafted neurons and the remaining neurons of the host, and (2) to provide additional neuronal targets for re-establishment of new synaptic contacts between sprouting trauma-injured descending motor axons.

As previously described, spinal grafting of NSI-566 in naive rat or rat and non-human primate L3 compression, complete or hemi-transected spinal cord, and spinal ischemic models has resulted in a significant improvement of neurological function and suppression of spasticity-characterized morphologically by extensive axonal sprouting and development of synaptic contacts with the host neurons (Cizkova et al., 2007; Lu et al., 2012; Rosenzweig et al., 2018; van Gorp et al., 2013).

Several phase I clinical trials to test the safety of spinally grafted human cells of different germ origin in peri-acute or chronic spinal trauma patients were previously reported. First, immuno-activated, autologous, peripheral blood macrophages have been transplanted intraspinally into the spinal cords of acute SCI patients using a hand-held microsyringe (Lammertse et al., 2012). Second, Feron et al. investigated the effect of olfactory ensheathing cells implantation and postulated that these cells may promote axonal regeneration. Dissociated and cultured cells from autologous olfactory mucosal tissues were transplanted into 12 chronic ASIA-A patients. Neither functional improvements nor adverse effects due to the treatment were noted at 3-year follow-up (Feron et al., 2005; Mackay-Sim et al., 2008). Third, autologous bone-marrow-derived mononuclear cells were injected intraspinally into the injury area of nine chronic ASIA-A patients (Deda et al., 2008). No complications were seen. Collectively, these initial clinical studies have established the feasibility of spinal cell delivery procedures in acute or chronic spinal trauma patients. Until now no human spinal-cord-derived neural precursors were tested in the clinical setting for treatment of SCI. The NSI-566 line is the only spinal-cord-derived human cell line recently FDA-authorized for testing spinal grafting in human patients.

From a safety perspective, there are several key points to note from our current study. (1) No surgery-related complications such as laminectomy site infection or CSF leakage was seen in any of the subjects. (2) Analysis of pain scores showed that patients experienced no spontaneous or evoked pain up to 18–27 months post-procedure. (3) MRI analysis showed no detectable post-cell injection inflammatory changes, swelling,
or fluid accumulation (indicative of syrinx formation) at or around the cell injection site.

Regarding secondary outcomes, analysis of neurological ISNCSCI level, EMG, and/or BMCA showed potentially positive changes in three subjects. Subject 001 reliably showed previously unrecorded activation of motor units in the triceps surae on BMCA, 27 months after injection, with reinforcement maneuvers (but not voluntarily). This change in the pattern of the EMG response is most consistent with an increased central state of excitability compared to the baseline recording and may be attributable to new activity in reticular circuitry, potentially via a functional reconnection of supraspinal motor centers within spinal circuitry. This transforms the patient status from a "complete" to an "incomplete" SCI status. Potential alternative explanations include a significant difference in patient effort between the studies or development of spasticity since the baseline recordings. However, patient effort is closely monitored by the examiner, and spasticity giving this result would be unusual given the chronicity of injury. Furthermore, the same subject showed a two-level sensory and motor improvement (ISNCSCI score) measured between 3 and 6 months post-procedure. Subject 010 demonstrated new voluntary muscle activity one level inferiorly on the right (T7) at 6 months post-cell grafting (compared to baseline or 4 weeks) which was re-confirmed at 18 months. Using the ISNCSCI score, a one to two level of motor and sensory improvement was seen 6 months after the procedure, supporting the EMG findings, but recognizing the caveats of interrater variability, sampling error, and multilevel innervation with paraspinal EMG. In subject 006, EMG showed perhaps the most robust change, recording previously unrecorded muscle activity in the left T7 and T8 paraspinal muscles at 12 months post-procedure as well as the development of new intolerance to needle EMG between T6 and T9 at 18 months post-procedure. However, no change in overall neurological ISNCSCI level was seen in this subject. The small changes seen in the level of injury on the left side of subject 006 and the small change seen at 27 months in subject 001 likely reflect the difficulty in assessing the exact motor and sensory level in the midthoracic spine and may indicate that one level of improvement may be within the margin of error of these assessments at the midthoracic level. Of note, it may be expected that subclinical reinnervation would be detected by EMG initially, prior to any manifestation of clinical improvement. This is due to EMG being capable of detecting motor units that are of insufficient power to be of clinical significance, as well as the fact that motor neurons newly under central control might be ineffective until maturation of central motor programming. The presence of new intolerance seen at T6–T9 is suggestive of alteration in spinal processing of afferent

Figure 5. Technique and Spinal Injection System to Perform Spinal Cord Cell Injections in Patients with Chronic SCI
(A and B) The safety and engraftment properties of NSI-566 cells, including synapse formation with neurons of the host, was extensively studied in naive-immunosuppressed pigs and spinally injured rodents.
(C) A sample of four subjects with chronic SCI classified as ASIA-A, a motor and sensory complete SCI, levels T2–T12, who met eligibility criteria were enrolled.
(D) Schematic drawing of exposed dorsal spinal cord depicting the location of six individual spinal cell injections with respect to the injured spinal cord region (dotted area).
(E) Spinal cell injections were performed using a free-floating cannula attached to an XYZ manipulator. The XYZ manipulator is mounted on a self-supporting platform attached to patient vertebral column (vertebral laminae) above and below the level of laminectomy using four stainless steel posts and spinal screws.
(F) Position of the injection floating cannula after placement of the injection tip into the spinal cord tissue. A stop-cock ring and floating portion of the cannula can now be seen (white arrow). The guiding stainless-steel tubing was retracted to permit the flotation of the injection cannula.
sensory signals and can be related to peripheral sprouting of sensory afferents. Electrophysiologically defined improvement seen in three of four of our subjects may reflect several mechanisms including improved myelinization and/or development of new synaptic contacts with the host neurons and descending motor tracts (Cizkova et al., 2007; Lu et al., 2012; Rosenzweig et al., 2018; van Gorp et al., 2013).

DTI was performed for research purposes. DTI enables both qualitative and quantitative assessment of the spinal cord (by measurement of the fractional anisotropy and apparent diffusion coefficient parameters). After functional disruption of the spinal cord in SCI, these tracts could be improved after stem cell implantation. No apparent evidence of remodeling and improvement was seen in the DTI imaging in this study. However, the stable appearance of DTI imaging suggests that no further disruption took place post-transplantation. Imaging was performed on 1.5T MRI machines in this study. The wider use of 3T scanners, as well as the development of improved acquisition and post-processing techniques, should result in an increased role of spinal cord DTI in both research and clinical SCI practice (Rajasekaran et al., 2012; Sasiadek et al., 2012).

Lastly, as described in the STAR Methods, the surgical procedure was a significant technical advance for this study (Figure S5). The use of a “floating cannula” allowed accuracy of delivery without suspension of respiration and other facets of homeostasis, including blood pressure and other vital signs (Video S1). This setup is different as compared to other injection devices that are fixed to the operating room bed and require suspension of ventilation to ensure proper injection.

Whether or not a higher degree of synapse formation and corresponding functional improvement would be achieved once higher cell doses are employed for spinal grafting is not defined at present. In addition to showing safety and tolerability, the dose of NSCs utilized was based on the safe and well-tolerated dose used for ALS and showed proof-of-concept results in this trial that are suggestive of functional improvement, supporting a further FDA-approved study in our cervical spine cohort.

Despite these potentially encouraging secondary data seen in our current clinical study, we emphasize that the study was designed as a safety trial without statistical power or a control group needed to evaluate any functional change resulting from cell grafting. Nonetheless, some of the clinical data at late time points after cell delivery are certainly intriguing and merit further investigation.

Conclusion and Future Directions

Our current clinical data demonstrate that spinal grafting of the human NSI-566 cell line in chronic spinal trauma patients is safe with no detectable side effects identified at 18–27 months after cell delivery. Published studies in this complex arena are usually small. Despite the small sample size, the key strengths of the study are the extensive follow-up period and the timeline of treatment, as everyone was treated after 1 year of injury when there is little to no chance of spontaneous recovery. Like other phase I studies, this study was intended to provide proof of safety and tolerability and proof-of-concept data that will justify the next cohort. This favorable human safety profile, in conjunction with signs of potential efficacy signal and promising prior animal studies, warrants future dose escalation studies in patients with chronic SCI.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes six tables, further methods (inclusion/exclusion criteria and study visit protocols), and one video and can be found with this article online at https://doi.org/10.1016/j.stem.2018.05.014.

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AUTHOR CONTRIBUTIONS


DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES


STAR METHODS

KEY RESOURCES TABLE

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CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Joseph Ciacci (jciacci@ucsd.edu). Cell source requests should be directed to and will be fulfilled by Karl Johe, Ph.D. (Neuralstem Inc., kjohe@neuralstem.com).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals Safety Study in Immunodeficient Spinally Injured Rats

Animal welfare: The study design and animal usage were reviewed and approved by the Institutional Animal Care and Use Committee (MC 0071, UCSD, 9500 Gilman Dr., La Jolla, CA). Animal welfare for this study was in compliance with the U.S. Department of Agriculture’s (USDA) Animal Welfare Act (CFR 9 - Parts 1, 2, and 3), and the Guide for the Care and Use of Laboratory Animals. Animals: Rats, Athymic Nude (rnu-/rnu-) - Hsd: RH-Foxn1rnu (Harlan, Madison, WI, USA) were used. A total of 90 male rats were enrolled, of which 8 were used for descending motor tracts labeling (Figure 1A). Animal ages at injury ranged from 10 to 13 weeks, and body weights varied between 309 to 345 g.

Animal housing: Animals were single-housed in cages with irradiated bedding. Acclimation times prior to the experiment varied but were at least 8 days. Environmental controls were set to maintain temperatures from 64° to 79°F (18° to 26°C), with fluorescent lighting on a 12 hour/12 hour on/off inverted cycle. Animals were fed irradiated Harlan Teklad Global 18% Protein Rodent Diet T.2918.15 (Harlan Laboratories, Placentia, CA) ad libitum. In addition, animals that were selected for addition behavioral testing (see below) also received Fruit Crunchies (BioServ product # S05798-1) and HydroGel (ClearH2O®, ME 04101). Animals were provided with purified water (Mountain Dairy, Riverside, CA, USA) ad libitum.

Clinical Trial Design and Patient Selection

Inclusion and exclusion criteria are listed in Methods S1. This was a Phase I safety study of human spinal cord-derived neural stem cell transplantation for the treatment of chronic spinal cord injury, defined as at least one year but no more than 2 years after traumatic SCI. A sample of 4 subjects with chronic SCI classified as AISA-A, a motor and sensory complete SCI, levels T2-T12, who met eligibility criteria were enrolled. No control group was included. Inclusion and exclusion criteria are listed in Methods S1. All subjects received spinal cord injections of human spinal cord derived neural stem cells (NSI-566). The trial was registered in ClinicalTrials.gov as NCT 01772810. IRB approval granted by UCSD Health Center, Human Research Protections Program (HRPP), 9452 Medical Center Drive, La Jolla, CA 92037

NSI-566 Neural Stem Cell Line

NSI-566, is a human spinal cord-derived neural stem cell line. Neural stem cells are the precursor cells present in the neuroepithelium along the neuraxis during mammalian fetal development. NSI-566 was derived from a single post-mortem spinal cord
of an 8-week gestational age fetus. This tissue was obtained in compliance with the National Institutes of Health (NIH) and Food and Drug Administration (FDA) Good Tissue Practice Guidelines, and under a protocol approved by an outside independent review board. Neural stem cells were isolated by dissociating a single piece of spinal cord tissue of lower cervical/upper thoracic region and expanding it as a single line. Three-tiered cell banks, consisting of a master cell bank (MCB), a working cell bank (WCB), and a clinical cell bank (CCB), were established and cryopreserved under cGMP. The current MCB is >99% nestin-positive, adherent neural stem cells established at passage 6. A WCB at passage 9 was manufactured from this MCB, and a CCB at passage 12 was manufactured from the WCB. All cell doses used in this study were prepared from the passage 12 CCB. Karyology of the clinical lot cells showed normal 44, X, and Y chromosomes. Greater details of the cGMP generation of clinical material was previously described (Glass et al., 2012).

For cell administration, NSI-566 was provided as a live-cell suspension, ready-to-inject, formulation, requiring no further manipulation. Briefly, NSI-566 cell suspension was prepared one day prior to each scheduled surgery at a cGMP facility. One or more vials of the cryopreserved CCB were thawed at once, washed of the freezing medium by repeated centrifugation in a hibernation medium (HM), and concentrated to a final concentration of \(2 \times 10^6\) cells/mL of HM. This target concentration had been established for being safe and adequate for intraspinal injections by series of preclinical (Usvald et al., 2010; van Gorp et al., 2013) and clinical studies ((Glass et al., 2012; Glass et al., 2016). The HM is a sterile, buffer solution free of preservatives and antibiotics developed for intraparenchymal injections into CNS, which was provided by Neuralstem, Inc. The cell suspension was then packaged in a custom-designed insulated shipping container that maintained the cell vials at 2°C–8°C, and shipped to the surgery site for overnight delivery by a commercial package courier (Federal Express). Prior shipping stability studies had validated >70% cell viability up to 60 hours under these conditions. The expiry window for this study was 48 hours from the time of preparation.

Before proceeding with cell administration, the cells suspension was inspected for cell viability of at least 70% using the method of trypan blue exclusion in order to proceed with the implantation. The viability ranged from 87%–92% and there were no failed deliveries or rejections due to out-of-range release specifications. Sterility and endotoxin level of each cell batch were verified by post hoc testing of retention sample kept at the manufacturing site, and the test result was notified to the study investigators within 14 days of surgery. There was no incidence of nonconformance in regard to sterility or endotoxin level.

The clinical lot of NSI-566 had undergone extensive preclinical safety and efficacy studies in various small and large animal studies, which had been reviewed by US FDA under an IND (Investigational New Drug) application (#014413). Potential of NSI-566 to form tumor cells was evaluated in 3 different animal models in 5 studies. In an accepted mouse model of tumorigenicity, there was no evidence of tumorigenicity 1 or 3 months following subcutaneous injection with \(1 \times 10^7\) cells. Four studies were conducted in which possible tumor formation was evaluated after injection of maximal feasible dose (0.45 – 0.6 \(\times 10^6\) cells) into the clinically intended target site, the spinal cord; 2 non-GLP 9-month survival studies, 1 GLP 6-month survival study, and 1 GLP 9-month survival study. No evidence of tumorigenicity due to NSI-566 was observed in any of these studies, as assessed either by mitotic activity or histopathology. In some of the rodent intraspinal injection studies, a 2-5-fold increase in the cell number was observed post transplantation. This increase is believed to represent a normal pattern of division of the spinal cord-derived human neural stem cells and their glial progenies, which gradually decline over time. The cell dose used in this study included such anticipated in vivo increase in graft size.

DNA of NSI-566 was subjected to high resolution sequencing analysis of major HLA loci: Class I locus A, B, and C; and Class II locus DRB1, DRB3/4/5, DPB1, DOA1, and DOB1. Based on the genotypes, followed antibodies in treated subjects were monitored: HLA-A2; HLA-A68; HLA-B62; HLA-B71; HLA-Cw7; HLA-DR9; HLA-DR12; HLA-DR52; HLA-DR53; HLA-DQ7, HLADQ9 (and the DQ alpha specificities 03 and 06); HLA-DP2 and HLA-DP6.

Each subject received total of 6 intraspinal injections (2 \(\times 10^5\) cells/injection delivered in 10\(\mu\)L of hibernation buffer). The injections were placed bilaterally into the remaining tissue lateral to the injury site and within the medial white matter-appearing tracts of approximately one segment below the injury site, as verified by intra-operative fluoroscopy imaging. Injections were made using a customized stereotactic cell injection device (Figure S5E), (Tadesse et al., 2014).

**METHOD DETAILS**

**Induction of spinal injury (SCI):** The day prior to surgeries the back of the animals was shaved. On SCI surgery days, each animal received lactated ringer’s (5 mL, s.c.). Animals were anesthetized with isoflurane (5% for induction, ~2% for maintenance, in air). The surgery site was wiped with alcohol and chlorhexidine diacetate solution (2%–4%). Rat body temperature was maintained by a water blanket heating system. The skin over the vertebral column was opened, paravertebral muscles were dissected away, and the animal was mounted onto a Stereotaxic frame (Stoelting Lab Standard Stereotaxic - Single, Cat# 51600 Lab Standard) with Spine Adaptors (Stoelting, Cat# 51695 Rat Spinal Adaptor). A thoracic spinal segment (Th10) was then exposed using a dental drill. A moderate injury was made by letting the rod of a MASCIS (NYU) Impactor dropped from a height of 12.5 mm onto the exposed surface of the spinal cord. Next, the impactor was removed immediately, the animal was detached from the frame, the surgical site was irrigated with sterile saline, layers were closed with absorbable Vicryl suture, and Bactracin/Neomycin/Polymyxin (triple antibiotic) ointment was applied to the incision site. Cefazolin (10mg/kg, s.c.) was also given at the day of surgery.

**Dosing surgery:** One week following SCI surgeries, prior to cell grafting, animals were allocated to groups by stratified randomization based on body weights. Animals were assigned to either the vehicle group (n = 45) or the cell-graft group (n = 45). There were no exclusion criteria for dosing surgery other than appearing healthy enough. Moribund animals or animals found dead were replaced.
The day prior to grafting the animals were anesthetized to remove any remaining skin sutures and to shave the back of the animals. The spinal cord was re-exposed. The dorsal aspect of the vertebra immediately caudal to the existing laminectomy was removed using the dental drill. Special care was taken to keep the dura intact. For the current safety assessment, a maximum feasible cell dose was estimated by several preliminary dose-range finding studies using subcutaneous and intraspinal delivery. The cell suspension (Group A) or vehicle/hibernation buffer-only (Group B) was injected as follows:

1) 20 injections peripheral from the injury epicenter (1 µL each; 2 µL/min) were made bilaterally into about 2 segments above and/or below the injury epicenter at 1 mm intervals.

2) 5 injections (5 µL each; 2 µL/min) were injected around the borders of injury epicenter. A total of 25 injections were made in each spinal cord, resulting in a total of 45 µL (cells suspended at 10^4 cells/µL, resulting in a total of ~4.5 x 10^6 cells).

The required volume of dosing for completing all injections of one animal was drawn into a 100 µL NanoFil syringe (World Precision Instruments) with a 33-gauge needle (World Precision Instruments, Cat# NF33BV). The syringe was mounted onto a manually controlled syringe holder/injector (David Kopf, Model 5000+5001) attached to the stereotaxic frame. The needle tip was lowered to a depth of 1-1.5 mm from the pial surface at the Dorsal Root Entry Zone (DREZ) into the spinal parenchyma, and the injection was made. After a thirty second pause, the needle was gently drawn out of the spinal cord. The syringe/needle ensemble was cleaned by repeatedly rinsing with and immersion in 70% isopropanol/water solution for a minimum of 15 minutes before and after each animal.

**GFP labeling of descending motor tracts:** A small group of SCI animals (n = 8; 4 with cell treatment and 4 vehicle-injected), was used to study the innervation of grafted cells by descending motor axons of the host. To label descending motor tracts animals were anesthetized and received four brain injections of recombinant Adeno-Associated Virus (AAV) engineered to express Green Fluorescent Protein under the human synaptophysin promoter (AAV.synGFP; neuronal specific expression). Bilaterally injected vectors were targeted into motor cortex and nucleus ruber. Stereotactic coordinates used for the injections were: motor cortex: bregma – 2.0 mm, lateral 3.0 mm, depth 1.5 mm, red nucleus: bregma – 5.8, lateral 0.8 mm, depth 7.1 mm. Each injection consisted of 5 µL of the virus suspension at 10^13 gc/mL and was injected over 5 min period using 32G stainless steel needle. Vector was injected 4-6 weeks before sacrifice.

**Post-surgical care:** All animals received Sulfamethoxazole and Trimethoprim oral suspension, (USP 200 mg/40 mg per 5 mL) in the water (5 mL per 250 mL) for 1-2 days before, up to 30 days after initial contusion, and as needed in case of infections. Animals were checked for bladder retention at least two times daily for the duration of the study. If full, bladders were emptied by manual expression. Consecutively, the surgical area was checked. Ketoprofen (4.0 mg/kg, s.c.) was given approximately one hour prior to surgery and approximately every 24 hours for up to 48 hours. Animals found ill were given up to 5 mL (s.c. < 2 daily) Lactated Ringer’s solution until improvement was observed. Hexa-Caine Spray and/or Bacitracin/Neomycin/Polymyxin triple antibiotic ointment was used to treat scabs or wounds.

**Cells:** The cells, named “NSI-566RSC,” were produced by Neuralstem Inc. (Rockville, MD, USA), as described before (Johe et al., 1996). The grafted cells were a clinical lot (Lot No. CRL471925-3, passage 12), a human neural stem cell line derived from fetal spinal cord of 8 gestational weeks. They were suspended in hibernation buffer at 10^4 cells/µL. Each batch (and vehicle) was prepared at Neuralstem Inc. (Rockville, MD, USA) one day before surgery and shipped by overnight service in temperature monitored containers.

**Post injury health observations, measurements, and specimens:** General Health Observations (GHOs) were performed on the dosing day, pre-dose, post-dose, and weekly thereafter. GHOs included weight, general appearance, stool appearance, toxicity symptoms and other appearing additional health issues. In addition, tumor presence was assessed by full body palpation. Any palpable cell mass was measured using a caliper. All animals were observed daily to ensure no loss of animals for necropsy.

**Behavioral testing:** Behavioral testing was performed under red light or dark conditions. Open field locomotor rating: Locomotor recovery after spinal cord contusion injury was monitored using the Bassoo, Beattie, and Bresnahan (BBB) open field locomotor rating scale. In the present study, the BBB score was obtained weekly until eight weeks post-injury, biweekly up until week 20, and every four weeks thereafter. Each examination was conducted by two experienced examiners and during five minutes. The animals selected for BBB testing comprised of the last cohorts of 20 animals in both therapeutic groups (n = 40 total).

Additional tests, performed only once near the end of the study: Additional behavioral testing was conducted on animals showing weight support during the last BBB assessments. Behavioral testing was conducted approximately 1.5 months prior to sacrifice. For training/testing, animals were transferred to the testing room in their original housing and allowed to habituate to the room for 30 minutes, during which white noise was generated. The following behavioral tests were conducted:

**Activity assessment in an open field:** One week prior to the beginning of the test trials, animals were adapted to the apparatus arenas (76 x 152 x 50 cm; W x L x H) for five minutes on each day. After habituation, the animals were placed in the center of a clean arena and tracked by a video tracking system under dark conditions (night/active portion of their diurnal rhythm), over a period of three hours. The distance traveled and a number of various movement variables was assessed (EthoVision, Noldus Technology, the Netherlands). Three separate observation periods were evaluated. Each observation period was separated by at least 24 hours. During testing, animals had access to Hydrogel and food pellets.

**Beam walk:** Rats were trained to traverse elevated (1 m) narrow beams (3, 2.5, and 2 x 200 inches; W x L) toward a darkened goal box (10 x 10 x 10 inches). Only motor impaired animals would produce foot faults (slips from beam), which number than represent a
motor deficit. Bright light and white noise were produced near the start to promote beam crossing. Rats were allowed to fall from the beam (onto a container with padding). For training, each rat was placed in goal box for two minutes and afterward placed on the balance beam for 1 minute per trial. The rat was considered trained when it was able to remain on the beam for three consecutive trials. Mean scores out of two trials for each beam were used (i.e., right and left hind paw foot faults and falls). The animal was allowed to take ~5 min to complete a crossing. In between runs an animal was placed in their home cage for ≥ 15 s. If an animal was able to cross a beam for at least two thirds, it was also tested on the narrower beam (i.e., 2.5 or 2 inch).

**Catwalk gait analysis:** The CatWalk apparatus (CatWalk 7.1, Noldus Technology, the Netherlands) was used to quantify gait parameters by footprint analysis during walkway crossings. Animals walk down a horizontal glass walkway \((109 \times 15 \times 0.6 \text{ cm}; L \times W \times H)\), of which the glass is illuminated along the long edge. The light only enters the (side of the) glass and reflects merely internally (when the glass is bordered by air). As an animal crosses the walkway, light reflects off of the animal’s paws, producing a series of bright footprints when viewed through the glass, which are recorded by a video camera. Hence, testing was performed in a darkened room. Walkway crossing was stimulated by rewarding the animal with little Fruit Crunchies, placed at the end of the walkway. In conjunction, animals were further motivated by food restriction prior to training/testing. Hence, food was removed from their cages approximately 18 hours before testing (and returned after training/testing). In addition, the following criteria concerning walkway crossings needed to be met: (1) the animal needed to walk uninterrupted across the walkway, at a constant pace, and (2) a minimum of three such crossings per animal were required. Data from three proper crossings was averaged for statistical analysis.

**Sensory function assessment (mechanical and thermal):** In this test, pain thresholds for a supraspinal response (vocalization or escape behavior) to a below-level evoked mechanical or thermal stimulus were assessed. The animals were habituated to the set-up, test room, and investigator for one week (no responses were elicited), twice daily for five minutes, prior to testing. The investigator held the animal in an upright position, fixed the tested hindpaw, and then the hindpaw was stimulated. When a response was elicited, the stimulus was stopped immediately and the maximal pressure elicited was recorded. Both hind paws were tested for four times while alternating between paws, with at least a 1-hour interval between trials. The first scores of each paw were removed.

The mechanical stimulus was created using a rigid tip mounted on a pressure transducer (IITC life sciences Electronic von Frey Anesthesiometer, Cat# 2393, Woodland Hills, CA), which was operated by a second investigator (SvG). The stimulus was applied on the dorsal side of a hind paw. Compressing the paw of the animal with the tip on the pressure transducer at the distal metatarsal or metacarpal area in a gradual incremental fashion (paw rests on table surface).

For eliciting the thermal stimulus an infrared beam apparatus (Ugo Basile, Cat# 37360, Collegeville, PA, USA) was used. For testing, the investigator held the animal’s plantar side of a hind paw over the infrared light beam mounted in the apparatus.

**Necropsy:** The post-grafting survival period varied between 272-274 days (~10 months). Animals were sacrificed by 2 mg pentobarbital and 0.25mg phenytoin (0.5mL of Beuthanasia-D, Intervet/Schering-Plough Animal Health Corp., Union, NJ, USA) followed by transcardial perfusion of saline, trailed by 4% paraformaldehyde (phosphate buffered). The central nervous system tissue was dissected out and preserved in 4% paraformaldehyde. The following tissues were also taken out and placed in 10% formalin: skeletal muscle (thigh), skin, adrenals, aorta, rectum, cecum, colon, duodenum, esophagus, epididymides, eyes with optic nerves, femur, Harderian gland, heart, ileum, jejunum, stomach, kidneys, spleen, liver, lung with bronchi, mesenteric lymph node, mammary gland, pancreas, thymus, thyroid with parathyroid glands, trachea, pulmonary, prostate, mandibular salivary glands, tongue, sciatic nerve, testes, seminal vesicles, and bladder.

At necropsy, any tissue with discoloration, lesion, necrosis, distension, malformation, and/or suspected tumor/mass were sent for external histopathological evaluations by a board-certified veterinary pathologist (J.E. Sagartz DVM PhD DACVP, Seventh Wave Laboratories LLC, Chesterfield, MO), as were the histological sections of the central nervous system. Evaluation was performed in a manner blinded to treatment nature. Severity grades for pathology diagnoses were based on a 4-point scale as follows: Grade 1 = minimal, Grade 2 = moderate, Grade 3 = marked and Grade 4 = severe. After completion of pathology analysis, the pathologist was unblinded and the final conclusion was made.

**Central nervous system histology:** Each cord was photographed against a ruler (cm) along with its identification number. The cords were bisected into approximately equal rostral and caudal pieces and embedded horizontally into a gelatin block \((6.5 \times 4.5 \times 2.5 \text{ cm L} \times W \times H)\). Spinal cords from 16 - 20 animals were embedded in 4 horizontal layers in a block 1. The rostral pieces were placed in the first and third layers and the caudal pieces were in the second and fourth layers, with the ventral side facing down. The layers were separated by ± 0.4 cm of gelatine. The cords (columns) were separated by ± 0.2 cm from each other. After solidification of the gelatin block, it was placed in 30% sucrose in 4% formaldehyde for 48 hours. Then, it was frozen in cold 2-methylbutane using dry ice, cryostat sectioned horizontally \((40\mu\text{m thick})\), and mounted with intervals of 12 sections. Whole brains were also embedded into gelatin blocks, cryostat sectioned coronally at 40 μm thickness. All stained slides were sent to a Board-certified veterinary pathologist for evaluation (J.E. Sagartz DVM PhD DACVP, Seventh Wave Laboratories LLC, Chesterfield, MO). For histopathological stainings every 24th section was used. The spinal cords used to assess the neuronal integration (see below) were not gelatin embedded but were frozen directly (after 48h in 30% sucrose) using isopentane (~80°C), mounted in OCT compound (Tissue-Tek®) and cryostat sectioned at 30 μm in the horizontal, sagittal, or transverse plane, and collected for free-floating immunofluorescence staining.
**Stainings used entailed:** hematoxylin & eosin (H&E), human axonal neurofilament antibody (h.HO14; rat 1:100; human specific axonal marker; gift from Dr. Virginia Lee, University of Pennsylvania, PA, USA), Glia Bifilary Acidic Protein antibody (h.GFAP; rabbit 1:500; human specific astroglial marker; Origene, Rockville, MD, USA), and Green Fluorescent Protein antibody (GFP; chicken 1:2000; Aves, Tigard, OR, USA), Neuron-Specific Enolase antibody (h.NSE; mouse 1:500; human specific neuronal marker; Vector Labs, Burlingame, CA, USA) Synaptophysin antibody (h.Syn; mouse 1:500; human specific synaptic marker; Millipore, Billerica, MA, USA), Human Nucleus antibody (h.HuMA; mouse 1:200; Millipore, Billerica, MA, USA), Glial Bifilary Acidic Protein antibody (GFAP; mouse 1:500; Sigma-Aldrich; St. Louis, MO, USA), Oligodendrocyte lineage transcription factor 2 antibody (Olig2; rabbit 1:1000; Abcam ab81093; Cambridge, MA, USA), Ki67 antibody (proliferation marker; Abcam ab16667; 1:100; rabbit), and DAPI (In Prolong®; Life Technologies, Carlsbad, CA, USA). Immunostainings were finished using fluorescent-conjugated secondary donkey antibodies (Alexa® Fluor 488 & 647; Jackson Immuno Research, West Grove, PA, USA; & Alexa® Fluor 555; Invitrogen; 1:500).

**Surgical and Cell-Grafting Procedure**

The intervention included placing an anesthetized subject in the prone position and sterile processing of the associated surgical trial materials. An approximately 10 - 15 cm incision was performed in the dorsal midline and a bilateral laminectomy performed over the injured spinal cord segments. All prior fusion hardware was removed during the surgical procedure to allow for optimum serial magnetic resonance imaging (MRI). Following laminectomy an incision of approximately 2-4 cm was made in the dura, which was then tacked up, allowing exposure of the spinal cord. The stereotaxic injection platform was then attached to 4 percutaneous posts through an approximately 1cm skin incision immediately above and below the laminectomy site (Figure 5E), (Tadesse et al., 2014). The injection device consisted of a Z-drive holding a 30-gauge beveled needle in perpendicular position over the exposed spinal cord. The top end of the needle was attached to tubing which was attached to a microprocessor-controlled syringe pump. The syringe is back-filled with mineral oil to eliminate air and to create an immiscible barrier against aqueous solution in the syringe. The syringe plunger is inserted into the syringe, pushed toward the end, and attached to the drive spindle of the injection pump. Separately, the injection cannula is manually filled with sterile injectable saline in order to eliminate air. The saline-filled cannula is attached to the Hamilton syringe. Using the injection pump in reverse, a small air space is created at the cannula tip to prevent mixing of the cell suspension with the saline. Using the injection pump in reverse, the cannula is loaded with the required volume of the cell suspension. The capacity of the cannula is at least 500 µL so that the injection volume never touches the tip of the syringe. Prior to initiating spinal cord injections, a 5 µL bolus (1 injection) is ejected under direct vision of the surgeon to ensure that the system is open and unobstructed. After the needle is inserted into the spinal cord, the guide sheath is retracted, converting the cannula into a “floating cannula.” This feature allows for accuracy of delivery without suspension of respiration and other facets of homeostasis, including blood pressure and other vital signs. This setup is different as compared to other injection devices that are fixed to the operating room bed and require suspension of ventilation to ensure proper injection. Bilateral injection positions were determined by preoperative MRI and target approximately 1mm lateral to the rim of the remaining tissue bordering the injury site. The needle was lowered into the spinal cord to the depth of approximately 4 mm from the pial surface (Figures 5D and 5F). The cell suspension was then injected using the syringe pump at flow rate of 5.0 µL per min for a period of 2 minutes. The needle was left in place for 1 minute after injection and then slowly pulled out of the cord, advanced to the next position along the cord avoiding visible blood vessels and the injection procedure was repeated. At this time once all injections had been completed the dura was then closed in a watertight fashion, and the posterior spinal fascia and skin closed in meticulous layers. Subjects were then extubated and recovered in a post-anesthesia care unit, followed by recovering in an intermediate level care unit of the acute care hospital.

