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Upfront

The Rise of ctDNA, Part One

New ctDNA assays could make more metastatic melanoma cases detectable

June 2017

A new blood plasma test that detects circulating tumor DNA (ctDNA) could help identify mutations in metastatic melanoma that are tough to spot using current methods, according to researchers at NYU Langone Medical Center, USA (1).

BRAF and NRAS mutations account for over half of the 50,000 melanoma cases diagnosed in the US – but what about the rest? Though telomerase reverse transcriptase (TERT) promotor sequence mutations appear in up to 85 percent of all metastatic melanomas (2), the high G-C content of the TERT sequence can make such mutations difficult to detect using more traditional sequencing technology. The problem – and potential – prompted David Polsky, senior investigator of the associated study, to try an alternative technology – mutation-specific droplet digital PCR – and successfully developed a pair of tests that can detect changes in two mutation hot spots in the sequence. The assays were able to detect TERT mutations with high sensitivity and specificity; in tumor and plasma samples from patients with and without metastatic melanoma, all cases were detected successfully, with no false positives – even with as little as 1 percent of the mutated ctDNA present in a 5 ml blood plasma sample.

The blood tests could offer an alternative to CT scans – and the resulting radiation exposure – and allow more convenient and frequent testing that covers a wider range of melanomas, explained Polsky (3). He is hopeful that, once validated, the tests will quickly see widespread use. "Our goal is to use these tests to make more informed treatment decisions and, specifically, to identify as early as possible when a treatment has stopped working, cancer growth has resumed, and the patient needs to switch therapy," he added.

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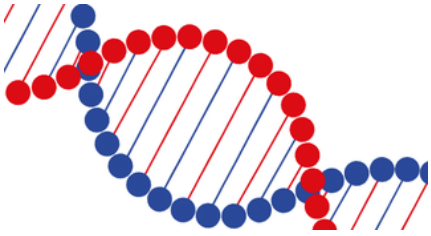
The Rise of ctDNA, Part Two

Circulating tumor DNA profiling can yield new insights into early-stage lung cancer evolution

June 2017

What do we know about the early stages of lung cancer? Not much, because most cases are only diagnosed in late stages, once the symptoms have become unmistakable – and even relapses are often missed at first. Given that lung cancer is both the most common cancer worldwide and the leading cause of cancer death, it's vital that we learn as much as we can about how the disease evolves – and what we may be able to do to detect and stop it early.



To that end, a group of researchers have performed circulating tumor DNA (ctDNA) profiling on the first 100 participants in the TRACERx (Tracking non-small cell lung Cancer Evolution through therapy) study, taking a tumor-specific, phylogenetic approach (1). What does that mean? The team were able to spot early predictors of ctDNA release, detect resistance to adjuvant chemotherapy, and identify patients likely to experience a relapse. But the method's power doesn't stop there – researchers were even able to keep track of the molecular profiles of recurrent and metastatic tumors, allowing them to observe the cancer's evolution and potentially opening the door to future personalized treatments.

The science isn't quite ready for prime time yet. Its sensitivity is constrained by tumor volume; the smallest tumors visible by standard imaging correlate with plasma ctDNA levels at the very extreme of current detection limits – and the cost of targeted ctDNA profiling is still a significant burden. But there's a clear need to improve current treatments, whose success rates are low and toxicities high. If ctDNA profiling can provide insights into which patients are most likely to relapse and which cancers are most susceptible to chemotherapy, then as technologies improve and costs drop, we may one day be able to offer every lung cancer patient the treatment most likely to yield a cure.

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Cracking a Cold Case

A 30-year-old medical puzzle leads researchers to develop a new molecular therapy

June 2017

Steven Francis, a patient at McGill University Health Centre, was at the center of a mystery. From an early age he had experienced fungal infections, an inflamed colon, shingles, respiratory problems, impeded growth, and a host of other problems. But no one could explain why.

At 33, he was referred to Donald Vinh, who went searching for answers. "When this patient was referred to me, I went over his entire file in detail, covering some 30 years and literally filling two large cardboard boxes. I also looked at his family history. Since the 1980s, many new immune deficiencies have been identified, and I was able to apply the knowledge from these advances to solve the case," he says.

And solve it he did – discovering that Steven had a mutation in ZAP-70. The ZAP70 protein helps to activate T cells and is critical for immune system function – and usually, mutations of the gene require a hematopoietic stem cell transplant for the patient to survive beyond early childhood. "Leaky" deficiencies in the gene are less common, with only a few cases reported in the literature. As stem cell transplants could prove risky for older patients, Vinh and his colleagues looked at a different approach: mutation-targeted molecular therapy.

Steven's specific mutation affects the splicing of ZAP-70, so the team designed an antisense morpholino oligonucleotide that targets the splice site generated by the mutation. This allowed the protein to be successfully synthesized ex vivo. If the treatment can be translated to humans, it could potentially improve immune system function.

Vinh is hopeful that the discovery of ZAP-70 mutations in adults, and the proof-of-concept study of a potential treatment, could lead to great advances in the field. "There are definitely more steps to take before we can test this treatment. For one thing, we have to convince the industry to support us. When Steven can finally get the benefit of the treatment, I'll be able to count this as a victory," he adds.

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Metabolic Mystery Revealed

A new genome-wide study shows that anorexia nervosa is not purely psychiatric – metabolic factors also play a role

June 2017

Anorexia nervosa is a devastating disorder – psychologically and physically damaging, tenacious in its grip on those diagnosed, and sometimes even fatal. It is usually diagnosed and treated by a psychiatrist – but now, new research asks: is the disorder exclusively a mental illness? A genome-wide association study (GWAS) conducted by researchers at the University of North Carolina School of Medicine has discovered strong correlations with psychiatric traits like neuroticism and schizophrenia – but, unexpectedly, also with metabolic features, such as insulin-glucose metabolism. Cynthia Bulik, Professor of Medical Epidemiology and Biostatistics at Karolinska Institutet and Founding Director of the UNC Center of Excellence for Eating Disorders, discusses her team's discovery of a significant locus for anorexia nervosa on chromosome 12 (1).

What's the importance of the newly discovered locus?

It's the first significant locus discovered for anorexia nervosa – in an area that has been previously associated with type 1 diabetes and autoimmune illnesses. As we have seen in other psychiatric disorders, the discovery of the first significant locus tends to mark an inflection point in genomic discovery. We are actively increasing sample size (13,000 cases currently queued for genotyping).

Anorexia nervosa has always been an enigma. Especially puzzling is how these individuals can reach and maintain such low BMIs. Moreover, we have had no explanation for how or why, after therapeutic re-nourishment, their bodies rapidly rebound to the previous low BMIs. It makes me wonder whether what we are seeing is, in essence, the opposite of obesity. Individuals who are obese and diet down to a lower weight are known to regain that weight (and more) – a phenomenon that has been described as a "high set point." It's possible that what we see in anorexia nervosa is essentially the opposite – the body returning to a low set point. To date, we have primarily turned to psychological explanations for this repeated loss of weight. Now, our data suggest that we need to explore metabolic factors as well. That was the biggest eye-opener for us. We hadn't anticipated that the associations with anorexia nervosa would be so strong.

