Surrogate Measures of Outcome in Clinical Trials: Clues Worth Examining?

The clinical trialist must not only ask, “Does my treatment work?” but also, “What is the best way to prove it within the constraints of the clinical trial framework?” Should the trialist look at purely clinical measures of success and, if so, which ones? Alternatively, can a biomarker or radiographic marker be an important determinant of effect? What if there is a discrepancy between the two, such as a positive result for the outcome surrogate but not the clinical outcome itself? Of course, an intervention that changes clinical outcome is more powerful and has the potential to change clinical practice immediately. A change in only a surrogate marker for outcome is more difficult to interpret, and more controversial, but is it worth examining?

Recently published results from two clinical trials of intracerebral hemorrhage (ICH) highlight this issue. The Intensive Blood Acute Cerebral Hemorrhage (INTERACT) study (Lancet Neurol 7:391–399, 2008) examined the effect of targeted lowering of blood pressure after ICH, and the Factor VII for Acute Hemorrhagic Stroke (FAST) trial (N Engl J Med 358:2127–2137, 2008) examined the effects of intravenous administration of recombinant activated coagulation Factor VII in noncoagulopathic patients after ICH. Hematoma expansion had been shown to correlate with morbidity and mortality after ICH, and the hypothesis of these two studies is that reduction in this expansion may provide clinical benefit. However, in either trial, there was no significant difference at 90 days, in terms of mortality or general disability scales (e.g., Barthel and Rankin Index).

But, were the studies completely negative? In both trials, the proposed therapy demonstrated the hypothesized reduction in hematoma growth after admission. The FAST trial showed that patients receiving 80 µg/kg of Factor VII had a smaller amount of hematoma expansion (14 versus 36%) compared with patients receiving placebo. In INTERACT, patients who received aggressive targeting of blood pressure to goals of systolic blood pressure of 140 mmHg also had a lower percentage of hematoma expansion than a control group with less rigorous blood pressure control (11 versus 20%). The trials were very similar in other ways: approximately 80% of the hemorrhages were in the basal ganglia or thalami, and the average age of patients was in the early 60s. In the FAST study, treatment initiation was within 4 hours of symptom onset, whereas in INTERACT, treatment was to be initiated within 6 hours. In the various treatment groups, mean baseline volume of hematoma was larger in FAST (22–24 mL) than INTERACT (13–14 mL) and, consistent with this, the average mortality was higher in FAST (18–21%) than INTERACT (10–13%).

Is this promising or not? For the caregiver seeking a revolution in ICH management, clearly the absence of a clear clinical benefit may seem disappointing. But, for those seeking an evolution of potential strategies or further study designs that may indeed culminate in a clinical benefit, the radiographic outcome data provided by the measurement of hematoma volume is useful. These data allow investigators to begin to hypothesize why the trials did not produce a positive clinical outcome. In the FAST trial, was it because thromboembolic events offset any potential benefit derived from reducing hematoma expansion? In the INTERACT trial, would expansion have been more greatly inhibited if the treatment had been started earlier? To what extent did FAST patients, treated primarily in the United States and Europe, already have a treatment paradigm that lowered blood pressure in a similar fashion to INTERACT, which was conducted primarily in Asia? Or, would there be clearer benefit when combining these therapies with each other?

The results motivate even more provocative questions. Could the outcome of these cases have been so heavily influenced by more deterministic prognostic factors, such as initial clot volume, age, or level of consciousness? Could cases with large ICH volumes and/or those with associated intraventricular hemorrhage (IVH) have so heavily weighed the outcome that they wiped out any modest benefit on growth of ICH? Would one or the other therapy prove more beneficial when complemented by subsequent hematoma reduction surgery, such as with minimally invasive approaches, or specific interventions for clearance of associated IVH?