**Immunosuppression**

All 4 subjects were initiated and maintained for 12 weeks on a combination cocktail of immunosuppressive (IS) regimen that had previously been used successfully in ALS trials with the same cell line (Glass et al., 2012; Tadesse et al., 2014). This included the use of three separate medications. Basiliximab (Simulect) 20 mg intravenous (IV) administered within 2 hours prior to transplantation surgery. A second dose of 20 mg was given on postoperative Day 3 or 4. No additional doses of Basiliximab were used. Tacrolimus was started on post-transplant Day 1. It was initially dosed at 0.1 mg/kg/day divided every 12 hours by mouth (PO). Trough levels were measured while the subjects were hospitalized and the dose of tacrolimus was adjusted as necessary to ensure maintenance of a trough serum level of 4-8 ng/ml. Following discharge, trough levels were measured at the 2-week post-operative visit and at scheduled visits thereafter. Mycophenolate mofetil was started on post-transplant Day 1 at 500 mg twice a day, on post-transplant Day 8 increased to 500mg in the morning and 1 g at night, and on post-transplant Day 15 increased to 1 g twice a day. In all 4 subjects Tacrolimus and mycophenolate mofetil were withdrawn after 12 weeks post-transplantation. The dose of both medications was reduced by half at Week 13 and by another half at Week 14, followed by complete cessation at Week 15. Presence of antibodies against donor HLAs were monitored during this period and at scheduled intervals thereafter. Because assessment of rejection is an efficacy metric rather than a safety metric, more comprehensive immunological workup thoroughly assessed in the Phase 2 study, including complement and interleukin levels. Changes in MRI intensity at the cell transplant area were also monitored before and after the IS withdrawal. All subjects tolerated the immunosuppressive regimen well. Subjects were monitored on a nearly weekly basis per our study protocol. See Methods S2.
**Outcome Measures**

Subjects were assessed for adverse events including pain and infection, motor function, and quality of life. Additional secondary outcome assessments were made to measure any postoperative changes from baseline in neurologic deficits, neurophysiology, imaging studies, bladder and bowel function, allodynia and neuropathic pain. ISNC-SCI (International Standards for the Neurological Classification of Spinal Cord Injury) examination was used to monitor neurologic deficits. Neurophysiological changes were monitored when feasible by needle electromyography (EMG) and/or surface poly-electromyography (Brain Motor Control Assessment (BMCA) (Lee et al., 2004; Sherwood et al., 1996). Imaging studies were done by standard 1.5T MRI for safety monitoring. Diffusion tensor imaging (DTI) imaging of the spinal cord was performed for longitudinal experimental studies (1.5T, TR 2500-5000ms, TE 64-95ms, slice thickness 3.5-4 mm, FA 90, DFOV 190-340, NSA 1-8). Bladder and Bowel Function and Pain and Allodynia questionnaires were administered. Quality of life was assessed by Functional Independence Measure (FIM) and Spinal Cord Independence Measure (SCIM) questionnaires. Subjects were followed postoperatively at 2 weeks, monthly for 6 months and at every 6 months thereafter in post-study safety are planned to be followed up for total 60 months post stem cell treatment. Patients did not receive any additional rehabilitation beyond their routine outpatient physical and occupational therapy.

**Study Oversight**

An independent Data Safety Monitoring Board (DSMB) was convened at approximately 4-week intervals to review the available safety data. The DSMB was charged with making specific recommendations regarding study continuation. It had not identified any safety issues which precluded continuation of the study.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

For pre-clinical animal studies, results were analyzed using ANOVA (one-way, or two-way group x time repeated-measures, and using a fixed-effect model), with a Bonferroni post hoc test for multiple comparisons. If the unequal variances were observed (Bartlett’s test), the Kruskal-Wallis test with Dunn’s post hoc comparisons were used. To analyze differences between the two groups, we used Student’s t tests or Mann-Whitney test (Non-parametric), or a repeated-measures ANOVA (when appropriate). All statistical analyses were performed two-tailed, and a p value of 0.05 was considered significant. Between-group variations are reported as cell-injected versus vehicle-injected.

**ADDITIONAL RESOURCES**

The trial was registered in ClinicalTrials.gov as NCT 01772810.
HDAC and PI3K Antagonists Cooperate to Inhibit Growth of MYC-Driven Medulloblastoma

Highlights
- High-throughput screening identifies inhibitors of MYC-driven MB
- HDACIs inhibit growth of murine and human MYC-driven MB cells
- HDACIs and PI3KIs cooperate to activate FOXO1 and suppress tumor growth in vitro
- HDACIs and PI3KIs inhibit growth of MYC-driven tumors in vivo

In Brief
MYC-driven medulloblastoma (MB) has a poor prognosis. Pei et al. report that HDAC inhibitors potently inhibit MYC-driven MB cell growth in vitro, in part by inducing the expression of FOXO1, and synergize with PI3K inhibitors to inhibit tumor growth in vivo.

Accession Numbers
GSE69410
HDAC and PI3K Antagonists Cooperate to Inhibit Growth of MYC-Driven Medulloblastoma

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SUMMARY

Medulloblastoma (MB) is a highly malignant pediatric brain tumor. Despite aggressive therapy, many patients succumb to the disease, and survivors experience severe side effects from treatment. MYC-driven MB has a particularly poor prognosis and would greatly benefit from more effective therapies. We used an animal model of MYC-driven MB to screen for drugs that decrease viability of tumor cells. Among the most effective compounds were histone deacetylase inhibitors (HDACIs). HDACIs potently inhibit survival of MYC-driven MB cells in vitro, in part by inducing expression of the FOXO1 tumor suppressor gene. HDACIs also synergize with phosphatidylinositol 3-kinase inhibitors to inhibit tumor growth in vivo. These studies identify an effective combination therapy for the most aggressive form of MB.

INTRODUCTION

Medulloblastoma (MB) is the most common malignant brain tumor in children. Current treatments include surgical resection followed by radiation and intensive chemotherapy. Despite improvements in survival, favorable outcomes for patients with MB lag behind many other pediatric cancers, and treatment is often associated with severe long-term side effects (Packer et al., 2012; Saury and Emanuelson, 2011). Moreover, treatment stratification is still mostly based on clinical variables such as age, metastatic stage, degree of surgical resection, and histopathological subtype, which results in some children with favorable prognosis being over-treated and some with very poor prognosis still dying of the disease. Improved patient stratification and development of safer and more effective approaches to therapy are urgently needed.

Recent genetic studies have demonstrated that MB is comprised of four molecular subgroups, WNT, Sonic Hedgehog (SHH), group 3 (G3), and group 4 (G4), that have distinct gene expression and methylation profiles, mutations, and prognosis.
Among the most aggressive forms of MB are G3 tumors, which exhibit amplification or overexpression of the MYC oncogene; patients with these tumors are more likely to exhibit metastasis, to relapse following therapy, and to die from their disease (Cho et al., 2011; Northcott et al., 2011). More effective therapies are especially important for these patients.

We recently generated a mouse model of G3 MB by overexpressing Myc and a dominant-negative form of Trp53 in cerebellar stem cells and transplanting these cells into the cerebellum of adult mice (Pei et al., 2012). Recipients develop tumors that resemble human MYC-driven MB in terms of both histology and gene expression patterns, making these animals a valuable tool for developing and testing new therapies. The goal of this study was to use our mouse model to identify more effective approaches to treatment of G3 MB.

RESULTS

High-Throughput Screen for Small Molecules that Inhibit Survival of MYC-Driven MB

To screen for compounds that inhibit the survival of MYC-driven MB, we isolated cells from MYC/DNp53 (hereafter called MP) tumor-bearing mice, plated them in 384-well plates, and evaluated cell viability. To optimize the assay, we first tested the effects of varying the cell culture period and cell density on cell viability. BEZ-235, a phosphatidylinositol 3-kinase (PI3K)/mTOR inhibitor that we previously identified as an inhibitor of MP tumor cells (Pei et al., 2012), was used as a positive control. When cells were cultured for 6–48 hr, we observed a significant inhibition of viability in BEZ-235-treated cells compared with control cells at each time point, with a maximal difference (5.9-fold) at 48 hr (Figure S1A). We also tested the effects of varying the number of cells per well. Maximal differences (6–7-fold) were observed with 8,000–16,000 cells (Figure S1B). Based on these results, we chose the 48 hr time point and used 10,000 cells/well for our screen.

For the screen, we tested compounds from seven focused small-molecule libraries (Figure 1A). All compounds were tested at 1 μM, and compounds were scored as active if they inhibited cell viability by at least 2-fold relative to control (DMSO). Of the 3,642 compounds tested, 142 met these criteria (Figure 1B). These compounds represent diverse drug classes (Table S1) including antineoplastic drugs, antibiotics, and inhibitors of histone deacetylases (HDACs), PI3K/mTOR, DNA topoisomerase, and 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase.

Identification of Compounds that Kill Human MYC-Driven MB Cells and Are Not Toxic to Normal Cerebellar Cells

To determine whether the compounds identified in our primary screen could also inhibit growth of human MYC-driven MB cells, we used cells from a patient-derived xenograft (PDX) representing human MYC-driven MB. Cells were isolated from PDX-bearing mice and treated with compounds for 48 hr before assessment of viability. Among the 142 compounds active against murine (MP) tumor cells, 50 also significantly inhibited survival of human MYC-driven tumor cells (Table S1 and Figure 1C). Many drugs that kill tumor cells also exhibit substantial toxicity to normal cells. To eliminate compounds that might be broadly toxic to normal cells, we tested our 142 hits on post-mitotic cerebellar granule neurons and astrocytes, two major populations of cells in the cerebellum. We found 107 compounds that were non-toxic to neurons and 128 compounds that were non-toxic to astrocytes (Table S1 and Figure 1C). Overall, 23 unique compounds (four of which appeared twice in our screen) met our criteria.

(Northcott et al., 2012). Among the most aggressive forms of MB are G3 tumors, which exhibit amplification or overexpression of the MYC oncogene; patients with these tumors are more likely to exhibit metastasis, to relapse following therapy, and to die from their disease (Cho et al., 2011; Northcott et al., 2011). More effective therapies are especially important for these patients.
HDAC Inhibitors Potently Inhibit Growth of MYC-Driven MB Cells In Vitro

Among the 23 unique compounds identified in our screen, four (apicidin, histone deacetylase inhibitor III, N-hydroxy-7-(2-naphthythio) heptanomide (HNHA), and scriptaid) were histone deacetylase inhibitors (HDACIs). To determine whether HDACIs might be effective for treating G3 MB, we investigated this class of compounds in more detail. We tested nine commercially available HDACIs, including several compounds that are in clinical trials for other diseases. Cells from MP tumors and from a human G3 PDX were treated for 48 hr and viability was assessed. All compounds significantly inhibited survival of both murine and human tumor cells (Figures 2A and 2B). The potency of these drugs varied greatly (half maximal inhibitory concentration [IC$_{50}$] range, 8–800 nM), but relative potencies were similar between mouse and human tumor cells. Notably, the pan-HDACI LBH-589 (panobinostat) was the most potent inhibitor of tumor cell growth in vitro. LBH-589 was at least 80-fold more potent than suberoylanilidehydroxamic acid (SAHA), a U.S. Food and Drug Administration-approved pan-HDACI that is in clinical trials for many types of cancer. LBH-589 was recently approved for treatment of multiple myeloma and is in trials for additional malignant and non-malignant diseases but has not been evaluated in the context of MB.

Having observed potent inhibitory effects of LBH-589 on murine and human G3 MB cells, we next tested its effects on normal cells. As shown in Figure 2C, LBH-589 inhibited survival of cerebellar granule neurons and astrocytes, but only at much higher concentrations than those required to inhibit murine and human MYC-driven MB cells (granule neuron IC$_{50}$ = 140 nM; astrocyte IC$_{50}$ = 110 nM). Doses that inhibited tumor cell survival by 50% (8–10 nM) had no effect on granule neurons and astrocytes. These data suggest that LBH-589 selectively inhibits survival of MB cells and might be a useful therapeutic agent for treatment of MYC-driven MB.

To determine whether LBH-589 was specifically active against G3 MB, we tested its effects on PDX lines representing other subgroups of MB. Although all PDX lines were sensitive to the drug, the median IC$_{50}$ for G3 PDX lines (14.44 nM) was lower than those for SHH (47.4 nM) and G4 (25.27 nM) lines (Figures 2D–2F). Notably, IC$_{50}$ values for all three subgroups of MB were much lower than those for granule neurons and astrocytes. These data suggest that LBH-589 is effective against multiple MB subgroups, with the greatest potency against G3.

### Gene Expression Profiling Predicts a Role for FOXO Proteins in HDACI-Mediated Inhibition

We performed expression profiling using Affymetrix Mouse Gene 2.0 ST Arrays to elucidate the mechanisms by which LBH-589 suppresses growth of MYC-driven MB. MP tumor cells from three different animals were incubated with DMSO or LBH-589 for 6 hr or 12 hr before RNA was isolated for microarray analysis. Unsupervised hierarchical clustering (Figure 3A) and principal component analysis (Figure 3B) revealed a high level of consistency across biological replicates (tumor cells from different animals). For each replicate, treatment with LBH-589 resulted in a marked change in gene expression, both at 6 hr and at 12 hr. Using the criteria of fold change ≥ 2 and p < 0.01, we found 681 genes differentially expressed (DE) at 6 hr and 792 genes differentially expressed at 12 hr. The complete list of DE genes is presented in Table S2.

To gain insight into the pathways regulated by LBH-589, we investigated the DE genes using Ingenuity Pathway Analysis (IPA) (Figures 3C and 3D). HDACs remove acetyl groups from core histones; this promotes formation of histone-DNA complexes, reducing access of transcription factors to DNA and thereby decreasing transcription. Conversely, HDACs increase histone acetylation and cause an overall increase in transcription. Consistent with this, a number of the top IPA biofunctions induced by LBH-589 were related to DNA transcription and promoter activation (Figure 3C and Table S3). Biofunction analysis also suggested that LBH-589 decreased expression of genes associated with stem cell proliferation (Figure 3C). We also used IPA to identify upstream regulators whose target genes are altered by LBH-589 (Figure 3D and Table S4).

### Table 1. Compounds that Kill Murine and Human MYC-Driven MB Cells but Are Not Toxic to Normal Cerebellar Cells

<table>
<thead>
<tr>
<th>Function</th>
<th>Drug Name</th>
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<tbody>
<tr>
<td>HDAC inhibitors</td>
<td>apicidin</td>
</tr>
<tr>
<td></td>
<td>histone deacetylase inhibitor III</td>
</tr>
<tr>
<td></td>
<td>HNHA</td>
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<tr>
<td></td>
<td>scriptaid</td>
</tr>
<tr>
<td>PP2A inhibitors</td>
<td>cantharidin</td>
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<tr>
<td></td>
<td>canthridic acid</td>
</tr>
<tr>
<td>HMG-CoA reductase inhibitors</td>
<td>cerivastatin Ca</td>
</tr>
<tr>
<td>DNA topoisomerase II inhibitors</td>
<td>camptothecin</td>
</tr>
<tr>
<td></td>
<td>doxorubicin hydrochloride</td>
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<tr>
<td></td>
<td>idarubicin hydrochloride</td>
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<tr>
<td></td>
<td>etoposide*</td>
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<tr>
<td>Antineoplastic agents</td>
<td>amsacrine hydrochloride*</td>
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<td>ancitabine hydrochloride*</td>
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<td></td>
<td>cytarabine*</td>
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<tr>
<td></td>
<td>homidium bromide</td>
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<tr>
<td></td>
<td>mycophenolic acid</td>
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<tr>
<td>Disruption of microtubule assembly</td>
<td>nocodazole</td>
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<tr>
<td></td>
<td>T113242</td>
</tr>
<tr>
<td>Antibiotic/antifungal agents</td>
<td>ciclopirox olamine</td>
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<tr>
<td></td>
<td>dactinomycin</td>
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<tr>
<td>IGF inhibitor</td>
<td>chromomycin</td>
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*Drug that appears more than once in different libraries.
surprisingly, targets of HDACs and BRD4 bromodomain proteins (readers of histone acetylation) were decreased in response to the drug (Bandopadhayay et al., 2014; Bhadury et al., 2014). In addition, targets of MYC were significantly decreased. Conversely, targets of the transcription factors FOXO3, HNF4A, and STAT4 were increased following treatment with LBH-589. FOXO and HNF4A proteins are important regulators of glucose and lipid metabolism (Gross et al., 2008; Hwang-Verslues and Sladek, 2010); thus, increased expression of their targets could explain the observation (Figure 3C) that genes associated with carbohydrate metabolism were increased, and those associated with insulin sensitivity and lipid metabolism were decreased following exposure of cells to HDACIs.

To determine which of the pathways regulated by LBH-589 might be important for inhibition of tumor growth, we compared the genes induced and repressed by LBH-589 with genes previously found to be altered during transformation of stem cells into MP tumors (Pei et al., 2012). Using gene set enrichment analysis (GSEA), we found 56 gene sets that were enriched in both datasets (Figure 3E; Tables S5 and S6). Among these, the set composed of genes with FOXO1 binding motifs near their transcriptional start sites was significantly increased in LBH-589-treated tumor cells (normalized enrichment score [NES] = 1.26), while expression of the same set of genes was decreased in MP tumors compared with neural stem cells (NES = −1.74) (Figure 3F). Analysis using Nextbio software also identified FOXO1 target genes as being significantly induced by LBH-589 and significantly repressed during transformation (Figure 3G; Tables S7 and S8). The fact that FOXO1 target genes decrease during development of MB and increase in response to HDACIs suggests that downregulation of FOXO1 may be important for transformation.

Having observed decreased expression of FOXO1 targets in MP tumors compared with normal neural stem cells, we sought to determine whether FOXO1 expression is also altered in human MB. Analysis of RNA sequencing data from more than 100 human MBs representing all four subgroups of the disease (Figures S2A–S2C) revealed that all subgroups of MB express lower levels of FOXO1 than normal fetal cerebellum, but the subgroups with the highest levels of MYC (WNT and G3) show especially low levels of FOXO1 (Figure S2C). Low levels of FOXO1 and high levels of MYC were also found in several human G3 MB PDX lines (Figures S2D and S2E). These data suggest that expression of FOXO1 and MYC is inversely correlated in both murine and human MB, consistent with the notion that FOXO1 may act to oppose the transforming effects of MYC (Bouchard et al., 2004; Peck et al., 2013).

**FOXO1 Is Induced by HDACIs and Can Inhibit Growth of MYC-Driven MB**

The observation that FOXO1 targets were increased in MP tumor cells following treatment with LBH-589 raised the possibility that expression of FOXO1 transcription factors themselves might be induced by the drug. The FOXO family consists of three
members (FOXO1, 3, and 4) with overlapping expression patterns, transcriptional activities, and biological functions (Paik et al., 2007). To determine which of these might be regulated by HDACIs, we analyzed their mRNA expression by qRT-PCR in MP tumor cells treated with DMSO or LBH-589 for 6 or 12 hr. As shown in Figure 4A, LBH-589 markedly increased expression of Foxo1 (24–34-fold compared with 6 hr DMSO) but had little effect on expression of Foxo3 or Foxo4 (1–1.2-fold and 1.5–1.8-fold respectively). FOXO1 protein also increased significantly within 6 hr of drug treatment at concentrations similar to those that induce histone acetylation at this time point (Figure 4B). Thus, LBH-589 potently induces expression of FOXO1.

To determine whether FOXO1 levels influence the effects of HDACIs on tumor cell viability, we infected murine MP or human PDX tumor cells with lentiviruses encoding shRNAs targeting FOXO1. As shown in Figure 4C, LBH-589 induced Foxo1 expression in cells expressing non-targeting (NT) shRNAs, and this induction was blunted by two different FOXO1 shRNAs (#1 and #2). After 48 hr, FOXO1 shRNA-expressing MP tumor cells showed reduced sensitivity to LBH-589 compared with cells expressing NT shRNA (Figure 4D). Similar results were observed in G3 PDX lines expressing FOXO1 shRNA (Figures S3A–S3C). To test whether FOXO1 itself is sufficient to impair growth or survival of MYC-driven MB, we generated retroviruses encoding full-length FOXO1 and used them to overexpress the protein in murine MP or human PDX tumor cells (Figures 4E and S3D). After 48 hr, FOXO1-expressing MP tumor cells showed a 35% decrease in viability and FOXO1-expressing PDX cells showed a 48%–51% decrease in viability compared with control (GFP-expressing) cells (Figures 4F, S3E, and S3F). Moreover, mice transplanted with FOXO1-overexpressing cells showed prolonged survival compared with mice transplanted with control virus-infected cells (Figure 4G). Together these results suggest...
that induction of FOXO1 contributes to HDACI-mediated inhibition of tumor growth.

**HDACIs Synergize with PI3K Inhibitors to Activate FOXO1 and Inhibit Growth of MYC-Driven MB**

The induction of FOXO1 by LBH-589 was notable for several reasons. First, as mentioned above, Foxo1 mRNA expression decreases during formation of MP tumors from NSCs, suggesting that high levels may be disadvantageous for tumor growth. Second, FOXO1 has been reported to function as a potent tumor suppressor. Not only can it promote cell cycle exit and apoptosis by inducing expression of CDK inhibitors and pro-apoptotic proteins but it has also been reported to directly oppose the effects of MYC by inducing expression of MYC antagonists and inhibiting expression of MYC target genes (Bouchard et al., 2004; Delpuech et al., 2007; Zhang et al., 2011). Finally, FOXO1 activity is regulated by the PI3K-AKT pathway (phosphorylation of FOXO1 protein prevents it from entering the nucleus) (Calnan and Brunet, 2008), and our previous studies demonstrated that antagonists of this pathway also suppress growth of MP tumors (Pei et al., 2012). The fact that two classes of drugs that inhibit growth of MYC-driven MB can both activate FOXO1 (HDACIs increasing its expression and PI3KIs promoting its nuclear localization) supports the importance of FOXO1 in regulating tumor cell growth and survival. Moreover, the fact that these drugs regulate FOXO1 by distinct mechanisms raised the possibility that they might cooperate to inhibit tumor growth.

We examined the effects of these drugs on FOXO1 expression and phosphorylation in MP tumor cells to determine whether PI3KIs could synergize with HDACIs to enhance activity of FOXO1. As shown in Figures 5A and 5B, LBH-589 increased the levels of FOXO1 protein, along with the levels of acetylated histones (H3K9 and H3K27). Notably, the FOXO1 protein induced by LBH-589 exhibited significant amounts of phosphorylation. The PI3KI BKM-120 had little effect on absolute levels of FOXO1, but it reduced phosphorylation of the FOXO1 protein.
that was induced by LBH-589 (Figures 5A and 5C). BKM-120 also inhibited phosphorylation of AKT and S6, known targets of PI3K signaling. Similar effects were seen in a G3 MB PDX line treated with LBH-589 and BKM-120 (Figures S4A–S4C). Thus, the combination of HDACIs and PI3KIs increases the levels of dephosphorylated FOXO1 in MYC-driven MB cells.

Since dephosphorylation of FOXO1 has been reported to increase its accumulation in the nucleus, we asked whether treatment with LBH-589 and BKM-120 affected nuclear localization of FOXO1. MP tumor cells were treated with the drugs for 6 hr, and then cytoplasm and nucleus were separated and examined by western blotting. Loading controls for the cytoplasmic and nuclear fractions were GAPDH and nuclear lamin A/C (LMNA), respectively. W, whole cell lysate; C, cytoplasmic fraction; N, nuclear fraction.

Relative nuclear FOXO1 protein expression was calculated from three independent western blots like the one shown in (D), by normalizing levels of total FOXO1 in the nuclear (N) fraction to LMNA and then comparing each treatment with DMSO control (set to 1). The unpaired, one-tailed t test was used; p values <0.05 were considered significant.

MP tumor cells were treated with the indicated doses of LBH-589 and BKM-120 for 48 hr before viability was assessed. Compusyn software (http://www.combosyn.com/) (Chou, 2010) was used to calculate combination indices (CIs). Criteria for synergy, additivity, and antagonism are shown below the chart. Error bars represent the mean ± SD.

See also Figure S4.
The ability of LBH-589 and BKM-120 to cooperate at a biochemical level prompted us to ask whether these drugs could synergize to inhibit survival of MYC-driven MB cells. To test this, we treated cells from murine MP tumors or from a human G3 PDX line with varying doses of LBH-589, BKM-120, or combinations of these two drugs. Cells were cultured for 48 hr and survival was assessed. The cytotoxicity of each treatment (% of the maximal cytotoxic effect seen with the highest dose of drug) was entered into Compusyn software to calculate the combination index (CI) (Chou, 2010). As shown in Figure 5F, at almost all doses tested, LBH-589 and BKM-120 were strongly synergistic in suppressing survival of MP tumor cells. For example, LBH-589 alone had an IC₅₀ of 5.6 nM, whereas the addition of 625 nM BKM-120 lowered the IC₅₀ to 0.605 nM (Figure 5G). The IC₅₀ for BKM-120 was also markedly lowered (from 1.9 μM to 21 nM) by addition of LBH-589 (Figure 5H). Potent synergy was also seen in human G3 PDX cells (Figures S4D–S4F). Consistent with these results, a dual HDAC/PI3K inhibitor, CUDC-907 (Qian et al., 2012), exhibited potent suppressive activity against both murine and human MYC-driven MB cells (Figures S4G and S4H). These data suggest that HDACIs and PI3KIs synergize to inhibit the growth of MYC-driven MB cells.

To determine whether FOXO1 is important for the efficacy of the combination treatment, murine MP and human PDX tumor cells were infected with retroviruses expressing a dominant-negative form ofFOXO1 (DN-FOXO1) (Nakae et al., 2000; Wang et al., 2011), then treated with either drug alone or with the two drugs in combination. As shown in Figures 6A–6D, DN-FOXO1-expressing cells were significantly more resistant to combination treatment than control cells. The same effect could also be seen in MP and PDX tumor cells expressing shFOXO1, compared with those expressing NT shRNA (Figures 6E and 6F). These findings suggest that the synergy between HDACIs and PI3KIs is mediated, at least in part, by activation of FOXO1.

**HDACIs and PI3K Inhibitors Cooperate to Inhibit Growth of MYC-Driven MB In Vivo**

In light of the potent inhibitory effects of LBH-589 and BKM-120 in vitro, we tested the efficacy of these compounds in vivo. We first determined whether systemically administered LBH-589 and BKM-120 could accumulate in intracranial tumors and affect their respective molecular targets. Consistent with our in vitro findings, we found that BKM-120 (alone or in combination with LBH-589) diminished phosphorylation of AKT and S6, whereas LBH-589 (alone or in combination with BKM-120) increased histone acetylation (Figure S5A). Moreover, animals treated with LBH-589, alone or in combination, showed an increase in total
FOXO1 protein; however, animals treated with the combination showed reduced levels of FOXO1 phosphorylation compared with treatment with LBH-589 alone (Figures S5B–S5D). These results suggest that HDACIs and PI3KIs can reach intracranial tumor cells and alter their protein targets, including FOXO1.

We generated cohorts of MP tumor-bearing mice to assess whether the combination of HDACIs and PI3KIs was effective at inhibiting intracranial tumor growth. Animals were subjected to weekly bioluminescence imaging, and when tumors were clearly detectable (signals in the range of $10^5$–$10^6$ rad/s), mice with equivalent bioluminescent signals were randomized into four treatment groups: vehicle, LBH-589, BKM-120, or LBH-589 + BKM-120. Animals were treated until they displayed tumor-associated morbidity and then euthanized. Animals treated with vehicle showed rapid tumor growth and had to be sacrificed 3–4 weeks after transplantation (median survival, 24 days) (Figures 7A and 7B). Treatment with BKM-120 or LBH-589 alone initially slowed tumor growth, but tumors eventually grew and animals had to be sacrificed (median survival, 33 and 40 days, respectively). The combination of BKM-120 and LBH-589 markedly inhibited tumor growth, and animals survived much longer (median survival, 64 days). The combination of BKM-120 and LBH-589 also potently inhibited tumor growth in flank allografts (Figures S6A–S6C).

To determine whether LBH-589 and BKM-120 were also effective against human MYC-driven MB, we tested these compounds on animals carrying G3 PDXs. Cells from the PDX line MED411FH (Morfouace et al., 2014) were infected with a lentivirus encoding GFP and luciferase and transplanted into the cerebella of NSG mice. When tumors were readily detectable by bioluminescence, mice were treated with vehicle, LBH-589, BKM-120, or a combination of the two drugs. Mice treated with vehicle exhibited a median survival of 51 days. BKM-120 or LBH-589 alone increased the median survival to 65 and 59 days, respectively. In contrast, animals treated with the drug combination exhibited a median survival of 78 days, a 27-day increase compared with animals treated with vehicle (Figures 7C and 7D). LBH-589 and BKM-120 also significantly prolonged survival in two other G3 PDX lines (p < 0.00001 for MB002 and ICb-1572; Figures S6D and S6E). These studies suggest that combining HDACIs and PI3KIs might represent an effective therapy for MYC-driven MB.

**DISCUSSION**

Despite aggressive multimodal therapy, the prognosis remains extremely poor for patients with MYC-driven MB (Cho et al., 2011; Northcott et al., 2011). One approach to identifying more effective therapies is high-throughput drug screening, which allows evaluation of large numbers of compounds with diverse biological activities. Such screening has been used to identify therapies for other cancers (Atkinson et al., 2011; Gupta et al., 2011; Northcott et al., 2011), but few screens have been done for MB (Morfouace et al., 2014; Triscott et al., 2013). By using our model of MYC-driven MB, we were able to identify drugs that might be effective for this extremely aggressive disease.

Unlike previous studies, which used MB cell lines or neurospheres that had undergone several passages in culture (Morfouace et al., 2014; Triscott et al., 2013), we assayed cells freshly isolated from tumor-bearing mice. By avoiding culture, which may select for subsets of tumor cells, our screen was designed to identify agents that would be effective against a large proportion of tumor cells. Moreover, by screening cells in serum-free media without growth factors, we hoped to avoid identifying agents that inhibit the effects of these growth factors, which are not necessarily present in the tumor microenvironment. Using these approaches, we found 142 compounds that significantly reduced survival of murine MYC-driven MB cells. The fact that more than one-third of these also potently inhibited survival of G3 PDX cells provides strong validation of these compounds as inhibitors of MYC-driven MB.

The compounds we identified included a number of chemotherapeutic agents that have been previously used for MB, such as etoposide (Ruggiero et al., 2010), camptothecin (Bomgaars et al., 2007), and cytarabine (Mastronuzzi et al., 2013). In
addition, our studies pointed to a number of compounds that have not been extensively evaluated for this disease. For example, inhibitors of protein phosphatase 2A (PP2A) were potent inhibitors of MYC-driven MB, consistent with recent studies suggesting that PP2A inhibitors can inhibit the growth of MB cell lines (Cimmino et al., 2012). Likewise, inhibitors of HMG-CoA reductase (statins) emerged as hits in our screen. In light of the role of cholesterol biosynthesis in hedgehog signaling, it has been suggested that statins might be effective against SHH-associated MB (Bar and Stearns, 2008). Moreover, a recent report demonstrated that lovastatin can repress MYC expression and function in MB cell lines (Takwi et al., 2012). Further investigation of these agents for treatment of MB is warranted.

For the current studies, we focused on HDACIs, since several of these agents emerged as potent inhibitors of MYC-driven MB. Our finding that HDACIs are effective against mouse and human MYC-driven MB cells is consistent with previous studies showing that these drugs can impede growth of MB cell lines, and can induce cell death in genetically engineered models of SHH-driven MB (Milde et al., 2012; Shu et al., 2006; Spiller et al., 2006). Notably, LBH-589 was more potent than all the other HDACIs we tested, including SAHA, one of the few HDACIs that has been in clinical trials for MB (Fouladi et al., 2010; Witt et al., 2012). To determine which HDAC isoforms are important for survival of MYC-driven MB cells, we also tested a panel of isoform-selective inhibitors (data not shown). Our studies suggest that inhibitors of class I HDACs (HDAC-1, -2, -3, and -8) are effective against MP tumor cells, although none of these compounds was as potent as LBH-589. In contrast, inhibitors of class II-A (HDAC-4, -5, -7, and -9) and class II-B (HDAC-6) showed minimal activity against MP tumor cells. This conclusion is supported by a recent study demonstrating that class I HDACs (especially HDAC1 and HDAC2) are overexpressed in G3 MB, and that inhibitors of these enzymes reduce growth of MB cell lines (Ecker et al., 2015). Thus, class I HDACs are likely to be the predominant mediators of tumor cell survival in our model of MYC-driven MB, and likely represent the key targets of the pan-HDAC inhibitor LBH-589.

Among the pathways most significantly regulated by HDACIs was the FOXO pathway. This was initially suggested by expression analysis, which indicated that FOXO target genes were increased in LBH-589-treated tumor cells. Further studies indicated that the levels of FOXO1 mRNA and protein were markedly increased by LBH-589. FOXO1 may contribute to HDACI suppression of tumor growth by interfering with MYC-associated transactivation and transformation (Bouchard et al., 2004; Peck et al., 2013). This interference may involve induction of MX1-SRxs, which can bind to MAX and titrate it away from MYC (Delpuech et al., 2007), or induction of mir-145, a microRNA that can silence MYC expression (Gan et al., 2010). Alternatively, FOXO proteins could act independently of MYC to inhibit tumor growth by inducing expression of cyclin-dependent kinase inhibitors such as CDKN1A (p21) and CDKN1B (p27) and proapoptotic proteins such as BIM, BNIP3, FAS ligand, and TRAIL (Zhang et al., 2011). Our observations that FOXO1 overexpression inhibits tumor growth while FOXO1 knockdown blunts responses to HDACIs provide additional support for the importance of this pathway in mediating the inhibitory effects of HDACIs. However, it is important to note that HDACIs activate multiple pathways in MB cells, and that other proteins besides FOXO1 are likely to contribute to HDACI-mediated growth inhibition.

