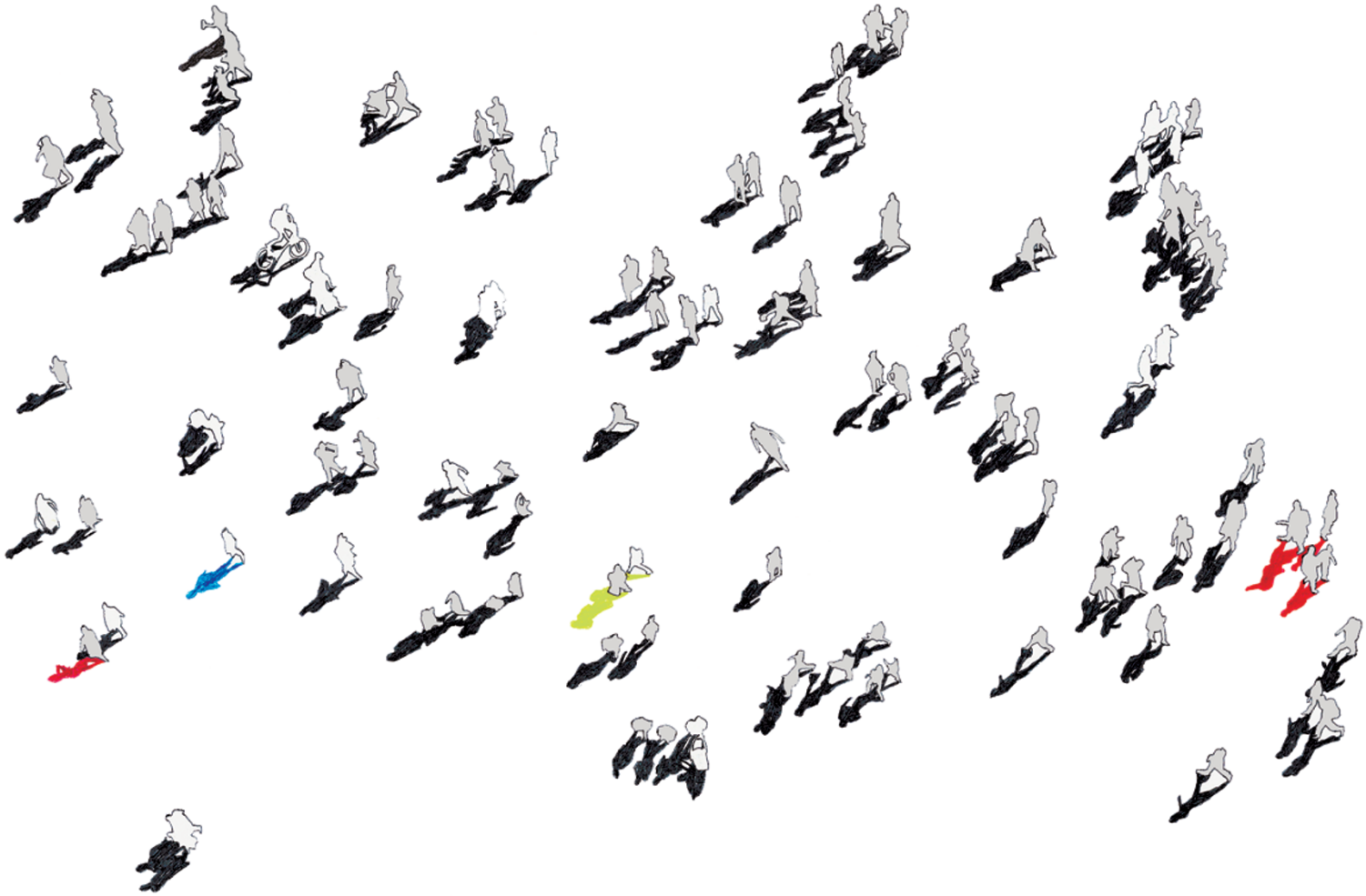
TECHNOLOGY FEATURE

A MOST EXCEPTIONAL RESPONSE

Sometimes a drug causes a tumour to completely recede, but only in a tiny percentage of people. Scientists want to decipher such outlier responses for the benefit of all patients.



