DOUPE LECTURE

Torsten O. Nielsen MD, PhD, FRCPC

Associate Professor
Pathology and Laboratory Medicine
University of British Columbia

Discovery research to clinical trial: A ten year journey

Abstract

Clinician-scientists have the training and motivation to translate basic science into tools for improved clinical care, but the road to achieve this is hardly straight forward, particularly for large scale genomic datasets. This year's Joe Doupe Young Investigator award winner, Dr. Torsten Nielsen, details successful examples of new scientific insights, diagnostics and clinical trials that have resulted from microarray-based gene expression profiling of sarcomas: TLE1 as a biomarker for synovial sarcoma, histone deacetylase inhibitor therapy for translocation-associated sarcomas of young adults, and CSF1 pathway inhibitors for tenosynovial giant cell tumors. Results from exciting, emerging next generation sequencing technologies will need to undergo similar validation and preclinical studies before they can be expected to impact patient care.

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Correspondence to:

Torsten O. Nielsen, MD, PhD, FRCPC
Anatomical Pathology, JP1502
c/o Rm 1401, JP Pavilion
Vancouver Hospital & Health Sciences Ctr
855 West 12th Avenue, Vancouver, BC (V5Z 1M9)
Email: torsten@interchange.ubc.ca

The year 2000 was full of excitement in the clinical research community, arising from the development of new microarray technologies that promised an entirely new and comprehensive view of the molecular biology of human disease. In many ways, the recent application of these next generation sequencing technologies to medical research has generated similar anticipation that major clinical breakthroughs are just around the corner. Clinician-scientists realize, however, that the actual clinical implementation of such basic science and discovery-based research is another matter entirely, requiring years of research and development, most of which are ultimately unsuccessful in changing clinical practice. The path is rarely direct or clear, requiring multiple steps not only in the science itself, but also in obtaining the training, funding, personnel, infrastructure and (what may be most difficult of all for clinician-investigators) time to organize and achieve these steps. In this article, I will relate the story of how a discovery research project I started as a resident eventually led to multi-institutional implementation of a new diagnostic test, and to two cancer-targeted therapy clinical trials that began accruing patients in autumn 2010.

Clinician-scientist training and research

I trained in the McGill MD/PhD program, but the key benefits of combined medical and scientific training can also be achieved by many other routes, including separate degrees, research-intensive residencies, clinician-investigator programs, and fellowship training. Clinical training confers a diverse knowledge and perspective on human disease, an understanding of the clinical thinking and decision-making process (including such mantras as first-do-no-harm, focus on diseases that are common and/or dangerous and/or fixable, and “if it doesn’t change your management, don’t order the test”), and an appreciation of the realities of patient care and clinical implementation of new technologies and ideas. Serious research training drives home the principles of proper experimental design (e.g., a positive result is meaningless without a negative control), imparts credibility as a researcher, develops practical skills in the writing and critical appraisal of papers and grants, provides plenty of experience in dealing with failure, and gives the trainee some extra time to observe mentors and role models before making critical decisions about what specialty to pursue and where to do it.

My inspiration as a child came from Terry Fox; I knew early on that I wanted to become a cancer researcher whose work would actually help people. Although I had not originally considered pathology, I came to realize that the day-to-day work in this specialty often concerns cancer. The pathology

profession offers access to vital tissue resources, relative control over my time, and places me squarely at the forefront of translational research because molecular biology advances translate most directly into diagnostics and biomarkers, relative to comparatively long timelines for drug development. For other diseases, different specialty choices might provide a better match between research and clinic. The UBC Anatomical Pathology program I joined had the additional advantage of offering large amounts of elective time for research.

Following the release of “The Chipping Forecast” special issue of *Nature Genetics*, detailing the impending microarray revolution [1], many researchers thought these new techniques might replace the traditional pathology techniques [2]. I was fortunate enough to do a research elective in England, in a laboratory developing and implementing its own microarray technology. Although this research time was productive [3,4], I quickly realized that experience was more important than equipment when it came to generating reliable results. Pat Brown at Stanford was one of the pioneers of cDNA microarray technology, and, with colleagues, had begun to apply these high throughput techniques to neoplastic diseases, leading to breakthroughs in lymphoma [5] and breast cancer [6], among others. By having credible credentials and by asking the right questions, I was able to transform a brief visit to California into a collaborative research project that quickly led to the world’s first large scale expression profiling study of soft tissue tumors [7]. This also exposed me to tissue microarrays [8], a new, complementary technology that opened up huge possibilities for translational studies that were easier to do in Canada than in the USA [9].

Among the key lessons I learned from these experiences during residency was the necessity of having both tissue specimens and linked patient data resources in order to perform effective translational research. Although new and exciting, few molecular tests could actually beat established clinical tools such as microscopic morphology. Time was the single most important resource to have, and I was able to leverage the successful residency research program into a short fellowship period to hone clinical subspecialty skills in a low volume, high interest area (sarcomas), and to write up my first grant applications, which secured an academic position with protected research time.