More than a dozen HDACIs are currently in trials for cancer and other diseases. Although these drugs have shown significant effects on their own in the context of cutaneous T cell lymphoma (Olsen et al., 2007), in most other cancers the benefits of HDACI monotherapy have been limited; thus, efforts have focused on evaluating combinations of HDACIs with other drugs (Thurn et al., 2011). Having observed that HDACIs function in part by inducing expression of FOXO1, and knowing that FOXO1 nuclear localization can be regulated by PI3K/AKT signaling, we speculated that PI3K inhibitors might cooperate with HDACIs in targeting MYC-driven MB. Our studies revealed that BKM-120 potently synergized with LBH-589 to activate FOXO1 and to inhibit tumor growth in vitro and in vivo. Importantly, previous studies have suggested that these drugs can cross the blood-brain barrier (Bendell et al., 2012; Pipalia et al., 2011). These findings suggest that the combination of HDACIs and PI3K inhibitors may have utility for patients with MYC-driven MB. In addition, our studies suggest that patients with other subgroups of MB may also benefit from this combination. In future studies it will be important to determine whether combining these agents with radiotherapy or other chemotherapeutic drugs will result in even greater effects on tumor growth.

The fact that MB and other pediatric cancers are relatively uncommon makes clinical trials for these diseases particularly challenging. Since each center sees only a few patients, trials require cooperation among several centers or consortia, and when only a small percentage of patients respond to a given therapy, results are difficult to interpret. Thus, rational selection of therapies and of patients who are likely to respond to them is essential. Our studies suggest that animal models of individual disease subgroups can be extremely valuable tools in this effort. High-throughput screening using these models can identify drugs that are effective for each form of the disease, and preclinical studies using these models can validate the efficacy of these agents and help select priorities for clinical trials. Although no model can predict with certainty which agents will be effective in the clinic, making informed choices about which agents to test can increase the likelihood that patients will benefit from their treatment.

**EXPERIMENTAL PROCEDURES**

**Animals**

C57BL/6J mice used as a source of cerebellar stem cells were obtained from the Sanford Burnham Prebys (SBP) Medical Discovery Institute Animal Facility. NOD-SCID IL2R-gamma null (NSG) mice used for intracranial tumor transplantation were purchased from Jackson Labs. CD-1 nu/nu mice for flank tumor transplants were from Charles River Labs. Mice were maintained in the animal facilities at SBP and at the Sanford Consortium for Regenerative Medicine. All experiments were performed in accordance with national guidelines and regulations, and with the approval of the animal care and use committees at SBP and at the University of California San Diego (UCSD).

**MP Tumor Generation and Tumor Cell Preparation**

Cerebellar stem/progenitor cells (Prom1+ cells) were purified by fluorescence-activated cell sorting (FACS) sorting from the cerebella of postnatal day 5–7 (P5–P7) C57BL/6J pups as previously described (Pei et al., 2012). To generate...
MP tumors, cells were infected with Myc-IRESP-Luciferase and DNp53-IRESP-GFP retroviruses and stereotaxically injected into the cerebellum of 6- to 8-week-old NSG mice. Animals were monitored weekly with in vivo bioluminescence imaging and euthanized when they showed signs of MB. Tumors were then dissociated and resuspended in NeuroCult medium with proliferation supplement (STEMCELL Technologies) for subsequent experiments. See Supplemental Experimental Procedures for retroviruses and in vivo bioluminescence imaging.

**Patient-Derived Xenografts and Normal Cerebellar Controls**

PDX lines used for this study include MB002 (G3) generated by the Cho lab (Bhandopadhayay et al., 2014); Icb-984 (SHH), Icb-1572 (G3), Icb-1487 (G3), and Icb-1299 (G3), generated by the Li lab (Zhang et al., 2012); Med-1712-FH (SHH), Med-411-FH (G3), and Med-211-FH (G3), generated by the Olson lab (Girard et al., 2015; Morfouace et al., 2014); and RCMB28 (G3), RCMB18 (SHH), RCMB32 (SHH), and DMB006 (G4) generated by the Wechsler-Reya lab (Blin et al., 2014; Kool et al., 2014). PDX lines were generated by implanting patient cells directly into the cerebellum of immune-compromised mice, and propagating them from mouse to mouse without in vitro passaging; the identity and subgroup of each line was validated by gene expression and/or methylation analysis. For all experiments, cells were isolated from tumor-bearing mice, resuspended in NeuroCult with proliferation supplement, and assayed as described below. Normal cerebella (P486 and P487) used as controls for qRT-PCR experiments were kindly provided by Charles Eberhart, Johns Hopkins University.

**Inhibitors**

Libraries used for high-throughput drug screening are described in Figure 1. Other compounds used include the HDAC inhibitors SAHA, HHNA, LBH-589, scriptaid, MS-275, givinostat, PDX101, LAQ-824, and MGCD0103 (all from Selleck), the PP2A inhibitors cantharidin and norcantharidin, the topoisomerase inhibitors camptothecan and topotecan (all from Sigma), the HMG-CoA reductase inhibitors cerivastatin (Sigma) and itavastatin (Sequoia Research Products Ltd. UK), and the PI3K/TOR inhibitors BEZ-235 and BKM-120 (both from Active Biochem). All inhibitors were dissolved in DMSO to 20 mM and further diluted to an appropriate final concentration in culture medium at the time of use.

**High-Throughput Drug Screening**

For primary screening, 10^4 MP tumor cells were plated in 25 μl of NeuroCult with proliferation supplement in 384-well plates (Corning) using an electronic dispenser (>20% weight loss), whereupon they were euthanized. See Supplemental Experimental Procedures for retroviruses and in vivo bioluminescence imaging.

**Statistical Analyses**

Statistical analysis was performed using GraphPad Prism software. All data are presented as means ± SD unless stated otherwise. Comparisons between different groups were made using Student’s t test or ANOVA as appropriate. The dose-response curves for various compounds were evaluated using Prism software to determine whether these curves were statistically different with respect to the fitted midpoints (log IC50) using the sum-of-squares F test. The statistical significance of Kaplan-Meier survival curves was assessed using the log-rank (Mantel-Cox) test. P values of 0.05 or lower were considered statistically significant for all experiments.

**ACCESSION NUMBERS**

The accession number for the gene expression microarray data reported in this paper is NCBI GEO: GSE69410. The accession number for the RNA-seq data reported in this paper is European Genome-Phenome Archive: EGAD00001001899.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, six figures, and eight tables and can be found with this article online at http://dx.doi.org/10.1016/j.cccel.2016.02.011.

**AUTHOR CONTRIBUTIONS**


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**REFERENCES**


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First-in-Man Clinical Experience Using a High-Definition 3-Dimensional Exoscope System for Microneurosurgery

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BACKGROUND: During its development and preclinical assessment, a novel, 3-dimensional (3D), high-definition (4K-HD) exoscope system was formerly shown to provide an immersive surgical experience, while maintaining a portable, low-profile design. OBJECTIVE: To assess the clinical applicability of this 3D 4K-HD exoscope via first-in-man surgical use. METHODS: The operative workflow, functionality, and visual haptics of the 3D 4K-HD exoscope were assessed in a variety of microneurosurgical cases at 2 US centers. RESULTS: Nineteen microneurosurgical procedures in 18 patients were performed exclusively using the 3D 4K-HD exoscope. Pathologies treated included 4 aneurysms, 3 cavernous malformations (1 with intraoperative electrocorticography), 2 arteriovenous malformations, 1 foramen magnum meningioma, 1 convexity meningioma, 1 glioma, 1 occipital cyst, 1 chiari malformation, 1 carotid endarterectomy, 1 subdural hematoma, 1 anterior cervical discectomy and fusion, and 2 lumbar laminectomies. All patients experienced good surgical and clinical outcomes. Similar to preclinical assessments, the 3D 4K-HD exoscope provided an immersive 3D surgical experience for the primary surgeon, assistants, and trainees. The small exoscope frame, large depth of field, and hand/foot pedal controls improved exoscope mobility, decreased need to re-focus, and provided unobstructed operative corridors. Flexible positioning of the camera allows the surgeon’s posture to be kept in a neutral position with uncompromised viewing angles. CONCLUSION: The first-in-man clinical experience with the 3D 4K-HD exoscope confirms its excellent optics and ergonomics for the entire operative team, with high workflow adaptability for a variety of microneurosurgical cases. Expanded clinical use of the 3D 4K-HD exoscope is justified.

KEYWORDS: 3D visualization, Ergonomics, Exoscope, Microneurosurgery, Neurosurgical education, Operating microscope

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he development of the operative microscope has been critical to the evolution of microneurosurgery. However, the small operative windows and limited approach angles inherent in accessing deep cranial structures continue to limit surgeon visualization with traditional microscopy. Neuroendoscopy can provide improved visualization of deep structures while maintaining surgeon ergonomics,1-3 but applications of this technique are limited by a short focal length, a small depth of field, and technical constraints imposed by the presence of the endoscope immediately adjacent to the operative field.

Extracorporeal telescope (“exoscope”) systems have been developed in an attempt to address this ongoing neurosurgical need.4-6 Combining the benefits of traditional neurosurgical microscopes and endoscopes, these systems possess wide operative fields and focal distances long enough
to allow nonobstructive positioning, and are easily maneuverable to simultaneously optimize operative angles and surgeon ergonomics. The entire surgical team also has the same view as the primary surgeon, facilitating operating workflow and trainee education. Early iterations of neuro-exoscopes were nonetheless limited by 2-dimensional views of the operative field, which reduced surgeon stereopsis as compared to traditional microscopy.

In a prior proof-of-concept cadaveric investigation, a novel 3-dimensional (3D), high-definition (4K-HD) exoscope was found to provide comparable surgical proficiency with improved ergonomics relative to the operative microscope. In this paper, we assess the operating room (OR) workflow, visual haptics, surgeon utilization, and general functionality of the 3D 4K-HD exoscope across a variety of microneurosurgical procedures, as part of its first-in-man clinical neurosurgical use. Shortly after this study was completed, we have reported, in a separate publication, on the safe and effective use of this system in spinal surgery.

METHODS

Patient Selection

Selected patients undergoing microneurosurgical operations at 2 US centers over a period of 6 wk were consented for intraoperative use of the exoscope. A traditional operating microscope was immediately available during all cases. The operative workflow, functionality, and visual haptics of the 3D 4K-HD exoscope were assessed by 5 primary surgeons and a multitude of assistant surgeons, supporting OR staff (including circulating nurses and scrub technologists), residents, and students. With institutional review board (IRB) approval, data on patient age, sex, diagnosis, surgery, and outcomes were prospectively recorded. For the purpose of this preliminary report, subjective assessments were obtained from those individuals and an overall consensus was reached among the coauthors regarding the advantages and disadvantages of this system. In one institution, a questionnaire was prospectively administered to all involved personnel, where each of the features and characteristics of the exoscope were assessed in a semiquantitative fashion, in head-to-head comparison with the standard microscope.

Exoscope Specifications

The attributes of the exoscope have been previously described. The exoscopic camera yields a high-resolution 3840 × 2160 pixel image (Sony Olympus Medical Solutions Inc, Tokyo, Japan) with the 4K-3D liquid-crystal display screen, viewable through polarized 3D glasses. Minimal-maximal magnification of 1.1-25.8×. Working-distance ranges from 220 to 550 mm. Field-of-view ranges from 7.5 to 171 mm. The exoscope is fixed to a counterweight apparatus allowing weightless maneuverability through tactile controls adjacent to the camera with regulation of focus/zoom via a pedal. The system utilizes a UNIX operating software which is controlled by touchscreen.

IRB Approval and Patient Consent

In one institution, IRB approval was obtained to allow prospective collection of patient data, including age, sex, diagnosis, surgery, and outcome. In the other institution, IRB approval was not required given that very limited data sets were collected, with no patient-specific information whatsoever. Patient consents were not required by the IRB in either institution, given that no identifying patient information is being presented.

RESULTS

During the study period, the 3D 4K-HD exoscope was utilized as exclusive means of intraoperative microscopy in 19 microneurosurgical procedures performed for a variety of pathologies in 18 patients.

Surgeries Included

- Four clip ligations of intracranial aneurysms (1 previously ruptured posterior communicating artery [PCoM] aneurysm with post-coiling recurrence, 1 unruptured PCoM aneurysm, 2 unruptured middle cerebral artery [MCA] bifurcation aneurysms)
- Three cavernous malformation resections including one with intraoperative electrocorticography (ECOG) strip placement (1 in right cerebellar peduncle, 1 right parasagittal parietal, 1 temporal)
- Two arteriovenous malformation resections (1 right occipital Spetzler-Martin grade 4, 1 in left Sylvian fissure Spetzler-Martin grade 2)
- Fenestration of a symptomatic right occipital cyst
- Resection of a frontal glioblastoma
- Resection of a convexity meningioma
- Two suboccipital craniectomies with C1 laminectomies (1 for resection of a foramen magnum meningioma, 1 for decompression of a Chiari I malformation)
- A carotid endarterectomy (CEA)
- Evacuation of a subdural hematoma
- An anterior cervical discectomy and fusion, and
- Two lumbar laminectomies

Surgeons were able to clearly visualize eloquent structures using the 3D 4K-HD exoscope in all 19 procedure and all 18 patients experienced good surgical and clinical outcomes. Both the primary and assistant surgeons wore 3D glasses and were able to operate simultaneously, relying on the same monitor. A second monitor was used for a limited number of cases in which the co-surgeon’s position resulted in a line of sight opposite that of the primary surgeon. While the use of a single screen did decrease the overall footprint of the OR, the use of dual monitors for spinal procedures required face-to-face monitor setup, thus increasing the OR footprint, as reported elsewhere. The OR staff generally felt that the compact size of the exoscope and its portable screens improved microscope maneuverability, while providing unhindered surgical and instrument-passing corridors. This setup allowed the primary and co-surgeons to be in a comfortable and ergonomic operating position, allowing unhindered horizontal gaze throughout the procedure. However, because of the need to optimize sight lines, surgical setup was forced to be altered in some cases. Normally, with the operating microscope, the placement of the surgical assistant was arbitrary and based on
surgeon preference. In contrast, to optimize the sight angle, the exoscope required placement of the assistant between the surgeon and the scrub technician. The large depth of field and easy-to-use hand/foot pedal microscope controls for zoom/focus allowed for an immersive 3D operative experience for the primary surgeon, co-surgeon, scrub technician, and other people in the room, including nonscrubbed residents, medical students, neuromonitoring staff, and anesthesia (Figures 1 and 2). As the primary surgeon’s operative field was shared with all OR staff, resident and student education was markedly enhanced.
Neurologists participating in an ECOG case found the visualization valuable for better understanding of electrode strip position. Visual quality was excellent for surgeons and observers, with a crisp operative field focus maintained up to the maximal zoom of 25.8× (Figure 3).

Additional advantages of the exoscope were noted by the primary and assisting surgeons across various operations, based on the anatomy encountered. For aneurysms, the low profile of the exoscope was particularly helpful for passing of long-handled instruments, such as clip appliers, between the surgeon and scrub technician. That being said, the placement of the assistant relative to the surgeon needed to be changed in some cases to maintain sight lines. The unobstructed field also facilitated co-surgeon assistance in the setting of a ruptured aneurysm (as occurred from an adjacent blister aneurysm during clipping of an MCA bifurcation lesion), by permitting steady assistant suctioning that did not obstruct the view of the primary surgeon while obtaining vascular control. Overall, visualization of the arterial tree with the exoscope was excellent during neurovascular procedures (Figure 4). Unfortunately, although the exoscope camera is infrared-capable, indocyanine green (ICG) videoangiography is not yet available in the US, pending FDA approval. Nevertheless, despite this temporary disadvantage, we have successfully used the exoscope in a hybrid OR setting, thus obviating the need for ICG videoangiography.

For parasagittal approaches, as was used for resection of a parasagittal cavernous malformation, the exoscope could be positioned orthogonal to the operative corridor while maintaining upright surgeon positioning. This minimized brain retraction while also allowing for a deeper cortical entry point in one of the trajectories used, decreasing the manipulation of normal cerebral tissue (Figure 5A). Similarly, for posterior fossa surgeries, the exoscope could be aimed in a caudal-to-rostral position, with the surgeon maintaining horizontal gaze. This provided optimal visualization of inferiorly accessed structures such as the obex, tela choroidea, inferior medullary velum, and fourth ventricle (Figure 5B). The exoscope’s large depth of field was also particularly useful in lateral posterior fossa surgery. This was highlighted during the removal of a foramen magnum meningioma, where high-resolution 3D visualization of the dense neurovasculature in this area facilitated safe tumor dissection (Figure 5C). The exoscope and its benefits were highlighted during spinal procedures (Figure 6). The exoscope system was positioned at the head of the bed, by the anesthesiologist, thereby minimizing OR footprint. Notably, the small frame of the exoscope as well as large depth of field allowed for optimization of ergonomic positioning during spinal procedures without the necessity for the surgeon to be in constant flexion. Similarly, for CEA, a procedure that the majority of surgeons perform under loupe magnification, surgical ergonomics and lighting were enhanced with the use of the exoscope (Figure 7). This was particularly valuable in the setting of a high carotid bifurcation, which usually creates disadvantages in maintaining adequate illumination and often results in challenging head and neck positions for the operating surgeon and assistant. In addition, the assistant, OR technician, and students were able to maintain a surgeon’s view throughout the CEA procedure. Across various approaches, the use of single hand/foot switches to maneuver the exoscope improved operative prowess by allowing the surgeon to remain attentive to the operative task at hand.

Despite the numerous advantages of this system, some technical difficulties were nonetheless uncovered. Specifically, optimal positioning of the surgical assistant relative to the surgeon and to the scrub technologist with the Mayo table seemed somewhat challenging at first, necessitating a period of gradual adaptation to this new setup by the various members of the OR team. Additionally, given that the screens essentially reflected the surgeon’s point of view, first assistants had to constantly adapt their hand movements to correct for the orientation angle between them and the surgeon. During spine and posterior fossa surgeries, the surgeon and assistant often stand facing each other across the operating table. In such instances, the screen image can be digitally rotated 180° so that both operators maintain their normal surgical orientation. Screen and exoscope positions had to be modified based upon side of surgery and patient position, which, at times, impacted the “usual and customary” positioning of scrub technologist, surgical assistant, and operative equipment. However, despite that early adjustment period, the majority of these variables were worked through and gradually resolved as more experience was acquired with the exoscope system.
In one institution, a standard questionnaire was administered, at the end of each of 10 procedures, to the following individuals: surgeon, surgical assistant, scrub technologist, circulating nurse, anesthesiologist, observers. The questionnaire solicited their subjective assessment of various measures, including image quality (everyone), posture (surgeon and assistant), fatigue (surgeon and assistant), ease of use (surgeon), focus quality (surgeon), zoom quality (surgeon), light intensity (surgeon), ease of sterile draping (scrub technologist and circulating nurse), ease of mobilization and positioning (circulating nurse), ease of storage (circulating nurse). Each measure was graded semi-quantitatively, relative to the standard microscope, from A to E (A: significantly better, B: somewhat better, C: same, D: somewhat worse, E: significantly worse). The results of this questionnaire came overwhelmingly in favor of the exoscope, as summarized below:

- Image quality was rated as same or better than microscope by 95% (57/60) of participants: A 71.7% (43/60), B 16.7% (10/60), C 6.7% (4/60), D 3.3% (2/60), E 1.7% (1/60).
- Posture was also rated as same or better than microscope by 95% (19/20) of participants: A 40% (8/20), B 50% (10/20), C 5% (1/20), E 5% (1/20).
- Fatigue was rated as same or better than microscope by all 20 participants: A 30% (6/20), B 30% (6/20), C 40% (8/20).
- Ease of use was rated as same or better than microscope by 95% (9/10) of participants: A 20% (2/10), B 30% (3/10), C 20% (2/10), D 30% (3/10).
- Focus quality was rated as same or better than microscope by 90% (9/10) of participants: A 20% (2/10), B 50% (5/10), C 20% (2/10), E 10% (1/10).
- Zoom quality was rated as same or better than microscope by all 10 participants: A 20% (2/10), B 50% (5/10), C 30% (3/10).
Adaptability of the exoscope to challenging microneurosurgical approaches. A, Interhemispheric approach: the exoscope allows visualization of the parasagittal cortex, while maintaining surgeon ergonomics due to the uncoupling of exoscope visual intake and output. B and C, Difficult to obtain views of the posterior fossa are also achieved with the surgeon maintaining upright positioning, as demonstrated by intraoperative images of the obex and floor of the fourth ventricle following a suboccipital craniectomy B, and images of the medulla and lower cranial nerves during resection of a foramen magnum meningioma C.

- Light intensity was rated as same or better than microscope by all 10 participants: A 30% (3/10), B 50% (5/10), C 20% (2/10).
- Ease of sterile draping was rated as same or better than microscope by all 19 participants: A 52.6% (10/19), B 26.3% (5/19), C 21.1% (4/19).
- Ease of mobilization and positioning was rated as better than microscope by all 10 participants: A 80% (8/10), B 20% (2/10).
- Ease of storage was rated as better than microscope by all 9 participants: A 66.7% (6/9), B 33.3% (3/9).

DISCUSSION

The favorable ergonomics, maneuverability, and immersive visual experience demonstrated by the 3D 4K-HD exoscope in preclinical work translated well to a variety of microneurosurgical procedures in the clinical setting. The educational advantages associated with a shared operative view by the primary surgeon and trainees, as well as its previously discussed competitive cost, reinforce the clinical utility of this system. While most contemporary neurosurgical microscopes provide the ability to project live microsurgical images onto large OR screens, allowing nonsurgeons and trainees to closely follow the surgical intervention, the images projected are generally 2 dimensional. In contrast, images projected from this exoscope system onto the large OR screens are 3D with a 4K-HD quality, thus providing a uniquely immersive experience to unscrubbed personnel, which is far superior to what is currently commercially available.

Many of the preclinically identified advantages of the exoscope were amplified in a real-world operative setting. One of the biggest limitations of the operative microscope is its large frame and fixed bulky design. These features not only take up valuable OR space and complicate the use of other intraoperative tools, such as the angiography c-arm or neuronavigation system, but also constrain the primary and co-surgeon to fixed positions around the operative field with limited visual angles. The 3D 4K-HD exoscope solves these problems by placing a small camera on a compact mobile stand, with visual output on a separate screen. This uncoupling of the exoscope input and visual output increases maneuverability of the camera within the OR and around the operative field, providing less of an obstruction to multiple device use and freeing the surgeon and assistants from constrained positions and postures. During long procedures, surgeon fatigue related to suboptimal ergonomic conditions can negatively impact operative performance.9-12 This is particularly true for operations in the posterior fossa, which provides a smaller operative space and more challenging approach angles than supratentorial surgeries. The dense neurovascular anatomy in the posterior fossa is also well visualized by the wide operative field and broad depth of focus of the 3D 4K-HD exoscope.

The unobstructed operative corridor of the 3D 4K-HD exoscope provided additional real-world advantages not previously identified in our preclinical study. Uncontrolled arterial hemorrhage, as encountered during an intraoperative aneurysm rupture or during complicated arteriovenous malformation surgery, is one of the most stressful situations a neurosurgeon can encounter, requiring high-level focus and coordination from all members of the surgical team. When this situation was encountered with the exoscope, all team members immediately became aware, due to their direct and shared visualization of the operative field. Required instruments, including long-handed clip applicers, were passed easily from the scrub technician to the primary surgeon, who was then able to work around the assistant surgeon tasked with keeping the operative field clear. However, the use of the exoscope allowed the assistant to enjoy the same view as...
the surgeon rather than being at an angle dictated by the microscope objective, an initial adaptation period was necessary before the assistant's positioning and hand movements became natural. The large depth of focus also limited the need for exoscope adjustments during this critical time, allowing arterial control to be rapidly obtained. Of note, the use of 3D glasses and light dimming in the OR has not been problematic in such emergency situations, since we typically keep 1 or 2 overhead lights on to facilitate the tasks of the scrub technologist and anesthesiologist.

Although the majority of cases were performed with a single viewing screen shared by the surgical team, positioning of the primary surgeon and co-surgeon on select cases required the use of a second screen. This primarily occurred during posterior fossa and spine operations, where the surgeon and assistant were positioned on opposite sides of the body with straightforward gazes 180° apart. In this situation, a single screen positioned orthogonally to the head of the bed could have also been used, but would have required the primary and co-surgeons to have their head and arms at differing angles to reach and see the operative field. While adaptation to this position would have been possible, a more ergonomic solution was the use of a second screen, each screen being positioned within the optimal gaze angle of the primary surgeon and co-surgeon, respectively. Placement of the screens was an underappreciated
problem initially and, while the footprint of the exoscope itself was a significant improvement over the operating microscope, the addition of 1 or 2 screens with a sizable footprint was somewhat cumbersome. Cables connecting the exoscope to the screens were also an additional step, which somewhat added to the complexity of the OR setup. Improvements in the monitors and their footprint and weight will be necessary in the long term to resolve this issue. Finally, the use of a foot pedal, while not mandatory with the microscope, is a standard feature of the exoscope. Theoretically, despite its low profile, this could result in some additional clutter in the OR. However, this has not been a significant issue in our experience, given that we routinely use the foot pedal with the microscope for hands-free zoom magnification.

CONCLUSION

The first-in-man experience with the 3D 4K-HD exoscope confirms its high-resolution, immersive optics, maneuverability, and surgeon ergonomics for microneurosurgical applications. Expanded clinical utilization of this system is justified based on functional advantages over existing neurosurgical microscope and endoscope systems. However, during the preliminary testing phase, it is advisable to have an operating microscope available.
in the room, until the OR staff becomes well accustomed to this new exoscope system. While it is acknowledged that this paper is largely based on preliminary empirical observations using the exoscope and that head-to-head clinical comparison with the microscope has not been performed, it does nonetheless suggest a promising role for this exoscope system as an adjunct or even potential replacement for the conventional neurosurgical microscope.

**Disclosures**

Drs Langer, Khaleesi, and Levy served as medical consultants for Sony Olympus. Exoscope availability was supported by an unrestricted gift from Sony Olympus Medical Solutions Inc (Tokyo, Japan).

**REFERENCES**


**COMMENT**

The 4K-3D exoscope is a new technology which may potentially provide ergonomics that better fit some surgeons. The ease of use is, to a large extent, subjective and based on a surgeon’s training and previous experience. There is definitely something to be said about its educational qualities, as well as its use in certain procedures in which a microscope becomes cumbersome (such as for example in sitting supracerebellar approaches, etc). On the other hand, it is hard to argue that any kind of digital resolution can ever be as good as direct optics, and it is highly questionable that this technology will be a game-changer as the high price-tags are becoming harder to justify in the increasingly financially-savvy medical environment.

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Tentorial sling for microvascular decompression in patients with trigeminal neuralgia: a description of operative technique and clinical outcomes

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OBJECTIVE Trigeminal neuralgia is a debilitating pain disorder most often caused by arterial compression of the trigeminal nerve, although there are other etiologies. Microvascular decompression (MVD) remains the most definitive treatment for this disorder, with cure rates reported between 60% and 80%. Traditional MVD techniques involve a retrosigmoid craniotomy with placement of an inert foreign material, such as Teflon, between the nerve and compressive vessel. Recurrence of trigeminal neuralgia after MVD has been associated with vessel migration, adhesion formation, and arterial pulsation against the Teflon abutting the nerve. Additionally, foreign materials such as Teflon have been reported to trigger inflammatory responses, resulting in recurrence of trigeminal pain. An alternative method for decompression involves the use of a sling to transpose the compressive vessel away from the nerve. Results of various sling techniques as a decompressive strategy are limited to small series and case reports. In this study, the authors present their experience utilizing a tentorial sling for MVD in patients with trigeminal neuralgia.

METHODS Institutional review board approval was obtained in order to contact patients who underwent MVD for trigeminal neuralgia via the tentorial sling technique. Clinical outcomes were assessed utilizing the Barrow Neurological Institute (BNI) pain intensity score immediately after surgery and at the time of the study.

RESULTS The tentorial sling technique was performed in 45 patients undergoing MVD for trigeminal neuralgia. In 41 of these patients, this procedure was their first decompressive surgery. Immediate postoperative relief of pain (BNI score I) was achieved in 80% of patients undergoing their first decompressive procedure. At last follow-up, 73% of these patients remained pain free. Three patients experienced recurrent trigeminal pain, with surgical exploration demonstrating an intact tentorial sling. The complication rate was 6.6%.

CONCLUSIONS Transposition techniques for MVD have been described previously in small series and case reports. This study represents the largest experience in which the utilization of a tentorial sling for MVD in patients with trigeminal neuralgia is described. The technique represents a novel method for decompression of the trigeminal nerve by transposition of the offending vessel without the use of foreign material. Although the authors’ preliminary results parallel the historical cure rate, further outcome data are required to assess long-term durability of this method.

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KEYWORDS tentorial sling; microvascular decompression; trigeminal neuralgia; MVD; Teflon granuloma; surgical technique; pain

TRIGEMINAL neuralgia is a debilitating facial pain disorder, with an annual incidence of 4–5 per 100,000 and a slightly higher occurrence in females. The most commonly accepted etiology for classic trigeminal neuralgia is vascular compression at or near the root entry zone. Microvascular decompression (MVD) is considered the most definitive and durable treatment for trigeminal neuralgia, as patients who undergo MVD can have complete resolution of pain immediately after surgery. Traditional decompressive methods involve placing foreign material between the nerve and the compressive blood vessel to maintain distance between the 2 structures. In this method, the foreign material acts as a cushion or buttress interposed between the 2 structures. Teflon is most
often used, but other materials such as cotton, small pieces of muscle, Gelfoam (Pfizer), Ivalon sponges (Fabco), and others have also been used.4,10,11

Long-term cure rates for trigeminal neuralgia after MVD range from 60% to 80%.2-4,9 In a large study by Barker et al., 1185 patients underwent MVD for trigeminal neuralgia; 75% of these patients were pain free at 1 year, and 64% were pain free 10 years after surgery.1 Historically, recurrent pain has been attributed to a number of factors: vessel migration back toward the nerve, adhesion formation, foreign material impingement against the nerve, blood vessel/Teflon complex pulsations abutting the nerve, and general failure of the operative technique.4,9

Teflon is the most used material for decompression of the trigeminal nerve because it is considered an inert substance.2,13,15 However, numerous reports of inflammatory reactions related to Teflon use have been described within the neurosurgical literature, as well as within other surgical subspecialties.2,4,5,9,10,15 It has been proposed that Teflon granuloma formation is the cause of delayed recurrence of facial pain after initial surgical success.2,4,5,9,10,15 Other foreign materials used in MVD procedures have also been found to cause inflammatory reactions.13

Alternative decompressive techniques for trigeminal neuralgia are described in the literature, including the use of a slinging method to reposition the compressive vessel away from the trigeminal nerve. The first report of this method was described by Fukushima in 1982, in which a Dacron sling technique was used.7 Other case reports and small case series describe similar methods, with various sling materials used to transpose the offending vessel away from the nerve. These studies have predominantly used foreign materials for sling creation, including Dacron, suture, Gore-Tex tape, aneurysm clips, and gelatin sponge.9,13 However, Melvill and Baxter described the use of an autologous dural sling created from the underside of the tentorium.11 This method was used in 7 patients with a follow-up period ranging from 1 to 8 months. Although the series is small and with short follow-up, the authors reported success, with 6 patients achieving complete cure of facial pain and 1 patient with significant improvement in facial pain.

Here, we report the largest series of patients who underwent MVD for trigeminal neuralgia in which a tentorial-based sling was used for vessel transposition.

**Methods**

Institutional review board approval was obtained from the University of California, San Diego, to review charts of patients who underwent MVD for trigeminal neuralgia by the senior author during the period of 2013–2016. All patients were older than 18 years and treated at University of California, San Diego, Medical Center. Prior to surgery, patients were evaluated in the clinic and gave their consent to undergo MVD for trigeminal neuralgia. We reviewed the operative reports to assess whether the tentorial sling method was used for MVD. A total of 45 patients underwent MVD with the use of a tentorial sling during a 2.5-year period starting in late 2013 and ending in 2016. During this time period, any patient undergoing MVD for trigeminal neuralgia and with a compressive artery amenable to tentorial sling transposition underwent this procedure. Patients who underwent MVD for trigeminal neuralgia with use of the tentorial sling were contacted to complete a clinical outcome questionnaire. Study outcomes included postoperative facial pain status, current medication usage for facial pain, subsequent interventions for facial pain, and complications. The Barrow Neurological Institute (BNI) pain intensity score, described by Rogers et al.,14 was used to assess facial pain status (Table 1).

**Operative Technique**

A standard retrosigmoid approach is performed. The junction of the tentorium and petrous bone is identified, and the petrosal veins are cauterized, as is standard practice in both our traditional and tentorial sling MVD procedures. Once the subjacent arachnoid is dissected off of the trigeminal nerve, the region is explored for vascular compression with specific focus on the nerve root entry zone. As part of the inclusion criteria for this study, a compressive artery must have been present and in a location amenable to slinging. Once the offending vessel is identified, it is dissected free from the trigeminal nerve and mobilized away from the nerve.

Attention is then turned toward creation of the tentorial sling (Fig. 1). A view of the underside of the tentorium in relation to the trigeminal nerve and compressive vessel is shown in Fig. 1A. Using a 15 blade, a 3- to 4-mm split-thickness incision is made along the underside of the superficial/lateral aspect of the tentorium (Fig. 1B). Blunt dissection continues between the 2 layers of the tentorium (Fig. 1C). A second incision is then carried out perpendicular to the initial incision, directed toward the medial edge of the tentorium. This perpendicular incision is created using microscissors, cutting within the split-thickness dural plane (Fig. 1D and E). Care is taken to avoid injury to the trochlear nerve while making the distal cut in this location (Fig. 1D and E). The tentorium is often highly vascular, and hemostasis can be accomplished with use of Surgicel and limited bipolar cauterization, as excessive cautery will shrink the tentorial sling. A third cut, parallel to the second, is completed, creating a 3- to 4-mm split-thickness strip of dura with a pedicle at the medial and deep aspect of the inferior tentorium (Fig. 1F). We found the optimal sling length to be 1.5–2 cm. The dural sling is then wrapped around the compressive vessel, becoming amenable to slinging.