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BY VIVIEN MARX

If Patient X were like most people with advanced bladder cancer, she would probably be dead by now. After her first diagnosis, she received standard chemotherapy. It failed. Then she entered a clinical trial for a drug that was originally approved to treat other tumour types: would it also work in metastatic bladder cancer? Apparently not — none of the other patients in the trial did well.

Yet Patient X thrived. Her tumour completely disappeared, says computational biologist Barry Taylor at Memorial Sloan Kettering Cancer Center (MSKCC) in New York, where Patient X was treated. Today, a little more than five years after treatment, she is healthy and has no evidence of disease[1].

Patient X (her identity is shielded to protect her privacy) is an exceptional responder, one of those rare individuals who have a dramatically positive response to a therapy that does little or nothing for most other patients. This response is not unique to cancer. Immunologists, for example, have discovered why some individuals can be HIV-positive and yet avoid the symptoms of AIDS.

By definition, exceptional responses are rare, which makes them hard to study. Their anecdotal nature seems to contradict the teachings on statistically sound results in biomedical research. In a clinical trial, even if there are several exceptional responders, a drug will ▶

fail to achieve approval because it does not improve the health of the majority of patients. This means there has been little incentive for researchers or drug companies to investigate thoroughly why a few people respond so well.

But that neglect is starting to be addressed as more cases of exceptional responses in cancer reach the published scientific literature and techniques emerge for profiling patients at the molecular level[2]. In Patient X's case, genome sequencing revealed a mutation in her tumour that explains why her cancer is specifically vulnerable to the drug she received on the clinical trial[1]. Such successes indicate that searching for and profiling these patients can potentially help researchers to predict many other patients' responses to potential therapies.

The relatively new ability to comprehensively characterize a tumour's genome, transcriptome (its gene expression) and metabolome (its metabolic processes) increases the chance of discovering the reasons behind outlier results, says Kenneth Kinzler, a cancer researcher at the Johns Hopkins Kimmel Cancer Center in Baltimore, Maryland. "The hope is that a signal seen in an exceptional responder will be seen in other cancer patients and be a predictor of therapeutic response regardless of tumour type," he says.

Exceptional responders can help scientists to predict the responses of many other patients with cancer.

MEMORIAL SLOAN KETTERING CANCER CENTER

THE EXCEPTIONAL PROFILE

There is no universally accepted definition of exceptional responders, says Barbara Conley of the US National Cancer Institute (NCI) in Rockville, Maryland. Conley directs the Exceptional Responders Initiative (ERI), which profiles these patients. The ERI considers a drug response to be exceptional when a tumour disappears or when a patient shows an exceptional response to treatment and lives longer than 90% of others treated similarly. In tough-to-treat and advanced cancers, an exceptional response is when treatment causes a tumour to regress by at least 30% for at least six months, but only in less than 10% of people on the same treatment.

"The hope is that a signal seen in an exceptional responder will be seen in other cancer patients."

In the case of Patient X, for example, her sequenced tumour genome revealed a mutation in a gene called tuberous sclerosis complex 1, which is one of several genes involved in a pathway that regulates cell growth and proliferation. The drug that worked for Patient X, but not for the other patients in the clinical trial, inhibits signalling in that pathway.

But that does not completely explain Patient X's exceptional response. Analysis of tumour samples from 13 other patients in her trial showed that four had a mutation in the same gene, but the drug gave them only a short reprieve. To get a better understanding of Patient X and other exceptional responders, the ERI wants to do comprehensive profiling of a wide variety of parameters, including patients' clinical history, DNA changes, RNA levels of different genes (which reflect their activity) and metabolic pathways.

Taylor and his colleagues have long encountered the critique that studying exceptional responders is merely generalizing anecdotes. But even though published studies on exceptional responders are few, he says, "I think the weight of evidence has now shifted that view."

