ineffective treatment to another," says Rosenberg. *–Jordan Calmes-Miller* ■

Neoantigen Quality Predicts Immune Response, Survival

It's the quality, not quantity, of tumor neoantigens that may best predict response to immunotherapy and the likelihood of long-term survival among patients with cancer.

A team led by Marta Łuksza, PhD, of the Institute for Advanced Study in Princeton, NJ, and Benjamin Greenbaum, PhD, of the Icahn School of Medicine at Mount Sinai in New York, NY, combined concepts from immunology, evolutionary biology, physics, and computer science to study how the immune system recognizes tumors, and how tumors mutate and evolve in response, especially in the face of checkpoint inhibition.

The researchers developed a mathematical model and tested it on three data sets—two cohorts of patients with melanoma given anti-CTLA4 therapy, and a group with non–small cell lung cancer given anti–PD-1 therapy (Nature 2017;551:517–20). They found that two main factors determine the importance of any tumor neoantigen in shaping responses to immunotherapy. First, the mutated peptide must have a greater binding affinity than its wild-type counterpart to a class I MHC molecule. T-cell receptors must then recognize the neoantigen as foreign, much as it might a pathogen, and mount an immune attack.

The likelihood of both these events happening is at the heart of the researchers' "neoantigen fitness" model. When they compared their model against one that simply tallied up the number of mutated peptides present on the surface of tumor cells, they showed that the fitness-based analysis, by capturing both neoantigen and tumor heterogeneity, better predicted survival outcomes in patients receiving immunotherapy.

"Our approach has consistent predictive value across the patient cohorts we studied, but we think its strength comes from its universality," Łuksza says. "It can be extended to include other factors as we learn more about how the immune system recognizes tumors under therapy."

A companion study, led by Vinod Balachandran, MD, of Memorial Sloan Kettering Cancer Center in New York, NY, further validated this model in long-term survivors with pancreatic ductal adenocarcinoma (Nature 2017;551:512–16). All had undergone surgery and some had received adjuvant chemotherapy, but immunotherapy was not part of the treatment regimen. Initially, the team found that tumors with the highest neoantigen number and the most abundant cytotoxic T-cell infiltrates—but neither alone—stratified patients with the longest survival. Digging into possible reasons, they showed that long-term survivors displayed lasting circulating T-cell reactivity to high-quality neoantigens, as defined by the fitness model.

Because this approach worked for three different tumor types, two flavors of checkpoint inhibitor, and two different clinical settings—with or without immunotherapy—"our data may be identifying some common principles on how the immune system recognizes mutations," says Balachandran.

Elizabeth Jaffee, MD, of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University in Baltimore, MD, isn't yet convinced. "It's an interesting model," she says, but points out that, in the context of pancreatic cancer, it's so far been tested only in a unique subset of patients that may not represent the population as a whole. How relevant is this going to be, she asks, for the 93% of patients who don't survive beyond 5 years with surgery alone, but may do so with newer therapeutic interventions?

Balachandran and his colleagues next plan to see if the model predicts response rates to immunotherapy among participants in the Pancreatic Cancer Action Network's Precision Promise trial. Additionally, his team is engaging with Genentech and Mainz, Germany–based BioNTech to determine how insights gleaned from this work can be applied to trials of personalized mRNA-based neoantigen vaccines.

"Pancreatic cancer is a challenge, given the relatively low number of mutations harbored by these tumors," says Ugur Sahin, MD, BioNTech's cofounder and CEO. It's also considered a "cold" tumor, with very few infiltrating T cells. The new findings upend this conventional wisdom, Sahin says, and "strongly suggest" that even pancreatic cancer might be responsive to a neoantigen-based therapeutic strategy. *–Elie Dolgin* ■

Wild Microbiome Stems Tumorigenesis in Lab Mice

Despite the many therapies that owe their foundation to findings in mouse models, there's a growing appreciation among scientists that typical lab mice—and, more specifically, the effects of their sterile environs—do not always accurately reflect real-world diseases. Now, a recent study has found that simply replacing their gut microbiome with the microbes of wild mice alters the animals' immune response, perhaps for the better (Cell 2017;171:1015–28).

The lab mice who received a microbial boost from their wild counterparts were more resistant to inflammation-driven diseases, the authors report, including colorectal cancer and flu.

"Our starting hypothesis was that in nature, the microbiome has co-evolved with its host for millions of years and probably has beneficial health effects that we do not see in laboratory mice," says study author Barbara Rehermann, MD, of the National Institute of Diabetes and Digestive and Kidney Diseases.

To test that hypothesis, the authors trapped more than 800 wild mice in barns in eight different locations around Maryland and Washington, DC. They characterized the gut microbiota of 98 of these mice using ribosomal RNA profiling and found that the barn animals' microbiomes, though similar to each other, were very different from those of the laboratory mouse strain C57BL/6. The lab animals' microbiomes were less complex and were deficient in certain bacterial species present in the wild animals, chiefly *Bacteroidetes* and *Proteobacteria*.

The researchers isolated wild gut microbiomes and those from laboratory animals and transplanted them into separate groups of laboratory mouse pups reared without microbes. Using a chemical mutagen and a colitis-inducing compound to trigger

colorectal cancer, the researchers found that mice with wild microbiomes developed fewer and smaller tumors than those with lab-derived microbiomes. The wild microbiomes also appeared to be protective against influenza—an intranasal injection of the virus killed 83% of the lab microbe–bearing animals, but only 8% of those with wild bacteria on board.