**TABLE 1. Barrow Neurological Institute (BNI) pain intensity score**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
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<tbody>
<tr>
<td>I</td>
<td>No trigeminal pain, no medication</td>
</tr>
<tr>
<td>II</td>
<td>Occasional pain, not requiring medication</td>
</tr>
<tr>
<td>III</td>
<td>Some pain, adequately controlled with medication</td>
</tr>
<tr>
<td>IV</td>
<td>Some pain, not adequately controlled with medication</td>
</tr>
<tr>
<td>V</td>
<td>Severe pain/no pain relief</td>
</tr>
</tbody>
</table>

Reprinted from Int J Radiat Oncol Biol Phys, vol 47, issue 4, Rogers CL, Shetter AG, Fiedler JA, Smith KA, Han PP, Speiser BL, Gamma knife radiosurgery for trigeminal neuralgia: the initial experience of the Barrow Neurological Institute, pp 1013–1019, copyright 2000, with permission from Elsevier.
with care to preserve any perforators, as too much tension may cause avulsion (Fig. 2A–C). Once the tentorial sling is wrapped around the compressive vessel, the complex is brought back up to the tentorium, thereby transposing the vessel away from the trigeminal nerve. The free edge of the tentorial sling is secured to the uppermost aspect of the sling or to the free cut edge of the tentorium with a Weck (Teleflex) clip (Fig. 2D). Once displaced away from the nerve, the vessel is inspected to ensure pulsatility and continued blood flow while also inspecting the nerve to ensure adequate decompression (Fig. 2E and F). Photographs obtained before and after vessel transposition utilizing the tentorial sling technique are directly contrasted, as shown in Fig. 3. A video of the tentorial sling microsurgical technique demonstrates the procedure performed in a cadaveric specimen (Video 1).

**FIG. 1.** Creation of a right-sided tentorial sling. A: View of the cerebellopontine angle, including the underside of the tentorium (Tent). The SCA is seen looping inferiorly, compressing the medial/superior aspect of the trigeminal nerve (TN). B: An initial 3-mm transverse incision is made using a 15 blade. C: Dissection is carried out in a split-thickness plane of the dura. D: Microscissors are used to cut perpendicular to the initial incision. E: A parallel cut, approximately 1.5–2 cm long, is made to complete the tentorial sling (SL). F: A view of the tentorial sling prior to wrapping around the compressive vessel. *Tentorial incision. Figure is available in color online only.

**FIG. 2.** Utilization of the tentorial sling to transpose the compressive vessel away from the nerve. A: The tentorial sling is mobilized toward the compressive vessel. B: The vessel is lifted to allow the tentorial sling to be positioned around it. C: The sling is manipulated around the vessel. D: The free edge of the sling is secured to the tentorium using a Weck clip. E: The transposed vessel is inspected to ensure no constriction of blood flow and adequate decompression of the nerve. F: Final view of the vessel repositioned away from the trigeminal nerve by the tentorial sling. The motor rootlet (MR) of the trigeminal nerve is now visible. Figure is available in color online only.

**VIDEO 1.** Video clip demonstrating the tentorial sling technique for decompression of the trigeminal nerve in a cadaveric specimen. CN = cranial nerve. Copyright Jeffrey A. Steinberg. Published with permission. Click here to view.

The dura is closed in a watertight fashion if permissible, with use of dural substitute as needed, followed by use of bone cement to fill the craniectomy defect. The soft tissue is then closed in sequential layers.

**Results**

A total of 45 patients met inclusion criteria for our study, having undergone MVD for trigeminal neuralgia with the use of a tentorial sling. Thirteen other patients underwent MVD for trigeminal neuralgia during the same period without the tentorial sling method. This included 5 patients who underwent revision MVD (3 from outside
institutions) and 8 patients without a direct arterial compressive vessel. Patient demographics for those who underwent the tentorial sling procedure included 14 males and 31 females. The mean age at the time of surgery was 59 years. Twenty-eight (62%) MVDs were right-sided and 17 (38%) were left-sided. Trigeminal neuralgia in all patients was categorized as classical trigeminal neuralgia as defined by the International Classification of Headache Disorders (13.1.1). The mean length of stay was 3.7 days with a median length of stay of 3 days.

For 41 patients (91%), MVD with use of the tentorial sling was the first decompressive procedure for trigeminal neuralgia. Seven of these patients had undergone prior palliative procedures: radiofrequency ablation in 1 patient, Gamma Knife radiosurgery in 2 patients, glycerol injection in 2 patients, balloon compression in 1 patient, and both Gamma Knife radiosurgery and glycerol injection in 1 patient. The other 4 patients had undergone prior MVD, 3 at outside institutions and 1 at the University of California, San Diego, by the senior author.

Immediate postoperative clinical outcomes are shown in Table 2 for the 41 patients who underwent their first MVD. Thirty-three patients (80%) achieved complete relief of facial pain (BNI score I). Two other patients had significant symptom relief, with only occasional pain and no medication usage (BNI score II). Two patients reported improvement, but still required medication to manage their facial pain (BNI score I or II). Although 78% of patients had a favorable result at last follow-up, 73% of patients remained completely pain free without medication use at the last follow-up (BNI score I). Two patients who maintained significant pain relief with only occasional mild pain (BNI score II), 78% of patients had a favorable result at last follow-up. Of the 7 patients with minimal or no improvement at last follow-up (BNI score IV or V), one had a history of prior glycerol injection and another a history of prior Gamma Knife radiosurgery.

Three patients underwent reexploration for continued pain after their initial MVD with a tentorial sling. In one of these patients, no new compressive vessel or lesion was found, and the tentorial sling transposition of the previously compressive artery was intact. Massage of the trigeminal nerve was performed, and the pain decreased to BNI score II postoperatively. Another patient initially had complete resolution of pain for 1 year; however, the pain recurred to a BNI score of IV. Reexploration demonstrated significant adhesions between the dura, cerebellum, trigeminal nerve, and surrounding structures. The tentorial sling–vessel construct was intact. After releasing the adhesions, the patient achieved resolution of pain (BNI score I). In the third patient, the sling remained intact; however, a portion of the ectatic vessel had come in contact with the nerve. This portion of the vessel was mobilized away from the nerve, and a small piece of Teflon was placed between the 2 structures. This revision MVD was performed in the only patient in the study in whom foreign material (Teflon) was used, with a final technical result utilizing the tentorial sling in the first procedure and Teflon in the second because of the ectatic vessel anatomy. Postoperatively, the patient’s pain improved to a BNI score of II. These 3 patients were all recorded as having a BNI score of IV or V at last follow-up for the study (Table 2). Examining outcomes after these 3 reexploration surgeries, 35 patients (85%) achieved complete pain relief or significant improvement in facial pain (BNI score I or II).

Of the 4 patients in the study who had undergone prior MVD without the use of a tentorial sling, 1 patient had a complete cure (BNI score I), and the other 3 achieved moderate improvement in pain (BNI score II). Although each of these patients had undergone prior MVD, a compressive vessel was discovered during the senior author’s repeat exploration, which was amenable to decompression by the dural sling transposition method.

The overall complication rate for the 45 procedures performed was 6.6%. Complications included 2 CSF leaks (one requiring CSF diversion), and 1 patient had a small cerebellar stroke within the superior cerebellar artery (SCA) territory, resulting in mild dysmetria. This patient had an ectatic, tortuous SCA that looped down around the trigeminal nerve. Mobilization of the vessel involved significant manipulation of the artery to decompress the nerve. The sling portion of the procedure was completed.
TABLE 2. Immediate postoperative outcome and outcome at last follow-up in patients who underwent MVD as their first decompressive procedure

<table>
<thead>
<tr>
<th>BNI Score</th>
<th>Immediate Outcome</th>
<th>Outcome at Last Follow-Up</th>
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<tbody>
<tr>
<td>I</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>V</td>
<td>2</td>
<td>3</td>
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</table>

without complication. The small postoperative stroke was attributed to the significant manipulation of the vessel. No postoperative facial numbness was encountered in any patient.

Discussion

Microvascular decompression remains the only definitive cure for trigeminal neuralgia. Traditional methods involve placement of a foreign material (most often Teflon) between the compressive vessel and nerve. Multiple studies have demonstrated that inflammatory responses to this material can result in granuloma formation and lead to pain recurrence and failure of the MVD. In a review by Capelle et al., the authors reviewed their series of 200 patients and found 3 with a Teflon granuloma during reexploration for recurrent facial pain. Additionally, the authors reviewed a small body of literature in which occurrence of Teflon granulomas was reported, citing an overall incidence of 1%–7%.

The present study is the first of its size to describe MVD with use of a tentorial sling for vessel transposition. For patients undergoing MVD with the use of a tentorial sling as their initial decompressive procedure, 80% achieved complete pain relief immediately following surgery, defined as a BNI pain score of I. Including the 2 patients who only experienced occasional facial pain after the procedure, 85% had significant improvement postoperatively. However, 3 patients did develop recurrence of pain. At the last follow-up, the cure rate was 73%, with 78% of patients achieving significant improvement of their facial pain. In one of the largest MVD clinical outcome studies for trigeminal neuralgia, Barker et al. reported an 82% rate of pain relief immediately following surgery and a 75% rate at 1 year. Additionally, maintenance of pain relief at 10 years was 64%, demonstrating the continued recurrence of facial pain over time.

Four patients underwent MVD with the use of a tentorial sling after previously undergoing traditional MVD. Results for these patients were mixed—1 patient had complete pain relief, and 3 achieved partial pain relief. In general, repeat MVD for trigeminal neuralgia is associated with variable outcome. Additionally, performing repeat MVD utilizing a sling method is only applicable for patients found to have a compressive artery against the nerve. All patients who underwent the tentorial sling procedure were found to have a compressive artery at the nerve root entry zone without other sources of compression. No foreign material was used in any of the patients undergoing MVD with use of the tentorial sling except for a patient who experienced pain recurrence; during reexploration, Teflon was used to augment the previous tentorial sling–SCA complex, which remained intact.

Although temporal durability of this procedure requires longer-term patient outcome data, we believe that it has 2 main advantages over the traditional MVD method. First, the sling method does not require the use of foreign material, aside from a Weck clip to hold the sling in place. A Weck clip is used to secure the tentorial sling as it provides efficient and permanent fixation. An alternative option of using suture to secure the tentorial sling is possible; however, because of the small corridor and deep location of the site, we believe that this would incur added operative time and challenge. In the absence of foreign material, the likelihood of an inflammatory response is minimized. This may lead to a lower rate of trigeminal pain recurrence over time. Second, by transposing the vessel away from the nerve, there is no vessel/foreign material complex near the trigeminal nerve. Instead, the artery is repositioned, leaving the nerve and root entry zone free from any compression, including that from inserted material. Because the vessel is wrapped, postoperative migration of the vessel back toward the nerve is unlikely. In the 3 patients who underwent reexploration in our study, we found that all tentorial slings were maintained in place with the vessel adequately repositioned and with good pulsatile flow. In one patient, an ectatic portion of the SCA had come in contact with the nerve. Another patient demonstrated significant adhesion formation. During reexploration in the third patient, no compression or adhesions of the nerve were found. Although facial pain recurred in these patients, the tentorial sling remained intact. These cases are a reminder that the pathophysiology of trigeminal neuralgia is complex and incompletely understood.

Although our results are similar to those of the traditional MVD technique, the unique tentorial sling method described here provides neurosurgeons an alternative surgical technique that may be used in cases in which buttressing with foreign material alone does not provide enough decompression.

The tentorial sling method does have limitations. As with any new procedure, there is a learning curve. Challenges with creating the tentorial sling include tearing of the dura, bleeding, and maintaining a split-thickness pedicle. We found that laying a piece of Gelfoam along the cerebellopontine angle when creating the sling prevents tentorial bleeding from accumulating at this site. Additionally, use of Surgicel and bipolar cautery help control bleeding from the cut edges of the tentorium. Care must be taken not to shrink the dural sling with excessive use of bipolar cautery. The sling procedure adds 30–45 minutes of surgical time initially when learning the technique; however, after becoming accustomed to the method, the procedure could be performed in approximately 20 minutes. In our practice, we regularly sacrifice the petrosal vein in both the traditional MVD technique and the novel method described here. We have not found that this leads to a higher complication profile, and we believe that sacrifice of the petrosal vein allows for improved operating...
Conclusions
This study represents the largest series of patients with trigeminal neuralgia who were treated using a decompressive sling technique. Although the long-term durability of this technique is unknown, it has potential advantages over traditional methods, as it avoids the use of foreign material and achieves complete transposition of the compressive vessel. Limitations include extending the surgical time and potentially dural bleeding. Although Teflon granuloma is reviewed in this study and described in the literature, it should be understood that the rate of complications from Teflon or other foreign material is relatively low, and traditional MVD techniques remain an overall very successful neurosurgical procedure. The tentorial slinging technique provides an alternative decompressive method for neurosurgeons who treat trigeminal neuralgia. We plan to assess clinical follow-up in our patients at 5- and 10-year time points to further assess temporal durability.

References

Disclosures
The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions
Conception and design: Steinberg, Sack, Weingarten, Alksne. Acquisition of data: Steinberg, Wilson. Analysis and interpretation of data: Steinberg, Sack, Wilson, Alksne. Drafting the article: Steinberg, Sack, Weingarten, Alksne. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Steinberg. Study supervision: Steinberg, Sack, Weingarten, Carter, Khalesi, Alksne.

Supplemental Information
Videos

Previous Presentations
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Abstract

Sonic hedgehog (SHH) medulloblastoma (MB) subtype is driven by a proliferative CD15+ tumor propagating cell (TPC), also considered in the literature as a putative cancer stem cell (CSC). Despite considerable research, much of the biology of this TPC remains unknown. We report evidence that phosphatase and tensin homolog (PTEN) and phosphoinositide 3-kinase (PI-3K) play a crucial role in the propagation, survival and potential response to therapy in this CD15+ CSC/TPC-driven malignant disease. Using the ND2-SmoA1 transgenic mouse model for MB, mouse genetics and patient-derived xenografts (PDXs), we demonstrate that the CD15+TPCs are (1) obligately required for SmoA1Tg-driven tumorigenicity (2) regulated by PTEN and PI-3K signaling (3) selectively sensitive to the cytotoxic effects of pan PI-3K inhibitors in vitro and in vivo but resistant to chemotherapy (4) in the SmoA1Tg mouse model are genomically similar to the SHH human MB subgroup. The results provide the first evidence that PTEN plays a role in MB TPC signaling and biology that PI-3K inhibitors target and suppress the survival and proliferation of cells within the mouse and human CD15+ cancer stem cell compartment. In contrast, CD15+ TPCs are resistant to cisplatinum, temozolomide and the SHH inhibitor, NVP-LDE-225, agents currently used in treatment of medulloblastoma. These studies validate the therapeutic efficacy of pan PI-3K inhibitors in the treatment of CD15+ TPC dependent medulloblastoma and suggest a sequential combination of PI-3K inhibitors and chemotherapy will have augmented efficacy in the treatment of this disease.
Introduction

Medulloblastoma (MB) is an aggressive cerebellar tumor and the most common pediatric brain malignancy [1, 2]. The current treatment for medulloblastoma includes resection of the tumor followed by radiation and chemotherapy which includes cisplatinum regimen. Although the cure rate is 50–80%, survivors suffer severe side effects including growth impairment, endocrine disorders, and marked neurocognitive deficits [3]. Thus, more effective and less toxic therapies for medulloblastoma are urgently needed. Recently, several groups [4–8] have performed gene expression profiling and DNA-copy-number analysis of MB, and have identified at least four major subtypes of the disease: WNT, Sonic hedgehog (SHH), Group C, and Group D. These molecular subtypes have distinct characteristics in terms of gene expression, mutational profiles, epidemiology, and prognosis. Among molecular subtypes, tumors associated with uncontrolled activation of SHH pathway are commonly defined as SHH MB. The SHH pathway is an essential embryonic signaling cascade that regulates stem-cell and progenitor-cell differentiation in multiple developmental processes [9]. Mutations in the SHH pathway suppressor Patched or alterations of other SHH pathway components result in its permanent activation and MB tumor formation [10, 11]. About 30% of MB exhibits uncontrolled activation of the SHH signaling pathway [11]. Although, several smoothened (SMO) antagonists including NVP-LDE225 & GDC0449 are currently being evaluated in clinical trials in patients with medulloblastoma, there is rapid development of tumor resistance [12, 13]. A study by Buonomici et al demonstrated that NVP-LDE225 resistance in MB is mediated by the activation of the phosphoinositide 3-kinase (PI3K) signaling pathway. [14]. Existing literature suggests that the tumor suppressor, PTEN and its target PI-3K are important in the pathogenesis of SHH-associated MB [15–20]. Recent genomic analysis of medulloblastoma tumors revealed that PI-3K mutation (PIK3CA, PTEN, PIK3CG) is frequent in SHH subgroup tumors [21, 22]. In one of the studies, out of 13 Hedgehog subgroup tumors profiled, 2 had loss-of-function mutations in PTEN, and another patient had an activating mutation in PIK3CA [22]. In another study, out of 133 SHH MB tumors profiled, PI-3K pathway is mutated in >5% of SHH MBs [21, 22].

Multiple reports indicate that MB is driven by treatment resistant stem cell-like cells, termed cancer stem cells/tumor propagating cells (CSC/TPCs). Landmark studies demonstrate that tumor samples extracted from murine genetic MB models, Sonic hedgehog (SHH-Patched) and from human MB, are propagated by cells expressing the progenitor marker CD15/SSEA [23, 24]. CD15 is a carbohydrate antigen that is expressed on both progenitors and stem cells in the embryonic and adult central nervous system [25, 26] and most notably is considered as an important marker for TPCs in the SHH subgroup of medulloblastoma [23].

TPCs are considered to be proliferative and a major contributor to tumor resistance and recurrence [27–30]. Several studies have demonstrated a role for the PI-3K/AKT pathway in proliferation and propagation of TPCs [31–33]. Reports have shown that blocking PI-3K activity suppresses the proliferation of TPCs in breast, ovary and various other cancers [34]. Hambardzumyan et al. suggested that the PI-3K pathway activity regulates survival of cancer stem cells following radiation in medulloblastoma in vivo. [35]. Thus, we hypothesized that targeting the PTEN-PI-3K signaling axis in the MB TPC compartment may provide long-term tumor control and/or eradication of medulloblastoma. Previously, our group has reported that heterozygosity of PTEN promotes tumorigenesis in both human and in the SmoA1 mouse model of medulloblastoma [15]. We reported that 61% of human medulloblastoma tumors have lost expression of the PTEN protein and this loss in PTEN is of prognostic significance in this disease (15). Herein, using the SmoA1Tg mouse model and primary human MB patient xenograft tumor samples (PDXs), we observed that tumor-propagating capacity of CD15+ TPCs in
SHH-driven MB is regulated at least in part by the PTEN-PI-3K signaling pathways, and that targeting this axis using PI-3K inhibitors may block the in vivo propagation of TPCs and induce apoptosis.

**Materials and Methods**

**Animal studies**

ND2:SmoA1 (SmoA1) transgenic mice were a gift from James Olson (University of Washington, Seattle, WA) [36]. Mice were maintained in the Moores Cancer Center vivarium at University of California, San Diego, and all experiments were performed using procedures approved by the University of California, San Diego IACUC committee.

**Stereotaxic implantation of tumor cells**

Stereotaxic implantation of tumor cells was performed as described before [23, 37]. Nude nu-nu mice were anesthetized using 60 mg/kg ketamine (Fort Dodge Animal Health) plus 20 mg/kg xylazine (Ben Venue Laboratories), and positioned in a stereotaxic frame with a mouse adapter (Kopf Instruments). An incision was made in the midline of the scalp over the cerebellum, and a small hole was made in the skull (3 mm to the right and 1 mm anterior to bregma) using a beveled (sharp point) 25 G needle. A 30-gauge Hamilton syringe loaded with cells was mounted on a micromanipulator and introduced through the hole to the surface of the right frontal lobe, at a depth of 4.5 mm. Freshly-sorted CD15+/- tumor (uncultured) cells were injected over the course of 2 minutes, and the needle was left in place for five more minutes to avoid reflux. Finally, the skin was closed with 6–0 fast absorbing plain gut suture using a 3/8 PC-1 cutting needle (Ethicon). Animals were monitored continuously during and in postoperative period to assure that mice have recovered from surgery and are ambulatory without evidence of discomfort. Potential painful and stressful effects of this survival surgery include: 1) poor feeding, 2) weight loss, 3) ruffled fur, 4) loss of mobility in cage, 5) evidence of infection at surgical site. The analgesic buprenorphine (0.1 mg/kg) was injected in case of pain or discomfort. Nonabsorbable sutures and/or staples was removed 10–14 days following surgery. If morbidity is not corrected by our interventions, mice were euthanized using CO2 followed by cervical dislocation.

**Cell isolation & flow cytometry**

Tumor cells were isolated from PDX as well as 4 to 6-month-old SmoA1Tg mice as follows. Briefly, tumor tissue was cut into small pieces, and incubated at 37°C for 30 min in digestion buffer consisting of Dulbecco’s PBS (DPBS, Life Technologies, Grand Island, NY) with 10 U/ml papain (Worthington, Lakewood, NJ), 200 μg/ml L-cysteine, and 250 U/ml DNase (Sigma, St. Louis, MO). The digestion buffer was then removed and replaced with DPBS containing 8 mg/ml soybean trypsin inhibitor (Boehringer Mannheim, Indianapolis, IN), 8 mg/ml bovine serum albumin (BSA, Sigma), and 250 U/ml DNase, followed by titration of tissue using pipettes of decreasing bore size to obtain a single-cell suspension. Cells were centrifuged at room temperature and resuspended in PBS containing 200 μg/ml BSA (PBS/BSA) and passed through a cell strainer (Becton Dickinson, Franklin Lakes, NJ) to remove debris. This suspension was centrifuged through a step gradient of 35% and 65% Percoll (Amersham Biosciences), and cells were harvested from the 35%–65% interface, washed in PBS/BSA. For sorting of CD15+ and CD15- cells, tumor cells were re-suspended in FACS buffer (DPBS + 2% FBS) and stained for 30 min with CD15 antibody (BD Biosciences, Cat no. 340850, primary antibody)
washed with FACS buffer, stained for 30 minutes with secondary antibody (FITC), and then analyzed or sorted using a FACSVantage SE flow cytometer.

Cell proliferation, BrdU incorporation, apoptosis and cell cycle analysis

Tumor cells were isolated as previously described [23] from human patient derived xenografts (PDX) as well as 4 to 6-month-old SmoA1 PTEN+/+ mice displaying physical and behavioural signs of medulloblastoma.

FACS sorted CD15+ and CD15− cells were plated at 4 × 10⁴ cells/well in ultralow binding 96-well plates in serum-free medium containing Neurobasal and B27 supplements. Cells were incubated overnight and treated with DMSO or inhibitors for 48 hr. Cell viability assay was performed using AlamarBlue® (Roche) according to manufacturer’s protocol. For BrdU incorporation studies, tumor cells were isolated from Smo A1 Tg model as described above. 2 million tumor cells per well were plated into 24-well plates in serum-free medium containing Neurobasal and B27 supplements. The cells were pulsed with BrdU for 30 minutes and then washed with media to remove any remaining BrdU. Cells were collected immediately after the pulse (“30 minutes”) and stained with CD15 antibody as described above. The cells were then fixed and stained using the FITC BrdU Flow Kit (BD Biosciences) and propidium iodide (PI) according to the manufacturer’s instructions. The analysis was performed using a FACS Calibur flow cytometer (BD Biosciences). For apoptosis studies, CD15+ and CD15− cells were treated with inhibitor for 24 hrs, followed by caspase-3 activity assay using kit (Roche) or staining with annexin VFITC antibody and propidium iodide (PI) according to manufacturer’s instructions (BD, Pharmingen, San Diego, CA). For cell cycle analysis DNA content was analyzed with FACS Calibur flow cytometer (BD Biosciences).

Western blot analysis

FACS sorted CD15+ or CD15− cells were treated with different concentrations of inhibitors or DMSO for 30 minutes and then stimulated with IGF 50 ng/ml for 30 minutes. Cells were lysed with RIPA lysis buffer (Pierce) containing protease inhibitor cocktail. Proteins were quantitated by the BCA protein assay (Pierce) and equal amounts of protein were resolved by polyacrylamide gels, transferred to nitrocellulose membrane and probed with following primary antibodies: p-AKT(Ser473) (cat no. 9271), p-AKT(Thr308) (cat no. 9275), AKT(cat no. 9272), p-P70S6K(Thr389) (cat no. 9205), p70S6K (cat no. 2708), p-4EBP1(Thr37/46) (cat no. 2855), 4EBP1 (cat no. 9452), pERK(Thr202/Tyr204) (cat no. 9101), p-PRAS40(Thr246) (cat no. 2997), PRAS40 (cat no. 2610), p27 Kip1 (cat no. 2552), p-MDM2(S166) (cat no. 3521), Bad (cat no. 9292) and PARP (cat no. 9542) (all from Cell Signaling Technologies); p21 Cip1/Waf1 (sc-397), Bax (sc-493) and β-actin (sc-47778) from SantaCruz.

Quantitative real-time analysis

RNA was extracted from CD15+ and CD15− cells using RNeasy Kit (Qiagen, Germantown, MD) according to manufacturer’s instructions. For RTPCR, cDNA was prepared from 1 μg RNA sample using iscript cDNA synthesis kit (Bio-Rad, Hercules, CA). cDNA was amplified by RT-PCR reactions with 1× SYBR green supermix (Bio-Rad, Hercules, CA) in 96-well plates on an CFX96 Real time system (Bio-Rad, Hercules, CA) using different primers for human or mouse genes. The sequence of the primers are as described in S1 Table. Relative expression levels were normalized to GAPDH expression according to the formula < 2^(Ct gene of interest- Ct GAPDH) >.
In-vivo BKM120 treatment

To study effect of BKM-120 on tumor growth in vivo, CD15+ and CD15- TPC were implanted intracranially into the cerebella of secondary NSG mice or subcutaneously in nu-nu mice. For subcutaneous tumors, 20 days after transplantation, mice were randomly separated into two groups: Group 1 was given vehicle (untreated) and Group 2 was given 30 mg/kg BKM-120 by oral gavage once daily for 21 days. Tumor dimensions were recorded every third day and tumor volume was measured using the following formula: volume = 0.5 x length x (width)^2. For intracranial tumors, 40–50 days after implantation, tumors were checked by MRI and divided into groups and treated as described for subcutaneous tumors. Measurement of tumor volume for subcutaneously implanted tumor was done by callipers and for intracranial implanted tumor was performed by Magnetic resonance imaging (MRI). MRI was performed using a 1.0-T MRI. Mice were anesthetized with 2% isofluorane and the mice were then imaged with an Aspect M2 1.0-Tesla small animal scanner (Aspect Imaging; Shoham, Israel). T2-weighted, images were obtained by using a repetition time of 2500 ms, an echo time of 60 ms, a slice thickness of 1 mm, field of view of 35 mm and a matrix size of 184 x 184 (in plane resolution of 35/184 = 0.19mm). For MR imaging studies, tumor volumes were measured by manually segmenting tumors using either Varian’s Image Browser software or the public domain program ImageJ Image (http://rsb.info.nih.gov/ij). T2-weighted images were sometimes used to help clarify tumor margins.

Human tumor isolation and propagation

Human MB tissue for patient-derived xenografts was obtained from surgical resection of tumors at Rady Children’s Hospital (San Diego, CA). All procedures using human tissue were approved by the Institutional Review Boards of Rady Children’s Hospital. Upon retrieval, the tissue was mechanically dissociated into a single-cell suspension, then immediately injected into the brain of NSG mice. When the mice became symptomatic, the tumors were again dissociated into single-cell suspensions and then re-transplanted back into the brain of naïve hosts to establish a propagated line for each patient-derived xenograft.

Microarray analysis

SmoA1Tg tumor cells were sorted into CD15+ and CD15- populations and used for RNA isolation using RNasy Kit (Qiagen, Germantown, MD). RNA integrity was assessed using an Agilent 2100 Bioanalyzer. Samples showing RNA integrity (RIN) greater than 8.0 were used for microarray analysis. RNA was labelled and hybridized to Affymetrix Mouse Genome 1.0 ST arrays. The array data were processed by the RMA software. Significance Analysis of Microarray (SAM) software was used to determine differential expression with a false discovery rate (FDR) <1% and a minimum fold-change of 2 unless otherwise stated. Heatmaps were generated using the R package. Gene expression data were transformed into Z-score and hierarchical clustering with an Euclidean distance was applied to generate the row and column dendrograms. Microarray data have been deposited in the GEO public database (http://www.ncbi.nlm.nih.gov/geo/), with GEO accession number is GSE41717.

Subclass and SUBMAP analysis to compare murine CD15+ TPCs to human MB subgroups

MB subclass and SUBMAP analysis, which allows for cross-platform and cross-species comparison of microarray data based on Kolmogorov-Smirnov statistics (33), was used to compare murine tumors to the human MB samples described in [4]. The Subclass Mapping module in
the Gene Pattern software package (www.broadinstitute.org/genepattern) was used to compare the mouse dataset to a gene expression dataset composed of primary human MB classified into molecular subtypes (c1–c6) as defined in Cho et al. and a series of normal cerebellum samples and atypical teratoid rhabdoid tumors [4]. Mapping results are represented as a subclass association (SA) matrix-filled with p-values for each subclass association.

**Gene set enrichment analysis (GSEA)**

GSEA was carried out as described in [38]. Briefly, genes were ordered based on their differential expression between two classes (CD15+ vs. CD15-). An enrichment score (ES) was then calculated for each gene set. Gene sets with a nominal p-value < 0.05 were included as significant. For this analysis, curated gene sets (c2) from MSigDB v.3.0 (http://www.broadinstitute.org/gsea/msigdb/index.jsp) were utilized. PCA analysis of the 22 leading edge genes identified in the SUBMAP evaluation were compared across species barrier in CD15+, CD15- and in human MB tumor gene expression database [4].

**Results**

**PI-3K signaling is highly elevated in CD15+ TPCs isolated from SmoA1 Tg medulloblastoma mouse model**

It is well established that CD15 is a marker for tumor propagating cells in Ptc+/- model of SHH driven medulloblastomas [23]. In the present study, ND2SmoA1 transgenic mouse model was used to characterize the signaling pathways required for proliferation of CD15+ TPCs. First, we performed preliminary experiments in our SmoA1 model to validate that CD15 is a cell surface marker for tumor propagating cells in this SHH driven medulloblastoma model. For this purpose, an orthotopic transplantation assay was established in which SmoA1 PTEN+/+ tumor cells were sorted into CD15+ and CD15− fractions, and 2 x 10^6 cells from each fraction were stereotaxically implanted into the cerebellum of nude/nu-nu mice. S1A Fig shows the FACS data validating that pure CD15+ population is isolated from the tumors. As shown in S1B Fig, implantation of only CD15+ TPCs resulted in secondary tumors within 10–12 weeks that histologically resembled the primary tumors from which the cells were derived (data not shown). In S1B Fig, we demonstrate Ki67 staining only in secondary tumors derived from CD15+ TPCs indicating the higher proliferative index of these cells relative to normal brain. Moreover, our findings demonstrate that only CD15+ cells from SmoA1 PTEN+/+ medulloblastoma can generate tumors in vivo. In order to further evaluate the stem cell like properties of CD15+ TPC, we performed real time PCR analysis of a number of stem cell markers and the results demonstrate that certain stem cell markers e.g. oct4, klf4, sox2, cccr4, pou5f1, nanog, nestin and musashi are highly enriched in the CD15+ TPC compartment in the SmoA1Tg mouse model (S1C Fig). In order to gain further insight into the tumor propagating properties of CD15+ cells, we performed a number of biochemical and genomic analyses of CD15+ and CD15− cell populations isolated from SmoA1 Tg tumors. Based on this analysis, we found that the CD15+ population isolated from SmoA1 Tg mouse model form neurospheres (data not shown), display a distinct expression pattern and are highly proliferative as revealed by increasing viable cell numbers over time (Fig 1A, left panel) and higher BrDU incorporation in CD15+ cells as compared to CD15− cells from the same tumor (Fig 1A, right panel). This result is also supported by higher H3 thymidine incorporation (data not shown) in CD15+ cells as compared to CD15− cells. Data presented in S2 Table and S2 Fig shows that genes related to proliferation and cell survival (S2A Fig), SHH signaling pathway (S2B & S2C Fig) and angiogenesis (S2D Fig) are highly elevated in the CD15+ population. Overall, these data indicate
that the tumor-propagating capacity of CD15+ cells is associated with an increased capacity to proliferate and a decreased tendency to undergo apoptosis and differentiation.