Vincent Miller, a former MSKCC oncologist, agrees that views about outliers are changing and thinks that many more such individuals might be found. Any oncologist has a handful of patients in whom cancer just melts away with no obvious explanation, says Miller, who is chief medical officer of Foundation Medicine in Cambridge, Massachusetts, a company that performs genomic analysis of samples from people with cancer. In January, the pharmaceutical company Roche, based in Basel, Switzerland, bought a majority stake in Foundation Medicine, which is also involved in the ERI.

The ERI encourages clinicians to get in touch if one of their patients has an exceptional reaction to a drug. At that point, a multidisciplinary review determines whether a more comprehensive profile is warranted, says Conley. In approved cases, and with the patient's consent, the physician sends in the complete medical record and a tumour sample. Around 160 submissions are currently under review. Conley and her team have been surprised to see submissions about established drugs as well as drugs still under development.

The ERI makes sense only because large-scale sequencing efforts such as The Cancer Genome Atlas (TCGA) now offer huge data stores, says David Wheeler, who leads the ERI genome-analysis team at the human genome sequencing centre of Baylor College of Medicine in Houston, Texas. From Baylor, genome data will go to a database that is accessible by the research community.

The first few ERI samples are now beginning to arrive at Baylor, and researchers there are all set to potentially perform whole-genome sequencing using their newly arrived equipment — HiSeq X Ten Illumina sequencers. Whole-genome sequencing is ideal, says Wheeler, because it provides the most complete genomic information. But it also requires enough sample and plenty of time and money; so when the samples are smaller or when only ones with lower tumour purity are available, the team will just focus on protein-coding genes, which make up the exome.

For now, the ERI is in a pilot phase. If it proves successful, it could be scaled up by, for example, helping cancer treatment centres to forage for exceptional responders in their biobanks. But the pilot faces a few challenges.

One key issue is time, says Kristen Leraas, who is the sample coordinator at the biospecimen processing facility of the Nationwide Children's Hospital in Columbus, Ohio, where all of the ERI's samples are processed

CLOCK-WATCHERS

The Nationwide Children's Hospital in Columbus, Ohio, deals with tumour samples from the Exceptional Responders Initiative of the US National Cancer Institute (NCI). Extracting DNA and RNA from the precious, tiny samples involves a race against the clock to avoid degradation of nucleic acid.

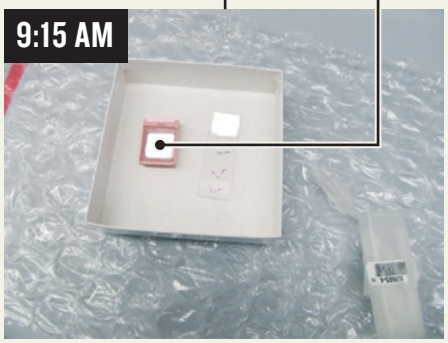
Processing of the newly arrived, tiny piece of tumour from an exceptional responder begins immediately

DAY 1

9:00 AM

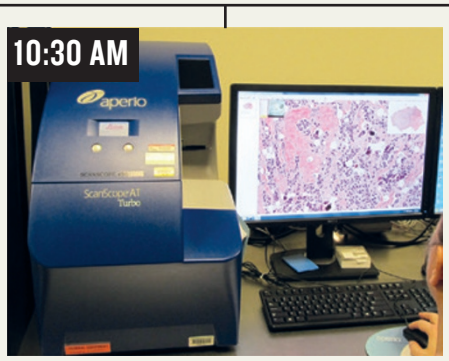
Treatment centres across the United States submit cases to the NCI. After a review, tumour samples from patients who show a truly exceptional response are sent to Nationwide.

9:15 AM



The tumour tissue often arrives fixed in formalin and embedded in paraffin. This process can damage nucleic acids but allows for pathology review and long-term storage.

10:30 AM



In one of many quality-control and preparation steps during the first day, the tissue slide is scanned so a pathologist can confirm the tumour type, assess its quality and see the amount of tumour in the sample.

(see 'Clock-watchers'). When a sample comes in, she says, scientists have to race against the clock to process, standardize and prepare it for sequencing: DNA and RNA have to be extracted from the sample quickly to avoid any kind of degradation. "We pretend our hair is on fire and we make sure we extract right away."