Starting a translational research program

The New Investigator funding, which I secured from the Terry Fox Foundation, seemed very appropriate for a sarcoma researcher, and I was most grateful for the chance to see if I could succeed as a clinician-scientist. Sarcomas are uncommon but

frequently large tumors, diagnostically challenging (encompassing nearly 100 subtypes), and, for the most part, they lack effective systemic treatment options. Nevertheless, sarcomas have a fascinating molecular biology that is highly favourable for translation into new clinical tools for diagnosis and treatment [10]. Our expression profiling studies [7,11] had helped crystallize the concept that nearly half of sarcomas have a well-defined molecular oncogenesis, often driven by specific point mutations or translocations. c-kit tyrosine kinase activating mutations had been shown to drive the biology of gastrointestinal stromal tumors [12], leading to the first effective drug therapy (imatinib) for this sarcoma [13,14]. I hoped to work toward similar advances for other sarcomas where such breakthroughs had yet to be made.

Among soft tissue sarcomas with a defined underlying molecular event, synovial sarcoma is the most common. This disease typically occurs in the limbs of young adults, and long term survival rates are disappointing (about 50%) due to the propensity for early and late metastases that are incurable with conventional chemotherapy [15]. Synovial sarcoma is characterized by a pathognomonic t(X;18)(p11;q11) event, fusing *SS18* (a chromatin remodeling protein associated with the SWI-SNF complex and transcriptional activation) to an *SSX* gene (which interacts with Polycomb proteins, leading to chromatin condensation and gene silencing) [16]. The net effect on transcription was unknown, nor were any inhibitors of *SS18* or *SSX* available.

In 2003, the research strategy for my new laboratory was to draw on expression profile data to identify key pathways, induced by *SS18-SSX*, that might be targeted by existing drugs never before tested in this disease. Immediately evident in the profile were genes involved in body segment patterning and mesenchymal differentiation, including retinoid-response genes [7,17,18]. These were of particular interest due to the breakthrough of retinoic acid-based differentiation therapy for promyelocytic leukemia [19], which also bears a fusion-transcription factor translocation (*PML-RAR α*). Although chemical and pharmaceutical retinoids were readily available for testing, it took well over a year for me to hire personnel, staff and equip my first lab, secure approvals and appropriate disease models, arrange materials transfer agreements, and generate results ... which all proved negative! My hypothesis was wrong. Neither retinoid agonists nor antagonists had significant effects on synovial sarcoma cell growth or death.

The value of the back-up plan

Fortunately my training in research and in grantsmanship had taught me at least two important things: each tested hypothesis

should have a back up plan in place in case it is wrong, and the research program should encompass multiple aims that are not dependent on each other's success. In leukemia, retinoid resistance can be overcome through the actions of histone deacetylase (HDAC) inhibitors, which, when tested by ourselves and others, appeared highly effective in synovial sarcoma preclinical models [20]. HDAC inhibitors are an emerging class of drugs that reverse epigenetic changes, frequently leading to reactivation of gene expression [21,22].

Concurrent aims to develop diagnostic tools for synovial sarcoma using tissue microarrays were making significant progress [23,24], identifying *TLE1* as the most sensitive and specific immunohistochemical marker yet known for synovial sarcoma [22]. This diagnostic test has since been adopted at multiple institutions worldwide [25]. Importantly, *TLE1* is known to function as a transcriptional corepressor in key developmental pathways. Thus, we began to view the critical function of the *SS18-SSX* oncoprotein as abnormal transcriptional repression rather than activation, and sought to identify repressed genes within synovial sarcoma gene expression profiles that might be critical to synovial sarcoma oncogenesis. By combining our primary tumor data with published *in vitro* model data [26], we were able to identify *EGR1* (a tumor suppressor gene with a role in muscle differentiation) as a direct target of the *SS18-SSX* oncoprotein [27]. Chromatin immunoprecipitation experiments demonstrated that treatment with HDAC inhibitors reversed *SS18-SSX*-mediated Polycomb recruitment and epigenetic gene silencing events, restoring expression of *EGR1*. *EGR1* then goes on to reactivate expression of the critical *PTEN* tumor suppressor gene, explaining the connection between synovial sarcoma oncogenesis and this well known cancer pathway for the first time [28]. Reactivation of *EGR1* appears critical to the mechanism of action of HDAC inhibitors in synovial sarcoma, as siRNA knockdown of *EGR1* abrogates drug-induced apoptosis. Thus, we finally had a drug that inhibits a direct action of the synovial sarcoma oncoprotein.

