Precision medicine requires precise targets and a community of patients

On April 29, Dr. Gerald Batist, Segal Cancer Centre, Jewish General Hospital, Montreal, presented the 2017 Cosbie Lecture

THE COSBIE LECTURE

Gerald Cosbie was a Canadian physician and pioneer in the treatment of gynecologic cancers. He cared for the first patients treated with radiotherapy at the Toronto General Hospital in 1921. He played an important role in the establishment of the Ontario Cancer Institute and served as Medical Director of the Ontario Cancer Research Foundation. The Cosbie lecture series, established by the Foundation and awarded to the Canadian Oncology Societies, was instituted in his honour in 1977. The series is currently sponsored by the Canadian Oncology Societies, Cancer Care Ontario and the Canadian Cancer Trials Group.

Gerald Batist, MD, CM, CQ, FRCPC, FACP, FCAHS
Dr. Gerald Batist is the former Chair of the Department of Oncology at McGill University and Director of the McGill Centre for Translational Research in Cancer. A major award from the Canada Foundation for Innovation led to the expansion of the Centre and its integration into the Segal Cancer Centre at the Jewish General Hospital, which he also directs. Dr. Batist is a clinician-scientist trained in medical oncology and molecular pharmacology. His work, both in his lab and clinical research, focuses on therapeutic resistance. This includes large consortia of biopsy-based clinical trials. In 2014, he co-led a successful application that resulted in the establishment of the Canadian National Centre of Excellence in Personalized Medicine, Exactis Innovations. The core feature is a program to build a massive biobank and database linked to a prospective longitudinal registry of cancer patients followed throughout the trajectory of their illness — a project called Personalize My Treatment. In 2016, Dr. Batist was appointed Member of the Order of Canada and Knight of the National Order of Quebec.

It is becoming increasingly difficult to justify some of the trials we continue to do worldwide, randomizing hundreds of thousands of unselected patients, and shocking that we define a gold standard when we achieve a 50% response rate in the study arm compared to 20% in the control group. Incrementalism and the trial-and-error approach to therapy has brought disappointing progress in improving cancer outcomes.

Today, we are thankfully changing our approach to patients with cancer, from a ready-to-wear, one-size-fits-all approach to a more personalized medicine, where we use biologic parameters to identify subgroups of patients and match them with therapies. This has brought some tremendous hits, for example with crizotinib: the first patient treated with crizotinib in Japan literally got up from an intensive care unit bed and walked out within a week or two. In patients with echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase (EML4-ALK) translocation-positive non-small cell lung cancer (NSCLC), treatment with crizotinib produced at least minor tumour shrinkage in 97% (Kwak et al 2010). The result is that drugs like this are getting approved following trials with just a few hundred patients. And that has some risks: the trial of vemurafenib, for example, showed very rapid clinical response in patients with BRAF-mutated metastatic melanoma, but response was short-lived: by the time the study was finished and the drug was approved, it was clear this was not the optimal drug for this disease.

In cancer immunotherapy, we have seen dramatic results when targeting specific proteins that are central to immune suppression. A very early study comparing nivolumab to dacarbazine (Robert C et al, *NEJM*) showed really dramatic effects, with progression-free survival (PFS) of 5.1 months with immunotherapy vs 2.2 months with the cytotoxic agent. These were early big wins, or low-hanging fruit, and invited the prospect that we would be able to segregate the cancer patient population and think of all kinds of cancers

as collections of uncommon or rare cancers. They would each have a molecular signature that would match them to a particular drug with very high response rates.

THE MOLECULAR MODEL OF DRUG DISCOVERY

In the approach to drug discovery that arose from these early wins, a molecule would be identified and drug producers would be searching the globe, enlisting cooperation from multiple hospitals to find enough patients in these rare subpopulations, analyze patients at a molecular level, and then test the molecule. That is a very long and expensive route to drug discovery. Pharma has gone along with the model, projecting hypermutated genes that expert opinion said were likely to be actionable mutations, and making drugs to target these mutations. Estimates based on the identification of hypermutated genes in different tumour sites in The Cancer Genome Atlas (TCGA) have been used to project how many drugs might be approved with these kinds of very high response rates.

New paradigms present new challenges. Three appear especially important today:

1. How do we rapidly and reliably identify these specific and uncommon patient subgroups? If we really want to make the case that drug costs should be reduced, we should be finding ways to recruit these patients in a more organized and rapid manner to enable these selected subpopulation trials.
2. How do we obtain comprehensive toxicity and "real-life use" data for drugs approved on the basis of around 150 patients treated with these drugs?
3. Can we truly define "drivers" that are the optimal therapeutic targets, and that may change over time?