Another challenge is that exceptional responses are unexpected, so the cancer centres sending tissue samples to Columbus do not collect them in a standardized way. One sample might be blood from someone with leukaemia, whereas another might come from a solid tumour. And unlike the case with the TCGA, it might arrive without a matched healthy tissue sample from the same person. A sample might be smaller than a pencil eraser and, in some cases — when it comes from a fine-needle biopsy, for example — it might even be invisible to the naked eye. TCGA samples weigh on average 260 milligrams, whereas "if we get 100 milligrams, that's a lot", says Jay Bowen, who directs logistics and data management at Nationwide's biospecimen processing facility. "Sometimes we make do with about 20 milligrams."

COMPREHENSIVE TESTING

The Nationwide laboratory's top priority with these samples is to extract enough nucleic acid to allow multiple analyses, including exome and messenger RNA sequencing. Some DNA is also sent to Foundation Medicine, where tests can detect and validate four classes of DNA alterations at once: substitutions of bases along the DNA strand, genetic insertions and deletions, changes in the number of copies of genes present in the genome and structural rearrangements[3]. Ideally, if the sample yields sufficient quantities of nucleic acid, whole-genome sequencing or other types of tests, such as analysis of DNA methylation, can be performed.

A potential complication is that tumour samples taken during surgery or biopsy are often fixed in formalin and then embedded in paraffin. These formalin-fixed paraffin-embedded (FFPE) samples are standard in medical centres and are preferred by pathologists, who can easily shave off a thin slice when they want to study the tumour's cellular morphology under a microscope as part of diagnosis.

But this process can crosslink nucleic acids, and can also oxidize and shear these molecules, says molecular biologist Erik Zmuda, who directs molecular characterization tasks at the Nationwide's biospecimen processing facility. There is a risk that a genomic signal indicative of an exceptional response is actually an FFPE artefact. Thus, for studying the tumour's genome, researchers much prefer frozen tissue.

Zmuda and his colleagues at Nationwide and other institutions think they see a way to allow pathologists to continue to use their preferred FFPE preservation method while providing molecular biologists with the ability to profile a sample at the resolution they need. The team's idea is to find a telltale signature of FFPE artefacts in tumour samples, which would allow them to computationally mask these effects in the data. The team is developing an algorithm that would correct for the artefacts and thus make it easier to compare data from FFPE and frozen samples. That, in turn, could open up possibilities to retroactively analyse patient samples from pathology departments in any hospital. As well as helping the hunt for signals in outlier genomes, this method could also be adapted for use in genome analysis more generally when diagnosing and treating patients.

Other fields have a longer tradition than cancer research does of looking at exceptional responders, says Stephen Friend, a former director of the oncology division of pharmaceutical company Merck in Kenilworth, New Jersey. Early in the AIDS epidemic, for example, immunologists noticed that some people can be HIV-positive but lack symptoms. This exceptional biology was found to result from a mutation that changes a protein on the surface of the immune-cell type that HIV infects, thus stopping HIV from entering the cell[4].

Such links between a specific mutation and disease have sometimes led to targeted drugs. But genomics is not a black and white world in which certain mutations lead to the same clinical course in all patients, says Friend. Environmental factors and other genetic variants play their part too. This may be why these targeted drugs do not work in 100% of the patients with that mutation, he says.

Friend co-directs the Resilience Project (http://resilienceproject.me), which is geared towards finding outliers in many diseases[5]. The goal is to find people who harbour DNA changes that cause severe and rare childhood diseases, or that heighten cancer risk, but who have lived into healthy adulthood in spite of their genomes.

The programme is run by the non-profit organization Sage Bionetworks, which is based in Seattle, Washington, and is devoted to setting up platforms through which scientists can collaborate and share data. The Resilience Project currently consists of researchers from the Icahn School of Medicine at Mount Sinai Hospital in New York (conversations are also under way with the Gurdon Institute in Cambridge, UK). DNA analysis is in progress on samples from

The clock starts ticking in earnest when scientists shave off slices of the paraffin block that contains the tumour to begin extracting DNA and RNA. Exposure to air can alter molecules and even change genetic sequences.