Translating the basic science into a clinical trial

The lack of effective systemic therapies for synovial sarcoma, coupled with the completion of phase I and II trials in hematopoietic neoplasms for several HDAC inhibitors, suggested an opportunity to move to human studies without necessarily requiring lengthy animal studies first to be performed. Synovial sarcomas are rare enough that no single institution will accrue enough cases for a reasonable trial design. Indeed, a major problem in sarcoma medical oncology has been the biologically-inappropriate lumping of all soft tissue sarcomas

TABLE 1. Translocation-associated sarcomas eligible for accrual into NCIC-CTG IND.200

Disease	5' partner	3' partner	Peak age	typical site	response to conventional chemotherapy
alveolar rhabdomyosarcoma	<i>PAX3 (or PAX7)</i>	<i>FKHR (or NCOA1)</i>	6-9	extremity (muscle)	+
alveolar soft part sarcoma	<i>ASPL</i>	<i>TFE3</i>	15-35	extremity (muscle)	-
clear cell sarcoma	<i>EWSR1</i>	<i>ATF1 (or CREB1)</i>	20-40	extremity (tendon)	-
desmoplastic small round cell tumor	<i>EWSR1</i>	<i>WT1</i>	20-30	abdomen	-
endometrial stromal sarcoma	<i>JAZF1 (or EPC1)</i>	<i>JJAZ1/SUZ12 (or PHF1)</i>	40-50	uterus	-
Ewing sarcoma	<i>EWSR1 (or FUS)</i>	<i>FLI1 (or ERG, ETV1, E1AF, FEV1)</i>	10-20	extremity (bone)	++
extraskelatal myxoid chondrosarcoma	<i>EWSR1 (or TAF2N)</i>	<i>CHN</i>	20-40	extremity (muscle)	-
low grade fibromyxoid sarcoma	<i>FUS</i>	<i>CREB3L2</i>	35	extremity (muscle)	-
myxoid liposarcoma	<i>FUS (or EWSR1)</i>	<i>DDIT3</i>	30-50	extremity (muscle)	-
synovial sarcoma	<i>SS18</i>	<i>SSX1 (or SSX2, SSX4)</i>	15-35	extremity (muscle)	+/-

together into studies, done to increase accrual rates and theoretical statistical power [29].

One compromise is to group only the most biologically-similar sarcomas together. Synovial sarcomas are part of a group of mesenchymal malignancies characterized by fusion transcription factor translocations (Table 1). All display a phenotype suggestive of blocked mesenchymal differentiation, and most present in the extremities of young adults. An evolving body of evidence suggests a role for abnormal transcriptional repression in these diseases [30,31]; indeed, as a group they appear highly sensitive to the action of HDAC inhibitors (based on results from our lab [32] and others [33,34]).

This work was presented at the NCIC-Clinical Trials Group meetings and caught the attention of the Sarcoma and Investigational New Drug committees. As a result of this attention and in collaboration with medical oncologists Quincy Chu and Elizabeth Eisenhauer, we have been able to initiate IND.200, a phase II study of the HDAC inhibitor SB939 in patients with translocation-associated sarcomas. This study has opened at multiple centres across Canada, with the first patients enrolled in October 2010. In addition to looking for objective response and progression free survival endpoints, correlative science projects embedded in the trial will determine the exact gene translocation present, and test expression of predictive biomarkers using tissue microarrays.

An even faster track to another clinical trial

The field of sarcomas incorporates many "orphan diseases," and gene expression profiling data almost certainly contains many other leads worthy of investigation and translation [35]. In the

case of the tenosynovial giant cell tumor, the combination of DNA and tissue microarray experiments, initiated in collaboration with Drs. West and van de Rijn from Stanford, led to the implication of aberrant macrophage colony-stimulating factor activity (induced by *COL6A3-CSF1* translocations) as the underlying biological event [36,37]. This finding immediately implied a potential therapeutic role for CSF1R inhibitors, another emerging drug class under intense development. Indeed, while we were developing preclinical models to test this hypothesis [38], a group in France jumped straight to "off label" treatment of refractory patients using imatinib (on the basis of its cross-inhibition of CSF1R), inducing at least one dramatic complete response [39]. A trial of the closely-related drug nilotinib began in autumn 2010 in the USA (NCT01207492) and in Europe for patients with relapsed or metastatic tenosynovial giant cell tumor, for which we will provide correlative science support.

Perspectives

By combining clinical knowledge of the problems that need addressing, with research skills on how to employ scientific tools to effectively answer these questions, clinician-scientists have the ability, and indeed the responsibility, to take on leadership roles in translational research. In some cases, where the scientific path is more straight forward, rare diseases may be more worth pursuing than common ones. Regardless, effective translation requires multidisciplinary collaborations including basic scientists, laboratory and diagnostic physicians, surgical and medical specialists, and statisticians. Actually implementing new advances into practice requires prospective studies that

often need to involve front line physicians, clinical trials groups, industrial collaborators, and health system policy considerations.

Microarray research drove many new discovery advances, and a decade later several have reached the point of clinical implementation in prospective studies. We are currently poised on the edge of a similar revolution, based on next generation sequencing technologies [40]. As costs rapidly decrease, medical scientists are gaining the ability to generate not only quantitative data about expression and copy number (in a digital fashion, superior to hybridization-based microarray methods), but also qualitative data about point mutations and other DNA sequence changes that underlie genetic contributions to disease. Generating vast volumes of data is easier than ever before, and the real trick again will be how to make sense of it all. Bioinformatics techniques play a large part, but in health research the ultimate meaning in the data will lie in improvements to patient care. This translational research will be one of the great opportunities and responsibilities for the new generation of clinician-scientists.

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