PI-3K/AKT pathway has been shown to be important for the proliferation of TPCs in both solid tumors and leukemia [31–33]. In order to examine the potential mechanistic role for the PI-3K signaling pathway in TPC propagation and survival in medulloblastoma, we determined the relative activation state of PI-3K/AKT in CD15+ TPCs vs. CD15- non-TPCs. Western blot analysis revealed that CD15+ cells have lower basal levels of expression of PTEN and have an activated PI-3K signaling axis compared to CD15- cells, showing substantial increase in phospho-AKT, phospho-S6 and phospho-4EBP1 (Fig 1B). These results were supported by the augmented levels of PTEN mRNA detected in CD15- population as compared to the CD15+ cells (Fig 1C). Taken together, these results suggest that PTEN expression is downregulated and PI-3K signaling is elevated in CD15+ TPC as compared to CD15- population.
Preferential targeting of TPC by PI-3K inhibitors in vitro

The above results demonstrate that PI-3K signaling is upregulated in CD15+ TPC as determined by the lower levels of PTEN and the activation of AKT. Hence, we hypothesized that treating CD15+ cells with PI-3K inhibitors would preferentially block the proliferation of CD15+ TPCs. For this, a panel of PI-3K inhibitors, SF1126 [39], BEZ-235 [40] (Selleck chemicals), PF4691502 [41] (Selleck chemicals) and BKM120 [42] (Novartis) were used. Cisplatinum, TMZ and NVP-LDE-225 were purchased from Selleck Chemicals. Results in Fig 2A show that although PI-3K inhibitors dose dependently reduce the proliferation of both CD15+ and CD15- TPCs isolated from SmoA1 Tg mouse model, it was more potent against CD15+ population. The IC50 for BKM120, BEZ, PF4691502 and SF1126 is 0.156 μM, 0.167 μM, 2.6 μM and 4.3 μM for CD15+ cells and 6.17 μM, 5.54 μM, 4.8 μM and 19.9 μM or CD15- cells, respectively. Previous work has suggested that BKM120 has excellent blood brain barrier

**Fig 2.** Preferential targeting of TPCs by PI-3K inhibitors in vitro (A) Effect of PI-3K inhibitors on proliferation of CD15+ and CD15- cells. CD15+ and CD15- cells were cultured in serum-free media containing no additive, DMSO (vehicle), BKM-120, BEZ-235, PF-04691502, and SF1126 at different conc. After 48 hr, AlamarBlue was added and plates were incubated at 37°C in 5% CO2 for 6 hours. Fluorescence signals were read as emission at 590 nm after excitation at 560 nm. (B) CD15+ and CD15- cells were treated with 100nM conc. of cisplatinum, TMZ, NVP-LDE-225 either alone or in combination with BKM 120 and analyzed for cell viability using Alamar Blue. (C) CD15+ TPCs were treated with BKM120 for 30 minutes followed by stimulation with IGF (50 ng/ml). Cell lysates were analyzed by Western blot for phosphorylation of substrates of PI-3K signalling. (D) Relative expression of SHH pathway genes in BKM120 treated (0.2, 2.0 μM) and untreated CD15+ TPCs. Relative expression levels were normalized to GAPDH. Graphs present mean ± SEM of 3–4 mice in each group for B and D. Statistical significance is assessed by two sample t-test where *denotes P<0.05, ** denotes P<0.01 and *** denotes P<0.001. Experiment was repeated 4–5 times with similar results.

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penetration [42]. Hence, we chose BKM120 for our in vivo studies. Current therapies for younger children with medulloblastoma have included the use of multiagent chemotherapeutic approaches including the chemotherapeutic agents, Temozolomide, cisplatinum [43, 44] and NVP-LDE225, a Smo antagonist developed by Novartis [45]. Hence, we examined the relative cytotoxicity of these drugs in CD15+ vs CD15- tumor cells. For these experiments, CD15+ and CD15- cells isolated from Smo A1 Tg model were treated with BKM120, cisplatinum, TMZ, NVP-LDE225 or combination of BKM120 with cisplatinum or TMZ or NVP-LDE225. Interestingly, cisplatinum (IC$_{50}$ 11.4 μM for CD15+ cells and 4.5 μM for CD15- cells) and TMZ (IC$_{50}$ 30 μM for CD15+ cells and 20 μM for CD15- cells) has no effect, while NVP-LDE-225 (IC$_{50}$ 2.8 μM for CD15+ cells and 2.5 μM for CD15- cells) has very less effect on survival of CD15+ cells (Fig 2B), suggesting that CSC/TPCs are resistant to conventional chemotherapy. S3A and S3B Fig shows dose dependent effect of cisplatin, and TMZ on CD15+ and CD15- cells. NVP-LDE-225 showed cytotoxic effects at high doses in CD15+ and CD15- cells, with no significant effect at 100 nM conc. (S3A & S3B Fig) Interestingly the combination of BKM120 & cisplatinum and BKM120 & TMZ did not result in augmentation of cytotoxicity activity in the CD15+ cells (Fig 2B). However, combination of BKM120 and NVP-LDE225 showed synergy in the CD15+ cells (Fig 2B). We next determined, if PI-3K inhibitors can block the elevated PI-3K signaling cascade in CD15+ TPCs. Treatment of CD15+ cells with different doses of BKM120 for 30 minutes, inhibited phosphorylation of AKT, its downstream target PRAS40 and mTOR substrates pS6, p4EBP1 in a dose-dependent manner (Fig 2C) exhibiting a dramatic decrease at 2.0 μM and almost complete inhibition at 10.0 μM. Real time PCR analysis demonstrated that 2 μM concentration of BKM120 completely suppressed the expression of SHH pathway genes viz., gli1, gli2, N-Myc, C-Myc, and cyclin-D1 (Fig 2D).

**Differential sensitivity of CD15+ vs CD15- cells to PI-3 kinase inhibitor; Inhibition of the PI-3K pathway suppresses proliferation by inducing cell cycle arrest and inducing apoptosis in CD15+ TPCs but not in CD15- cells**

We next investigated if PI-3K inhibitors can induce cell cycle arrest in the CD15+ TPC compartment. For this, we first evaluated the baseline percentages of CD15+ and CD15- cells in different phases of cell cycle and found that 67% & 26% of the CD15- TPCs were in G0-G1 versus S phase respectively, while CD15+ cells comprise 48% and 45% of the cells in G0-G1 versus S phase, respectively (Fig 3A). Moreover treatment of CD15+ TPCs with BKM120 resulted in cell cycle arrest with a proportional increase in G0–G1 and a decrease in the number of cells in the S phase of the cell cycle (Fig 3A, left panel), while the treatment of CD15- cells with BKM120 showed no change in the percentage of cells in G0-G1 versus S phase of cell cycle (Fig 3A, right panel). The G1 arrest induced by BKM120 was correlated with the up-regulation of p27$^{kip1}$ and p21$^{cip1}$ in BKM120 treated CD15+ TPCs (Fig 3B). Furthermore, the expression of cyclin A2, B1, B2, F, aurora kinase A, B, and CDK1 in CD15+ were completely suppressed by BKM120 at 2 μM concentration (Fig 3C). In order to determine if the cell cycle arrest phenotype was associated with the induction of apoptosis, Annexin-V FITC staining and caspase 3 activity assay were performed. Fig 3D reveals that BKM120 treated CD15+ TPCs induced a marked apoptotic response compared to untreated controls, while treatment of CD15- cell with BKM120 did not induce apoptosis (P >0.05). Consistent with this, fluorimetric caspase 3 enzyme assay showed that caspase 3 activity was increased in BKM120 treated CD15+ cells and not in CD15- cells (Fig 3E).

The AKT kinase is known to phosphorylate cytoplasmic MDM2 on serines 166 and 186, which promotes translocation of MDM2 from the cytoplasm into the nucleus where it
mediates the degradation of p53 [46]. BKM120 was observed to potently block the phosphorylation of MDM2 at S166 in a dose-dependent manner with its complete inhibition at 10 μM concentration (Fig 3F). In addition, BKM120 also induced apoptosis in CD15+ TPCs by elevating the expression of pro-apoptotic protein p53 which was associated with the increased transcriptional up-regulation of downstream targets BAX and BAD (Fig 3F). BKM120 treatment increased levels of BAX in a dose dependent manner leading to caspase 3 activation as confirmed by Western blotting (Fig 3F).

BKM120 inhibits tumor growth and preferentially blocks proliferation of CD15+ TPCs in vivo

To address the functional relevance of up-regulated PI-3K signaling in TPC population, we asked whether BKM120 could block tumor growth in CD15+ TPCs xenografts grown subcutaneously in nude mice. As we expected, tumor growth was robust in the vehicle treated control group while growth was markedly suppressed (86% inhibition) in the BKM120 treated experimental group (Fig 4A). In order to characterize the antitumor effects of BKM120 in vivo,
CD15+ and CD15- fractions were sorted from the treated and untreated tumors and used for apoptosis, cell cycle and proliferation studies. We observed that the proportion of CD15+ cells was much lower in the BKM120 treated group (45% reduction) (Fig 4B), indicating that treatment with PI-3K inhibitor BKM120 specifically depleted the CD15+ TPCs. These CD15+ TPCs isolated from BKM120 treated tumors showed less proliferation (S4A Fig) and increased apoptosis (S4B Fig) as compared to vehicle treated controls. Furthermore, the expression of genes related to cell cycle and SHH pathways were also down-regulated in CD15+ TPCs isolated from BKM120 treated tumors as compared to vehicle treated controls (S4C & S4D Fig).

Collectively, these results confirm the pharmacodynamic activity of BKM120 and indicate that BKM120 can function as a pro-apoptotic and an anti-proliferative agent for the CD15+ TPC compartment in vivo. In order to confirm our results that only CD15+ cells are capable of generating tumors, the CD15+ and CD15- cells isolated from the subcutaneous tumors were injected intracranially into NOD SCID mice. The MRI as well as H & E staining data clearly demonstrate that only CD15+ cells are capable of generating tumors (Fig 4C). Next, we evaluated the effect of BKM120 on the survival of mice, injected intracranially with CD15+ TPCs. For this, 2 million CD15+ TPC were injected into nude mice; after 40 days when mice displayed neurological symptoms c/w an advanced stage of intracranial tumor...
formation, they were divided into two groups. One group was treated with vehicle and another group was treated for 21 days with 30 mg/kg dose of BKM120. Fig 4D shows that BKM120 prolonged the survival of mice with advanced intracranial MB tumors (p < 0.05).

Comparison of SmoA1 PTEN+/+ CD15+ TPC genomic signature to human MB subgroups

To determine whether CD15+ population isolated from SmoA1 PTEN+/+ tumors resembled human SHH-driven MB at a molecular level, we performed gene expression analysis on CD15+ and CD15- tumor cells isolated from SmoA1 PTEN+/+ tumors and compared the resulting gene expression profiles with the gene expression profiles of recently identified 6 molecular subgroups of human medulloblastoma tumor samples (c1-c6) defined by Cho et al [4]. In this classification scheme, the c3 subgroup shows marked enrichment of genes associated with SHH signaling. Utilizing a subclass mapping algorithm [47] we generated a similarity metric between SmoA1 PTEN+/+ tumors and the MB subgroups defined by Cho et al [4]. As expected in Fig 5, this analysis revealed a high degree of similarity between CD15+ population from SmoA1 PTEN+/+ tumors and the ‘c3’ subtype of human MB, which is characterized by gene expression signatures indicative of SHH signaling pathway [4]. An unexpected finding was that the gene expression profile of the CD15- population correlates with ‘c7’ which represents gene expression in normal cerebellum (Fig 5A). To further verify that the CD15+ TPC population isolated from SmoA1 PTEN+/+ tumors resemble ‘c3’ subtype of human MB, we used the leading edge analysis tool within GSEA [38] to identify and group related gene sets between the mouse and human genomes, i.e. those in which the significance is driven by an overlapping subset of genes (the "leading edge"). These results further verified that the CD15+ TPC population isolated from SmoA1 PTEN+/+ resemble SHH driven c3 subtype of human medulloblastoma (Table 3). Using the submap algorithm, we found 22 leading edge genes that putatively support the association between mouse CD15+ with SHH driven c3 subtype of human medulloblastoma. ComBat was used to remove the systematic variations between the two datasets and for generating the principal components analysis (PCA) plot. The plot shows that CD15+ and CD15- cells were readily distinguishable from one another (Fig 5B). From the PCA plot using the 22 leading edge genes (listed in Fig 5C) from the Submap analysis, we can see that the c3 subgroup is separated from the other subgroups, and that CD15+ associates with the c3 subgroup. Hence this plot graphically illustrates the association found in the Submap analysis. In contrast, comparison of CD15- gene expression signature aligns more with the remaining MB subgroups in this analysis. We extended our study to predict candidate drugs that might either repress or up regulate an expression signature, by using a publicly available resource Connectivity Map (CMAP). This resource is based on a reference collection of gene-expression profiles from cultured human cells treated with bioactive small molecules, together with pattern-matching software to mine these data [48]. Notably, analysis of human SHH-driven (Group c3) tumors using CMAP, suggested that genes regulated by PI-3K/mTOR (LY294002, Sirolimus) and MAPK/MEK (U0125) inhibitors are also enriched in these tumors (Table 4), which is in close agreement with our results showing the elevated expression of these pathways in CD15+ TPCs. Furthermore this analysis revealed the identification of many other compounds predicted to repress the SHH driven tumors (Table 4). The list included several compounds that are either approved or undergoing clinical trials in different cancer types. Several drug classes which are listed top on the list include topoisomerase inhibitors (camptothecin, ellipticine, doxorubicin, etoposide, thioguanine, thioguanosine), multiple histone deacetylase inhibitors (trichostatin A, vorinostat, 5162773, 5186223), PARP inhibitors (1,5-isoquinolinediol, 3-aminobenzamide, phenanthridinone), proteasome inhibitors (MG132,
Fig 5. Similarity of CD15+ population from SmoA1 PTEN +/+ tumors to the 'c3/SHH' subtype of human MB. (A) The heatmap shows the degree of similarity as quantified by the SubMap method [47] between gene expression levels of murine SmoA1 Tg tumors (n = 5 paired CD15+ and CD15 samples) and 199 human tumors previously classified into 6 MB subtypes and normal control samples [4]. Each block in the heatmap corresponds to the p-value for similarity between the row category (mouse model samples, CD15+ or CD15-) and the column category (human tumor samples, classed by subtype). Blue suggests no similarity and red suggests strong similarity in gene expression levels. Note that the CD15- murine samples show the strongest similarity with human tumors subtypes c4 (Group 4) and c7 (normal CBL), while the CD15+ samples show similarity with c1 (Group 3) and c3 (SHH) subtypes. (B) Upper panel shows two dimensional Principal Components Analysis (PCA) plot comparing expression levels of the 22 leading edge genes from the SubMap analysis of Fig 5A. Data are from n = 5 paired CD15+ and CD15- murine SmoA1 PTEN+/+ tumors (open diamonds) and n = 199 human tumor samples (solid dots) previously classified into one of 6 MB subtypes [4] and reference normal samples. The human tumors are labeled according to subytypes as in Cho et al [4]. The first Principal Component, PC1, is plotted as the x-axis, and shows the major mode of variation in the data. PC1 is observed to separate the human tumors into three major groups, horizontally from right to left: c3 (purple, SHH subtype), c6 (orange, WNT subtype), and the combined group c1/c2/c4/c5/c7. Importantly, the CD15+ mouse samples (purple diamonds) are observed to associate with the human samples of SHH subtype (group c3), while the CD15- mouse samples (green diamonds) are clustered with the leftmost combined group c1/c2/c4/c5/c7 group containing normal human samples. The second PC, PC2 (y-axis) shows the next largest mode of variation in the data, and is seen to further divide the human samples in the c1/c2/c4/c5/c7 combined group. The human normal samples (c7, grey) are clustered at the extreme high values of PC2, are largely distinct from the human tumor samples. Human subtypes c2 (black) and c4 (green) are highly overlapped as expected, but some separation is observed between human subtypes c1 (brown) and c5 (yellow), suggesting that these subtypes have distinct molecular phenotypes. Lower panel shows the major classification of medulloblastoma in 4 major subgroups by Taylor et al.[55] (C) List of 22 leading genes obtained from submap analysis.

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1,4 chrysenequinone) and cyclin-dependent kinases (CDKs) inhibitors (alsterpaullone, 02974170002B, GW-8510).

Block the PI-3K signaling pathway inhibits proliferation of CD15+ TPC in primary human medulloblastoma and in a MBSHH PDX model

Finally, we validated our hypothesis by testing these PI-3K inhibitors in primary human medulloblastoma tumor cells and a patient-derived xenograft (PDX) SHH MB model. For this, we first analyzed the primary human MB tumor and PDX by H & E staining and by RT PCR analysis of stem cell marker genes (S5A and S5B Fig). These results and previous report [49] suggest that this MB PDX is a SHH subgroup tumor. Histopathologic diagnosis of MB was confirmed by attending pediatric pathologist at Rady Children’s Hospital. MB tumor cells were isolated and evaluated using FACS analysis for CD15 expression (Fig 6A). In order to evaluate potential contamination of CD15+ neutrophils in our experiments using human medulloblastoma PDX, we performed FACS analysis using CD15 and CD66 antibodies. An almost undetectable level of human neutrophils (<0.04% of total CD15+ population) was detected (data not shown). We evaluated the expression of stem cell markers in the tumor cells isolated from PDX and found significantly higher expression of oct4, sox2, nanog, klf4, cxcr4, musashi, CD133 and ngfr in patient samples when compared to normal cerebellum which served as a control (S5B Fig). Most notably, the expression of Pten is lower in the tumor cells isolated from this PDX as compared to control. A further characterization of the CD15+ population from the PDX reveals TPC properties and a higher proliferative capacity (Fig 6B, S5C Fig). Moreover, BKM120 potently inhibit the proliferation of the CD15+ TPC population by 21 fold, while there is minimal effect on CD15- population (IC50 for CD15+ cells is 0.218 μM and for CD15- cells it is 7.178 μM) (Fig 6C). Therefore, we investigated if exposure of cytotoxic agent, cisplatinum, NVP-LDE-225 and TMZ has a similar effect on the CD15+ TPC population isolated from PDX. Interestingly, cisplatinum and TMZ has no effect, while NVP-LDE-225 has very minimal effect on proliferation of CD15+ cells isolated from PDX (S5D Fig). Furthermore, BKM120 at 10 μM concentration completely blocked the phosphorylation of AKT, and its downstream targets, PRAS40 and mTOR substrates pS6, p4EBP1 in human MB TPCs (Fig 6D). The higher expression of p27 and p21 protein levels and cleavage of PARP upon treatment with BKM120 suggests that BKM120 increase cell cycle arrest and induce apoptosis in CD15+ cells isolated from human medulloblastoma (Fig 6D). Overall, these results suggest that the inhibition of PI-3K will be potently inhibitory for TPC survival in vivo. We confirm the in vivo efficacy of BKM120 in the PDX and we observed that this inhibitor blocked tumor growth and enhance survival of mice as shown in MRI images (Fig 6E). Finally, we subclassified the tumor obtained from the primary tumor sample as well as from PDX specimen for the expression of specific marker genes restricted to SHH, Wnt, Non SHH/Non Wnt pathway. We found that SHH pathway genes are similarly upregulated in the primary tumor as well as in PDX (Fig 6F). Importantly, these analyses were completed within 3–4 months in “real time” while patient received standard of care for MB which includes chemotherapy and radiotherapy which is associated with 30 percent recurrence rate.

Discussion

The PI-3K/AKT pathway has been shown to be important for the proliferation of TPCs in both solid tumors and leukemias [31–33]. The role of PTEN-PI-3K/AKT pathway in the pathogenesis and tumorigenicity of medulloblastoma has also been extensively studied in bulk tumor [14–16, 50, 51]. Herein, we report that, PI-3K signaling is highly elevated in CD15+ TPCs isolated from Smo AITg model of medulloblastoma and is required for the proliferation of TPCs.
PI-3K inhibitors exert a preferential effect on CD15+ TPCs isolated from Smo A/ITg model of medulloblastoma and in human patient derived xenografts, with minimal to no effect on CD15- population. In contrast, the cytotoxic chemotherapeutic agent, cisplatinum, TMZ and SHH inhibitor, NVP-LDE-225 do not display *in vitro* cytotoxicity against CD15+ TPCs isolated from this mouse model or a SHH subgrouped human patient derived MB xenograft. In contrast, BKM120 as a single agent, blocked the proliferation of CD15+ TPCs with minimal effect on CD15- cells. Most notably BKM120, was observed to: 1) induce p21waf1, p27kip1
and p53 expression 2) suppress proliferation and the expression of proliferative markers, cyclin D, MycN, gli-1, gli-2 3) induce apoptosis in the CD15+ TPC compartment and in the SHH subgroup of patient derived tumor cells (PDX) and 4) suppress tumorigenesis and increase host survival in an in vivo CD15+ TPC xenograft model.

Various clinical reports suggest that SHH-driven medulloblastoma patients treated with Smo antagonists, initially show dramatic tumor regression followed by rapid tumor recurrence [12, 13]. Buanamici et al has reported the upregulated PI-3K signaling as one of the potential mechanism of resistance developed in SHH driven medulloblastoma [14]. They reported that BKM120 in combination with Smo antagonist LED225 showed delayed tumor growth in Patch+/−p53−/− mouse model [14]. In our experiments, BKM120 treatment caused a substantial tumor reduction and most notably it suppressed the percent of CD15+ cells in the subcutaneous tumor, by inducing them to undergo apoptosis. Our findings have implications for the clinical development of PI-3K inhibitors including BKM120 in the treatment of SHH driven medulloblastoma. The observation that CD15+ TPC exhibited 10- to 20- fold greater sensitivity to PI-3K inhibitors than CD15-TPCs (Fig 2A) suggests that clinical trials designed with TPC-directed endpoints may facilitate demonstration of efficacy at sub-MTD doses. Current first-line chemotherapy generally consists of cytotoxic agents, such as platinum agents (cisplatinum) and etoposide. NVP-LDE-225, another drug we used in our study is in Phase II clinical trials for patients with hedgehog pathway activated relapsed medulloblastoma. While these agents may effectively debulk tumors and control disease initially, tumors invariably recur due to ineffective control of TPC. In the present study, we observed that the CD15+ TPC isolated from Smo A1 model as well as human PDX are markedly resistant to cisplatinum, TMZ and NVP-LDE-225 whereas CD15- population is more sensitive to these agent. Surprisingly, no synergy was noted when BKM120 was combined with cisplatinum and TMZ suggesting independent mechanisms for cellular cytotoxicity of cisplatinum in CD15- cells. It is interesting to speculate that CD15+ cells will arise in humans following treatment with cisplatinum chemotherapy due to this resistance pattern and that this population of cells would be sensitive to BKM120 treatment in sequence. Hence, treating SHH driven MB with TPC-targeting agent viz. PI-3K inhibitors is expected to block CD15+ TPC mediated tumor recurrence observed in MB if combined with standard of care agents. Our result that the CD15+ TPC population display stem cell markers and form large robust neurospheres in vitro suggest that PI-3K inhibitors preferentially target the TPC/cancer stem cell compartment. In agreement with our study, recent study has shown that PI-3K/mTOR inhibitor VS5584 preferentially target the aldefluor positive cancer stem cell compartment [34].

In conclusion, we have identified a role for PI-3K signaling in the proliferation and survival of TPC dependent c3 SHH subtype of MB. Our results provide the first evidence that PI-3K inhibitors have cancer stem cell disease modifying activity in vitro and in vivo. We expect that these findings will positively impact on our understanding of the signaling pathways operational in the cancer stem cell which promotes its tumorigenicity, survival and resistance. Bioinformatic analysis comparisons of the genomic signature of the CD15+ TPC population isolated from murine tumors reveals a similarity with the c3 subgroup of SHH driven human MB tumors. These results suggest that the research conducted on murine SmoA1Tg model will be potentially applicable in SHH driven human MB patients. Recent report by Pei et al also used the similar methodology to compare the data from murine Myc MB model to expression profiles from a distinct set of human samples [52]. Consistent with this, there are other reports which validate the use of this methodology to compare murine data with human samples [53, 54]. An important distinction between these reports and our work relates to our focus to compare the gene expression pattern within the CD15+ TPC cells to the different MB subgroups and to ultimately compare effects of genetic loss in PTEN using these methods on the TPC cell.
phenotype in an effort to discover resistance mechanisms to PI-3 kinase inhibitory regimens. There are no reports in the literature comparing mRNA gene expression in CD15+TPCs vs CD15- nonTPCs and/or comparing the CD15+ TPC signature to the human medulloblastoma tumor mRNA expression in subgroups of MB. Taken together, our study provides new avenues to perform genomic manipulations and multiple ‘omic’ analyses on the drug treated murine CD15+ TPC to determine possible mechanisms of resistance in the TPC compartment and thereby discover more efficacious treatments for this and other cancer stem cell driven diseases. Given the paucity of gain of function mutations in PIK3CA or loss of function mutations in PTEN observed in medulloblastoma tumors as determined by whole genome sequencing (1–3%), we envision the application of PI-3K inhibitors as adjuncts to existing chemotherapy and radiotherapy regimens in the future treatment of MB once Phase I trials are completed in pediatric oncology. Finally, we performed within 1–3 months of diagnosis in vivo molecular profiling, synthetic lethality and drug sensitivity screening of a primary highly anaplastic MB tumor (Fig 6) and its corresponding PDX in “real time” as a potential “clinical proof of concept” for the application of personalized pediatric oncology in a high risk disease setting.

Supporting Information

S1 Fig. SmoA1 tumors are propagated by CD15+ cells. (A) FACS data showing the isolation of pure population of CD15+ cells from Smo A1 tumors. (B) CD15+ and CD15- cells were implanted intracranially into nude/nu-nu mice. Upper panel shows H&E staining of Secondary tumor from a nu-nu host that received 2 × 10^6 CD15+ (Left panel) and CD15- cells (Right panel). Small box in Upper right panel shows H&E at 20X. Lower panel shows Ki67 staining of same tumor. Scale bar = 200 μm. (C) Relative gene expression of stem cell markers in the CD15+ and CD15- population isolated from SmoA1 tumors. Graphs represent mean ± SEM. Statistical significance is assessed by two sample t-test where * denotes P<0.05, ** denotes P<0.01 and *** denotes P<0.001.

(TIF)

S2 Fig. CD15+ cells from SmoA1 tumors have a distinct expression profile with increased proliferation and cell survival capacity. (A) Figure shows relative expression levels of genes related to proliferation and cell survival in CD15+ vs. CD15- population isolated from SmoA1 tumors (n = 3). (B) Heat map showing activation of SHH pathway genes in CD15+ cells (n = 7) compared to CD15- (n = 5). Colors illustrate fold changes, Red: up-regulation; green: down-regulation; black: no change. The bar code on the bottom represents the color scale of the log 2 values. (C) Left panel shows validation of differential gene expression for SHH pathway genes in CD15+ vs. CD15- population by RTPCR. Right panel shows Western blot revealing high expression of gli1, gli2 and cyclin D1. (D) Heat map showing activation of genes related to angiogenesis in CD15+ cells (n = 7) compared to CD15- (n = 5). Data are representative of three independent experiments. Values are mean ± SEM (n = 6–8) (A & C). Statistical significance is assessed by two sample t-test where * denotes P<0.05, ** denotes P<0.01 and *** denotes P<0.001.

(TIF)

S3 Fig. Dose dependent effect of Cisplatin, NVP-LDE-225 and TMZ on CD15+ and CD15- cells isolated form Smo A1Tg mice (A & B) CD15+ and CD15- cells were treated with different conc. of Cisplatin, NVP-LDE-225 and TMZ (0.1 μM, 1.0 μM and 10.0 μM). After 48 hr, AlamarBlue® was added and plates were incubated at 37°C in 5% CO₂ for 6 hours. Fluorescence signals were read as emission at 590 nm after excitation at 560 nm.

(TIF)
S4 Fig. BKM120 suppresses tumor growth by increasing apoptosis of CD15+ TPC and down regulating the expression of cell cycle and SHH pathway genes. (A) AlamarBlue data validating the less proliferation tendency of CD15+ cells derived from BKM120 treated tumors as compared to untreated ones. (B) BKM120 reduces cell proliferation in CD15+ CSC by inducing apoptosis. CD15+ and CD15- cells isolated from BKM120 treated and untreated tumors were assayed for apoptosis by using the Annexin V FITC assay. (p = 0.06). (C & D) BKM120 suppresses tumor growth by targeting cell cycle and SHH genes in CD15+CSC population. Expression of cell cycle genes (C) and SHH genes (D) in CD15+ cells derived from BKM120 treated and untreated subcutaneous tumors. Expression data is normalized to GAPDH. Values are mean ± SEM (n = 6–8) (A–D). Statistical significance is assessed by two sample t-test where * denotes P<0.05, ** denotes P<0.01 and *** denotes P<0.001.

(TIF)

S5 Fig. Characterization of CD15+ isolated from medulloblastoma tumor patient sample. (A) A small portion of the patient tumor was fixed in formalin, paraffin embedded and used for H & E staining. (B) Relative gene expression of stem cell markers in the tumor cells isolated from PDX. RNA isolated from normal cerebellum was used as a control. (C) In-vitro cell proliferation of total tumor cells, CD15+ and CD15- cells obtained from patient tumor. Total tumor cells and FACS sorted CD15+ CSCs have the ability to form neurospheres in the culture. Cells are cryopreserved and evaluated for sensitivity against kinome panel and siRNA screens for patient specific synthetic lethality effects in combination with PI-3K inhibitors. (D) CD15 + cells isolated from PDX were treated with different conc. of cisplatin (Left panel). Right panel shows the cell viability of CD15+ cells treated with 100nM conc. of cisplatinum, TMZ, NVP-LDE-225 either alone or in combination with BKM 120.

(TIF)

S1 Table. List of primer sequence used in Real Time PCR analysis.

(DOC)

S2 Table. Differentially expressed genes of different pathways analyzed by microarray data in CD15-vs. CD15+ FACS sorted population from SmoA1 x PTEN+/+ tumors.

(XLS)

S3 Table. Table shows leading edge genes that were used to perform PCA analysis and examine the similarity between the human MB sub classified tumors and CD15+ CSCs derived from SmoA1 PTEN+/+ Mouse MB.

(XLS)

S4 Table. Connectivity map analysis: Table shows compounds whose gene expression signatures closely match those of human Group c3 tumors. Among the top 50 compounds are several PI3K, MAPK/MEK and mTOR inhibitors (highlighted). These results are consistent with analysis of murine SHH tumors, which suggests activation of the PI3K/mTOR & MAPK/MEK pathway.

(XLS)

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Author Contributions
Conceived and designed the experiments: ARS SJ DLD. Performed the experiments: ARS SJ. Analyzed the data: LB KM YJC. Contributed reagents/materials/analysis tools: JRG GAM MLL JC RN DM ARS MZ MA DLD. Wrote the paper: SJ ARS DLD.

References


The Potential Impact of “Take the Volume Pledge” on Outcomes After Carotid Artery Stenting

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RESEARCH—HUMAN—CLINICAL STUDIES

BACKGROUND: The “Volume Pledge” aims to centralize carotid artery stenting (CAS) to hospitals and surgeons performing ≥10 and ≥5 procedures annually, respectively.

OBJECTIVE: To compare outcomes after CAS between hospitals and surgeons meeting or not meeting the Volume Pledge thresholds.

METHODS: We queried the Nationwide Inpatient Sample for CAS admissions. Hospitals and surgeons were categorized as low volume and high volume (HV) based on the Volume Pledge. Multivariable hierarchical regression models were used to examine the impact of hospital volume (2005-2011) and surgeon volume (2005-2009) on perioperative outcomes.

RESULTS: Between 2005 and 2011, 22215 patients were identified. Most patients underwent CAS by HV hospitals (86.4%). No differences in poor outcome (composite endpoint of in-hospital mortality, postoperative neurological or cardiac complications) were observed by hospital volume but HV hospitals did decrease the likelihood of other complications, nonroutine discharge, and prolonged hospitalization. From 2005 to 2009, 9454 CAS admissions were associated with physician identifiers. Most patients received CAS by HV surgeons (79.2%). On multivariable analysis, hospital volume was not associated with improved outcomes but HV surgeons decreased odds of poor outcome (odds ratio [OR] 0.76, 95% confidence interval [CI] 0.59-0.97; \( P = .028 \)), complications (OR 0.56, 95% CI 0.46-0.71, \( P < .001 \)), nonroutine discharge (OR 0.70, 95% CI 0.57-0.87; \( P = .001 \)), and prolonged hospitalization (OR 0.52, 95% CI 0.44-0.61, \( P < .001 \)).

CONCLUSION: Most patients receive CAS by hospitals and providers meeting the Volume Pledge threshold for CAS. Surgeons but not hospitals who met the policy’s volume standards were associated with superior outcomes across all measured outcomes.

KEYWORDS: Hospital volume, Surgeon volume, Carotid artery stenting, Centralization, Outcomes, Health policy

H
igh-volume hospitals (HVHs) and high-volume surgeons (HVSs) have been associated with improved outcomes for a variety of complex procedures, supporting efforts for the centralization of surgical care.1-4

To translate these findings into policy, 3 major academic health systems established the “Take the Volume Pledge” initiative in 2015.5 The Pledge set minimum hospital and surgeon volume standards for 10 complex gastrointestinal, cardiovascular, and orthopedic operations, challenging other health systems to join in order to promote optimal outcomes.6

Carotid artery stenting (CAS) was among the selected procedures of the Pledge, establishing a minimum volume of 10 annual procedures for hospitals and 5 annual procedures for surgeons.5 Clinical trials have identified the endovascular procedure as an efficacious and safe therapeutic alternative to carotid endarterectomy for the treatment of carotid artery stenosis in select patients.7,8 Most prior studies have demonstrated lower rates of mortality and stroke after carotid stenting procedures performed by HV institutions and providers.9-11 Notably, among Medicare beneficiaries, patients treated by low-volume (LV) operators were twice as likely to die

ABBREVIATIONS: CAS, carotid artery stenting; CI, confidence interval; CREST, Carotid Revascularization Endarterectomy Trial Versus Stenting Trial; LOS, length of stay; LVH, low-volume hospital; LVS, low-volume surgeons; HVH, high-volume hospital; ICD-9, International Classification, Ninth Revision; NIS, Nationwide Inpatient Sample; OR, odds ratio

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within 30 d after stenting compared to patients treated by HVs.\textsuperscript{14}

While the Pledge aims to improve patient outcomes, providers and policymakers have raised concerns regarding the potential unintended consequences that centralization policies may have on access to high-quality surgical care.\textsuperscript{16,17} Minorities and underinsured patients are less likely to receive complex surgery at HV centers,\textsuperscript{18} and an increase in travel burden decreased surgical access for rural communities, and barriers in the continuity of care may further exacerbate health disparities in already vulnerable patient populations.\textsuperscript{19}

To date, limited knowledge exists regarding the potential impact of Volume Pledge on access to CAS care and outcomes if it were broadly implemented. The primary objective of this study was to evaluate the impact of the Pledge’s minimum volume standards for hospitals and surgeons on outcomes after CAS. We also sought to determine differences in patient characteristics and the proportion of patients who receive care by hospitals and surgeons that meet or do not meet the Volume Pledge.