Nucleic acids must be isolated from the slices within 24 hours of the shaving to prevent possible damage caused by exposure to air.

Nucleic acids are quality controlled and made ready for shipment. The extracted nucleic acids can be sent on for sequencing and analysis.

more than half-a-million donors, says Friend, who also directs Sage Bionetworks.

If the first analysis of the donor DNA reveals a mutation that could have killed the carrier, researchers can dig deeper into that person's genetics and biochemistry in an effort to understand their resilience. If one mutation is decisive, analysis can be quick, says Friend. But a mutation might act in conjunction with secondary mutations elsewhere in the genome. Searching for such mutation combinations is difficult, he says. But with an outlier genome in hand, researchers are at least trawling through a bucket of data, not an ocean of data.

Scientists tend to keep findings under wraps until they publish. But Friend thinks that analysis should be a collaborative task that is spread across multiple laboratories. This would increase the speed at which scientists can decipher which factors — be they genetic, immunological, environmental or a combination — have protected resilient individuals. "What I'm hoping is that we can get scientists to take it on as a sort of crowd-sourced federated approach," says Friend. "No one is paid to do that, no one owns the data."

RARE SIGNALS

In a clinical trial, scientists strive for numbers: making sure there are sufficient cases of disease and controls to see whether a drug is having an effect, for example. They look for global trends rather than focus on the outliers, says Gustavo Stolovitzky, a researcher for the technology firm IBM in Yorktown Heights, New York, who runs the Dialogue for Reverse Engineering Assessment and Methods, a research venture and competition that, for example, looks at how well different algorithms predict the reaction of cancer cells to drugs[6].

By definition, outliers are too rare to have much statistical power, Stolovitzky says, and are usually dismissed as flukes. But conversely, he says, an exceptional response is a strong signal that is hard to miss. If many scientists hunt for exceptional responders in data from the ERI or the Resilience Project, perhaps 20 or even 50 cases can emerge. "That's starting to be something," says Stolovitzky. "It's a number we can do statistics with." If so, it may be possible to glimpse patterns that can help to explain how exceptional responders beat the odds.

In profiling outliers, scientists will not know which of the molecular signals is decisive, which is why comprehensive profiles are needed for everything — genome sequencing data, gene expression data, clinical data and other assay results. Comparing these profiles is tricky: for example, it can often be a challenge to compare genomic sequence, says Trey Ideker, a computational biologist at the University of California, San Diego. "We sequence this individual and they're a snowflake," he says — showing patterns that are unique even though the patients have the same type of cancer.

Ideker says that one approach to address that diversity is to view cancer as a disease of pathways, in which groups of genes act together to perform functions in the cell. When analysed on a pathway level, he says, patterns do emerge. For example, researchers may find that dissimilar-seeming mutations in a cancer all fall in a certain pathway, meaning that they all impair the same cell function.

These network patterns are not complete biochemical explanations of an exceptional response in cancer treatment, says Ideker, but they are indications of what to explore next. Crucially, he says, by considering pathways, an exceptional responder becomes part of a group. Even if it is not a large group, the person is no longer an outlier.

Many patients could benefit from ventures to decipher the molecular profile of exceptional responders. A physician might realize that a drug that was not expected to do well in a given patient might actually be surprisingly suitable, says Taylor. This approach to cancer treatment complements an emerging idea that rather than focusing on the organ in which the tumour originated, treatments should be targeted to the molecular profile driving a given cancer.

For research on outliers to be of greatest help, the outlier cases must be rigorously selected. Only then can the analysis deliver sound results despite the fact that it remains a profile of only one person, says Friend. Taylor agrees, pointing out that molecular analysis of tumours from patients is increasingly possible and that there is growing acceptance of studying outlier patients. "Nevertheless," he says, "it requires that we stay focused on exploring the most significant outlier responses to ensure the greatest return for patients." ■

Vivien Marx *is technology editor for* Nature *and* Nature Methods.

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