A study presented at ASCO 2015 exemplifies the limitations encountered in thinking that a mutation represents the activation of a gene. In a selected subgroup of patients, there was no correlation between the presence of any known activated mutation of phosphoinositide 3-kinase (PI3K), or of genes that would activate the pathway, and response to PI3K catalytic subunit alpha (PIK3CA) inhibitors. Such studies are piling up now, failing to show anticipated high response rates. We need to find out why.

MOVING BEYOND GENES TO PROTEINS

In the past couple of years, a few papers have gone back to TCGA archives to look not just at the genome-based data, but also at proteomic information. A study published about 2 years ago in breast cancer used reverse protein arrays to look at their correlation: for the most part, there was a misalignment of predicted mutations based on genomic structural changes, with expression at the RNA level and certainly with the proteins. This means that treating on the basis of prediction may not be correct. A study of a protein kinase B (Akt) inhibitor, in which patients were selected based on next-generation sequencing (NGS) analysis of their tumour, revealed that patients with a mutation displaying increased Akt that was phosphorylated and activated represented true-positive patients who might be expected to respond. Patients who were mutation-negative and did not show increased levels of Akt level were true negatives and would not be expected to respond at all. However, not all patients allow such clear-cut distinctions: What happens if there is a mutation, and protein is present, but is degraded for some reason? What happens if there is activation at an epigenetic level that does not show up on an NGS mutation analysis, and more protein is seen? These would be false-negative and false-positive selections. It is possible that many patients who would benefit from these treatments are screening as false negatives for the mutation. This leads to the idea that monitoring Akt protein expression and activity directly will more accurately identify responders.

Christoph Borchers is currently working with both the University of Victoria in BC and McGill University to develop a proteogenomics platform based on the understanding that the protein, and not the gene, is where the drug works. We began with a very sophisticated technology called iMALDI (immuno-matrix-assisted laser desorption/ionization) to measure AKT1 and 2 in tissue and cell lines, and are using a clinical trial in which patients are selected on the basis of NGS analysis to see whether looking at the proteins in those same samples enables us to predict response with greater precision. Proteogenomics figures prominently in an international deployment of the White House Cancer Moonshot program to accelerate progress against cancer. McGill and the University of Victoria are part of this international effort, working on standardizing this still-very-young technology through an organization called the Clinical Proteomic Tumour Analysis Consortium at the National Institutes of Health (NIH). The technology is allowing us to move from global proteomics to specifically targeted proteomics to precisely define the target.

RELAPSE

Another challenge with the big wins in targeted therapies is that the dramatic response obtained when we correctly match patient to drug is inevitably followed by eventual

relapse. We need a much better understanding of the molecular signatures and mechanisms if we hope to delay or overcome therapeutic resistance. My colleagues and I have focused our attention on metastatic tumours and the profile of therapeutic resistance. We developed a number of consortia, one in Quebec called Q-CROC, and part of that has been taken on the road through the Terry Fox Research Institute. The work involves biopsy-driven clinical trials in a variety of tumours: colon cancers, lymphomas, and triple-negative breast cancer. At the onset of metastatic disease, and before the start of standard therapies, patients have biopsies of their tumours and are then followed regularly. Some respond to therapy while others do not, but in either case, another biopsy is taken at relapse so we have tissue to compare at 2 timepoints, as well as with the primary tumour. These samples are analysed at a number of levels, and are used in mouse xenografts. My colleagues and I, along with my partner in this work from the beginning, Dr. Luc Bélanger in Québec City, who we lost this past year, have built a variety of organizations over the past 10 years to initiate this type of clinical trial in colorectal cancer, lymphoma, and breast and lung cancer.

Q-CROC-01 was the first study, and aimed to prospectively identify the molecular signature of clinical resistance to standard first-line therapy in patients with metastatic colorectal cancer. At the start, patients were receiving FOLFOX (leucovorin, fluorouracil, oxaliplatin) and bevacizumab, but that has since evolved. In the course of the study, we quickly understood the need to develop biospecimen expertise,becausee preanalytical quality control of the samples was incredibly important. Appropriate handling of samples can be very tumour-specific.

Once these platforms are built, they become a gift that keeps on giving, and we continue to learn from them. In the first 85 samples from the QCROC-01 study, we were able to find a large number of novel significantly mutated genes that are being characterized. Looking at the RNA sequencing level, a preliminary analysis allowed us to find one gene called regenerating family member 1 alpha (REG1A). Pre- and posttreatment samples show significantly increased levels of REG1A mRNA in the patients after treatment. In a multi-omics approach, we see protein significantly increased in posttreatment vs pretreatment biopsies. Validation with independent omic methods is a critical way to approach the identification of proteins, using novel approaches to analyzing the same samples in a study that continues to accrue samples, following patients through standard therapy. Cytoscan analysis can detect copy number aberrations, which are very common in colorectal cancer, and we can actually start to see subregion gains and losses that are associated with significant effects on PFS. Each of these subregions contains a variety of genes, and we are exploring all of them.