Will this help diagnose or stratify patients with the disorder?

That's our hope. We have been notoriously ineffective in treating anorexia nervosa, especially in adults. There are no medications that effectively treat the illness, nor any that target the underlying biology (because, until now, it has been poorly understood). Of course, we hope that genomic discovery will lead us in the direction of biologically or genetically informed therapeutic options.

In the future, using other genomic techniques, we may discover that some cases of anorexia nervosa are more strongly metabolic than others – or more strongly psychiatric. The ability to distinguish between different "subtypes" could potentially help guide our therapeutic approach.

First, though, we need a much more thorough understanding of the disorder's genetics. The next step is to increase sample size and conduct additional analyses. We expect, based on GWAS for other disorders, that we will discover additional significant loci.

How did you bring together such a large collaboration?

The Psychiatric Genomics Consortium (med.unc.edu/pgc) was founded in 2007. It first focused on schizophrenia, major depressive disorder, autism, ADHD, and bipolar disorder. I watched their progress and decided that it was essential to develop an eating disorders working group. I could see that it was important to rapidly unite researchers and clinicians around the world in a quest to discover the genes that contribute to eating disorders.

What we've achieved so far is a brilliant example of what can be accomplished through global collaboration. It's so clear that we are scientifically stronger as a team than we could ever be individually. I hope other laboratory medicine professionals take the same route – together, we can accomplish so much!

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Hurdles to HIV Survival

Why are so many patients still dying too young?

June 2017

A recent study in The Lancet has reported that – thanks to the latest HIV drugs – young adults with the virus can now enjoy a near-normal life expectancy. Today, a 20-year-old who began antiretroviral therapy (ART) in the last few years is estimated to live 10 years longer than a 20-year-old who began ART between 1996 and 1999. But for many patients, barriers to treatment prevent access to this increased life expectancy.

"I became involved in studying HIV survival in the first job I had after my master's degree," says Adam Trickey, lead author of the associated paper (1). "The Antiretroviral Therapy Cohort Collaboration is one of the largest HIV cohorts in the world, an enormous network across the US and Europe that has been running for more than a decade and contains over 100,000 patients – so I leapt at the opportunity to work with them," he says. "Despite being new to the field when I started working on this paper, I was able to quickly learn the ropes as my many co-authors are all world-leading academics and clinicians in this area, with thousands of peer-reviewed publications between them."

Trickey and his colleagues analyzed data from 18 European and North American cohorts – a total of just over 88,500 patients. Some of the barriers to treatment they observed included high financial costs, people being unaware that they had HIV, and the stigma associated with HIV, which prevented people from attending treatment centers for fear of discovery.

For patients who did have access to treatment, adherence was another potential issue. "Survival appeared to improve for all groups of HIV positive people, with the exception of those who contracted the disease through injecting drug use, which tends to be a marker of social marginalization," says Trickey. "Other research has shown that this group tend to have worse adherence to treatment programs and more comorbidities, suggesting the need for interventions increasing access to opioid substitution therapy and treatment for Hepatitis C virus infection."

Another factor affecting life expectancy was how soon people started treatment. The research team estimated that those who had a high CD4 cell count one year after starting treatment – indicating that they started treatment early ¬– had a life expectancy approaching that of the HIV negative population.

"Today's antiretroviral drugs are clearly very effective, and more efforts and funding should be invested at both national and regional levels to provide this treatment for all people living with HIV," says Trickey, adding that focusing on screening could make a big difference; those people not receiving treatment account for the majority of AIDS-related deaths.

Research also indicates that there is another gain to public health to be made: those who receive treatment are far less likely to transmit the virus to others.

"I hope that our research reduces the stigma suffered by people living with HIV and increases the awareness amongst the HIV-positive population that by starting treatment and adhering to the regimens prescribed by their clinicians, they are likely to live a longer life," adds Trickey.

Antiretroviral Therapy Cohort Collaboration, "Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies", Lancet HIV, [Epub ahead of print], 2017. PMID: 28501495.

Stem Cell Stepping Stones

A world-first treatment for STAT1 mutations has a disappointingly low success rate – but shows real potential

June 2017

Researchers recently conducted the first study assessing hematopoietic stem cell transplant as a treatment for patients with gain-of-function (GOF) STAT1 mutations.

STAT1 plays an important role in the immune system, and GOF mutations can lead to an autoimmune response and bacterial and viral infections of varying severity. Most patients with mutations have mild or moderate symptoms, but around one in ten cases are life-threatening.

Who?

Fifteen patients between the ages of 13 months and 33 years took part in the trial. The study was carried out by an international group of researchers from countries including the US, Japan, the UK and Germany. Satoshi Okada, one of the authors of the associated paper (1), discovered the effect of GOF STAT1 mutations in 2011 (2).

How?

Treatment was given at a number of different centers across the world; a variety of donor sources and conditioning regimens prior to stem cell transplant were used.

Results?

The treatment had a success rate of 40 percent (see Figure 1); only six patients survived – five disease free, and one remaining symptomatic.

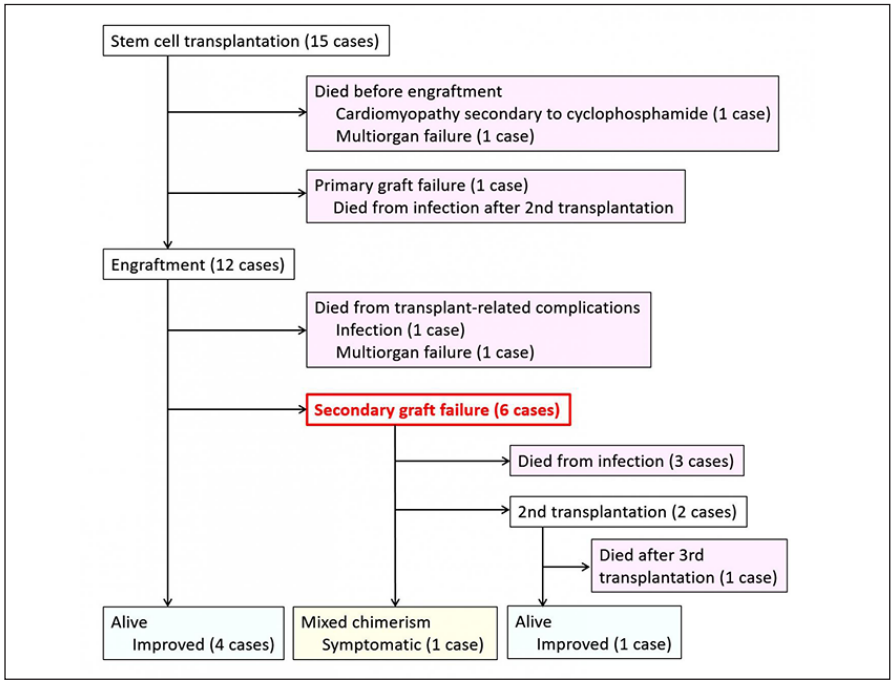

Figure 1. The prognosis of the 15 patients at completion of the stem cell treatment. Credit: Satoshi Okada.