METHODS

Inclusion and Exclusion Criteria

We queried the Nationwide Inpatient Sample (NIS) from 2005 to 2011 for adult patients who underwent CAS for carotid artery stenosis. The NIS is the largest all-payer inpatient administrative database comprised of patient discharge data from a 20% stratified sample of US nonfederal hospitals.\textsuperscript{20} Cases with an International Classification, Ninth Revision (ICD-9) diagnosis code of asymptomatic (443.10, 433.30) and symptomatic (433.11, 433.31) carotid artery stenosis that underwent CAS (ICD-9 00.63) were extracted.\textsuperscript{21} This study was exempt from institutional review board approval as the NIS is a publicly available database and contains no personal identifying information.

Hospital Volume and Surgeon Volume

Our 2 primary exposures of interest were hospital volume and surgeon volume. Based on the volume standards established by the Volume Pledge for CAS, hospitals were categorized as LV or HV if they performed <10 or ≥10 annual procedures, respectively. Similarly, surgeons were categorized based on the Volume Pledge as LV surgeons (LVSs) and HV surgeons (HVSs) if they performed <5 and ≥5 procedures per year, respectively.

Outcomes

The primary outcome of interest was poor outcome: a composite endpoint of either in-hospital mortality or postoperative neurological (iatrogenic ischemic stroke or intracranial hemorrhage; ICD-9997.0-09) or postoperative cardiac complications (cardiac arrest, failure, or insufficiency; ICD-9997.10). Secondary outcomes included other peri-procedural complications, nonroutine discharge, and prolonged length of stay (LOS). Peri-procedural complications included respiratory failure (518.4, 518.5, 518.8, 518.81), pneumonia (482.3, 482.8, 486, 581), myocardial infarction (410.00-410.9), venous thromboembolism (451.1, 451.2, 451.81, 451.9, 453.1, 453.2, 453.8, 453.9), gastrointestinal hemorrhage (530.82, 531.00-531.21, 531.40, 531.41, 531.60, 531.61, 532.00-532.21, 532.40, 532.41, 532.60, 532.61, 533.00-533.21, 533.40, 533.41, 533.60, 533.61, 534.00-534.21, 534.40, 534.41, 534.60, 534.61, 535.01, 535.11, 535.21, 535.31, 535.41, 535.51, 535.61, 578.9), acute kidney injury (584.5, 584.6, 584.7, 584.91, urinary tract infection (559.0, 509.09), and surgical site infection (998.5, 998.51, 998.59, 998.6). Discharge disposition was categorized as routine (home with or without home healthcare) or nonroutine (discharge to skilled nursing, short-term recovery, rehabilitation hospitals or institutions or in-hospital mortality). Prolonged LOS was defined as LOS ≥ 75th percentile for the study cohort.\textsuperscript{22-24}

Patient and Hospital Variables

The NIS provides patient demographic information such as age, sex, race/ethnicity, insurance status, emergent vs nonemergent admission, and hospital characteristics including teaching status, urban or rural location, bed size and geographic region. Patients were categorized into three age groups: 18 to 64, 64 to 79, and 80+ yr of age. The Charlson Comorbidity Index was calculated to assess for patient comorbidities.\textsuperscript{25} We also controlled for hypertension, coronary artery disease, congestive heart failure, dyslipidemia, diabetes mellitus, atrial fibrillation, valve disease, obesity, coagulopathy, alcohol abuse, tobacco use, chronic kidney disease, and chronic lung disease. Patients were categorized as symptomatic if they had an ICD-9 of 433.11, 433.31 corresponding to carotid artery stenosis with stroke or a diagnosis of carotid artery without stroke (443.10, 443.30) with a subsequent code for transient ischemic attack (435).\textsuperscript{21}

Statistical Analysis

Patients treated by LVHs and HVHs and HVSs were compared using Wilcoxon-rank sum tests for continuous variables and Pearson chi-square tests for categorical variables. Hierarchical mixed-effects logistic regression models were used to examine the association between Volume Pledge threshold and outcomes (poor outcome, complications, nonroutine discharge, prolonged hospital stay), and to account for hospital clustering. All multivariable regression models were adjusted for patient demographic, clinical, and hospital characteristics. The impact of hospital volume on outcomes was analyzed between 2005 and 2011. To analyze the effect of surgeon volume threshold on outcomes, an analysis was conducted between 2005 and 2009 as the NIS only provides unique identification numbers were excluded (n = 12 761, 57.4%) from surgeon volume analysis. Three hierarchical multivariable logistic regression models were performed to examine the effect of surgeon volume: Model 1 adjusted for surgeon volume without hospital volume, model 2 adjusted for hospital volume without surgeon volume, and model 3 adjusted for both surgeon and hospital volume. Analyses were performed using Stata SE Version 15.1 (StataCorp LLC, College Station, Texas). All tests were 2-sided with \( P < .05 \) considered statistically significant.

RESULTS

Hospital Volume Pledge Threshold

A total of 22 215 CAS admissions met inclusion criteria. The majority of patients underwent CAS at HVH (86.4%), accounting for 43.2% of the total 815 hospitals captured in our analysis (Table 1). LVH treated 13.6% of cases and constituted a larger proportion of hospitals (n = 463, 56.8%). The median
## TABLE 1. Baseline Characteristics Stratified by Hospital Volume Pledge Thresholds (2005-2011)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Carotid artery stenting (n = 22215)</th>
<th>LVH (n = 3025, 13.6%)</th>
<th>HVH (n = 19190, 86.4%)</th>
<th>P value</th>
</tr>
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<tr>
<td>Annual volume, median [IQR], cases/yr</td>
<td>6 [4-8]</td>
<td>34 [21-53]</td>
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<td>Hospitals</td>
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<td>72 [65-78]</td>
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<td>Race/ethnicity, n (%)</td>
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<td>White</td>
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<td>Symptomatic, n (%)</td>
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<td>Emergent admission, n (%)</td>
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<td>Charlson Comorbidity Index (CCI), n (%)</td>
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<tr>
<td>CCI ≥ 3</td>
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<td>6649 (34.6)</td>
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<td>11 142 (58.1)</td>
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<td>Coagulopathy</td>
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<td>Coronary artery disease</td>
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<td>Congestive heart failure</td>
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<tr>
<td>Atrial fibrillation</td>
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<td>Valve disease</td>
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<td>Obesity</td>
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<td>Chronic kidney disease</td>
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<td>Chronic pulmonary disease</td>
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<tr>
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<td>Tobacco use</td>
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<td>6050 (31.5)</td>
<td>.053</td>
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<tr>
<td>Missing</td>
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<td>133 (0.8)</td>
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<tr>
<td>Hospital Location, n (%)</td>
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<td>Rural</td>
<td>209 (6.9)</td>
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<td>Urban</td>
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<td>18 581 (96.8)</td>
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<tr>
<td>Hospital bedsize, n (%)</td>
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<td>Small</td>
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<td>1692 (8.8)</td>
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<td>Medium</td>
<td>844 (27.9)</td>
<td>3293 (17.2)</td>
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<tr>
<td>Large</td>
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<td>14 072 (73.3)</td>
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</tr>
<tr>
<td>Missing</td>
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<td>133 (0.8)</td>
<td></td>
<td></td>
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<tr>
<td>Geographic region, n (%)</td>
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<td>Northeast</td>
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<td>Midwest</td>
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<td>South</td>
<td>1105 (36.5)</td>
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<tr>
<td>West</td>
<td>648 (21.4)</td>
<td>3444 (17.9)</td>
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<td></td>
</tr>
</tbody>
</table>

HVH, high-volume hospital; IQR, interquartile range; LVH, low-volume hospital.
LV patients were more likely to have chronic kidney disease, 51.7%) were more common in patients treated by HVS, while artery disease (32.6% vs 29.3%) and dyslipidemia (56.5% vs 50.7%) were more common in patients treated at HVH. In contrast, LV patients were more likely to have obesity, chronic kidney disease, and coagulopathy. A larger proportion of HVH were large teaching centers and LVH were more likely to be located in rural areas (2.5% vs 6.9%).

Rates of in-hospital mortality, postoperative neurological complications, and postoperative cardiac complications did not differ between HVH and LVH (Table 2). No significant differences between HVH and LVH were observed for the composite endpoint poor outcome (4.9% vs 4.7%, P = .62, respectively). However, LVH had a higher proportion of other periprocedural complications (9.5% vs 7.6%, P < .001), nonroutine discharge (11.7% vs 8.8%, P < .001), and prolonged LOS (30.8% vs 25.6%, P < .001). Patients treated at LVH experienced higher rates of pneumonia, acute kidney injury, and urinary tract infections.

On adjusted analysis, HVH did not significantly predict decreased odds of poor outcome (odds ratio [OR] = 0.90, 95% confidence interval [CI] 0.73-1.10; P = .31; Table 3). However, treatment at HVH decreased the likelihood of other complications (OR 0.84, 95% CI 0.70-0.99, P = .044), nonroutine discharge (OR 0.80, 95% CI 0.68-0.94; P = .008), and prolonged LOS (OR 0.84, 95% CI 0.72-0.97, P = .014).

Surgeon Volume Pledge Threshold

A total of 9454 patients treated between 2005 and 2009 had a corresponding surgeon identification number to meet inclusion criteria. Of these, the majority were treated by HVS (n = 7448, 79.2%), representing a third of surgeons included in the analysis (Table 4). The median CAS annual volume for LVS and HVS was 2 [1-3] and 17 [9-32], respectively. Of patients treated by LVS, 63.7% were treated at HVH. In contrast, 95.2% of patients treated by HVH underwent CAS at HVH (P < .001).

Similar results found in the hospital volume analysis were observed for surgeon volume (Table 4). A greater proportion of black (5.0% vs 3.6%), Hispanic (5.4% vs 3.6%), emergent (37.8% vs 28.2%), and symptomatic (12.0% vs 6.9%) patients, as well those with a greater comorbidity burden (37.1% vs 32.0%) underwent CAS by LVS. Compared to LV patients, coronary artery disease (32.6% vs 29.3%) and dyslipidemia (56.5% vs 51.7%) were more common in patients treated by HVS, while LV patients were more likely to have chronic kidney disease, coagulopathy, and use tobacco. A similar proportion of LVs and HVSs were associated with teaching and rurally located hospitals. However, LVS were more likely to operate at large hospitals (74.0% vs 69.5%).

The rate of poor outcome was lower for HVS (4.3% vs 6.5%; P < .001; Table 5). HVS had lower rates of in-hospital mortality (0.8% vs 1.3%; P = .03) and postoperative neurological complications (1.9% vs 3.2%, P < .001). LVS were associated with higher rates of respiratory failure, pneumonia, myocardial infarction, acute kidney injury, urinary tract, and surgical site infection (all P < .001). Rates of any adverse complication (12.2% vs 5.9%, P < .001), nonroutine discharge (12.5% vs 4.3%, P < .001), and prolonged hospitalization (38.1% vs 22.3%, P < .001) were higher among patients treated by LVS.

On multivariable analysis, HVS predicted decreased odds of poor outcome (OR 0.79, 95% CI 0.62-0.99; P = .042), complications (OR 0.61, 95% CI 0.50-0.75, P < .001), nonroutine discharge (OR 0.72, 95% CI 0.59-0.88; P = .001), and prolonged LOS (OR 0.53, 95% CI 0.45-0.62, P < .001) compared to LVS (model 1, Table 6). On adjusted analysis without surgeon volume, hospital volume was not associated with differences in outcomes (model 2). After adjusting for both surgeon and hospital volume, HVS remained predictive of improved outcomes (model 3).

DISCUSSION

Hospital and surgeon volume have long been recognized as a metric for the quality and safety of surgical care.1-4 To optimize patient outcomes, 3 major academic health systems announced “Take the Volume Pledge”—a policy that aims to centralize surgical care to hospitals and providers meeting minimum volume standards for complex procedures.5,6 In this study, we assessed the potential impact of the Pledge on outcomes after CAS if the policy were broadly implemented. We found that the overwhelming majority of patients underwent CAS by hospitals and surgeons meeting the Pledge’s volume thresholds for the endovascular intervention. Superior outcomes were largely driven by the Pledge’s volume standards for surgeons and were significantly associated with a decreased risk of poor outcome, periprocedural complications, nonroutine discharge, and prolonged hospitalization.

The impact of the Pledge’s volume threshold for hospitals was less clear. In our analysis of hospital volume, hospitals that met the Pledge’s criteria did not improve our primary endpoint. Although they were significantly associated with a lower risk of other complications, nonroutine discharge, and prolonged hospitalization, these benefits were not observed in our analysis of surgeon volume. Despite this, 95% of patients who underwent CAS by HV providers were also cared for at a HV center. In comparison, about two-thirds of patients who were treated by LV operators were managed at HV centers. Our data suggest that the Pledge’s surgeon volume standards may be more important in improving outcomes compared to their

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Carotid artery stenting (n = 9454)</th>
<th>Volume pledge category</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVSP (n = 1966, 20.8%)</td>
<td>HVSP (n = 7488, 79.2%)</td>
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<tr>
<td>Annual surgeon volume, median [IQR], cases/yr</td>
<td>2 [1-3]</td>
<td>17 [9-32]</td>
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<td>Surgeons</td>
<td>981 (69.0)</td>
<td>441 (31.0)</td>
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<td>Annual hospital volume, median [IQR], cases/yr</td>
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<td>37 [22-57]</td>
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<td>Hospital volume (≥10 procedures/annually)</td>
<td>1254 (63.7%)</td>
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<tr>
<td>Hospitals</td>
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<tr>
<td>Age, median [IQR], yr</td>
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<td>72 (65-78)</td>
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<td>Female, n (%)</td>
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<td>2901 (38.7)</td>
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</tr>
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<td>Race/ethnicity, n (%)</td>
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<tr>
<td>White</td>
<td>1513 (77.0)</td>
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<td>Other</td>
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<td>Symptomatic, n (%)</td>
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<td>Emergent admission, n (%)</td>
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<td>2111 (28.2)</td>
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<td>Charlson Comorbidity Index (CCI), n (%)</td>
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<td>CCI = 1</td>
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<td>CCI ≥ 3</td>
<td>730 (37.1)</td>
<td>2395 (32.0)</td>
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<td>Hypertension</td>
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<td>Tobacco use</td>
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<td>Teaching hospital, n (%)</td>
<td>1202 (61.1)</td>
<td>4394 (58.7)</td>
<td>.060</td>
</tr>
<tr>
<td>Missing</td>
<td>12 (0.6)</td>
<td>62 (0.8)</td>
<td>.24</td>
</tr>
<tr>
<td>Hospital location, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>53 (2.7)</td>
<td>240 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>1901 (96.7)</td>
<td>7186 (96.0)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>12 (0.6)</td>
<td>62 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Hospital bedsize, n (%)</td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Small</td>
<td>137 (7.0)</td>
<td>830 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>363 (18.5)</td>
<td>1395 (18.6)</td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>1454 (74.0)</td>
<td>5201 (69.5)</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>12 (0.6)</td>
<td>62 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Geographic region, n (%)</td>
<td></td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Northeast</td>
<td>472 (24.0)</td>
<td>2082 (27.8)</td>
<td></td>
</tr>
<tr>
<td>Midwest</td>
<td>261 (13.3)</td>
<td>1003 (13.4)</td>
<td></td>
</tr>
<tr>
<td>South</td>
<td>1010 (51.4)</td>
<td>3283 (43.8)</td>
<td></td>
</tr>
<tr>
<td>West</td>
<td>223 (11.3)</td>
<td>1120 (15.0)</td>
<td></td>
</tr>
</tbody>
</table>

HVH, high-volume hospital; IQR, interquartile range; LVH, low-volume hospital.
TABLE 3. Unadjusted Outcomes Stratified by Hospital Volume Pledge Thresholds (2005-2011)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Carotid artery stenting (n = 22215)</th>
<th>Volume pledge category</th>
<th>LVH (n = 3025, 13.6%)</th>
<th>HVH (n = 19190, 86.4%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>30 (1.0)</td>
<td>166 (0.9)</td>
<td>.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurological complication</td>
<td>72 (2.4)</td>
<td>399 (2.1)</td>
<td>.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac complication</td>
<td>62 (2.0)</td>
<td>412 (2.1)</td>
<td>.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite outcome</td>
<td>149 (4.9)</td>
<td>906 (4.7)</td>
<td>.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>64 (2.1)</td>
<td>345 (1.8)</td>
<td>.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>37 (1.2)</td>
<td>156 (0.8)</td>
<td>.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>61 (2.0)</td>
<td>323 (1.7)</td>
<td>.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous thromboembolism</td>
<td>9 (0.2)</td>
<td>41 (0.2)</td>
<td>.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal hemorrhage</td>
<td>11 (0.4)</td>
<td>78 (0.4)</td>
<td>.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute kidney injury</td>
<td>107 (3.5)</td>
<td>484 (2.5)</td>
<td>.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>95 (3.1)</td>
<td>468 (2.4)</td>
<td>.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical site infection</td>
<td>3 (0.1)</td>
<td>20 (0.1)</td>
<td>.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1 complication</td>
<td>287 (9.5)</td>
<td>1449 (7.6)</td>
<td>&lt;.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Discharge disposition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Routine discharge</td>
<td>2672 (88.3)</td>
<td>17 492 (91.2)</td>
<td>.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonroutine discharge</td>
<td>353 (11.7)</td>
<td>1698 (8.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOS, median, d</td>
<td>1 [1-4]</td>
<td>1 [1-3]</td>
<td>&lt;.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prolonged LOS (≥ 3 d)</td>
<td>932 (30.8)</td>
<td>4910 (25.6)</td>
<td>&lt;.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI, confidence interval; LOS, length of stay; HVH, high-volume hospital; LVH, low-volume hospital; OR, odds ratio.

a Denotes very small number, ≤ 10

Our results are consistent with prior literature documenting improved mortality and morbidity after CAS performed by HV operators. Among Medicare beneficiaries treated between 2005 and 2007, very LVSs (<6 annual procedures) had higher 30-d risk-adjusted mortality rates (OR 1.9) compared to HV operators (≥24 procedures). Another study of Medicare patients (2005-2009) demonstrated decreased 30-d mortality rates with increasing operator volume. In a pooled analysis of symptomatic patients from European randomized clinical trials comparing the efficacy of CAS and endarterectomy, operators performing <6 annual CAS procedures experienced a higher 30-d risk of stroke or death. Our data support the previously analysis of the NIS found that hospitals performing <38 CAS procedures per year were associated with a 19% increased risk in poor outcome (composite of in-hospital mortality, postoperative stroke, and discharge to a long-term facility). Similarly, Medicare beneficiaries experienced lower 30-d mortality at HV centers (≥40 procedures annually) compared to LVH (<10 procedures). In contrast, a study in Germany found no differences between HV (≥27 procedures for a 2-yr period) and LV centers in postoperative mortality or major stroke. Differences in the study population, endpoints analyzed, and methodologies used to determine volume emphasize the difficulty in establishing an optimal volume cut-off for hospitals that will consistently deliver superior outcomes.
documented inverse relationship between surgeon volume and patient outcomes following CAS, and validates the Pledge’s volume standards for operators as an adequate target in improving patient outcomes.

Carotid stenting is a technically demanding procedure associated with a substantial learning curve. In addition to increased operative experience, utilization of greater technological services and enhanced patient selection may contribute to superior outcomes observed in HVs. Among Medicare beneficiaries, Nallamothu et al found that LV operators were significantly less likely to use embolic protection devices for CAS. In another study of Medicare claims data, HVs were more likely to

### TABLE 5. Outcomes by Surgeon Volume Pledge Thresholds (2005-2009)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Carotid artery stenting (n = 9454)</th>
<th>Volume pledge category</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LV (n = 1966, 20.8%)</td>
<td>HV (n = 7488, 79.2%)</td>
<td></td>
</tr>
<tr>
<td>Outcome</td>
<td>In-hospital mortality</td>
<td>26 (1.3)</td>
<td>60 (0.8)</td>
</tr>
<tr>
<td></td>
<td>Neurological complication</td>
<td>62 (3.2)</td>
<td>144 (1.9)</td>
</tr>
<tr>
<td></td>
<td>Cardiac complication</td>
<td>49 (2.5)</td>
<td>156 (2.1)</td>
</tr>
<tr>
<td></td>
<td>Composite outcome</td>
<td>127 (6.5)</td>
<td>325 (4.3)</td>
</tr>
<tr>
<td>Complications</td>
<td>Respiratory failure</td>
<td>61 (3.1)</td>
<td>94 (1.3)</td>
</tr>
<tr>
<td></td>
<td>Pneumonia</td>
<td>29 (1.5)</td>
<td>48 (0.6)</td>
</tr>
<tr>
<td></td>
<td>Myocardial infarction</td>
<td>65 (3.3)</td>
<td>97 (1.3)</td>
</tr>
<tr>
<td></td>
<td>Venous thromboembolism</td>
<td>4 (0.3)</td>
<td>12 (0.2)</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal hemorrhage</td>
<td>11 (0.6)</td>
<td>20 (0.3)</td>
</tr>
<tr>
<td></td>
<td>Acute kidney injury</td>
<td>76 (3.9)</td>
<td>151 (2.0)</td>
</tr>
<tr>
<td></td>
<td>Urinary tract infection</td>
<td>69 (3.5)</td>
<td>150 (2.0)</td>
</tr>
<tr>
<td></td>
<td>Surgical site infection</td>
<td>4 (0.4)</td>
<td>4 (0.1)</td>
</tr>
<tr>
<td></td>
<td>≥1 complication</td>
<td>240 (12.2)</td>
<td>445 (5.9)</td>
</tr>
<tr>
<td>Discharge disposition</td>
<td>Routine discharge</td>
<td>1720 (87.5)</td>
<td>7163 (95.7)</td>
</tr>
<tr>
<td></td>
<td>Nonroutine discharge</td>
<td>246 (12.5)</td>
<td>325 (4.3)</td>
</tr>
<tr>
<td></td>
<td>LOS, median, d</td>
<td>1 [1-5]</td>
<td>1 [1-2]</td>
</tr>
<tr>
<td></td>
<td>Prolonged LOS (≥ 3 d)</td>
<td>750 (38.1)</td>
<td>1673 (22.3)</td>
</tr>
</tbody>
</table>

LOS, length of stay; HV, high volume; LV, low volume.

*Denotes very small number, ≤ 10


<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Model 1a</th>
<th>Model 2b</th>
<th>Model 3c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgeon HV vs LV</td>
<td>OR (95% CI)</td>
<td>P value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Poor outcome</td>
<td>0.79 (0.62-0.99)</td>
<td>.042</td>
<td>–</td>
</tr>
<tr>
<td>Complication</td>
<td>0.61 (0.50-0.75)</td>
<td>&lt;.001</td>
<td>–</td>
</tr>
<tr>
<td>Nonroutine discharge</td>
<td>0.72 (0.59-0.88)</td>
<td>.001</td>
<td>–</td>
</tr>
<tr>
<td>Prolonged LOS (≥ 3 d)</td>
<td>0.53 (0.45-0.62)</td>
<td>&lt;.001</td>
<td>–</td>
</tr>
<tr>
<td>Hospital HV vs LV</td>
<td>OR (95% CI)</td>
<td>P value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Poor outcome</td>
<td>–</td>
<td>–</td>
<td>1.00 (0.72-1.39)</td>
</tr>
<tr>
<td>Complication</td>
<td>–</td>
<td>–</td>
<td>0.96 (0.75-1.22)</td>
</tr>
<tr>
<td>Nonroutine discharge</td>
<td>–</td>
<td>–</td>
<td>0.91 (0.70-1.21)</td>
</tr>
<tr>
<td>Prolonged LOS</td>
<td>–</td>
<td>–</td>
<td>0.82 (0.64-1.03)</td>
</tr>
</tbody>
</table>

CI, confidence interval; LOS, length of stay; HV, high volume; LV, low volume; OR, odds ratio.

*Model 1: adjusted for surgeon volume only.
*Model 2: adjusted for hospital volume only.
*Model 3: adjusted for both surgeon and hospital volume.
performed CAS on healthier patients compared to LV providers. This is consistent with our data in which LV operators were more likely to perform CAS on patients who were symptomatic, emergently admitted, and had a higher comorbidity burden. While this may be due in part to more aggressive management by LVSs prior to medical optimization, it may also reflect higher disease severity in the patient population that these providers serve.

While there is no doubt that the Volume Pledge is intended to improve patient outcomes, providers and policymakers have raised concerns regarding unintended consequences of centralization policies. We found that minorities and rural patients were more likely to undergo CAS at hospitals or with providers that did not meet the Volume Pledge, supporting prior literature documenting decreased access to HV centers for vulnerable patient populations. Efforts to regionalize CAS should also aim to support strategies that mitigate potential barriers in access such as travel burden, limited health literacy, and discontinuity of care. While volume remains a useful metric for improved outcomes, it should not be an exclusive measure of the quality and safety of surgical care. HV centers have greater access to experienced surgeons, multidisciplinary teams, well-resourced intensive care units, and greater technological services; however, other structural processes play a critical role in improving outcomes such as the utilization of protocols or checklists and quality improvement initiatives. Furthermore, development of these policies should adequately reflect both health system and patient-centered outcomes.

Limitations

This study carries limitations inherent to a large administrative database such as a retrospective study design, coding inaccuracies, and selection biases. Potential confounding factors such as the degree of carotid stenosis, use of antiplatelet/anticoagulation or other medical therapy, angiographic data, and use of stent protection devices are not available in the NIS. Our study was limited to short-term in-hospital outcomes, and we were unable to assess longitudinal periprocedural endpoints assessed in clinical trials. Beginning in 2012, the NIS changed its sampling design to 20% of all patients from 20% of all hospitals. Thus, we were unable to calculate hospital volume after 2011 to include design to 20% of all patients from 20% of all hospitals. Thus, efforts to regionalize CAS should also aim to support strategies that mitigate potential barriers in access such as travel burden, limited health literacy, and discontinuity of care. While volume remains a useful metric for improved outcomes, it should not be an exclusive measure of the quality and safety of surgical care. HV centers have greater access to experienced surgeons, multidisciplinary teams, well-resourced intensive care units, and greater technological services; however, other structural processes play a critical role in improving outcomes such as the utilization of protocols or checklists and quality improvement initiatives. Furthermore, development of these policies should adequately reflect both health system and patient-centered outcomes.

CONCLUSION

The majority of patients underwent CAS at hospitals and by surgeons meeting the Volume Pledge threshold. Our study validates the Volume Pledge’s surgeon thresholds for CAS as surgeons that met the policy’s volume standards were associated with superior outcomes. The relationship between the Pledge’s volume standards for hospitals on outcomes after CAS is less clear. Volume policies should aim to address potential disparities in access to HV centers resulting from the regionalization of surgical care. Further research is warranted to elucidate the volume-outcome relationship in CAS that will optimize outcomes for all patients.

Disclosures

The project described was partially supported by the National Institutes of Health, Pre-doctoral Grant T35TR001443 awarded to MGB. The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

REFERENCES

The authors report their findings of outcomes of carotid artery stenting (CAS) procedures from a large national database. They sought to determine if centers and operators who meet the “volume pledge” for CAS have superior outcomes. The volume pledge for CAS was determined to be >10 CAS per center per year and >5 CAS per surgeon annually. The manuscript is thoughtful and well-written. The authors found that complications related to CAS were not significantly different between low and high volume centers. However, the experience of the surgeon was a significant factor in outcome. It was interesting that the majority of patients who “underwent CAS by high-volume providers were also cared for at a high-volume centers...about two-thirds of patients who were treated by low-volume operators were managed at high-volume centers.” In other words, treatment at a high-volume center does not guarantee a highly experienced operator. It is therefore difficult, based on the authors’ findings to advocate all patients requiring CAS be transferred to a high-volume center when the experience of the surgeon may be in question. In these cases, it stands to reason that the more experienced surgeons at high-volume centers should be performing CAS. Whether that means taking a “volume pledge” or just having a departmental volume requirement for each surgeon, that makes sense for patients. In addition, many of the “low volume” centers are now covered by operators from high-volume centers who may take call at smaller community or rural hospitals. These operators have the experience and can bring their protocols for post-op care to the centers to avoid postoperative complications. We are seeing this increasingly as more hospitals want to bring stroke care to their communities. The sprawl of experienced surgeons to community hospitals may change this picture a bit as well. The authors report responsibly on this topic and based on their findings it seems feasible that experienced operators can provide safe care at smaller, low volume centers if basic infrastructure and protocols are in place.

It is a challenge when making determinations about volume and whether or not surgeons/centers should be performing certain operations. The volume requirement is often arbitrary, does not take into account operator’s prior years of experience, and can serve to limit access to patients in rural areas or without access to nearby high-volume centers. Although rural or low-volume centers may have operators who can perform the procedure, the surgeon may not offer it due to a perceived liability of not meeting arbitrary volume requirements. That said, as the authors state, it is established for a number of procedures, that outcomes are better in centers that are high volume with very experienced surgeons. Therefore, it can be difficult to know how to achieve the best patient outcomes while also giving patients the best access to care. Strict guidelines serve as just that—a guideline—but are often imperfect and impractical. This manuscript describes that well.
Training Guidelines for Endovascular Stroke Intervention: An International Multi-Society Consensus Document

Background

Ischemic stroke is a leading cause of death and disability worldwide. Much of the long-term disability occurs in patients with Emergent Large Vessel Occlusion (ELVO). In fact, in these patients, occlusion of a major intracerebral artery results in a large area of brain injury often resulting in death or severe disability [1]. Until recently, intravenous tissue plasminogen activator (t-PA) was the only proven treatment for ELVO.

However, the landscape of stroke treatment has changed with the publication of five randomized multicenter controlled clinical trials. These trials provide Class 1, Level A evidence that endovascular thrombectomy (ET) is the standard of care for patients with ELVO. In particular, thrombectomy results in significantly better clinical outcomes compared to best medical therapy in patients with acute occlusion of the intracranial internal carotid artery (ICA) and/or M1 segment of the middle cerebral artery (MCA) [2–6]. These results have led to guideline recommendations advocating for endovascular treatment in addition to t-PA for patients with ELVO. In addition, ET is now offered as first line therapy for patients that are not eligible for intravenous thrombolysis [7–9]. However, achieving the best possible clinical outcomes with endovascular stroke treatment mandates structured training and education of those physicians who are providing endovascular stroke treatment. On this regard, a recent meta-analysis of these five clinical trials showed that the vast majority of thrombectomies were performed by experienced neurointerventionalists. These include interventional neuroradiologists, endovascular neurosurgeons, and interventional neurologists who routinely perform neuroendovascular procedures [10]. None of the studies allowed physicians without previous experience in mechanical thrombectomy to enroll patients. The centers participating in these trials offered endovascular stroke therapy 24 h a day (with the exception of those in the EXTEND-IA trial) with expertise in vascular neurology and neurocritical care in a comprehensive stroke center. On-site expertise in vascular neurology and neurocritical care is paramount to achieving good clinical outcomes.

Geographical limitations to rapid access to acute stroke centers providing mechanical thrombectomy have led some to suggest physicians without prior experience or formal neuroendovascular training should consider providing coverage for these procedures. A multidisciplinary British Intercollegiate Stroke Working Party put forth a document outlining the safe delivery of mechanical thrombectomy, which highlights that operators should not normally carry out procedures with which they are unfamiliar and that they should recognize ad-hoc arrangements are not in the best interest of patients [11].

It is also important to recognize that modern endovascular stroke therapy focuses on direct clot removal with mechanical devices, as compared with previous paradigms where intra-arterial thrombolytic infusion was an acceptable treatment option for large vessel occlusions [12]. The technical skills needed to safely deliver devices into the intracranial circulation are significantly more involved than simply placing a catheter for medication infusion. Catheter skills from other circulations do not replace the need for formal training in safe intracranial microcatheter navigation and device placement.

Acute ischemic stroke is a complex disease and successful endovascular treatment is based on the comprehensive ability to rapidly integrate multiple pieces of information, including: the patient’s history, clinical examination, neuroradiological studies, and to subsequently formulate a treatment plan. Both patient selection and procedural expertise are critical to achieve a good clinical outcome. Hence, there is a clear rationale for formal training in both clinical neuroscience and interventional neuroradiology.

The purpose of this document is to define what constitutes adequate training for physicians who can provide endovascular treatment for acute ischemic stroke patients. These training guidelines are modeled after prior standards of training documents such as the
training, competency and credentialing standards for diagnostic cerebral angiography, carotid stenting and cerebrovascular intervention [13] and the performance and training standards for endovascular ischemic stroke treatment [14], written and endorsed by multispecialty groups. In addition, the importance of organ specific training, rigorous quality improvement benchmarks, and minimum volume requirements needed to maintain high quality care has been extensively described for acute myocardial infarction, an analogous time sensitive disease [15].