NEW OPPORTUNITIES

Colorectal cancer seems to be particularly resistant to the type of immunotherapy checkpoint inhibitors currently in use, pointing to the likelihood of different immune suppression factors. Dr. Simon Turcotte at the Centre Hospitalier de l'Université de Montréal (CHUM) and Dr. Claudia Kleinman at The Segal Centre used the Q-CROC-01 data to generate a consensus clustering, based on the literature and some experimental data to search the RNA sequencing data, look for an immune-reactive subgroup of patients, and classify a variety of aspects of immune therapy. From this work, we are beginning to understand that these factors are entirely independent from one another, with, for example, lymphocytic infiltration being distinct from lymphocytic activation, etc.

Work is also attempting to move from surrogate measures of mutational load to more specific description. Dr. Turcotte is looking at neoantigen load and trying to identify specific and novel neoantigens, because we know there are proteins, predicted to be present by the genetic modification, that are not present; there are also proteins that are present, but are not predicted by the genome. These may be highly neogenic antigens useful in cancer therapeutics. Dr. Turcotte has developed a variety of validation tools, mostly using external databases; in one cluster of 431 genes curated from the literature, he finds an immunoreactive cluster in 26% of patients, and this is now being validated in other larger datasets. There is tremendous value in building these large platforms: they continue to receive patient samples, and as the technology advances, will provide us more and more information.

VALIDATION TOOLS

To truly define drivers that are the optimal therapeutic targets and identify mechanisms of therapeutic resistance requires a number of validation steps. One ideal preclinical platform is the use of patient-derived xenografts (PDX), or mouse avatars. Researchers have taken samples from Q-CROC-01 and other trials, put them into mice and grown them, and found that PDX mutations are truly representative of the tumours in the patients.

One illustrative case involves a 78-year-old man with gastric cancer who presented with metastases on imaging in the lung, liver and bones, with the primary gastric cancer still in place. It was strongly human epidermal growth factor receptor 2 (HER2)-positive, using the usual criteria for diagnosis. The patient received some chemotherapy, but the backbone of his therapy was trastuzumab, resulting in radiologic complete response in all metastases, except the primary gastric cancer, and this was stable for 6 years on maintenance trastuzumab. After that, the stomach mass started to grow and bleed, and even though everything else was stable, resection was undertaken to relieve symptoms, and a sample was taken from the primary tumour and put in a mouse. Ultimately the cancer recurred in the patient's stomach, as well as his lung and liver.

After growing in a mouse for a long period, this man's stomach cancer showed resistance to trastuzumab, and was then tested with a variety of agents, with trastuzumab emtansine (T-DM1) showing considerable effectiveness. T-DM1 uses an inactive HER2 as a target to deliver a cytotoxic cargo. It was also possible to analyze these resistance

samples and see that HER2 itself was not mutated; HER2 is therefore not resistant to trastuzumab by virtue of a loss of functional mutation. It may be that T-DM1 is effective because the protein is there as a target, but other targets also emerge, some of which, including kinase mutations, have been suggested in the literature, and are testable in these PDX animals. The patient has since been treated with 2 cycles of T-DM1 and, while it is early, his chest x-ray has improved, and cell-free DNA for amplified HER2 is precipitously dropping.

REVISITING ASSUMPTIONS

Findings like this are forcing us to reconsider some of the things we take for granted, such as that trastuzumab and HER2 are matched in a 1-to-1 fashion. Trastuzumab marked the dawn of one of the major accomplishments of selecting patients, and it was quite brave of Roche at the time to cut their market down to 15% of breast cancer patients through selection. Looking back at the trial that led to the adjuvant approval of trastuzumab, where all patients were seemingly identical in their amplification of HER2, and were randomized so one group got chemotherapy and the other got chemotherapy and trastuzumab, what becomes interesting today is the persistence of a plateau suggesting that, even in patients with amplified HER2, HER2 is not the sole driver. Other genes are involved and need to be identified.

THE CENTRE OF EXCELLENCE IN PRECISION THERAPEUTICS

As mentioned earlier, two major challenges in the new paradigm involve finding ways to rapidly and reliably identify specific and uncommon patient subgroups, and obtaining comprehensive toxicity and "real-life use" data for drugs approved on the basis of a small number of cases.