In a press release (3), Okada said, "Overall, this result is disappointing – but the fact five patients were cured proves that treatment with stem cells can work, and we now need to learn from these 15 individual cases."

Why?

The researchers offered three possible reasons for the low success rate. First, following transplantation, the number of healthy cells shrank with time, allowing host bone marrow to reform and resulting in transplant rejection. Second, the type of conditioning treatment used also played a role; some harsh treatments extensively damaged patient tissue. Finally, younger patients had more positive outcomes than older ones, potentially because their immune systems were less weakened by repeated infection.

What's next?

The research team now plan to explore ways to improve the success of the treatment, including optimizing the methods used to eradicate host bone marrow, and aiming to perform transplants as early as possible.

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CEA: Overlooked and Underused

Could an existing blood test improve treatment choices – and outcomes – for some colon cancer patients?

June 2017

A Mayo Clinic study has found that a simple blood test could improve treatment for nearly one fifth of patients with stage II colon cancer; but many who could benefit from the test are not receiving it.

The test measures levels of carcinoembryonic antigen (CEA), which has an established link to patient prognosis, and can help to predict recurrence-free survival. But researchers found that it could also play an important role in selecting the most appropriate therapy for some patients (1).

Using information from the National Cancer Database, the team studied over 40,000 patients and found that the results of the CEA test could improve colon cancer staging predictions, raising the risk from average to high in 17 percent of stage II patients. The new classification could have affected potential treatment options, including the decision to use chemotherapy. The researchers also found that adjuvant chemotherapy following surgery appeared to reduce the increased mortality associated with stage II patients with an elevated CEA level.

"The decision to give a patient chemotherapy after surgery is not a light one, and physicians must weigh the risks and benefits," said Kellie Mathis (2), a Mayo Clinic colon and rectal surgeon and senior of the study. "There is no good reason for a physician to omit this blood test, and more work needs to be done to ensure that all patients receive it."

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From Acne to MS

A recent clinical trial finds a new use for an old antibiotic

June 2017

Multiple sclerosis (MS) is a demyelinating disease with no cure. Some therapies are available to alleviate symptoms, which range from the physical to the psychiatric, but adverse reactions and drug costs mean that improvements are needed. We spoke to one researcher who helped to uncover a new potential therapy for MS: minocycline, a broad-spectrum antibiotic that is commonly used to treat acne. Luanne Metz, first author of the associated paper (1), tells us more.

Where did the idea to treat MS with an acne drug come from?

My collaborator, V Wee Yong, studies mechanisms of pathogenesis in MS. He was studying the way that inflammatory cells cross from the bloodstream into the brain. Specifically, he was studying enzymes called matrix metalloproteinases that facilitate this migration, and he found a report that these enzymes are inhibited by minocycline. He tested this in the lab, and we performed several human trials that confirmed the benefit of minocycline. By the time we started this recent trial, it would have been unethical to do a placebo-controlled trial in relapsing-remitting MS, so we studied patients who had suffered one clinically isolated demyelinating event (such as optic neuritis or myelopathy) – also known as a clinically isolated syndrome (CIS) – and a change in their MRI scan. We knew these candidates had a very high risk of MS but did not yet have the disease.

Why are new treatments needed?

Current options for MS depend highly on where you live and what insurance you have, and prices can vary tremendously. In Canada and the US, we have interferon beta and glatiramer acetate for CIS but Canadian provincial insurance doesn't cover either in many areas. For relapsing-remitting MS, we have injectable, oral and infused therapies, but they are expensive (in Canada from CA$18,000–34,000 per year, and in the US over US$60,000 per year). Inexpensive therapies are desperately needed, both in North American and worldwide, as many people can't afford existing treatments. By comparison, in Canada the cost of minocycline is less than CA$600.00 per year, and can be prescribed by any physician on the day of diagnosis. And from the results of our trial, the effects of using minocycline are similar to those of first line approved therapies – however, we will need further studies to determine if the benefit holds at 24 months.

What potential impact could this medication have on patients with MS?

This medication can now be prescribed to people experiencing a first event that is suggestive of MS to potentially prevent further disease activity, such as another relapse or evidence of ongoing brain inflammation seen using MRI. These relapses leave behind permanent deficits 40 percent of the time. MRI activity indicates higher risk of further relapse, and MRI lesions can accumulate and contribute to cognitive dysfunction. But early treatment has the potential to be preventative – and an affordable, widely available drug could mean that many more people will have access to it.

What are the next steps?

One challenge we will face going forward is funding – because this is a cheap generic drug, it will be hard to find funding outside of governments or not-for-profit organizations. But we are hoping to obtain funding to confirm our results and demonstrate that minocycline has a longer-term benefit for patients. We also hope to explore the effect of minocycline on the gastrointestinal microbiome, and to do another study trialing minocycline along with glatiramer acetate.

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In My View

Embracing the Proteogenomic Toolkit

To win the war on cancer, we need to put proteomics on an equal footing with genomics.

By Andreas Hühmer, Director, Proteomics and Metabolomics Marketing, Chromatography and Mass Spectrometry, Thermo Fisher Scientific, USA.

June 2017

Advances in our understanding of cancer biology through gene expression analysis have resulted in major steps towards the goals of reliable and effective cancer diagnosis, prognosis and treatment. But despite the progress we've made over the past few decades, many would justifiably argue that genomics has not fully lived up to its promise.

Although a number of cancer-driving gene mutations have been identified through the genomic characterization of tumor tissue by large-scale projects such as the Cancer Genome Atlas, the widespread identification of targetable cancer drivers remains a significant hurdle. For metastatic breast cancer, for instance, few validated oncogenic drivers exist (1). Moreover, establishing whether gene mutations are cancer "drivers" or "passengers" continues to be a challenge – and is difficult to determine based on genomic assessment alone.

Genomics has taught us that cancer is far more complex than we previously thought. The tumor microenvironment is highly heterogeneous, with significant variability even between individual cancer cells (2). This complexity is compounded by the fact that cancer is dynamic; taking a tumor sample and sequencing its genetic contents merely produces a snapshot, not the blueprints for future tumor growth. The apparent lack of correlation between the genome and phenome highlights the need for a complementary proteomic approach to unravel cancer's complexity.