This document represents the cumulative work of the societies listed below, and represents an international consensus on adequate training to safely and effectively perform these procedures:

- American Academy of Neurological Surgeons/Congress of Neurological Surgeons (AANS/CNS)
- American Society of Neuroradiology (ASNR)
- Asian Australasian Federation of Interventional and Therapeutic Neuroradiology (AAFITN)
- Australian and New Zealand Society of Neuroradiology – Conjoint Committee for Recognition of Training in Interventional Neuroradiology (CCINR) representing the RANZCR (ANZSNR), ANZAN and NSA
- Canadian Interventional Neuro Group (CING)
- European Society of Neuroradiology (ESNR)
- European Society of Minimally Invasive Neurologic Therapy (ESMINT)
- Japanese Society for Neuroendovascular Therapy (JSNET)
- Sociedad Ibero Latino Americana de Neurroradiologia (SILAN)
- Society of NeuroInterventional Surgery (SNIS)
- Society of Vascular and Interventional Neurology (SVIN)
- World Federation of Interventional and Therapeutic Neuroradiology (WFITN)

**Physician Qualifications**

Physicians providing intra-arterial treatment for acute stroke are required to have appropriate training and experience for the performance of neuroangiography and interventional neuroradiology.

We recognize that the specific training pathways may differ across nations, but the consensus is to mandate adequate training to perform emergent endovascular stroke intervention. These cognitive requirements consist of baseline training and qualifications as well as ongoing professional education, which are essential for safe and efficient patient management.

It is also important to point out that these qualifications are for new practitioners who are not currently performing acute stroke intervention with mechanical thrombectomy. We understand that there are current practitioners (who are board certified or board eligible in radiology, neurology or neurosurgery) who may have trained prior to the establishment of formal training pathways, and have acquired the necessary skills listed below to safely and effectively treat these complex patients. We would still expect the same requirements for maintenance of qualifications as listed below.

**I. Baseline Training and Qualifications**

1. **Residency training** (in radiology, neurology or neurosurgery) which should include documented training in the diagnosis and management of acute stroke, the interpretation of cerebral arteriography and neuroimaging under the supervision of a board-certified
neuroradiologist, neurologist or neurosurgeon with subsequent board eligibility or certification. The residency program and supervising physicians should be accredited according to national standards as they pertain to the countries involved. Those physicians who did not have adequate such training during their residencies must spend an additional period (typically one year) by training in clinical neurosciences and neuroimaging, focusing on the diagnosis and management of acute stroke, the interpretation of cerebral arteriography and neuroimaging prior to their fellowship in neuroendovascular interventions.

2 Dedicated training in Interventional Neuroradiology (also termed Endovascular Neurosurgery or Interventional Neurology) under the direction of a Neurointerventionalist (with neuroradiology, neurology or neurosurgical training background), at a high-volume center. It is preferred that this is a dedicated year, which occurs after graduating from residency (i.e., a fellowship). A training program accredited by a national accrediting body is also strongly preferred but not required. Published standards exist for various countries [16–22]. Within these programs, specific training for intra-arterial therapy for acute ischemic stroke should be performed, including obtaining appropriate access even in challenging anatomy, microcatheter navigation in the cerebral circulation, knowledge and training of the use of stroke specific devices and complication avoidance and management.

While various national standards will have differing procedure requirements, we encourage practitioners to meet their national minimum procedural and training standards. Fellowships which are not accredited by national credentialing bodies should still have adequate training to meet their local minimum procedure requirements. In addition, we expect that minimum training numbers for stroke thrombectomy may increase in future revisions of these standards given the recent developments in the field.

II. Maintenance of Physician Qualifications

It is vital that the physician have ongoing stroke specific continuing medical education. A minimum of 16 h of stroke specific education every 2 years is suggested. Individual physician outcomes should conform to national standards and institutional requirements. In addition, the physician should participate in an ongoing quality assurance and improvement program. The goals of this quality assurance program for stroke therapy would be to monitor outcomes both in the peri-procedural period and at 90 days. The quality assurance program must review all emergency interventional stroke therapy patients. In addition, participation in a national quality improvement registry, when available, is also encouraged. Outcomes should be tracked and recorded. While threshold levels for recanalization, complication rates, etc. have yet to be established, we suggest the following as a minimum:

1. Successful recanalization (modified TICI 2b or 3) in at least 60% of cases.
2. Embolization to new territory of less than 15%.
3. Symptomatic intracranial hemorrhage (i.e. Parenchymal Hematoma on imaging with clinical deterioration) rate less than 10%.

Hospital Requirements

Successful treatment of the ELVO patient does not occur in a vacuum, but rather with the framework of a multi-disciplinary team. As such, we feel it is critical that the patients be treated in a center, which has 24/7 access to the following:

1. Angiography suites suitably equipped to handle these patients, as well as equipment and capability to handle the complications.
2. Dedicated stroke and intensive care units (preferably dedicated neurointensive care unit), staffed by physicians with specific training in those fields.
3 Vascular neurology and Neurocritical care expertise.
4 Neurosurgery expertise, including vascular neurosurgery
5 All relevant neuroimaging modalities (CT/CTA, MR/MRA, Trans-cranial Doppler [TCD]), including 24/7 access to CT and MRI.

Summary

We, as a group of international multi-disciplinary NeuroInterventional societies involved in the endovascular management of acute ischemic stroke, have put forth these training guidelines. We believe that a neuroscience background, dedicated neurointerventional training, and stringent peer review and quality assurance processes are critical to ensuring the best possible patient outcomes. Well-trained neurointerventionalists are a critical component of an organized and efficient team needed to deliver clinically effective mechanical thrombectomy for acute ischemic stroke patients.

References


Purpose—The aim of this guideline is to provide a focused update of the current recommendations for the endovascular treatment of acute ischemic stroke. When there is overlap, the recommendations made here supersede those of previous guidelines.

Methods—This focused update analyzes results from 8 randomized, clinical trials of endovascular treatment and other relevant data published since 2013. It is not intended to be a complete literature review from the date of the previous guideline publication but rather to include pivotal new evidence that justifies changes in current recommendations.

Members of the writing committee were appointed by the American Heart Association/American Stroke Association Stroke Council’s Scientific Statement Oversight Committee and the American Heart Association/American Stroke Association Manuscript Oversight Committee. Strict adherence to the American Heart Association conflict of interest policy was maintained throughout the consensus process. Recommendations follow the American Heart Association/American Stroke Association methods of classifying the level of certainty of the treatment effect and the class of evidence. Prerelease review of the draft guideline was performed by 6 expert peer reviewers and by the members of the Stroke Council Scientific Statement Oversight Committee and Stroke Council Leadership Committee.
Since the publication of the most recent “Guidelines for the Early Management of Patients With Acute Ischemic Stroke” in 2013, substantial new high-quality evidence on the clinical efficacy of endovascular treatments of acute ischemic stroke has become available. This focused update on endovascular treatment of acute ischemic stroke analyzes results from 8 randomized, clinical trials of endovascular treatment and other relevant data published since 2013 while taking into account the previous evidence summarized in the 2013 guidelines. This focused update is not intended to be based on a complete literature review from the date of the previous guideline publication but rather to include pivotal new evidence that justifies changes in current recommendations. When there is overlap, the recommendations made here supersede those of previous guidelines.

Members of the writing committee were appointed by the American Heart Association (AHA)/American Stroke Association Stroke Council’s Scientific Statement Oversight Committee and the AHA/American Stroke Association Manuscript Oversight Committee, representing various areas of medical expertise. Strict adherence to the AHA conflict of interest policy was maintained throughout the consensus process. Panel members were assigned topics relevant to their areas of expertise, reviewed the stroke literature with emphasis on publications since the prior guidelines, and drafted recommendations in accordance with the American College of Cardiology/AHA’s Level of Evidence grading algorithm (Table 1). All recommendations were unanimously approved by the members of the writing group.

Treatment With Intravenous Recombinant Tissue-Type Plasminogen Activator

Rapid administration of intravenous recombinant tissue-type plasminogen activator (r-tPA) to appropriate patients remains the mainstay of early treatment of acute ischemic stroke.1 Timely restoration of blood flow in ischemic stroke patients is effective in reducing long-term morbidity. For patients who meet national and international eligibility guidelines, intravenous r-tPA improves functional outcomes at 3 to 6 months when given within 4.5 hours of ischemic stroke onset and should be administered. Every effort should be made to shorten any delays in the initiation of treatment because earlier treatments are associated with increased benefits. If patients who are eligible for intravenous r-tPA do not have intracranial vascular imaging as part of their initial evaluation, they should begin receiving intravenous r-tPA before being transported for additional imaging and before being transferred for endovascular treatment. This approach will help minimize onset-to-treatment times, a key driver of efficacy for r-tPA.

New Randomized, Clinical Trials of Endovascular Stroke Treatment

Studies With Primarily Intra-Arterial Fibrinolysis or First-Generation Mechanical Embolectomy Devices

Three randomized controlled trials of endovascular treatment of acute ischemic stroke with primarily intra-arterial fibrinolysis and/or first-generation mechanical embolectomy devices were carried out from 2004 to 2012 (Tables 2–4). Intra-arterial Versus Systemic Thrombolysis for Acute Ischemic Stroke (SYNTHESIS Expansion) was a prospective, randomized, open-label, blinded-end-point (PROBE), 2-arm superiority trial that enrolled 362 patients with ischemic stroke who were eligible for intravenous r-tPA within 4.5 hours of onset and for whom endovascular treatment was possible within 6 hours. No imaging other than nonenhanced computed tomography (CT) was required. The patients were randomized 1:1 to standard-dose intravenous r-tPA 0.9 mg/kg or endovascular therapy (intra-arterial r-tPA, mechanical clot disruption or retrieval, or a combination of these approaches). Only 8% had posterior circulation strokes. Median onset to treatment time interval was 165 minutes in the intravenous r-tPA group and 225 minutes in the endovascular group. Among the patients who received endovascular treatment, 66% underwent infusion of intra-arterial r-tPA and thrombus fragmentation with a guidewire only; in 34%, a device was also deployed. Stent retrievers were used in 14%. Data on rates and efficacy of recanalization were not published. There was no difference in the primary end point of the percentage with good outcome defined as a modified Rankin Scale (mRS) score of 0 or 1, death at 3 months, or symptomatic intracerebral hemorrhage (sICH) at 7 days. There were no significant differences in outcomes in subgroups, including time to treatment (0–3 or 3–4.5 hours), baseline National Institutes of Health Stroke Scale (NIHSS) score (<11 or ≥11), and age (≤67 years or >67 years).

The Interventional Management of Stroke Trial III (IMS III) was a PROBE, 2-arm superiority trial that enrolled patients with a major ischemic stroke defined by NIHSS score ≥10 who received intravenous r-tPA within 3 hours and were likely to or known to have occlusion of a major cerebral artery. Those who showed clear hypodensity in greater than one third of the middle cerebral artery (MCA) territory on nonenhanced CT were excluded. No other imaging was required. An amendment midway through the trial allowed screening with CT angiography (CTA) for patients with NIHSS score >8. More than 95% received a clinical diagnosis of anterior circulation stroke. Patients were randomly allocated 1:2 to standard-dose intravenous r-tPA (0.9 mg/kg) or to intravenous...
r-tPA 0.6 mg/kg followed by endovascular therapy with a device and/or intra-arterial r-tPA if occlusion persisted and if the endovascular intervention could be started within 5 hours and completed within 7 hours of onset. In the endovascular group, groin puncture occurred at a mean±SD of 208±47 minutes after stroke onset. Endovascular therapy was administered in 77% randomized to this treatment group. Intra-arterial r-tPA alone was used in 41%, and a device with or without intra-arterial r-tPA was used in 59%; in only 1.5% were stent retrievers used. Recanalization occurred 325±52 minutes after stroke onset, achieving Thrombolysis in Cerebral Infarction (TICI) grade11 2b/3 in 41%. The trial was stopped early for futility after 656 of the projected 900 subjects were enrolled. There was no significant difference in outcome between the intravenous r-tPA–only group and the endovascular group for the primary end point of the percentage of patients with a good outcome as measured by an mRS score of 0 to 2 or for death at 90 days. In the endovascular group, there was no difference in

<table>
<thead>
<tr>
<th>CLASS (STRENGTH) OF RECOMMENDATION</th>
<th>LEVEL (QUALITY) OF EVIDENCE‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLASS I (STRONG) Benefit &gt;&gt; Risk</td>
<td>LEVEL A</td>
</tr>
<tr>
<td>Suggested phrases for writing recommendations:</td>
<td></td>
</tr>
<tr>
<td>- Is recommended</td>
<td>- High-quality evidence‡ from more than 1 RCTs</td>
</tr>
<tr>
<td>- Is indicated/useful/effective/beneficial</td>
<td>- Meta-analyses of high-quality RCTs</td>
</tr>
<tr>
<td>- Should be performed/administered/other</td>
<td>- One or more RCTs corroborated by high-quality registry studies</td>
</tr>
<tr>
<td>- Comparative-Effectiveness Phrases†:</td>
<td></td>
</tr>
<tr>
<td>- Treatment/strategy A is recommended/indicated in preference to treatment B</td>
<td></td>
</tr>
<tr>
<td>- Treatment A should be chosen over treatment B</td>
<td></td>
</tr>
<tr>
<td>CLASS IIa (MODERATE) Benefit &gt;&gt; Risk</td>
<td>LEVEL B-R (Randomized)</td>
</tr>
<tr>
<td>Suggested phrases for writing recommendations:</td>
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</tr>
<tr>
<td>- Is reasonable</td>
<td>- Moderate-quality evidence‡ from 1 or more RCTs</td>
</tr>
<tr>
<td>- Can be useful/effective/beneficial</td>
<td>- Meta-analyses of moderate-quality RCTs</td>
</tr>
<tr>
<td>- Comparative-Effectiveness Phrases†:</td>
<td></td>
</tr>
<tr>
<td>- Treatment/strategy A is probably recommended/indicated in preference to treatment B</td>
<td></td>
</tr>
<tr>
<td>- It is reasonable to choose treatment A over treatment B</td>
<td></td>
</tr>
<tr>
<td>CLASS IIb (WEAK) Benefit = Risk</td>
<td>LEVEL B-NR (Nonrandomized)</td>
</tr>
<tr>
<td>Suggested phrases for writing recommendations:</td>
<td></td>
</tr>
<tr>
<td>- May/might be reasonable</td>
<td>- Moderate-quality evidence‡ from 1 or more well-designed, well-executed nonrandomized studies, observational studies, or registry studies</td>
</tr>
<tr>
<td>- May/might be considered</td>
<td>- Meta-analyses of such studies</td>
</tr>
<tr>
<td>- Usefulness/effectiveness is unknown/unclear/uncertain or not well established</td>
<td></td>
</tr>
<tr>
<td>CLASS III: No Benefit (MODERATE) (Generally, LOE A or B use only)</td>
<td>LEVEL C</td>
</tr>
<tr>
<td>Suggested phrases for writing recommendations:</td>
<td></td>
</tr>
<tr>
<td>- Is not recommended</td>
<td>- Randomized or nonrandomized observational or registry studies with limitations of design or execution</td>
</tr>
<tr>
<td>- Is not indicated/useful/effective/beneficial</td>
<td>- Meta-analyses of such studies</td>
</tr>
<tr>
<td>- Should not be performed/administered/other</td>
<td>- Physiological or mechanistic studies in human subjects</td>
</tr>
<tr>
<td>CLASS III: Harm (STRONG) Risk &gt; Benefit</td>
<td>LEVEL E</td>
</tr>
<tr>
<td>Suggested phrases for writing recommendations:</td>
<td></td>
</tr>
<tr>
<td>- Potentially harmful</td>
<td>Consensus of expert opinion based on clinical experience when evidence is insufficient, vague, or conflicting</td>
</tr>
<tr>
<td>- Causes harm</td>
<td></td>
</tr>
<tr>
<td>- Associated with excess morbidity/mortality</td>
<td></td>
</tr>
<tr>
<td>- Should not be performed/administered/other</td>
<td></td>
</tr>
</tbody>
</table>

COR and LOE are determined independently (any COR may be paired with any LOE).
A recommendation with LOE C or E does not imply that the recommendation is weak.
Many important clinical questions addressed in guidelines do not lend themselves to clinical trials. Although RCTs are unavailable, there may be a very clear clinical consensus that a particular test or therapy is useful or effective.

* The outcome or result of the intervention should be specified (an improved clinical outcome or increased diagnostic accuracy or incremental prognostic information).
† For comparative-effectiveness recommendations (COR I and IIa; LOE A and B only), studies that support the use of comparator verbs should involve direct comparisons of the treatments or strategies being evaluated.
‡ The method of assessing quality is evolving, including the application of standardized, widely used, and preferably validated evidence grading tools; and for systematic reviews, the incorporation of an Evidence Review Committee.

COR indicates Class of Recommendation; LOE, Level of Evidence; NR, nonrandomized; R, randomized; and RCT, randomized controlled trial.
outcome between those treated <90 minutes and those treated >90 minutes from intravenous r-tPA to groin puncture. The proportion of patients with an mRS score of 0 to 2 at 90 days increased with increasing recanalization.12

MR and Recanalization of Stroke Clots Using Embolectomy (MR RESCUE) was a PROBE, 2-arm superiority trial that enrolled 118 patients with large-artery occlusion and anterior circulation ischemic stroke within 8 hours who were ineligible for intravenous r-tPA or had persistent vessel occlusion after intravenous r-tPA. Patients were divided into 2 subgroups by pretreatment CT or magnetic resonance imaging (MRI) into those with a favorable or those with an unfavorable penumbral pattern with the use of imaging criteria based on a previous study.13 Patients were randomly allocated 1:1 to standard medical care or endovascular therapy (MERCI [Mechanical Embolus Removal in Cerebral Ischemia] or Penumbra device with optional intra-arterial r-tPA). Onset to groin puncture in the endovascular group was 381±74 minutes.

Studies With Primarily Stent Retrievers

Five randomized controlled trials of endovascular treatment of acute ischemic stroke with primarily stent retrievers were carried out from 2010 to 2015 (Tables 2–4). The Multicenter Randomized Clinical Trial of Endovascular Treatment for
Acute Ischemic Stroke (MR CLEAN) was a PROBE, 2-arm superiority trial that studied 500 patients with acute ischemic stroke caused by an proximal intracranial occlusion in the anterior circulation (distal intracranial carotid artery, MCA [M1 or M2], or anterior cerebral artery [A1 or A2]) established with CTA, magnetic resonance angiography (MRA), or digital subtraction angiography and a score of ≥2 on the NIHSS. The steering committee recommended that neuroimaging studies to assess vessel patency should preferably be done before or simultaneously with treatment with intravenous r-tPA. Initiation of endovascular treatment within 6 hours of stroke onset had to be possible. There were different specific exclusion criteria for patients with coagulation abnormalities, previous ischemic stroke, ICH, or severe head trauma, depending on whether intra-arterial fibrinolysis was contemplated. Patients who were eligible in agreement with national guidelines received intravenous r-tPA. Those with a nonfavorable response were eligible for inclusion. There was no specified time for observation to determine the response to intravenous r-tPA, nor was there an exact definition of what constituted a nonfavorable response, although recovery to a level that would not result in administration of intravenous r-tPA was suggested. Patients were

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Age, Mean±SD (IQR), y</th>
<th>NIHSS Score, Median (IQR)</th>
<th>Territory, %</th>
<th>ASPECTS, Median (IQR)</th>
<th>Device Deployment in Active Group</th>
<th>Onset to IV r-tPA, Mean±SD, Median (IQR), min</th>
<th>Time Onset to Groin Puncture, Mean±SD, Median (IQR), min</th>
<th>Time to Recanalization, Median (IQR), min</th>
<th>TICI Grade 2b/3</th>
<th>Time to Reperfusion Mean±SD, Median (IQR), min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SYNTHESIS Expansion</td>
<td>181/181</td>
<td>66±11/67±11</td>
<td>13 (9–17)/13</td>
<td>88/94 anterior</td>
<td>91%</td>
<td>IA r-tPA alone 66% Device added 34% 14% stent retriever</td>
<td>165 (140–200)</td>
<td>225 (194–260) to clot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMS III</td>
<td>434/222</td>
<td>69/68</td>
<td>17 [7–40]/16 [8–30]</td>
<td>97/97 anterior (clinical)</td>
<td>56.9%/59.0% (8–10)</td>
<td>77% IA r-tPA 41% IA r-tPA+device 38% IA r-tPA+device 21% device only 1.5% stent retriever</td>
<td>122±34/121±34</td>
<td>208±47</td>
<td>41</td>
<td>325±52</td>
<td></td>
</tr>
<tr>
<td>MR RESCUE</td>
<td>64/54</td>
<td>66±15</td>
<td>17 (13–21)</td>
<td>ICA 20/13 M1 61/72 M2 19/15</td>
<td>95% 58% MERCI 22% Penumbra 16% both</td>
<td></td>
<td>381±74</td>
<td>27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR CLEAN</td>
<td>233/267</td>
<td>66 (56–76)/65 (56–76)</td>
<td>17 (14–21)/18 (14–22)</td>
<td>4–38</td>
<td>IC A 0.4/1.1 IC A+M1 25.3/28.2 M1 66.1/82.0 M2 7.7/7.9 A1/A2 0.4/0.8</td>
<td>83.7% 81.5% stent retriever IA TICI 21%</td>
<td>85 (67–110)/87 (65–116)</td>
<td>260 (210–313)</td>
<td>59</td>
<td>332 (279–394)</td>
<td></td>
</tr>
<tr>
<td>ESCAPE</td>
<td>165/150</td>
<td>71 (60–81)/70 (60–81)</td>
<td>16 (13–20)/17 (12–20)</td>
<td></td>
<td>ICA+M1 27.6/26.5 M1/all M2 68.1/71.4 M2 3.7/2.0</td>
<td>91.5% 72.7% stent retriever</td>
<td>110 (80–142)/125 (89–183)</td>
<td>72.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SWIFT PRIME</td>
<td>98/98</td>
<td>65±13/66±11</td>
<td>17 (13–20)/17 (13–19)</td>
<td></td>
<td>ICA 18.3/16.0 M1 67/77 M2 14/6</td>
<td>88.8% All stent retriever</td>
<td>110.5 (85–156)/117 (80–155)</td>
<td>224 (165–275)</td>
<td>88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EXTEND-IA</td>
<td>35/35</td>
<td>69±12/70±12</td>
<td>17 (13–20)/13 (9–19)</td>
<td></td>
<td>ICA 31/31 M1 57/51 M2 11/17</td>
<td>77% All stent retriever</td>
<td>127 (93–162)/145 (105–180)</td>
<td>210 (166–251)</td>
<td>86</td>
<td>248 (204–277)</td>
<td></td>
</tr>
<tr>
<td>REVASCAT</td>
<td>103/103</td>
<td>66±11/67±10</td>
<td>17 (14–20)/17 (12–19)</td>
<td></td>
<td>ICA 0/1 M1 65/64 M2 10/8</td>
<td>95% All stent retriever</td>
<td>118 (90–150)/105 (86–138)</td>
<td>269 (201–340)</td>
<td>66</td>
<td>355 (269–430)</td>
<td></td>
</tr>
</tbody>
</table>

ASPECTS indicates Alberta Stroke Program Early CT Score; ESCAPE, Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion With Emphasis on Minimizing CT to Recanalization Times; EXTEND-IA, Extending the Time for Thrombolysis in Emergency Neurological Deficits–Intra-Arterial; IA, intra-arterial; IAT, intra-arterial therapy; ICA, internal carotid artery; IMS III, Interventional Management of Stroke Trial III; IQR, interquartile range; IV, intravenous; MR CLEAN, Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke; MR RESCUE, MR and Recanalization of Stroke Clots Using Embolectomy; NIHSS, National Institutes of Health Stroke Scale; r-tPA, recombinant tissue-type plasminogen activator; REVASCAT, Endovascular Revascularization With Solitaire Device Versus Best Medical Therapy in Anterior Circulation Stroke Within 8 Hours; SWIFT PRIME, Solitaire FR With the Intention for Thrombectomy as Primary Endovascular Treatment of Acute Ischemic Stroke; and TICI, Thrombolysis in Cerebral Infarction.
randomly allocated 1:1 to either usual care alone or intra-arterial treatment plus usual care. Intra-arterial treatment consisted of arterial catheterization with a microcatheter to the level of occlusion and delivery of a fibrinolytic agent, mechanical thrombectomy, or both. The method of intra-arterial treatment was left to the discretion of the local interventionist. Sixty-four percent of participants had M1 occlusion alone, and an additional 27% had occlusion of M1 and the internal carotid artery (ICA). Of the 195 patients in the endovascular group of 233 who received endovascular treatment, onset to groin puncture was 260 minutes (interquartile range, 210–313 minutes), a stent retriever was used in 81.5%, and TICI grade 2b/3 recanalization was achieved in 59%. The treatment effect was estimated as an odds ratio (OR), adjusted for prespecified prognostic factors that intra-arterial treatment would lead to lower mRS score at 90 days, compared with usual care alone (shift analysis). The adjusted OR was 1.67 (95% confidence interval [CI], 1.21–2.30) in favor of intervention. There was an absolute difference of 13.5% (95% CI, 5.9–21.2) in the rate of functional independence (mRS score, 0–2) in favor of the intervention (32.6% versus 19.1%). There were no significant differences in mortality or the occurrence of sICH. Most patients (445 of 500) received intravenous r-tPA and showed benefit in subgroup analysis. There were too few patients who did not receive intravenous r-tPA to draw any conclusions.15 In a subsequent presentation at the 2015 International Stroke Conference, the MR CLEAN investigators reported a stroke onset–to–reperfusion time of 332 minutes (interquartile range, 279–394 minutes) and demonstrated a marked decline in clinical benefit with time so that the benefit was no longer statistically significant if reperfusion occurred after 6 hours 19 minutes.16

The Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion With Embolus Pulling and Stenting (ESCAPE) was a PROBE, 2-arm superiority trial of 316 patients with disabling acute ischemic stroke (NIHSS score >5) who could be randomized up 12 hours after the onset. Groin puncture had to be possible within 60 minutes of CT/CTA. Nonenhanced CT and CTA (preferably multiphase) were performed rapidly with a target door-to-imaging time of 25 minutes to identify participants with a small infarct core (by Alberta Stroke Program Early CT Score (ASPECTS)6–10 or CT perfusion), an occluded proximal intracranial artery in the anterior circulation (internal carotid, M1 MCA, or carotid terminus occlusion). If CTA or MRA was part of the entry criteria and 125 patients under the revised imaging entry criteria. Sites without perfusion imaging used ASPECTS (ASPECTS >6 was required). A total of 71 patients were enrolled under the initial imaging entry criteria and 125 patients under the revised imaging entry criteria. Perfusion imaging was performed and used for selection in 82.6%. Seventy-three percent of participants had M1 occlusion, and 17% had ICA occlusion. Intravenous r-tPA was administered at an outside hospital in 35%. Participants were randomized 1:1 to receive guideline-based care alone or guideline-based care plus endovascular treatment with the use of available thrombectomy devices. The use of retrievable stents and suction through a balloon guide catheter during thrombus retrieval was also recommended. Participants in both groups received intravenous r-tPA within 4.5 hours after onset if they met accepted local guidelines. The primary outcome was the OR that the intervention would lead to lower scores on the mRS at 90 days (shift analysis). After the release of the MR CLEAN results, an interim analysis conducted earlier than planned showed that a stopping criterion based on the prespecified O’Brien-Fleming stopping boundary had been crossed, and the trial was stopped. For the primary end point, the adjusted OR (indicating the odds of improvement of 1 point on the mRS) was 3.1 (95% CI, 2.0–4.7) favoring endovascular intervention. The proportion of patients with an mRS score of 0 to 2 at 90 days was 53.0% in the intervention group and 29.3% in the control group (P<0.001). Mortality at 90 days was 10.4% in the intervention group and 19.0% in the control group (adjusted rate ratio, 0.5; 95% CI, 0.3–0.8). The rate of sICH clinically determined at the study sites was 3.6% in the endovascular intervention group and 2.7% in the control group (adjusted rate ratio, 1.2; 95% CI, 0.3–4.6). Of the 165 participants randomized to endovascular intervention, retrievable stents were used in 130 of the 151 (86.1%) who underwent an endovascular procedure. TICI grade 2b/3 recanalization was observed in 72.4% in the endovascular group. In subgroup analysis, similar benefit was observed in the 235 patients who received intravenous r-tPA (OR, 2.5; 95% CI, 1.6–4.0) and the 76 who did not (OR, 2.6; 95% CI, 1.1–5.9). Only 49 participants (15.5%) underwent randomization ≥26 hours after symptom onset, too few to assess efficacy in the 6- to 12-hour time window.18

Solitaire FR With the Intention for Thrombectomy as Primary Endovascular Treatment of Acute Ischemic Stroke (SWIFT PRIME) was a PROBE-design trial that randomized 196 patients with acute ischemic stroke and NIHSS scores of 8 to 29 who received intravenous r-tPA within 4.5 hours of onset and had CTA or MRA confirmation of intracranial ICA, M1, or carotid terminus occlusion. If CTA or MRA was part of the local standard of care, it was performed at initial evaluation before intravenous r-tPA was started; if not, it was performed after review of the initial imaging and signing of informed consent. Groin puncture had to be possible within 6 hours of stroke onset. There were exclusion criteria for coagulopathies. Initially, CT perfusion or multimodal MRI was required, and enrollment was restricted to patients with the target mismatch profile (as assessed by specialized software16) and defined as follows: The ischemic core lesion measured ≤50 mL; the volume of tissue with a time to maximum delay of >10 seconds was ≤100 mL; the mismatch volume was at least 15 mL; and the mismatch ratio was >1.8. Midway through the trial, the inclusion criteria were modified to accommodate sites with limited perfusion imaging capability. Sites with perfusion imaging were encouraged to continue to use the target mismatch criteria. Sites without perfusion imaging used ASPECTS (ASPECTS >6 was required).
Table 4. Selected Clinical Outcomes for Recent Randomized, Clinical Trials of Endovascular Treatments for Acute Ischemic Stroke

<table>
<thead>
<tr>
<th>Study</th>
<th>Primary End Point</th>
<th>Death (90 d/3 mo)</th>
<th>Symptomatic ICH</th>
<th>mRS 0 to 2 at 90 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active, Control, %</td>
<td>Active, Control, %</td>
<td>Active, Control, %</td>
<td>Active, Control, %</td>
</tr>
<tr>
<td>SYNTHESIS</td>
<td>mRS 0 to 2 at 90 d</td>
<td>30.4 34.8 0.71</td>
<td>14.4 9.9 0.22</td>
<td>7 d 6 6 0.53</td>
</tr>
<tr>
<td>Exp.</td>
<td>1 at 3 mo</td>
<td>(0.44 to 1.14)*</td>
<td>P=0.22</td>
<td>P=0.53</td>
</tr>
<tr>
<td>IMS III</td>
<td>mRS 0 to 2 at 90 d</td>
<td>40.8 38.7 1.5</td>
<td>19.1 21.6 0.52</td>
<td>30 h 6.2 5.9 0.83</td>
</tr>
<tr>
<td>MR RESCUE</td>
<td>Mean mRS</td>
<td>3.9 3.9 0.099</td>
<td>19 24 0.75</td>
<td>7 d 5 4 0.24</td>
</tr>
<tr>
<td>MR CLEAN</td>
<td>Improvement in mRS</td>
<td>1.67 1.21 to 2.3*</td>
<td>21 22 90 d 7.7 6.4</td>
<td>32.6 19.1 2.16</td>
</tr>
<tr>
<td></td>
<td>at 90 d (shift analysis)</td>
<td></td>
<td>(1.21 to 2.3)*</td>
<td>(1.39 to 3.38)*</td>
</tr>
<tr>
<td>ESCAPE</td>
<td>Improvement in</td>
<td>3.1 2.0 to 4.7*</td>
<td>10.4 19 0.5 (0.3 to 0.6)**</td>
<td>90 d 3.6 2.7 1.2 (0.3 to 4.6)**</td>
</tr>
<tr>
<td></td>
<td>mRS at 90 d</td>
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<tr>
<td>SWIFT PRIME</td>
<td>Improvement in</td>
<td>P=0.001</td>
<td>9 12 0.74 (0.33 to 1.68)**</td>
<td>27 h 0 3 60 35 1.7 (1.23 to 2.33)**</td>
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<td></td>
<td>mRS at 90 d</td>
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<td>5 and 6 combined</td>
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<tr>
<td></td>
<td>(shift analysis)</td>
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<tr>
<td>EXTEND-IA</td>
<td>Median reperfusion</td>
<td>100 37 4.7 (2.5 to 9.9)**</td>
<td>9 20 0.45 (0.1 to 2.1)*</td>
<td>36 h 0 6 6 71 40 4.2 (1.4 to 12)*</td>
</tr>
<tr>
<td></td>
<td>at 24 h</td>
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<tr>
<td></td>
<td>Decrease in NIHSS</td>
<td>37 40 6.0 (2.0 to 18.9)**</td>
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<tr>
<td></td>
<td>or NIHSS 0, 1 at 3 d</td>
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<tr>
<td>REVASCAT</td>
<td>Improvement in</td>
<td>1.7 (1.05 to 2.8)*</td>
<td>18 16 1.2 (0.6 to 2.2)**</td>
<td>90 d 2 2 2 2 18 0.53 3.7 (1.1 to 4.0)***</td>
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<tr>
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<td>mRS at 90 d</td>
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<td>5 and 6 combined</td>
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<tr>
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<td>(shift analysis)</td>
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ASPECTS indicates Alberta Stroke Program Early CT Score; EC, extracranial; ESCAPE, Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion With Emphasis on Minimizing CT to Recanalization Times; EXTEND-IA, Extending the Time for Thrombolysis in Emergency Neurological Deficits–Intra-Arterial; ICA, internal carotid artery; ICH, intracerebral hemorrhage; IMS III, Interventional Management of Stroke Trial III; IV, intravenous; MR CLEAN, Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke; MR RESCUE, MR and Recanalization of Stroke Clots Using Embolectomy; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; r-tPA, recombinant tissue-type plasminogen activator; REVASCAT, Endovascular Revascularization With Solitaire Device Versus Best Medical Therapy in Anterior Circulation Stroke Within 8 Hours; SWIFT PRIME, Solitaire FR with the Intention for Thrombectomy as Primary Endovascular Treatment of Acute Ischemic Stroke; T, terminus (of the internal carotid artery); and TICI, Thrombolysis in Cerebral Infarction.