These challenges form the basis of work at the Centre of Excellence in Precision Therapeutics — called Exactis Innovation — a not-for-profit organization that was incorporated in 2014 as a result of a public-private partnership/competition of the Network of Centres of Excellence (NCE). It is funded by the Canadian NCE (CIHR/SSHRC/NSERC), pharma and biotech companies, and public research funding organizations, and is partnering with hospitals and academic institutions across the country. The idea is to improve cancer survivorship through personalized innovative research. At the core of this initiative is the Personalize My Treatment (PMT) initiative, which involved considerable struggle with the Research Ethics Board that was eventually resolved. Patients consent to having their tumour tissue or other biospecimens molecularly profiled; to share their coded (molecular and clinical) data and biospecimens for research; and to have their clinical trajectory recorded throughout their lifetime. Target subpopulations are at high risk but are not presently in dire condition, and can be followed prospectively. Most importantly, we can recontact patients for possible participation in clinical trials aimed at offering a targeted therapeutic option, or at learning more about the disease and patient experience of the disease and treatments. Given the restrictions governing clinical research, this last ability is highly exceptional and is certainly not an option available to pharma. The strategy is very focused: patient recruitment and consent, clinical data collection, biospecimen collection and molecular profiling using emerging technologies for DNA alterations, RNA-based signatures, proteomics, and liquid biopsies.

Personalize My Treatment provides a variety of opportunities in clinical trial matching and in silico clinical trial design (we may eventually be able to help those who want to design a clinical trial identify where the patients are). It also enables post-marketing studies to collect toxicity data over a longer period of time. In discussion with regulatory agencies, there is some prospect that data we collect in a systematic way may be useful to pharma, who are charged, when they obtain conditional approvals, with finding ways to follow patients longitudinally. Drug discovery, diagnostic kit development and health econometrics represent further opportunities. This approach shortens that long route for a molecule to find its way to a subpopulation, with some facilitation by Exactis. And by reducing the cost of drug discovery, it may help make the case with pharma to reduce the costs of drugs.

CONCLUSION

Cancer should be viewed as a collection of uncommon or rare subgroups of patients.

Identifying these subgroups is a major challenge in developing novel drugs, ensuring that the correct target is measured, and recognizing alternative drivers and mechanisms of resistance. Our definition of a driver has really been quite loose, based on what drugs are available and on laboratory-based studies, rather than on what is happening in these patients. Optimal molecular profiling must include multiplexed and multi-omic analysis.

The Exactis Personalize My Treatment longitudinal prospective project offers the opportunity to rapidly identify patients and offer them molecular-based therapies, and to provide critical pharmacovigilance for new drugs approved after limited experience.

Patient engagement is a key driver of the successful transformation of cancer care to precision medicine. As patients give their consent prospectively — when they are at high risk but not currently ill — they become part of a community, which may be a very interesting way of improving accrual in clinical trials. Unlike other biobanking models, Personalize My Treatment provides the prospect of direct benefit to patients who join this community.

Find out more

To find out how you can work with Q-CROC, see www.qcroc.ca

For information on benefits of joining the Exactis Network, see www.exactis.ca

TIME TO ACT UP FOR CANCER!

During the question period following the Cosbie lecture, Dr. Batist expressed some impatience with the barriers to progress in effective treatment of cancer. "As cancer care providers, we know we're dealing with a lethal disease, but have been a little too sheepish in demanding improvements." He paid tribute to the activism of his recently departed colleague, Dr. Mark Wainberg: "I think we need to remember the panic, angst and anger that the Act Up organization used to move HIV drug development agenda forward and really bent the curve of the regulatory agencies. Somehow in cancer, it is acceptable that people still die of this disease, when we have all this knowledge and investment. We need a little bit more panic."

ACT UP ACCOMPLISHMENTS

The AIDS Coalition to Unleash Power (ACT UP) was founded in New York City in 1987 as a political action group in response to the AIDS crisis. Over the next 25 years, the 140 worldwide chapters of ACT UP have led successful protests to improve access to effective treatment, including protests against:

- pharmaceutical companies for charging astronomically high prices for AIDS medicine
- regulatory agencies for slow drug approval processes
- national research institutes for their failure to prioritize diverse types of treatment, and underrepresentation of women and minorities in clinical trials
- governments for failing to fund universal access to treatment
- insurance companies for discrimination

Dr. Mark Wainberg was Director of the McGill University AIDS Centre. Along with research to identify and test new antiviral agents that have played an important role in preventing progression of HIV to AIDS, Dr. Wainberg was an important advocate for patient involvement in scientific meetings, research competition juries and clinical trial design; for regulatory reform; and for access to effective therapies, in both developed and developing countries. He died in a swimming accident in 2017. A year before his death, following the 2016 Durban AIDS conference, he said: "When I look back on my career, I always feel the most important contribution of my life was political and not scientific."