Meanwhile, our ability to map out the proteomic landscape within tumor tissue has steadily grown over the past two decades. Advances in mass spectrometry and informatics now allow us to study protein samples on an unprecedented scale. The latest liquid chromatography-mass spectrometry (LC-MS) technologies, coupled with new multiplexed proteomics approaches based on isobaric labeling and advances in data processing, are leading to improvements in the depth and speed of quantitative proteome profiling – all while using ever smaller sample volumes (3).

But it's when these two approaches are used in combination that we can make the most progress. Proteomics techniques are now being used alongside genomic analysis to help unlock new cancer immunotherapies far more quickly than conventional approaches (4). Traditionally, the search for targetable cancer antigens was a time-consuming and error-prone process, involving DNA sequencing and mutation prediction algorithms, followed by large-scale immunological assays. Using mass spectrometry to profile peptides directly, we can reduce that timeline to a matter of weeks.

Advances in proteomics technologies are also driving improvements in

cancer biomarker discovery. Recently, groundbreaking research by the Karolinska Institute in Sweden demonstrated the potential of integrating both protein and genetic markers in a single test for prostate cancer (5). And one goal of the US' National Cancer Moonshot program's Blood Profiling Atlas project is to develop a readily accessible database of blood biomarkers that will make it easier for oncologists to diagnose patients using liquid biopsies.

Genomics will continue to play an important role in cancer research. However, it is becoming clear that gene expression analysis alone is unable to sufficiently advance our understanding of cancer biology necessary for truly effective patient stratification and personalized therapy. A decade of technological development has made proteomics research-ready; we must now fully use the whole proteogenomics toolkit to truly make inroads on the fight against cancer.

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Targeting Tuberculosis

Existing drugs can – and should – be repurposed to fight neglected diseases.

By Santiago Ramón-García, Principal Investigator at the Research Agency of Aragon (ARAID), Zaragoza, Spain, and Charles Thompson, Principal Investigator at the Thompson Lab at the University of British Columbia, Canada.

June 2017

Although entering a new era of innovative and personalized medicine in industrialized countries, we still rely on drugs developed more than 50 years ago to treat neglected diseases such as tuberculosis (TB). Since then, only two new drugs, Sirturo (bedaquiline) and Deltyba (delamanid), have been approved for treating TB. Because they are not known to be more effective than traditional frontline TB antibiotics, they are only used to treat multidrug or extensively drug resistant cases, which sometimes are incurable. In recent years, governments and pharmaceutical companies are recognizing an urgent need to improve current TB treatments. In addition to the well-recognized challenges of drug development, TB antibiotic development is particularly limited for a number of reasons, including:

- The causative agent of TB, Mycobacterium tuberculosis, is intrinsically resistant to most available antibiotics.
- TB is an airborne infectious disease that requires research facilities equipped with expensive, biosafety level 3 infrastructure, as well as dedicated, trained personnel.
- TB mainly affects developing countries lacking in resources and infrastructure. It was not until recently that major US-based organizations invested in basic and applied TB research. Unfortunately, the European Union neglected TB funding in its Horizon 2020 program.
- The current reward system for drug development is based on company profits from blockbuster drugs that are developed to treat chronic diseases in the industrialized world. Antimicrobials in general are not a good business investment under this model because treatment typically involves inexpensive drugs for just a few days or weeks – and in the case of TB and other neglected diseases, the cost of treatment must be minimal.
- There are only a handful of pharmaceutical companies with TB research in their current portfolio – many others have discontinued TB projects over the past decade.

In view of these challenges, new innovative approaches need to be introduced to quickly deliver new effective therapeutics to patients in need. To minimize the cost of developing new treatments for TB we combined two innovative concepts: drug repurposing and synergy. These concepts for treating TB originated more than ten years ago in the laboratory of one of the authors – Charles Thompson, at the University of British Columbia, Canada. Santiago Ramón-García joined him there in 2007 as a postdoctoral fellow to start the drug discovery program, searching for inhibitors of mycobacterial proteins that confer intrinsic antibiotic resistance. In 2011, we demonstrated that antibiotics with no significant activity against M. tuberculosis could be repurposed for TB therapy if administered in synergistic

combinations (1). A screen of a library of FDA-approved drugs, including around 150 antibiotics, identified lead compounds that increased the activity of an antibiotic (spectinomycin) that M. tuberculosis was able to resist. This led to the realization that available drugs, especially antibiotics, commonly act in synergy with one another against M. tuberculosis. In some cases, compounds used for other therapies had also their own inhibitory activities against M. tuberculosis. Recently, we also reported in vitro activity of cephalosporins alone and in combination with other antibiotics (2).

After a long period of screening, discovery, characterization, and development, we received funding from the Tres Cantos Open Lab Foundation to further develop this program. Santiago worked for two years in Spain at the GlaxoSmithKline (GSK) screening facilities with a focus on repurposing beta-lactams (and in particular cephalosporins) for TB therapy. Our observation showing that first-generation cephalosporins were active against M. tuberculosis was remarkable because they have been available for over 50 years – but no one previously noted their potential against TB. There is now a vast space to explore, including investigations of other beta-lactam families (a recent clinical trial led by GSK validated the potential of beta-lactams for clinical use [3]) and a vision: to translate in vitro activities of cephalosporins into clinical efficacy.

Cephalosporins could be effective antibiotics; however a single drug will be insufficient to control TB, especially in the long term, and we need to continue to fill the development pipeline. Clearly, more funding and commitment from all stakeholders (including funding agencies, governments, academics and the industrial sectors) are needed if we are to reach the WHO's goal of TB elimination. To this end, it is imperative to foster public-private partnerships such as the TB Drug Accelerator (TBDA) initiative, a groundbreaking partnership between pharmaceutical companies, research institutions, and the TB Alliance.

Given that drug development is a long and expensive process, repurposing old drugs for neglected diseases is a promising avenue. However, this area is neither sufficiently profitable to attract companies nor appealing to academic scientists supported by research grants that rely on publication and short term public disclosure. We believe more efforts and funding should be dedicated to this largely ignored and unexplored avenue, not only for TB, but also for other neglected diseases.

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Getting Smart with Clinical Trials

The use of liquid biopsy technology could help identify more qualified candidates, speed up recruitment, and even boost trial success.

By Joy Yucaitis, Senior Director, Oncology Strategy, Novella Clinical

June 2017

For oncology trials, liquid biopsy technology holds great promise. Applications include screening patients for trial enrollment, monitoring the success of therapy, and diagnosing disease recurrence. Beyond clinical trials, as the technology advances, it could be used to find cancers in their most nascent stages and inform prognoses.

Liquid biopsy involves analyzing blood or other bodily fluids, such as urine, saliva, or cervical fluid, for genetic information that provides indications of disease state. In cancer, the most frequently used sources of this information are circulating tumor cells and circulating tumor DNA (ctDNA).