In the FAST trial, earlier Phase II study had shown more compelling treatment effect on outcome, essentially forcing early termination of that earlier study (N Engl J Med 352:777–785, 2005). And, there may have been a modest benefit from treatment on outcome at 15 days, but this disappeared at 90 days. Could this mean that families and treating teams were initially enthusiastic about potential effects of therapy and invested in aggressive interventions, but later may have become more disappointed with quality of life, providing less aggressive support after discharge from the hospital? Could more uniform standards of skilled nursing care and rehabilitation have preserved or enhanced the treatment benefit? These questions reflect the extraordinary complexity of this disease and issues that will have to be tackled as novel therapies are introduced and tested.

Certainly, the use of the surrogate radiographic marker of hematoma volume leaves the door to these and other relevant questions open and may help guide future trial design for medical treatment of ICH. In the end, the ICH problem may require a multifaceted approach to make a difference in clinical outcomes. Trial designs that incorporate surrogate markers along with clinical outcome can shed useful light on complex dimensions of the disease.

**Science Times Editorial**
SATB1: The Convergence of Carcinogenesis and Chromatin Conformation

Nearly 16 years ago, researchers from the lab of Kohwi-Shigematsu first reported the discovery of a novel protein (Cell 70:631–645, 1992) that preferentially bound A/T-rich sequences of deoxyribonucleic acid (DNA), the special A/T-rich binding protein 1 (SATB1). SATB1 turned out to be more than a simple DNA-binding transcription factor. In the nuclei of cells, DNA is tightly compacted into chromatin by looping it around A/T-rich regions bound to SATB1. Cage-like networks of these proteins serve as anchors to the nucleoskeleton for millions of DNA base pairs (Nature 419:641–645, 2002; Nature Genet 34:42–51, 2003). Within each of these regions, SATB1 serves to coordinately activate or repress gene expression by controlling the conformation and accessibility of associated chromatin for remodeling and transcription (Nature Genet 38:1278–1288, 2006).

These findings would normally relegate SATB1 as a protein of interest primarily for molecular biologists, were it not for the recent revelation by Kohwi-Shigematsu’s group that its expression in human breast tumors is highly correlated with their metastatic potential (Nature 452:187–193, 2008). An initial study of 24 breast epithelial cell lines demonstrated that SATB1 expression was restricted only to metastatic phenotypes; the closely related homologue, SATB2 showed no such correlation. The authors then examined SATB1 expression in 1318 breast carcinoma specimens from patients with long-term clinical follow-up data and found that increased SATB1 expression was associated with shorter survival times by Kaplan-Meier analysis (Figure). Notably, multivariate analysis revealed that SATB1 expression was a poor prognostic factor for breast cancer independent of tumor stage, nodal stage, and histological grade.

Next, the effect of SATB1 downregulation and overexpression was examined in vitro and in vivo in human breast cancer cell lines. SATB1 levels were knocked down in the highly-metastatic MDA-MB-231 cell line by using ribonucleic acid (RNA)-interference through stable transfection. Reduction of SATB1 levels in these cells triggered decreased cell proliferation, restored anchorage-dependent growth, and reverted cells to a polarized morphology, all pointing towards a reversal of the metastatic phenotype. In vivo in athymic mice, MDA-MB-231 SATB1 knock-down cells showed markedly reduced lung nodule formation when systemically injected; tumor growth was similarly impaired when implanted in athymic nude mouse mammary fat pads. Complementary experiments involving ectopic expression of SATB1 in non-metastatic SKBR3 human breast epithelial lines in vivo demonstrated promotion of tumorigenesis in mammary fat pads and the formation of lung nodules in athymic mice when administered intravenously.

The most interesting data from this study derive from comparing the global expression profiles of SATB1-depleted and control MDA-MB-231 cells. SATB1 expression coordinates the upregulation of 409 genes, including cell adhesion, TGF-1 (transforming growth factor-1) signaling, and cell cycle families and the down-regulation of 456 others, including the tumor suppressors BRMS1, KAI1, NME1, and KISS1. The researchers went on to identify 40 genes implicated in human cancer metastasis specifically upregulated by SATB1, including metastasin (S100A4), VEGFB, matrix metalloproteases 2, 3 and 9, TGFBI and endothelial growth factor receptor subfamily members, including HER-2/NEU, SATB1 binding and association with MARs embedded in the upstream and downstream sequences of a number of these metastasis-associated proteins was confirmed by using the urea-chromatin immunoprecipitation assay.