Although the study did not investigate the exact immunologic mechanism behind these differences, "that this extremely diverse microbiota had protective effects [against tumorigenesis] was actually extremely interesting and calls for further in-depth analysis," says Mathias Heikenwälder, PhD, of the German Cancer Research Centre in Heidelberg, who was not involved in the study.

The findings do not imply that all researchers should add wild microbiomes to their mouse models, says Stephan Rosshart, MD, first author of the study. However, for translational research, he and Rehermann believe the "chimeric meta-organism" they've created not only maintains many of the benefits of the traditional laboratory mouse, but may be more reflective of humans, who also have complex and diverse microbiomes. So far, the transplanted microbiomes have proven to be stable in the lab animals for four generations, even under typical laboratory conditions.

"There is no equivalent in the human population" to lab-reared mice, says Rosshart. "We thought we could preserve everything that is great about the laboratory mouse and optimize it by giving one part back, which is a natural microbiome." *–Rachel Tompa* ■

Mainstreaming Cryo-EM in Cancer Research

Over the last several years, cryo-electron microscopy (cryo-EM) has steadily gained ground on X-ray crystallography as a tool for visualizing proteins and other molecules in fine detail. That cryo-EM's pioneers were awarded the 2017 Nobel Prize in Chemistry is therefore both timely and exciting, says Sriram Subramaniam, PhD, of the NCI.

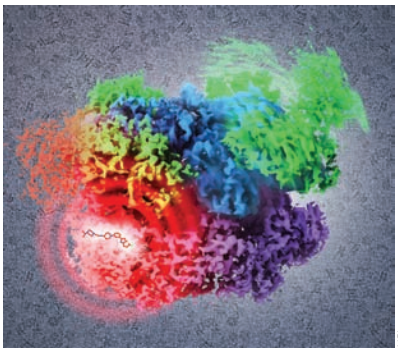
The three winners—Jacques Dubochet, PhD; Joachim Frank, PhD; and Richard Henderson, PhD—"laid the foundation, back in the 1980s, for what's become modern cryo-EM," he says. Today's scientists can study biological molecules in near-native states, at atomic resolution, by flash-freezing a given sample in under a millisecond and bombarding it with electrons. A special camera then captures 2-D images from different angles that are aligned into a 3-D map.

Until recently, however, cryo-EM remained on the fringes, Subramaniam observes, because it produced fuzzy and blob-like images on film that required development in a darkroom. The advent of direct electron detectors in 2013 revolutionized cryo-EM's data quality and inspired other technical advances in the field. Taking advantage of these improvements, Subramaniam's group became the first to solve the single-particle cryo-EM structures of beta-galactosidase, followed by the protein p97 and several dehydrogenases.

"The latter examples provided proof of principle that cryo-EM could be applied to clinical targets," he says. The therapeutic potential of blocking p97, a regulator of protein homeostasis—on which cancer cells can become overly dependent—is being investigated. Earlier attempts to study p97 using X-ray crystallography were limited to a resolution of 3.5 Å; Subramaniam's team successfully imaged it at 2.3 Å, and "we'll be using this structural information to inform better drug design," he says.

Besides running his own cryo-EM lab, Subramaniam recently established the National Cryo-Electron Microscopy Facility (NCEF; www.cancer.gov/research/resources/cryoem) at the NCI's Frederick National Laboratory for Cancer Research (FNLCR). "The idea is to democratize access to this technology," he explains. "There are dozens of cancer targets that should be worked on, but expense is a big bottleneck—cryo-EM infrastructure costs millions of dollars, and it takes months to get these instruments installed and working properly."

NCEF, which officially launched in May, currently houses one powerful, state-of-the-art Titan Krios microscope; another will be added in June 2018. This facility is one of the NCI's "national mission projects" based



The protein p97 is trapped in an inactive state by a new inhibitor (red) and the molecule cannot proceed into its normal reaction cycle.

at FNLCR, with another being the RAS Initiative, says Sara Hook, PhD, the program officer for NCEF. "We recognized the need for an extramural resource so researchers across the country—with or without NCI funding—could easily use cryo-EM for their work, without cost being a barrier." Any researcher can submit a request to use NCEF for free, she says.

"We ask them to briefly describe why their work is relevant to cancer," Subramaniam says, "but there's no need for a lengthy proposal, and no extended committee review process." That said, he adds, "researchers can't be purely aspirational—they need to use a lower-end feeder microscope, which is cheaper and much more widely available, to produce evidence that they have a specimen ready for top-of-the-line imaging and data collection at NCEF."

Centralized cryo-EM resources such as NCEF have been a growing trend for some time, Subramaniam observes. Howard Hughes Medical Institute's service facility in Ashburn, VA, and the Electron Bio-Imaging Center in Oxfordshire, UK, were among the first to emerge. The New York Structural Biology Center's National Resource for Automated Molecular Microscopy is another facility providing access to researchers who seek to apply this method to important biological problems.

"Cryo-EM is a rapidly evolving field, so it's important to have a shared resource run by qualified experts who can keep pace," he says. "We'll see further advances over the next few years, whether it's hardware, software, or biochemistry. This is just the beginning; it's ready for takeoff." *–Alissa Poh* ■