Tissue biopsies and imaging are the current gold standards for cancer diagnosis. Imaging tests can identify masses, but they can't find microscopic metastases or characterize a solid tumor's cellular composition. For that, you need tissue biopsy: a sample of tissue is removed using a needle, endoscope, or surgery, and is prepared, either as formalin-fixed paraffin-embedded or frozen samples. Tissue gathered in this way allows for histological analysis of cell shape, location, and concentration. It can also be used to determine mutation composition.

However, tissue biopsies have drawbacks – they're invasive for the patient and time-consuming for the scientist. A recent study reported a median span of 27 (1) days between ordering a test on non-small cell lung cancer (NSCLC) patients with acquired resistance, to receiving the results. Liquid biopsy, on the other hand, is less invasive, less expensive, and significantly faster, taking just three days from the blood draw. It's also more revealing than imaging; ctDNA, which can foreshadow resistance, can show up

as early as 10 months before the tumor's mass is captured by imaging (2).

That said, liquid biopsy also has downsides. For instance, similar to tissue biopsy, it can produce false negatives. Tumors are not homogenous, and not all tumor material finds its way into bodily fluids. Therefore, it is still necessary for clinical trials to corroborate liquid biopsies with tissue biopsies and imaging.

In clinical trials, I believe liquid biopsy would provide the most immediate value in the screening of patients for studies for targeted therapy, especially when tissue samples are not available. Currently, patients who are not eligible for needle biopsy and do not have adequate archival tissue samples are excluded from studies of targeted therapies, as the available methods cannot confirm that their cancer carries the targeted mutation. Therefore, liquid biopsy could potentially expand the number of patients who could benefit from targeted treatment studies.

Another area in which I see liquid biopsies being successfully implemented is in monitoring cancer progression. Liquid biopsy can be used to reassess patient response to treatment with each blood draw. Because it can detect tumor progression or shrinkage long before imaging, it allows for earlier treatment modification or intervention. In fact, a recent study (3) showed that liquid biopsy could determine the effectiveness of immunotherapy within two weeks of treatment. The researchers saw an increase in ctDNA levels in the blood, which indicated the tumor cells were dying. Because immunotherapy can often result in the appearance of the tumor growing larger ("pseudo-progression") liquid biopsy may be a useful tool for physicians by providing a more objective assessment of treatment response. Providers may also be able to identify the persistence of micro-metastases that increase the risk of recurrence, but cannot be identified through standard medical imaging.

Beyond speed and sensitivity, liquid biopsy can help physicians identify cancer patients at the greatest risk for recurrence, and use that information to inform treatment decisions. In a recent study, investigators harnessed the technology to determine the prognoses of patients with Stage II colon cancer with no metastasis (4). After surgery, they found that the presence of target ctDNA correlated with relapse. Physicians could use this information to either reassure patients who are unlikely to experience a relapse, or more expediently start post-surgical treatment on those with an increased risk of recurrence. Such data may push clinicians to more confidently adopt liquid biopsy technology.

And for companies hoping to commercialize liquid biopsy tests? They must demonstrate to regulatory agencies that liquid biopsy-guided treatment positively affects patient outcomes.

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The Importance of Chiral Metabolomics

Chiral amino acids, metabolites long overlooked as "unnatural," are now under the spotlight – as biomarkers for kidney disease.

By Tomonori Kimura, Department of Nephrology, Osaka University Graduate School of Medicine, Osaka, Japan.

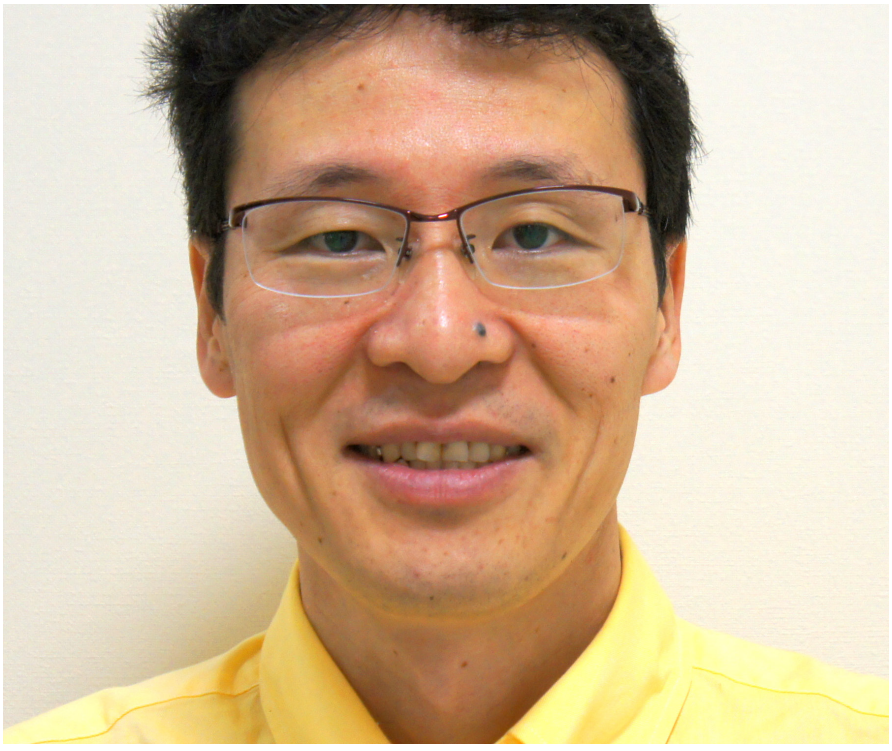
June 2017

Chronic kidney disease (CKD) is a highly-prevalent, global health problem; for example, in Japan, it is estimated that about 10 percent of the population have CKD. The number of patients with worsening kidney functions, eventually requiring costly kidney replacement therapy or transplantation, is increasing. In addition, the risk of life-threatening cardiovascular diseases increases with the progression of CKD stages. Preventing CKD patients from progressing to end-stage kidney disease is therefore critical, but unfortunately there are no effective methods to predict the progression of CKD. Currently, prediction relies on kidney functions estimated from serum creatinine and some additional information, such as proteinuria, but these are insufficient. Naturally, nephrologists are earnestly searching for better biomarkers.

Could amino acids, those vital components of human bodies, help provide the answer? The levels of amino acids (which comprise 20 percent of the body) are influenced by the functions of many organs, including the kidneys; kidneys regulate the body's amino acid balances via reabsorption. Scientists

have been studying amino acids ever since their discovery – for more than a hundred years. But because people only detected L-forms in nature, D-amino acids were regarded as unnatural and have not been studied vigorously. The presence of D-amino acids started to be reported sporadically, including in the blood of patients with kidney diseases. Some studies also indicated the physiological roles of D-amino acids in bodies (for example, D-serine is also known as a neurotransmitter of NMDA receptors in neurons) but once again these reports were sporadic – mainly because of the measurement challenge; typically, the amount of D-amino acids in human bodies are present at trace levels, and the chemically similar nature of amino acid enantiomers makes it difficult to separate them and measure them simultaneously. And because reliable methods to measure D-amino acids are lacking, their functions and presence in tissues have remained a mystery.