For many years, the study of cancer biology has focused on the sequential accumulation of gene mutations that promote the transformation of a tumor progenitor into a metastatic phenotype. Here, Han et al. elegantly define SATB1 as the first protein able to promote tumor progression and metastasis by globally remodeling the chromatin structure and transcriptional profiles of hundreds of genes. Not only does this make SATB1 relevant as a diagnostic and therapeutic target in breast cancer, it also raises the possibility that chromatin organizers likely participate in the transformation of other malignant tumors. Intriguingly, such “genome organizers” may play a role in oncogenesis within the central nervous system, as tightly-regulated SATB1 expression has been identified in the developing fetal brain, as well as in the dorsal spinal cord (Dev Biol 292:555–564, 2006).

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Carotid Stenting versus Endarterectomy in High-risk Cases: What Do “High-risk” and “Equivalent” Mean?

Carotid endarterectomy (CEA) has traditionally been viewed as the standard of care for symptomatic carotid artery stenosis, proven to reduce the incidence of stroke in this population. Endovascular stenting offers an alternative treatment that avoids the need for general anesthesia, surgical intervention, and potentially shortens hospital stay. Several studies have evaluated the short-term safety of carotid angioplasty and stenting (CAS), with the Stenting and Angioplasty with Protection in Patients at High Risk for Endarterectomy (SAPPHIRE) trial demonstrating comparable 1-year outcomes between CEA and CAS (N Engl J Med 351: 1493–1501, 2004). Although these results appear to validate the efficacy of stenting, evaluation of long-term follow-up is critical. To this end, Gurm et al. recently assessed 3-year outcomes in the randomized SAPPHIRE trial cohort (N Engl J Med 358: 1572–1579, 2008).

The SAPPHIRE trial cohort consists of carotid stenosis patients deemed to be at high risk of surgical complications. Of all patients originally enrolled in the SAPPHIRE trial, clinical follow-up data at 3 years was available for 85.6% of patients who received stenting (143 of 167 patients) and 70.1% (117 of 167) of patients who underwent an endarterectomy. The cumulative incidences of myocardial infarction within 30 days of the procedure or death/ipsilateral stroke within 3 years of the procedure was 24.6% (41 of 167) for patients in the stenting group and 26.9% (45 of 167) for the endarterectomy group. The majority of deaths in both groups were nonneurological, with neurological causes of death occurring in only three (1.8%) stenting patients and four (2.4%) endarterectomy patients. Over the 3-year span, a total of 15 strokes occurred in each of the two groups, with ipsilateral strokes occurring in 11 patients from the stenting group and nine from the endarterectomy group. Target vessels revascularization was required in 97.0% of patients in the stenting group and 92.9% of patients in the endarterectomy group. Most revascularization consisted of percutaneous treatment, with only one patient in each group requiring endarterectomy.

The results of this study demonstrate no significant difference between protected CAS and CEA with respect to the incidence of stroke, revascularization, and major adverse events within 3 years of treatment. Although these results appear promising, they are not corroborated by other recent investigations. One such study, the EVA-3S trial conducted by Mas et al., was prematurely terminated secondary to increased risk associated with stenting (N Engl J Med 355: 1660–1671, 2006). This discrepancy may be due, however, to differences in methodology. The two trials used different patient selection criteria. For example, all patients with a stenosis between 50 and 80% had to be symptomatic, whereas patients with stenosis of greater than 80% could be asymptomatic for inclusion in the SAPPHIRE trial. In contrast, the inclusion criteria of the EVA-3S trial included all patients to be symptomatic and exhibit a stenosis of at least 60%, and did not include patients at high surgical risk. Previous criticism of the EVA-3S trial also pointed out surgeon inexperience and low case volume at participating medical centers.