(Continued)

demonstrated that the prespecified criteria for stopping the trial at the first interim analysis were met. The 2 simultaneous success criteria used for the primary end point were both in favor of endovascular intervention: improved distribution (shift analysis) of mRS score at 90 days (P<0.001) and increased proportion with mRS score of 0 to 2 at 90 days (60% in the endovascular group and 35% in the nonendovascular group; risk ratio, 1.70; 95% CI, 1.23–2.33). There were no significant differences in death or sICH. TICI grade 2b/3 recanlization was observed in 88% of the endovascular group.20

The Extending the Time for Thrombolysis in Emergency Neurological Deficits–Intra-Arterial (EXTEND-IA) was similar in design to SWIFT PRIME. Seventy participants who were eligible with the use of “standard criteria” to receive intravenous r-tPA within 4.5 hours of stroke onset were randomized in a PROBE design to receive either intravenous r-tPA only or intravenous r-tPA plus endovascular therapy with a stent retriever. Groin puncture had to be within 6 hours, and endovascular treatment had to be completed within 8 hours after stroke onset. CT or MRI had to be performed before intravenous r-tPA was started. Occlusion of the ICA, M1, or M2 on CTA was required. In addition, CT or MRI perfusion imaging had to show a mismatch ratio of >1.2, an absolute mismatch volume of >10 mL, and an infarct core lesion volume of <70 mL as assessed with specialized software.19 There were specified exclusion criteria for coagulopathies. Occlusion of the ICA and M1 was present
### Table 4. Continued

<table>
<thead>
<tr>
<th>IV r-tPA Subgroups</th>
<th>Time Subgroups</th>
<th>ASPECTS Subgroups</th>
<th>NIHSS Subgroups</th>
<th>Age Subgroups</th>
<th>Vessel Subgroups</th>
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<td>Comparison</td>
<td>ASPECTS n</td>
<td>NIHSS n</td>
<td>Comparison</td>
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<td>treatment</td>
<td>(0.33 to 1.88)*</td>
<td>(0.27 to 1.2)*</td>
<td>(0.54 to 2.37)*</td>
<td>(0.14 to 1.57)*</td>
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<td>8 to 10</td>
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<td>1.57</td>
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<td>≤6 h</td>
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<td></td>
<td>≤180 min</td>
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<td>2.6</td>
<td>(1.5 to 4.5)[‡]</td>
<td>(1.11 to 2.34)[‡]</td>
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<td>2.6</td>
<td>(1.1 to 5.9)[‡]</td>
<td>≤8</td>
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<td>&gt;6 h</td>
<td>1.7</td>
<td>(0.7 to 7.9)[‡]</td>
<td>(0.7 to 7.9)[‡]</td>
<td>(1.1 to 5.3)[‡]</td>
</tr>
<tr>
<td>All</td>
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<td>1.62</td>
<td>8 to 10</td>
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<td>≥180 min</td>
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<td>≥4.5 h</td>
<td>71</td>
<td>1.4</td>
<td>(0.7 to 4.0)[‡]</td>
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<td>Yes</td>
<td>150</td>
<td>1.4</td>
<td>(0.8 to 2.6)[‡†]</td>
<td>8 to 10</td>
<td>142</td>
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<tr>
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<td>≤4.5 h</td>
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<td>1.8</td>
<td>(1.0 to 3.4)[‡]</td>
<td>(1.1 to 4.4)[‡]</td>
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<tr>
<td>No</td>
<td>56</td>
<td>2.7</td>
<td>(0.6 to 3.3)[‡]</td>
<td>≥8</td>
<td>101</td>
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</table>

*Adjusted odds ratio (95% confidence interval [CI]).
†Adjusted difference, 95% CI.
‡Relative risk, 99% CI.
‡‡Adjusted rate ratio, 95% CI.
[†]Odds ratio, 95% CI.
[‡]Risk ratio, 95% CI.
**Relative risk, 95% CI.
††Adjusted risk ratio, 95% CI.

in 31% and 54%, respectively. The coprimary outcomes were reperfusion at 24 hours and early neurological improvement (≥8-point reduction on the NIHSS or a score of 0 or 1 at day 3). The mRS score at 90 days was a secondary outcome. After the release of the MR CLEAN results, an unplanned interim efficacy analysis was implemented on the basis of a Haybittle-Peto stopping rule. The results of the interim analysis showed that the stopping criteria for efficacy were met, and the trial was halted. The percentage of ischemic territory that had undergone reperfusion at 24 hours was greater in the endovascular therapy group than in the intravenous r-tPA–only group (median, 100% versus 37%; \(P=0.001\)). Endovascular therapy, initiated at a median of 210 minutes (interquartile range, 166–251 minutes) after the onset of stroke, increased early neurological improvement at 3 days (80% versus 37%; \(P=0.002\)). More patients achieved functional independence in the endovascular group (score of 0 to 2 on the mRS, 71% versus 40%; \(P=0.01\)). There were no significant differences in rates of death or sICH. Recanalization to TICI grade 2b/3 was achieved in 86% of patients in the endovascular group at a median of 248 minutes (interquartile range, 204–277 minutes) after stroke onset.\(^21\)

Endovascular Revascularization With Solitaire Device Versus Best Medical Therapy in Anterior Circulation Stroke Within 8 Hours (REVASCAT) was a PROBE-design trial
randomizing 206 patients with acute ischemic stroke and an NIHSS score of ≥6 who had intracranial ICA or M1 occlusion by CTA, MRA, or digital subtraction angiography. Patients who had received intravenous r-tPA were eligible if there was no significant neurological improvement (criteria specified in the protocol) at 30 minutes after initiation of the infusion and vascular imaging at this time confirmed an eligible occlusion. Groin puncture had to be possible within 8 hours of stroke onset. There were exclusion criteria for coagulopathies. The main exclusion criteria on imaging were ASPECTS of <7 on nonenhanced CT or <6 on diffusion-weighted imaging–MRI. After the enrollment of 160 patients, the inclusion criteria were modified to include patients up to the age of 85 years (initially, 80 years was maximum allowed) with an ASPECTS of >8. Twenty-six percent had ICA occlusion, and 65% had M1 occlusion. Participants were randomized 1:1 to receive either medical therapy alone or thrombectomy with a stent retriever. Intravenous r-tPA was administered to 73%. When results of other similar trials became known, the Data Safety Monitoring Board recommended the recruitment be stopped because the emerging results showed that equipoise was lost, although the interim results did not reach the prespecified stopping boundaries. The masked steering committee agreed. Because just 1 analysis was performed, adjustment for multiple comparisons was no longer performed, and 95% CIs were reported. The primary outcome analysis showed a common OR of improvement in the distribution of the mRS score (shift analysis) favoring endovascular treatment (adjusted OR, 1.7; 95% CI, 1.05–2.8). The proportion of patients with an mRS score of 0 to 2 at 90 days was 43.7% in the intervention group and 28.2% in the control group (adjusted OR, 2.1; 95% CI, 1.1–4.0). There were no significant differences in death or sICH. Ninety-five percent of those in the endovascular group underwent thrombectomy. TICI grade 2b/3 recanalization was observed in 66% of the endovascular group. Across the prespecified subgroups, there were no significant interactions according to NIHSS score, vessel occlusion site, baseline ASPECTS, administration of intravenous r-tPA, age, or time of randomization, although for time of randomization dichotomized at 4.5 hours; the $P$ value for interaction was 0.9 with the latter group doing worse. No data are given for those who underwent groin puncture after 6 hours.

**Analysis and Conclusions**

None of the 3 earlier studies carried out with primarily intra-arterial fibrinolysis or first-generation mechanical embolec- tomy devices showed a benefit of endovascular treatment over intravenous r-tPA in intravenous r-tPA–eligible patients either as a substitute for initial treatment (SYNTHESIS Expansion) or as subsequent intervention in those with persistent large-artery occlusion after intravenous r-tPA (IMS III and MR RESCUE). MR RESCUE also showed no benefit for other patients treated within 8 hours even if selected by multimodal neuroimaging criteria. These studies, using almost exclusively intra-arterial r-tPA and first-generation endovascular devices alone or in combination, achieved recanalization rates of 27% to 41%. The subsequent trials using stent retrievers almost exclusively demonstrated improved results for both recanalization rates and outcome. Studies have shown that clinical outcome improved with increasing effectiveness of recanalization. Those with partial recanalization (TICI grade 2a) did not do as well as those with nearly complete or complete recanalization (TICI grade 2b/3) reflected as both differences in discharge disposition (41.0% of the TICI grade 2b/3 group discharged home versus 17.4% of the TICI grade 2a group) and functional outcome (34% with a TICI grade of 2a had an mRS score of 0 to 2 at 90 days versus 49% with a TICI grade of 2b/3).12,23 TICI grade 2b/3 recanalization was achieved in 59% to 88% of endovascularly treated subjects in the 5 stent retriever trials, whereas in the previous 3 studies, the rate had been 25% to 41%, as mentioned above. All 5 stent retriever studies showed clinical benefit in the endovascular group.

Of the 5 stent retriever trials, MR CLEAN, ESCAPE, and SWIFT PRIME permitted use of salvage intra-arterial fibrinolytic drugs, whereas EXTEND-IA and REVASCAT did not. These data do not establish the benefit of intra-arterial fibrinolytic salvage, nor can they establish lack of benefit. Such salvage techniques may be reasonable to use in some clinical circumstances.

The MR RESCUE trial enrolled patients up to 8 hours from symptom onset and showed no benefit from endovascular therapy with first-generation devices regardless of pen- umbral imaging pattern. Three of the 5 stent retriever studies specified a 6-hour window after stroke onset (2 specified 6 hours to groin puncture; the third specified 6 hours to start treatment). Aggregate data from REVASCAT and ESCAPE with treatment permitted out to 8 and 12 hours show a benefit, but ESCAPE enrolled too few patients after 6 hours to provide useful data, and REVASCAT provides no data about patients who underwent groin puncture between 6 and 8 hours. How much the overall positivity in these 2 trials was completely driven by those treated at shorter times is unknown at this time. The only time-dependent data are from the MR CLEAN presentation, which are not consistent with a benefit of treatment beginning after 6 hours. It will take patient-level meta- analyses to sort this out.

Every or nearly every patient in the 5 stent retriever studies first received intravenous r-tPA. Only REVASCAT stipulated the specific guidelines to be used to determine intravenous r-tPA eligibility (“guidelines provided by the European Stroke Organization”). EXTEND-IA refers to “standard criteria,” and the 3 other trials used “national guidelines.” Because it is not the purpose of this update is to address eligibility criteria for intravenous r-tPA, we have used the phrase “guidelines from professional medical societies” to address this issue in our recommendations. Too few data are available from the small number of those who did not receive intravenous r-tPA, for either time-based or non–time-based exclusion criteria, to determine with certainty whether there are characteristics that identify those who benefited from endovascular treatment. Two trials (MR CLEAN and REVASCAT) stipulated waiting for a period of time after beginning the administration of intravenous r-tPA before proceeding to endovascular therapy, whereas 3 trials (ESCAPE, SWIFT PRIME, and EXTEND-IA) did not. On the basis of these data, a waiting period is not necessary to achieve beneficial outcome in these patients.
All of these studies enrolled participants ≥18 years of age. There are no randomized trials of endovascular therapy in patients <18 years of age. Ischemic stroke resulting from large-vessel occlusion is rare in children and young adults relative to older individuals, posing challenges to rigorous study of this clinical scenario. Case reports and case series have documented that high rates of recanalization and favorable outcomes in young patients can be achieved with endovascular therapy.24–26 Ideally, appropriate trials would be done to test the efficacy of endovascular therapy in young patients. Studies in the United States, the United Kingdom, Australia, and Canada have shown median times from onset of symptoms to initial brain imaging for pediatric stroke of 8.8 to 16 hours.27 This problem of diagnostic delay will need to be addressed if trials of endovascular treatment for acute ischemic stroke are to be conducted successfully in this population.

Four stent retriever trials used NIHSS scores as eligibility criteria (>2, >5, 8–29, and >5), and the fifth enrolled patients with a similar distribution of NIHSS scores. From these trials, there are insufficient data in patients with NIHSS scores ≤0 to determine whether there is an overall net benefit from endovascular therapy in this population. Further randomized trials in patients with low NIHSS scores may be warranted. An NIHSS score of ≥6 was the minimum score used in 2 trials, thus fulfilling the AHA’s Level of Evidence grading algorithm for Level A evidence.

Four of the 5 stent retriever trials used a prestroke function eligibility criterion. REVASCAT and SWIFT PRIME used a prestroke mRS score of 0 to 1; EXTEND-IA used mRS scores of 0 to 2; and ESCAPE used Barthel scores of ≥90 to 100. MR CLEAN did not set a threshold and did not provide data on prestroke function. Thus, there are good data from 4 trials for patients with good baseline function (including 2 that required an mRS score of 0 to 1) and very few data for those without good baseline function.

All 5 stent retriever studies required baseline nonenhanced CT or MRI. MR CLEAN did not use a specific ASPECTS criterion for eligibility; it was the only positive trial that permitted enrollment of patients with ASPECTS <6. Although the treatment effect in that trial favored intervention in all 3 ASPECTS subgroups of 0 to 4 (28 patients), 5 to 7 (92 patients), and 8 to 10 (376 patients), the point estimate in the subgroup with an ASPECTS of 0 to 4 was close to unity with wide CIs (adjusted common OR, 1.09; 95% CI, 0.14–8.46). In the ESCAPE trial secondary analyses based on ASPECTS, the risk ratio favoring intervention was 2.6 (95% CI, 1.7–4.1) for patients with an ASPECTS of 8 to 10 and 2.7 (95% CI, 1.0–7.2) for those with a score of 6 to 8. EXTEND-IA did not report secondary analyses based on ASPECTS. SWIFT PRIME reported similar benefit for those with ASPECTS of 8 to 10 (OR, 1.62; 95% CI, 1.17–2.24) and 6 to 7 (OR, 1.98; 95% CI, 0.73–5.33), although the small number of 43 patients in the latter group produced wide confidence bounds. REVASCAT reported greater benefit for those with ASPECTS ≥8 (OR, 2.2; 95% CI, 1.1–4.4) than for those with ASPECTS <8 (OR, 1.4; 95% CI, 0.7–2.9). On the basis of these data, the benefit from endovascular therapy in patients with ASPECTS <6 is uncertain, and further randomized, controlled trials are warranted. An ASPECTS ≥6 was the minimum score used in 2 trials, thus fulfilling the AHA’s Level of Evidence grading algorithm for Level A evidence.

Each of the 5 stent retriever trials used different strategies of imaging-based selection criterion in addition to nonenhanced CT or MRI. Common to all was required demonstration, usually with a noninvasive vessel imaging study (CTA or MRA), of a large-vessel occlusion before randomization. MR CLEAN and REVASCAT also allowed digital subtraction angiography screening to identify a target occlusion. Two trials required noninvasive imaging to be performed at initial evaluation before intravenous r-tPA was started (combined occurrence of no clot at endovascular intervention in 12 of 200 [6.0%]); a third recommended the same (no clot at endovascular intervention in 8 of 233 [3.4%]); and a fourth stipulated that it be done at all centers for which this was part of local standard of care but otherwise after consent was obtained (no clot at endovascular intervention in 7 of 98 [7.1%]). REVASCAT stipulated that the imaging study must be completed no more than 90 minutes but ideally within 60 minutes before groin puncture, and for patients who received intravenous tPA, an imaging study assessing vessel patency must be obtained at a minimum of 30 minutes after that start of intravenous r-tPA infusion (no clot at endovascular intervention in 5 of 103 [4.9%]). The REVASCAT strategy did not result in a decrease in the number who failed to have a clot present at the time of endovascular intervention compared with the other studies. The goal of intravenous r-tPA and of endovascular therapy is to recanalize the occluded vessel as soon as possible. After the initiation of intravenous r-tPA, some patients will experience successful recanalization, obviating the need to pursue follow-on endovascular therapy.28 However, because recanalization occurs in only a minority of patients with large-vessel occlusion receiving intravenous r-tPA alone (e.g., 37.3% in the ESCAPE trial), noninvasive intracranial vascular imaging should proceed without delay before or immediately after initiation of r-tPA to identify the majority of patients who will benefit from follow-on endovascular therapy and to expedite its performance. This approach was explicitly taken by investigators in the ESCAPE trial, helping them achieve a median CT–to–groin puncture time of only 51 minutes.

The ESCAPE, EXTEND-IA, and SWIFT PRIME trials were initially designed with the intent to select and enroll only patients with small regions of ischemic cores and the presence of salvageable brain tissue (SWIFT PRIME and EXTEND-IA) and/or adequate collateral flow (ESCAPE). In ESCAPE, nonenhanced CT and CTA (preferably multiphase) were used to select patients with a target occlusion, small infarct core (ASPECTS 6–10), and moderate to good collateral circulation (filling of ≥50% of pial arterial circulation visualized on CTA). EXTEND-IA required demonstration of potentially salvageable brain tissue on perfusion CT (mismatch ratio of >1.2, absolute mismatch volume of >10 mL) and ischemic core <70 mL (relative cerebral blood flow <30% of normal). All images were processed on site with a specialized software package.29 Penumbral tissue was defined as regions with time-to-maximum (Tmax) perfusion values >6 seconds that were
not included in the ischemic core. SWIFT PRIME excluded patients with evidence of frank ischemia in greater than one third of the MCA territory or involving >100 mL of tissue. For the first 71 patients enrolled, an additional inclusion criterion was the presence of target mismatch defined as infarct core ≤50 mL (as assessed by specialized software⁴¹) and ischemic penumbra ≥15 mL with a mismatch ratio >1.8. After the enrollment of the first 71 patients, the investigators switched to the criterion to ASPECTS of ≥6 for sites that did not have CT perfusion capability. To date, subgroup analyses with the various imaging criteria have not been published. In these trials, the use of advanced imaging selection criteria had the potential advantage of increasing the likelihood of showing treatment benefit by enhancing the study population with patients most likely to respond to therapy. However, the inherent disadvantage of this study design is the possibility that patients who may have responded to therapy were excluded. In contrast, the MR RESCUE trial was designed specifically to validate imaging biomarkers as a selection tool for endovascular therapy. However, the trial was unable to demonstrate an overall benefit from endovascular therapy with first-generation devices or in the subgroup with a favorable penumbral pattern. None of the 5 stent retriever studies was designed to validate the utility of the advanced imaging selection criteria themselves in either the early or late time windows. Thus, the role of these techniques for patient selection requires further study.

The overwhelming majority of patients in the stent retriever trials had ICA or proximal MCA (M1) occlusion. The number of patients with isolated M2 lesions was small; ESCAPE, REVASCAT, and SWIFT PRIME excluded patients with isolated M2 occlusions, although small numbers of these patients were enrolled in these trials. The distinction of M1 from M2 can be difficult in some patients because of early branches of the M1 such as the anterior temporal branch. Inadequate numbers of patients with occlusion of other vessels, including M3 and anterior cerebral artery occlusions and those in the vertebrobasilar circulation, also were enrolled to allow assessment of clinical efficacy in these territories.

The usefulness of mechanical thrombectomy devices other than stent retrievers is not well established, either for technical efficacy or for clinical benefit. Most of the patients in MR CLEAN and ESCAPE and all of the patients in EXTEND-IA, SWIFT-PRIME, and REVASCAT who underwent an endovascular procedure were treated with a stent retriever (81.5% in MR CLEAN, 86.1% in ESCAPE). These trials were not designed to demonstrate the superiority of stent retrievers over other devices such as snares or suction aspiration systems. Therefore, the recommendation that stent retrievers are preferred over MERCI is unchanged from the previous guidelines based on the SWIFT and TREVO 2 (Trevo Versus Merci Retrievers for Thrombectomy Revascularisation of Large Vessel Occlusions in Acute Ischaemic Stroke) studies.⁴²⁻⁴³ At the time the present guidelines were written, there were no published randomized, clinical trials demonstrating clinical benefit or comparing the relative effectiveness of other devices versus stent retrievers.

None of these studies specified requirements for the use of a proximal balloon guide catheter, large-bore distal-access catheter, or cervical guide catheter alone or in conjunction with stent retrievers. The concomitant use of distal-access suction catheters during stent retriever mechanical thrombectomy has been described in retrospective case series.⁴²⁻⁴⁴ The advantages of the combined stent-aspiration technique include a flexible large-bore catheter in a triaxial technique, which provides stability for the stent-retriever; flow reversal to prevent distal embolization during stent retrieval of the thrombus; and the potential synergistic effect of both techniques of suction aspiration and stent retrieval used simultaneously.⁴²⁻⁴⁴ Clinical experience has shown the combination of balloon guide catheters or distal-access/aspiration catheters and stent retrievers to provide rapid, effective, and safe recanalization.⁴⁵⁻⁴⁶

All the stent retriever trials allowed the inclusion of patients with proximal cervical carotid stenosis, and all but 1 trial allowed the inclusion of patients with complete atherosclerotic cervical carotid occlusion (SWIFT PRIME). One difficulty with this exclusion is that differentiating complete cervical carotid occlusion from a distal ICA occlusion is often not possible on CTA or MRA.⁴⁷ The number of patients with cervical carotid occlusion or stenosis was not consistently reported but was substantial, ranging from 18.6% (REVASCAT) to 32.2% (MR CLEAN). Stenting of the underlying stenosis or occlusion was discouraged in the ESCAPE protocol. Thirty of the 75 patients with carotid stenosis or occlusion in the intervention arm were stented during the thrombectomy procedure in MR CLEAN. Nine of the 19 patients with carotid occlusion in REVASCAT were stented at the time of thrombectomy. The management of the underlying lesion was not reported in the other trials. Outcomes for the subgroup of patients with cervical carotid occlusion were reported in ESCAPE (OR, 8.7; 95% CI, 1.9–39.4) and MR CLEAN (adjusted OR, 1.43; 95% CI, 0.78–2.64). Although thrombectomy for patients with cervical ICA occlusion is clearly indicated by these data, the optimal management of the underlying stenosis is not clear. There are several potential advantages and disadvantages for angioplasty and stenting at the time of thrombectomy. Although immediate recanalization may reduce the risk of recurrent stroke, urgent stenting generally requires antiplatelet prophylaxis, which has been associated with intracranial hemorrhage in this setting. Carotid stenting and intracranial thrombectomy for the treatment of acute stroke resulting from tandem occlusions with aggressive antiplatelet therapy may be associated with a high incidence of intracranial hemorrhage.⁴⁸⁻⁴⁹ In addition, there is some risk for thromboembolic stroke at the time of stenting. Further studies are indicated.

General anesthesia with intubation and conscious sedation are the 2 most frequently used anesthetic approaches for patients with an acute ischemic stroke receiving endovascular therapy.⁵⁰ No dedicated randomized, controlled, clinical trials have addressed this issue. The MR CLEAN investigators have reported that the outcomes of the 79 patients in the endovascular group who received general anesthesia were not different from the outcomes of the 267 nonendovascular control patients (adjusted OR, 1.09; 95% CI, 0.69–1.71), whereas the outcomes for the 137 endovascular patients who did not receive general anesthesia were better than the outcomes for
the 267 control patients (adjusted OR, 2.13; 95% CI, 1.46–3.11).41 Similar data showing worse outcomes in those undergoing general anesthesia compared with conscious sedation for endovascular were reported in a recent meta-analysis of 9 nonrandomized studies comprising 1956 patients (814 received general anesthesia, 1142 received conscious sedation), with the largest study having 1079 patients and the smallest study having 66 patients.42 In this meta-analysis, compared with conscious sedation, general anesthesia was linked to lower odds of a favorable functional outcome (OR, 0.43; 95% CI, 0.35–0.53; P<0.01), higher odds of mortality (OR, 2.59; 95% CI, 1.87–3.58; P<0.01), and more adverse respiratory events (OR, 2.09; 95% CI, 1.36–3.23; P<0.01). No significant differences in the rates of asymptomatic ICH, sICH, or other vascular complications were seen between the groups. Furthermore, mean time to groin puncture, mean procedure time, and mean time from symptom onset to revascularization were not significantly different between the 2 techniques. There was substantial heterogeneity (I²>50%) across the included studies for the outcomes of functional status (I²=55%), time to revascularization (I²=60%), time to groin puncture (I²=83%), and procedure time (I²=91%). In most of the included studies, patients who received general anesthesia were typically in worse clinical condition at baseline, as reflected by their comparatively higher NIHSS scores. Only 6 of the 9 studies included information on baseline NIHSS score. Adjusting for NIHSS score by the use of meta-regression for the odds of having good functional outcomes yielded an OR of 0.38, which was similar to the unadjusted estimate of 0.43; however, the 95% CI became statistically insignificant (0.12–1.22). Thus, even after adjustment for initial stroke severity, the possibility of selection bias cannot be completely excluded. Patients with more severe strokes or poorer baseline conditioning may have received general anesthesia or may have been intubated before the procedure because of an actual or expected inability to maintain airway patency. Moreover, it is possible that the lower recanalization rates observed with general anesthesia in some studies were attributable to greater numbers of more technically difficult vascular occlusions in those who received general anesthesia. On balance, published data broadly indicate that conscious sedation might be safer and more effective than general anesthesia in the setting of endovascular therapy for acute ischemic stroke. However, specific randomized, controlled trial data are warranted to definitively establish conscious sedation as the preferred anesthetic technique in patients receiving endovascular treatment for acute ischemic stroke. Clinical trials are ongoing (http://www.clinicaltrials.gov; NCT01872884, NCT02317237).

The AHA’s Level of Evidence grading algorithm requires high-quality evidence from >1 randomized, controlled trial for Level of Evidence A. In accordance with this algorithm and the results from the 5 recent studies with stent retrievers summarized above, we concluded that the data supported Class I, Level of Evidence A recommendations but only for a carefully defined group of patients (see Recommendation 2). Subsequent meta-analysis of patient-level data may allow these recommendations to be expanded.

Recommendations

Endovascular Interventions

1. Patients eligible for intravenous r-tPA should receive intravenous r-tPA even if endovascular treatments are being considered (Class I; Level of Evidence A). (Unchanged from the 2013 guideline)

2. Patients should receive endovascular therapy with a stent retriever if they meet all the following criteria (Class I; Level of Evidence A). (New recommendation):
   a. Prestroke mRS score 0 to 1,
   b. Acute ischemic stroke receiving intravenous r-tPA within 4.5 hours of onset according to guidelines from professional medical societies,
   c. Causative occlusion of the ICA or proximal MCA (M1),
   d. Age ≥18 years,
   e. NIHSS score of ≥6,
   f. ASPETCS of ≥6, and
   g. Treatment can be initiated (groin puncture) within 6 hours of symptom onset

3. As with intravenous r-tPA, reduced time from symptom onset to reperfusion with endovascular therapies is highly associated with better clinical outcomes. To ensure benefit, reperfusion to TICI grade 2b/3 should be achieved as early as possible and within 6 hours of stroke onset (Class I; Level of Evidence B-R). (Revised from the 2013 guideline)

4. When treatment is initiated beyond 6 hours from symptom onset, the effectiveness of endovascular therapy is uncertain for patients with acute ischemic stroke who have causative occlusion of the ICA or proximal MCA (M1) (Class IIb; Level of Evidence C). Additional randomized trial data are needed. (New recommendation)

5. In carefully selected patients with anterior circulation occlusion who have contraindications to intravenous r-tPA, endovascular therapy with stent retrievers completed within 6 hours of stroke onset is reasonable (Class IIa; Level of Evidence C). Inadequate data are available at this time to determine the clinical efficacy of endovascular therapy with stent retrievers for those patients whose contraindications are time based or not time based (eg, prior stroke, serious head trauma, hemorrhagic coagulopathy, or receiving anticoagulant medications). (New recommendation)

6. Although the benefits are uncertain, the use of endovascular therapy with stent retrievers may be reasonable for carefully selected patients with acute ischemic stroke in whom treatment can be initiated (groin puncture) within 6 hours of symptom onset and who have causative occlusion of the M2 or M3 portion of the MCAs, anterior cerebral arteries, vertebral arteries, basilar artery, or posterior cerebral arteries (Class IIb; Level of Evidence C). (New recommendation)

7. Endovascular therapy with stent retrievers may be reasonable for some patients <18 years of age with acute ischemic stroke who have demonstrated large-vessel occlusion in whom treatment can be initiated (groin puncture) within 6
11. The use of a proximal balloon guide catheter or a large-bore distal-access catheter rather than a cervical guide catheter alone in conjunction with stent retrievers may be beneficial (Class IIa; Level of Evidence C). Future studies should examine which systems provide the highest recanalization rates with the lowest risk for nontarget embolization. (New recommendation)

12. The technical goal of the thrombectomy procedure should be a TICI grade 2b/3 angiographic result to maximize the probability of a good functional clinical outcome (Class I; Level of Evidence A). Use of salvage technical adjuncts, including intraarterial fibrinolysis, may be reasonable to achieve these angiographic results if completed within 6 hours of symptom onset (Class IIb; Level of Evidence B-R). (New recommendation)

13. Angioplasty and stenting of proximal cervical atherosclerotic stenosis or complete occlusion at the time of thrombectomy may be considered, but the usefulness is unknown (Class IIb; Level of Evidence C). Future randomized studies are needed. (New recommendation)

14. Initial treatment with intra-arterial fibrinolysis is beneficial for carefully selected patients with major ischemic strokes of <6 hours’ duration caused by occlusions of the MCA (Class I; Level of Evidence B-R). However, these data are derived from clinical trials that no longer reflect current practice, including the use of fibrinolytic drugs that are not available. A clinically beneficial dose of intra-arterial r-tPA is not established, and r-tPA does not have US Food and Drug Administration approval for intra-arterial use. As a consequence, endovascular therapy with stent retrievers is recommended over intra-arterial fibrinolysis as first-line therapy (Class I; Level of Evidence E). (Revised from the 2013 guideline)

15. Intra-arterial fibrinolysis initiated within 6 hours of stroke onset in carefully selected patients who have contraindications to the use of intravenous r-tPA might be considered, but the consequences are unknown (Class IIb; Level of Evidence C). (Revised from the 2013 guideline)

16. It might be reasonable to favor conscious sedation over general anesthesia during endovascular therapy for acute ischemic stroke. However, the ultimate selection of anesthetic technique during endovascular therapy for acute ischemic stroke should be individualized on the basis of patient risk factors, tolerance of the procedure, and other clinical characteristics. Randomized trial data are needed (Class IIb; Level of Evidence B-R). (New recommendation)

**Imaging**

1. Emergency imaging of the brain is recommended before any specific treatment for acute stroke is initiated (Class I; Level of Evidence A). In most instances, nonenhanced CT will provide the necessary information to make decisions about emergency management. (Unchanged from the 2013 guideline)

2. If endovascular therapy is contemplated, a noninvasive intracranial vascular study is strongly recommended during the initial imaging evaluation of the acute stroke patient but should not delay intravenous r-tPA if indicated. For patients who qualify for intravenous r-tPA according to guidelines from professional medical societies, initiating intravenous r-tPA before noninvasive vascular imaging is recommended for patients who have not had noninvasive vascular imaging as part of their initial imaging assessment for stroke. Noninvasive intracranial vascular imaging should then be obtained as quickly as possible (Class I; Level of Evidence A). (New recommendation)

3. The benefits of additional imaging beyond CT and CTA or MRI and MRA such as CT perfusion or diffusion- and perfusion-weighted imaging for selecting patients for endovascular therapy are unknown (Class IIb; Level of Evidence C). Further randomized, controlled trials may be helpful to determine whether advanced imaging paradigms using CT perfusion, CTA, and MRI perfusion and diffusion imaging, including measures of infarct core, collateral flow status, and penumbra, are beneficial for selecting patients for acute reperfusion therapy who are within 6 hours of symptom onset and have an ASPECTS <6. Further randomized, controlled trials should be done to determine whether advanced imaging paradigms with CT perfusion, MRI perfusion, CTA, and diffusion imaging, including measures of infarct core, collateral flow status, and penumbra, are beneficial for selecting patients for acute reperfusion therapy who are beyond 6 hours from symptom onset. (New recommendation)

**Systems of Stroke Care**

1. Patients should be transported rapidly to the closest available certified primary stroke center.
or comprehensive stroke center or, if no such centers exist, the most appropriate institution that provides emergency stroke care as described in the 2013 guidelines (Class I; Level of Evidence A). In some instances, this may involve air medical transport and hospital bypass. (Unchanged from the 2013 guideline)

2. Regional systems of stroke care should be developed. These should consist of the following:
   a. Healthcare facilities that provide initial emergency care, including administration of intravenous r-tPA, such as primary stroke centers, comprehensive stroke centers, and other facilities, and
   b. Centers capable of performing endovascular stroke treatment with comprehensive periprocedural care, including comprehensive stroke centers and other healthcare facilities, to which rapid transport can be arranged when appropriate (Class I; Level of Evidence A). (Revised from the 2013 guideline)

3. It may be useful for primary stroke centers and other healthcare facilities that provide initial emergency care, including administration of intravenous r-tPA, to develop the capability of performing emergency noninvasive intracranial vascular imaging to most appropriately select patients for transfer for endovascular intervention and to reduce the time to endovascular treatment (Class IIb; Level of Evidence C). (Revised from the 2013 guideline)

4. Endovascular therapy requires the patient to be at an experienced stroke center with rapid access to cerebral angiography and qualified neurointerventionalists. Systems should be designed, executed, and monitored to emphasize expeditious assessment and treatment. Outcomes for all patients should be tracked. Facilities are encouraged to define criteria that can be used to credential individuals who can perform safe and timely intra-arterial revascularization procedures (Class I; Level of Evidence E). (Revised from the 2013 guideline)

Disclosures

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