It is only recently that methods have been devised to measure D-amino acids precisely via a metabolomic approach. Kenji Hamase of Kyushu University in Japan and his colleagues went to great lengths to develop a metabolomic platform – based on micro-2D-HPLC – that can detect whole sets of chiral amino acids from human samples with precision. In the first dimension of HPLC, 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F)-labelled amino acids are separated by reverse-phase separation. Then, the fraction of each NBD-derived amino acid is automatically transferred to the enantioselective (chiral-selective) column for chiral separation. The 2D-HPLC system is powerful enough to detect all amino acid enantiomers from clinical samples ranging from around 1 fmol to 100 pmol – quantitatively.



Our research group from Osaka and Kyushu University searched for prognostic biomarkers of CKD by using such chiral amino acid metabolic profiling. Our study revealed that D-amino acids, particularly D-serine and D-asparagine, were robustly associated with the progression of CKD to end-stage kidney disease. The risk of progression to ESKD was elevated from two- to four-fold in those with higher levels. What is more interesting is that this trend is only seen in D-amino acids, and not in L-amino acids. The fact that just a trace portion of amino acids have a stronger relationship with disease processes and prognosis strongly supports the importance of chiral separations.

A D-amino acid test could provide a powerful tool for clinicians, helping them identify high-risk CKD patients for intensive care. The development of a device suitable for clinical use – designed to increase throughput – is currently under way. Another important direction for the future will be undertaking further detailed research to study the physiology and metabolism of D-amino acids, both of which are poorly understood, so that we can enrich our understanding of kidney diseases. Through chiral metabolomics, I believe that the mysterious world of D-amino acids will turn out to be a fruitful one for clinicians.

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Translated

How to Disappear Completely

Developing stents for coronary artery disease that don't outstay their welcome.

June 2017

In July 2016, the FDA approved the first absorbable stent for coronary artery disease: the Absorb GT1 Bioresorbable Vascular Scaffold (BVS) system. The Absorb GT1 is a non-metallic stent that releases the drug everolimus to limit the growth of scar tissue, ¬and – as its name suggested – is gradually absorbed by the body after implantation. Here, Richard Rapoza Divisional Vice President of R&D for Abbott Vascular, discusses the road to approval...

How did you come to work on Absorb?

After an undergraduate degree in chemical engineering, I started to consider further study in biology or medicine. So I went back to grad school and signed up for a PhD program in biomaterials, and studied the interactions of polymers with blood. After I graduated, I got a job creating coatings for medical devices that would prevent blood clots from forming on their surfaces. Since then, I've had the opportunity to work on various cardiovascular implants, with the latest being Absorb.

How long have you been a part of the project?

It started in 2003, and I joined the team in 2006 when we had the six-month first-in-human results. We had a small set of patients, mainly in New Zealand and Europe, and at six months the imaging results looked very good; the company was ready to commit to a higher-level effort. They assigned me to make that happen and, over time, the group developed from the original 30 people to almost 350.

What attracted you to the device?

Actually, when I learned about it, my first thought was "it will take a miracle to get this working!" I could see a technical path to implementing it, but I knew there would be many variables to adjust, and I couldn't see any easy answers as to where those different variables might land.

We took it one step at a time, at first making best guesses as to what needed to be controlled and how that could be achieved. We soon learned that there were several key parameters, and recognized that, if we kept those under control, everything else would fall into place.

Can you tell us more about the development process?

First, we asked ourselves about the properties of metallic stents that make them effective. Long term, you're really only wanting the stent to remain stable while not causing any adverse reactions. The short term is all about performing its crucial functions: it has to push the plaque out of the way, and to deliver enough drug to control the tissue reaction. Then, as with any permanent foreign body, the best you can hope for is that the body will adjust to it.

Next, the question became: how long does our stent need to look, feel and act like a metallic drug-eluting stent, before the body can take over? Our literature reviews suggested that we needed to keep the diameter of the blood vessel

constant for the first six months, and the vessel could then take over. It was key that the polymer structure would need to stay intact, even in the presence of degradation, for at least six months.

What are the long-term drawbacks to metallic stents?

As we discovered in our research, blood vessels only need support for a certain amount of time, and the presence of the implant past that window is actually detrimental to the healing of the artery. A permanent metallic cage prevents the vessel from dilating when you engage in physical activity – the diameter you're left with will always stay the same. You could make the argument that the target demographic (mainly elderly people) don't necessarily engage in a lot of difficult physical activity, but the reality is that the age of the population in need of this intervention is going down. So it's important to get the implant out of the way as soon as possible to restore activity in the vessel.

A more practical consideration is that many patients with metallic stents experience restenosis and may need repeat procedures, which are not easy to perform when there is already a metal cage in the vessel. You can run out of room because you can't keep dilating the blood vessels with more implants – after two or three stents you run out of options. But with an absorbable stent, the patient can be treated four or five years later as if their lesion is brand new.

What were the biggest challenges?

Perhaps surprisingly, the biggest issue wasn't technical. Instead, it was the mindset we faced. Once you get used to a technology that works (in this case, ten years of using metal stents) it affects everything. The methodology, all of the specifications, what's considered good or bad – it's all defined around a permanent structure. A good example is that most trials have looked at the diameter of the metal at implantation, then gone back to remeasure at six months. You then subtract new diameter from the original diameter and, if you assume the metal is not corroding or disintegrating, the difference between the two is the amount of tissue that has grown inside. But with a polymer implant, when you go in six months later, lo and behold, the body may actually have made the stent bigger ¬– so subtracting diameters means nothing. Interpretation of our data has to be different, and this can perplex physicians, who ask us how it can grow – it's not a permanent diameter and it's not metallic, we reply. The wrong mindset can throw even the best idea into a spin. We had to change everything – the interpretation of our clinical trial and engineering data, and also how we think and speak about the stent to people – so that our efforts wouldn't be hampered by stagnant thinking.

Is there potential for the technology to be applied to other areas?

Absolutely. We were approached about using the material in infants with fluid accumulation in the ear canal. In theory, a smaller version of the product could be implanted through the throat into the back of the ear canal to drain the fluid that's causing the pressure imbalance, and possibly also deliver an anti-inflammatory drug to the canal to ease the swelling. We haven't had the bandwidth to pursue the idea yet, but I do think it could be a brilliant approach.

Looking purely at drug delivery, because the stent is temporary, there are many possibilities. We have looked at an application for glaucoma – putting a temporary implant behind the eye that could deliver the drug where it is needed, but then eventually disappearing. We'd have to reconfigure the geometry of the implant, but it's another really appealing idea.