It was initially thought that such high-risk patients might ultimately be better served by CAS rather than CEA. This notion, however, is now coming under question, with at best equivalent results in strictly selected cohorts. Numerous randomized prospective trials including thousands of patients have provided in incontrovertible Class I evidence in support of the standard of care, accrediting the benefits of CEA in symptomatic and asymptomatic patients, including many trials which included various so called “high-risk” patients (Lancet 361:107–116, 2003). Questions arise as to who is a “high-risk” patient, and who decides this. In the SAPPHIRE trial, this decision was based on strict criteria, including assessment by experienced carotid surgeons before eligibility to randomize the cases. In clinical practice, false generalization is rampant, where “high risk” categorization may be made by primary care physicians without surgical input, and worse, insinuation of similar equivalency between CAS and CEA in non-high risk cases. This practice cannot be supported by current scientific evidence. Until additional prospective studies are completed, CEA remains the “gold standard” of care for most cases with significant carotid stenosis, and surgeon experience and institutional case volume should strongly influence management paradigms in “high-risk” cases.

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Technical & Clinical Research
Interlukin-6 Receptor Inhibitor Tocilizumab: A New Treatment Option in Rheumatoid Arthritis?

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease characterized by pain, fatigue, progressive joint damage, and disability. The exact cause of the disease is unknown, but there is evidence that proinflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin (IL)-1, and IL-6 produced by immune cells are basic to its pathogenesis. Over the past 20 years, multiple clinically effective therapies have been developed targeting inflammatory pathways including antirheumatic drugs (e.g., methotrexate) as well as biological agents that target the action of inflammatory cytokines (e.g., TNF-α or IL-1), or limit T-cell costimulation or B-cell function. Yet, there is a significant subset of patients who have poor or no response to current treatments.

The search for new treatment options in RA has recently centered on IL-6, a pleiotropic cytokine that is found in abnormally high concentrations in synovial tissue, serum, and synovial fluid of patients with active RA. In a recent issue of The Lancet (371:987–997, 2008), results of the tocilizumab Pivotal Trial in Methotrexate Inadequate Responders (OPTION) study, a double blind, randomized, placebo controlled trial investigating the effects of tocilizumab, a humanized IL-6 receptor inhibitor on symptoms of RA, are reported. In the study, a total of 622 patients with active moderate to severe RA with incomplete response to methotrexate were randomized into three groups: placebo, tocilizumab (4 mg/kg intravenously), or tocilizumab (8 mg/kg intravenously). Tocilizumab was administered at baseline and every 4 weeks thereafter for a total of 24 weeks. The patients were continued on their baseline methotrexate therapy, but all other disease-modifying drugs were discontinued.

The results of the study showed that IL-6 receptor inhibitor tocilizumab can ameliorate multiple symptoms of RA and potentially promote a remission of the disease. The 8 mg/kg group showed the greatest response to therapy, as 59% achieved a response of at least 20% reduction of joint swelling and pain on the American College of Rheumatology criteria (ACR20 response) compared with a 26% reduction in patients treated with placebo. In addition, 27% of patients went into remission compared with 0.8% of patients in the placebo group. The treatment groups saw a decline in the erythrocyte remediation rate and c-reactive protein blood levels and increase in the hematocrit and hematocrit, suggesting that the drug had an impact on the systemic inflammatory response underlying the disease.

Adverse effects of the drug were carefully monitored and reported in the study. Transient increases in hepatic enzymes and serious infections were noted to be slightly increased in the treatment group compared with the placebo. Importantly, there was a small but significant increase in the lipid levels of patients taking tocilizumab. Concerns have been raised that this therapy would confer a long-term increased risk of adverse cardiac events.