You've now been working on Absorb for over ten years – how have you found the process?

When you look back it seems like an awfully long time, but when you're in the middle of it, it seems like that's the way it should be! We had a lot of steps to work through – the first six month's results improved our understanding of what was happening inside the artery. It took us about a year to make the technical corrections required – changes to the chemistry and geometry of the stent, for example. After that, we were ready for a larger clinical study. It was great to see from the six-month results for the second set of patients that our corrections had been effective. At that point, we were ready to invest in a larger effort – getting pivotal trials underway, scaling up manufacturing – everything required to make the device commercial. And we weren't aiming for one or two countries, we wanted to gain approval in the EU, in the US, and in China and Japan.

So you can imagine: one year of technical adjustments, six months of follow up on those patients plus a year to enroll them, and you're already at two and a half years. Add on FDA negotiations, setting up a US trial, more follow up...

How did it feel to get it out into the clinic?

It was wonderful to see patients being treated. You really lose track of what you're doing when only analyzing data table after data table, while figuring out what experiments to do next.. It's completely different when you actually shake hands with patients – it's a different world. My first opportunity to meet a patient was in Italy. He had participated in a trial, and came back to do an interview with the press. The physician who implanted the device was attending, and they very graciously invited me to come along. He was a young guy of only 42, but his father and uncle had both died of heart attacks. He had a relatively simple lesion with some symptoms, but had not yet suffered a heart attack. For him, it was the perfect solution– it fixed his problem but didn't come with the longer-term risks and potential complications of a permanent implant.

What's your biggest lesson learned?

If I think back ten years, I kept envisioning the point where we would get approval in a major country. When we finally got EU approval, it was so satisfying to see all the discussions and details pay off. In the US, you have to have a panel meeting with experts who judge your data and reach a conclusion about recommending approval, and I imagined myself in that meeting many, many times. So one big lesson learned is that you have to visualize where you're going. But perseverance is the most important thing – with the right mindset, I believe you can achieve almost anything.

Toolbox

Pulling Back the Pre-Clinical Curtain

Are mouse models the best option we have?

June 2017

The pre-clinical mouse model is an essential part of many translational medicine journeys, providing preliminary efficacy results that often determine if treatments are able to make the leap into clinical trials. With the growing availability of tools and techniques for genetic recombination, mouse models appear to allow access to increasingly relevant results... or do they? Some researchers argue that alternatives to animal models are overdue, others believe that they're still the best pre-clinical option.

Can we gain more insight about the effectiveness of modern mouse models by 'pulling back the curtain' to see how these living pre-clinical pathways are created? Here, we speak with John Couse, portfolio director at Taconic Biosciences, to discuss how the shifting techniques and technologies in bioscience affect – and are affected by – mouse models.

Could you walk us through a typical mouse model process?

It begins when a customer liaises with a team of scientists to determine exactly the kind of traits they want to configure. A discussion on the best possible solution ensues; there are many various permutations and options for each particular need. Model generation takes a long time for a couple of reasons. Firstly, many pharma companies, biotechs, and even academics understand the model they're looking for, but often don't understand how they might get there. Secondly, extreme care is needed; a small misstep could lead to a minimum setback time of three to six months. Caution is all-important.

Once you've decided on the particular tool that you're going to use, whether it's CRISPR, homologous recombination, targeted transgenic, or something else, the process becomes more straightforward.

When you get down to designing a model to meet a customer's specifications, it's actually a relatively predictable process; the DNA level of the procedure, once underway, can be generally quite smooth, but the complexity can soon ramp back up.

Overall, it can be a lengthy process, with initial model generation taking 6–18 months, and the breeding portion taking an additional 9–24 months.

When breeding a genetically engineered mouse model, in many cases – at least for us – it's the first time that they've been generated, so they are absolutely novel models. There's no option to go back into the literature and say "this animal's going to behave this way", so the breeding and production portion of the process becomes a lot more unpredictable. Though our scientists are hugely knowledgeable and have seen just about every possible problem, even their combined expertise can't predict every downstream permutation during breeding, so there is a little trepidation at that stage.

There are clear difficulties when translating mouse model results to humans...

In the end, they are only mice. Even rats, which are arguably physiologically closer to humans, are far from the ideal scenario. I think difficulty in translation will always exist, and the gap between

Profile: John Couse

From a research standpoint, I grew up at NIH, as I had the opportunity of staying within the same field and laboratory for almost 18 years. It was a wonderful experience where I carried out basic research, but after all that time I reached a point in my career where I wanted new challenges and growth opportunities. I wanted to see a different part of science and how it applied to medicine and human health – to see how it was applied towards drug delivery, which led me to Taconic.

As portfolio director of Taconic's custom scientific services, I essentially lead the services focused around generation of genetically engineering mouse models from the initial idea and conception, all the way through to breeding and generation of study cohorts that will eventually feed a pipeline of research for those models.

the model you're using and how you want to apply the data to improve and impact human health is always going to be relatively large.

Despite that gap, I think it's certainly easier to translate mouse models to clinically relevant results than it is to convert in vitro data. In the last 10 years, the field has really taken huge strides to develop models that decrease the disparity, such as the generation of humanized mice – cases where we're able to put large human genomic inserts into the mouse genome, while simultaneously knocking out the endogenous mouse locus. And when you start to develop animals that have multiple insertions of human genes, your model presumably becomes even more predictable of human physiology. But again, it is just a mouse model so there are limitations on how many – and which – human gene inserts you can feasibly include. Overall, I think the ultimate goal for any scientist working with pre-clinical models is to close the gap as much as possible.

Some say mouse models are outdated because of that gap. What's your view?

Although I don't agree, I can understand the argument. But with the constraints of time, budget, and resources, it is tempting to focus on in vitro tools only and avoid the use of rodent models. However, the limitations of in vitro tools are as obvious as those related to mouse models.

Right now, at our disposal we have in vitro and in vivo tools via CRISPR, homologous recombination, organ-on-chip devices and more. They all have their advantages and disadvantages, but being able to access all those tools is a definite benefit.

A researcher may argue that organ-on-chip has advanced away from animal models in certain fields – for example, toxicology – and I'd tend to agree. But when you start to move into other therapeutic areas that require a more physiological system, such as endocrinology, it's very difficult to mimic within a chip. The disadvantages are even more evident when looking at the fields of behavior and neuroscience, because what you can't necessarily do on a chip is look at the developmental and activational roles of genes – something that is much more attainable in an animal model.

Would I say the mouse is outdated? No. Would I say that there are limitations on the use of mice? Absolutely. But again, there are limitations on every tool when viewed in isolation.