The OPTION study introduces a new promising treatment tool in a subgroup of patients with inflammatory rheumatoid arthritis. Long-term safety needs to be established and other measures of efficacy such as effect on radiographic progression of joint disease need to be studied. Furthermore, therapy with tocilizumab needs to be compared with treatment using other proven drugs such as therapies targeting TNF-α. Indeed, this may be the arrival of a new therapeutic agent in the treatment of methotrexate-resistant moderate to severe RA; however, the optimism surrounding the new class of IL-6 inhibitor drugs should be tempered with caution, as further research is needed to completely understand its efficacy, side effects, and therapeutic potential.

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Spine Research

Cortical Control of a Prosthetic Arm for Self-feeding

The complexity of brain machine interface (BMI) design, the almost “sci-fi” impact of the potential end product, and the merging of neuroscience, neurosurgery, and bioengineering in these pursuits combine to make the BMI motor prosthetic field one of the most high profile and exciting areas of scientific research. Advances in this field are frequently published in some of the highest impact journals and are widely disseminated by the popular press. It is important in evaluating this field to look at each incremental step in motor prosthetic development beyond the “wow” factor in order to determine how much closer we are to developing a functioning prosthesis to help paralyzed patients.

The quest to develop a thought-controlled motor prosthetic limb combines a number of difficult technical requirements. A brain signal with enough specificity and complexity to be trained and translated into multidimensional movements is necessary. The recorded neural activity must be maintained over time, without signal dropout due to glial scarring or loss of the recorded cells. The electronic technology will ideally need to be both miniaturized and also completely implantable, with remote transmission of information from the brain recording to the motor prostheses. Finally, sensory feedback from the prosthetic limb should be able to scale the motor output through the prosthesis.

The latest development in this field, published in Nature by Velliste et al. (453:1098–1101, 2008), represents a striking advance in one area of BMI motor neuroprosthetics. Researchers working at the University of Pittsburgh used intracortical microelectrode arrays implanted in the motor cortex of monkeys to record populations of single- and multiunit neuronal spiking activity. The authors translated the modulations in motor cortex neuronal activity that occurred when food was presented at different target locations to multidimensionally control a prosthetic arm. Amazingly, the monkeys rapidly learned how to control their own neuronal activity to feed themselves using the prosthetic arm. They were able to alter prosthetic trajectory when the food target was unexpectedly moved. They even learned novel behaviors with the prosthesis such as “finger” licking of the gripper portion of the prosthesis. This emergent behavior was not part of the experimental paradigm and represents embodied control of the prosthesis by the monkey.

This work by Velliste et al. in the Schwartz lab provides compelling justification for the enthusiasm accompanying BMI motor prosthetic development. It represents the state-of-the-art in multidimensional prosthetic motor control. However, significant requirements remain in all areas of...
As brain tumors have become the leading cause of cancer-related deaths in children under the age of 15 years, the emerging strategy of using the tumor tracking capacity inherent in many stem cell populations to deliver therapeutic agents to brain cancer cells has gained momentum among neuroscientists and neurosurgeons working on understanding these malignancies. The authors of the recent article, "A Novel and Generalizable Organotypic Slice Platform to Evaluate Stem Cell Potential for Targeting Pediatric Brain Tumors" advocate for a new brain slice assay within which to study the honing and migration of different stem cell populations to reach brain tumors (Cancer Cell Int 8:9–16, 2008).

The idea of using the tumor tracking capacity inherent in stem cells has broad potential applications from hematopoietic stem cells to human brain derived neural stem cells to umbilical cord blood derived stem cells. It has been known for some time that neural stem cells, when implanted into rodents harboring experimental gliomas, hone to the tumor beds (Proc Nat Acad Science USA 97:12846–12851, 2000) and may even suppress the proliferation of some tumor models. However, beyond this, little is known with respect to the mechanism of action through which this modulatory behavior is affected. This lack of understanding, combined with the well-described idea of using the cells as Trojan horses to deliver drugs or toxins, creates a huge experimental niche of in vivo experiments that are too costly and time consuming to perform in large scale animal trials.