On the other side, mouse models are relatively easy to produce. We understand the mouse better than any other laboratory animal model when it comes to genetic engineering – especially using homologous recombination – and it's relatively inexpensive, compared with other animal models. So whether the mouse model is outdated is not necessarily relevant. Despite one's view here, we should always be searching for better or complementary alternative solutions to generate better translational data. Maybe that lies with another animal model – or something else entirely. But until we get there, I think a mouse model is one of the best tools in a scientist's arsenal.

What is the most exciting development in the pre-clinical research space?

There's a lot of excitement about CRISPR/Cas-9, and I think it's absolutely warranted. The technology is really quite amazing, both in its simplicity and its power – which I don't think we have a full understanding of yet.

In regards to CRISPR's application in research, it allows the potential to quickly mimic certain human diseases,

especially the rare ones, in mice and rats. Human conditions that we believe are attributed to specific point mutations in human genes. In the past, these disease models were less often generated because of the difficulty and expense of trying to create them through homologous recombination. But CRISPR is specifically adept at producing these single point mutations quickly and inexpensively, allowing for recapitulation of rare diseases of genetic cause.

The advantages become especially clear when you start to see that these models can be applicable and affordable to the academic community. And when you start to give the larger scientific community access to a model of one particular disease, it's amazing what can happen in terms of improving our understanding over a very short period of time. As soon as we're able to harness the power of that technology, I think we might see an explosion in the development of these models.

John Couse is portfolio director of custom scientific services at Taconic Biosciences.

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Sitting Down With

From Bench to Bedside and Back Again

Sitting Down With... James Wilson, Professor and Director, Rose H. Weiss Orphan Disease Center, Professor of Medicine and Pediatrics, University of Pennsylvania.

June 2017

What inspired you to study medicine – and what drew you to genetics?

As an undergraduate, I was interested in the physical sciences, and had planned on attending graduate school to do chemistry. However, my father and grandfather were both physicians, and I made the decision that if I was going to have a career in science, I wanted the results of my research to be closely connected to improving outcomes for patients. So I decided to enter a combined MD/PhD program at the University of Michigan.

My interest in genetics derived from my work as a young scientist and physician – my training really set the stage for my entire career. My motivation came from the interactions I had with patients during my research, as well as in my clinical rotations in pediatrics. I got to know three different patients who had really awful genetic diseases – a young man with Lesch–Nyhan syndrome, and one who had metachromatic leukodystrophy. The third patient – one who left a lasting impression on me – was a young boy with a severe form of epidermolysis bullosa.

How close – or far – are we from treating such genetic conditions?

I began my career studying genetic diseases and made the decision, literally on the spot, that I was going to focus on gene therapy. Of course, I didn't realize at the time how complicated that would be, or how long it would take to get to a point where we were having an impact on patients! It's been a 30-year journey, and now we're at the point where we're seeing gene therapies being approved for patients with rare diseases.

The fact is that we're only at the beginning, and I have to remind myself – and those who are affected by what we do – that this still experimental science. But we're at the stage where the translational investigator can play a key role. We're taking our discoveries from the bench to the bedside, but the most important step in the entire process is learning the potential and limitations of our technology in the clinic, and bringing it back to the bench. The future is bright, and there will be successes, but there will also be failures. One thing is certain: we need to continue to innovate.

What other projects are you working on?

One interesting area I'm involved in is the use of AAV as a vector, not necessarily to treat a genetic disease, but as a better way to deliver therapeutic proteins that otherwise require repeated infusion. The notion is to take the gene encoding the therapeutic protein, clone it into an AAV vector, and inject the vector. This will program the patient's

cells to express the therapeutic protein, possibly providing longer-term expression of the protein, and potentially achieving more localized, effective and safe outcomes.

Right now, we're focusing on using an AAV to express antibodies against infectious diseases, which could result in a new paradigm for vaccines. Our model doesn't require the patient to have an immune system, because we've programmed non-immune cells to express antibodies against a pathogen. We're getting the most traction in this area through funding by the federal government – in particular, the Department of Defense – as a way to develop countermeasures against pandemics, such as influenza, and possible biothreats.

Your work sounds challenging – but also rewarding...

One thing that I enjoy about my job is the challenge of integrating both medicine and science on a daily basis. I find it helpful to view myself as a bit of a generalist, both in terms of clinical practice and research. I try not to stay confined to the disciplines I've trained in. I've also been able to benefit greatly from the input of the incredibly bright and talented young scientists and trainees in my lab, who help me to expand my horizons.

A critical factor for success is the ability to appreciate the other aspects of translational medicine that you need to move into the clinic – the ones that are not necessarily related to the science. You need to figure out what they are, learn about them, and take as much control over them as you can. For example, interfacing with the biopharmaceutical industry, and the various aspects of technology transfer. I often see scientists defer these important parts of the process to others. Learning what these issues are and getting involved has given me more influence and control over the trajectory of my work, and that has been pivotal to my success.

What advice would you give to young translational researchers?

There are three areas to consider. The first is to establish a goal, and do whatever you need to do to achieve it. Also you must realize that you may be forced to become knowledgeable about (or even a master in) areas of science or medicine that you previously had no direct experience of.

Secondly – and crucially – place yourself in an environment that is truly committed to translational research. I see the word translation virtually everywhere, but very few institutions really support the development of careers in translational research, or support bench to beside and first-in-human studies, which is where our impact can be. Find that institution, and get there.

Finally, our field is complicated, and involves many different stakeholders and contributors. As a translational scientist, you can be the glue that brings them all together – the "missing link". So you have to be interested in networking and building teams. You need to be a leader, and pull together many diverse individuals, many of whom don't report to you. It's a real skill, but essential for your success.

What are your career highlights?

I still think I'm waiting for the true highlight. My dream since I was a young student was to change the course for patients with genetic disease, and I think we're getting closer and closer. These families had no hope, but now I think we're providing a little. We want to go further by providing real solutions.

Having said that, I love being a scientist, and I have been lucky enough to have some eureka moments in the lab – and believe me, they are few and far between. Two that really stand out to me are a late night I spent as a graduate student working to identify the molecular basis for Lesch–Nyhan syndrome. I was looking at the chromatography read out from a protein taken from a patient, and discovered that he had an altered peptide that I subsequently showed was due to an amino acid difference. That was an incredible high for me as a young scientist. But in a way it was also a low point because I appreciated soon thereafter, when speaking to the parents of this patient, that this discovery was not really going to impact their son. And that led me to gene therapy.

My next eureka moment occurred about 25 years later, when we discovered a new family of AAVs that we then developed as a vector. We found that they were 50 to 100-fold more efficient than what we already had – and I knew that discovery would change the trajectory of my career.

If you could begin your career again, would you do anything differently?

I just feel blessed to have had the opportunity to use my training in medicine and in science to work towards a translational goal. My boss recently asked me what part of my day or my career I don't like, and I told him "There's nothing about what I do that I don't enjoy – I'm living the dream!"

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