An in vitro slice assay overcomes several inherent problems in large scale animal models. One such problem is the difficulty in tracking the stem cells once they are introduced into animals. These slices can be maintained for many months, and stem cells can be introduced and manipulated in a serial fashion. The cells can be tracked as they migrate and hone to tumor transplants. The foundation of the author’s assay is to establish a model which closely mimics the in vivo milieu, first to maintain stem cells in a quiescent state and then induce stem cells to produce targeting molecules and enzymes. The concept, however, has broad implications as a potential intermediate step between in vitro assays and rodent assays. These can be used to study inherent cellular, molecular and developmental stem cell properties which control the honing, adhesion, integration and engraftment of these multipotential cells within tumor cell populations.

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STEM CELL RESEARCH
On May 28, 2008, the National Institute of Neurological Disorders and Stroke (NINDS) announced a new program that may provide a much needed source of support for research training for neurosurgery residents. The ideal of the neurosurgeon as an “operating neuroscientist” has roots dating back to the initial conception of the “new specialty of Neurosurgery” by Harvey Cushing in the 1930s. Indeed, the incorporation of “protected/required” research time as an integral part of residency training is virtually ubiquitous in our residency training programs. It is believed that the future of our specialty is critically dependent on basic and translational investigation conducted by those who are uniquely qualified to address aspects of certain neurological diseases. However, this paradigm is constantly besieged by a multitude of factors, both economic and logistic. The amount of information that residents are required to assimilate is growing exponentially. For example, in addition to the traditional knowledge-base of neurological diagnosis, intensive care unit management, neurology, neuropathology, neuroradiology, and basic spinal, peripheral nerve, and cerebral surgery, neurosurgical residents are now required to develop a working knowledge of the “complex spine,” stereotactic radiosurgery, and endovascular neurosurgery. Rather than focusing efforts on acquiring research training during the research years of residency, many trainees are opting for “infolded fellowships.” In addition, dwindling department and institutional resources due to reduced compensation for services and the need to hire physician extenders have limited financial support for research. This is compounded by severe restrictions on the number of hours worked by residents. Without question, if our field considers that the concept of the neurosurgeon-scientist is still relevant in contemporary neurosurgery, the future of neurosurgery may be dependent on producing at least an adequate number of such neurosurgeon scientists.

Neurosurgery organizations such as the Congress of Neurological Surgeons and American Association of Neurological Surgeons have fellowship and research programs aimed at providing support for this critical period in the training of neurosurgeons. However, these programs are extremely competitive due to limited resources. The new NINDS program (RFA-NS-09-001) is aimed at providing support for mentored research for residents in neurosurgery, pathology, and radiology. A total of $1.5 million per year has been set aside to fund a total of 5 to 15 projects in the first year. The successful programs will be led by established neurosurgeon or neurologist scientists and will offer a structured experience in research training, as well as access to mentored projects. The trainee must begin the experience for at least 9 months during residency, and there is the option of continuation of funding during the fellowship. A total of $70,000 of direct cost can be granted. The deadline for application is September 10, 2008. This is a very timely, unique, and novel new funding mechanism for the resident experience. More information on this program can be found online at http://grants.nih.gov/grants/guide/rfa-files/RFA-NS-09-001.html.

The overall goal of NINDS’s research training and research education programs is to ensure that highly trained scientists will be available to conduct basic, clinical, and translational research on the mechanisms, cure, and treatment of neurological disorders. NINDS provides mentored career development awards (K08 and K23 mechanisms) to highly qualified clinician-scientists who have early training and experience in research, and who generally have one or more significant, original research publications. However, there is a need for a mechanism to support the early training of clinicians during the residency/fellowship period in research and related skills, which would enable them to compete successfully for the mentored career development awards. This Research Education Program is designed to foster the development of neuroscience researchers through both research training and educational experiences that will prepare clinicians to successfully compete for individual fellowships or mentored career development awards.

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Research Training and Funding