perhaps better known for two things: products that purify DNA or RNA from samples, and clinical assays that can determine a patient's mutation status and help physicians tailor treatments accordingly.

These companion diagnostics (CDx) include Qiagen's four "therascreen" tests, which variously detect mutations in *EGFR*, *FGFR3*, *KRAS*, and *PIK3CA* to guide treatment decisions for seven different therapies including afatinib (Gilotrif; Boehringer Ingelheim), panitumumab (Vectibix; Amgen), erdafitinib (Balversa; Janssen), and alpelisib (Piqray; Novartis).

The company also sells a *JAK2* assay designed to help clinicians accurately diagnose polycythemia vera by screening for a DNA substitution found in more than 94% of patients with the myeloproliferative neoplasm, and a test for high-risk strains of human papillomavirus to aid in screening for cervical cancer. Plus, Qiagen offers research-grade kits for profiling tumor mutation burden and for analyzing circulating tumor cells.

Yet, no one product in Qiagen's oncology product line—from its sample prep and analytic tools to downstream bioinformatics solutions—is likely as important to Thermo as the regulatory know-how that the firm brings to the table in the CDx arena, says Stephane Budel, PhD, a founding partner at DeciBio, a life sciences consulting firm in Los Angeles, CA. "Don't even think about the specific items in the portfolio," he advises. "One of the most underrated drivers of the acquisition on the oncology side is Qiagen's expertise in pushing CDx through the FDA."

Thermo, for its part, offers a series of complementary research tools for studying solid tumors, analyzing immune–tumor interactions, and performing liquid biopsies. The company also has two FDA-cleared clinical diagnostics: the SPOT-Light test for *HER2* amplifications linked to breast cancer, and the Oncomine Dx Target Test, the first next-generation sequencing (NGS)–based assay approved for non–small cell lung cancer.

Oncomine involves screening tumor samples for DNA and RNA variants associated with 23 genes, including three—*BRAF*, *ROS1*, and *EGFR*—validated to inform patient eligibility for three different treatment regimens. Looking to expand the reach of the diagnostic aid, Thermo recently signed two collaborative research deals: one with Eli Lilly centered on *RET* alterations to identify patients who stand to benefit from selpercatinib (LOXO-292), a RET inhibitor now under review by regulatory agencies around the world; and one with Janssen focused more generally on investigational lung cancer therapeutics.

NGS has been less of a priority at Qiagen, which has doubled down on targeted PCR-based assays. Last year, the company halted development of its GeneReader sequencing system and instead allied with Illumina, an industry leader in NGS technologies, in a 15-year research pact geared toward making diagnostic kits for use on Illumina's MiSeq and NextSeq platforms.

Should the Qiagen deal go through, it is unclear whether Thermo Fisher, maker of the competing Ion Torrent sequencer, will maintain ties with Illumina. Thermo Fisher declined to comment. *–Elie Dolgin* ■

CAR Engineering Comes to Macrophages

First, there were chimeric antigen receptor (CAR) T cells. Then came CAR natural killer cells. Now, scientists at the University of Pennsylvania (Penn) Perelman School of Medicine in Philadelphia have outfitted human macrophages with CAR constructs, creating cancer-homing Pac-Man–like cells with the ability to infiltrate solid tumors, ingest malignant tissue, and stimulate adaptive immunity in mouse models (Cancer Discov 2020;10:484).

In mice implanted with HER2-expressing ovarian cancer cells, a team led by Saar Gill, MD, PhD, and Michael Klichinsky, PharmD, PhD, showed that macrophages transfected with a HER2-targeting CAR could decrease tumor burden and prolong survival (Nat Biotechnol 2020 Mar 23 [Epub ahead of print]). The therapeutic benefit derived not only from direct antigen-specific phagocytosis, but also from indirect proinflammatory effects, including enhanced antigen processing and cross-presentation to tumor-specific T cells.

"They have the ability to flip a cold tumor into a warm tumor if they are properly engineered," Klichinsky says.

Getting the engineering to work was not trivial. Because the lentiviral and retroviral vectors typically used for CAR transduction don't work well with primary macrophages, the researchers developed an adenoviral system that allowed them to deliver the CAR transgene while inducing the cells to adopt the "M1" phenotype associated with producing high levels of proinflammatory cytokines. Even in the face of cytokines that normally skew macrophages toward the "M2" suppressive state, the CAR-transfected cells maintain their antitumor activity. "They seem to be locked into this phenotype," Klichinsky says.

M2-type macrophages are often one of the most abundant cells in tumor infiltrates, "so the idea that you would want to add more macrophages to the tumor microenvironment isn't immediately obvious," says Meghan Morrissey, PhD, of the University of California, San Francisco (UCSF), who was not involved in the research. As such, this "suggests that macrophages can be reprogrammed to go from being a negative all the way to being a positive."

Saar and Klichinsky's work underpins Carisma Therapeutics, a company they founded in 2016 that has raised approximately $60 million to date. According to Klichinsky, who serves as vice president of discovery research, Carisma's lead CAR-macrophage candidate, an autologous HER2-targeted therapy called CT-0508, is slated to enter human testing before the end of the year; programs centered on targeting mesothelin and PSMA remain in earlier stages of discovery.

Two competing firms have similar technologies in development. Thunder Biotech spun out of the laboratory of Kim O'Neill, DPhil, of Brigham Young University in Provo, UT, who has shown that mesothelin-targeted CAR macrophages can shrink tumors in a mouse model of triple-negative breast cancer. Meanwhile, Myeloid Therapeutics—whose scientific founders include UCSF's Ron Vale, PhD, and Siddhartha Mukherjee, MD, DPhil, of Columbia University in New York, NY—is engineering myeloid cells for vaccine and tumor-killing purposes.

According to Myeloid's Chief Scientific Officer Daniel Getts, PhD,

these undifferentiated myeloid cells traffic more effectively to tumors than terminally differentiated macrophages. In contrast to Carisma, both Myeloid and Thunder are using nonadenovirus transduction systems.

Yet, perhaps the most notable difference between the three companies' technologies lies in the intracellular signaling domain chosen for each CAR construct: CT-0508 takes advantage of CD3ζ, part of the T-cell antigen receptor and the same domain used in first-generation CAR T-cell therapies, whereas Thunder's and Myeloid's products incorporate signaling domains from Toll-like receptors and phagocytotic receptors, respectively.

Judging by the Penn team's results, CD3ζ, despite its usual role in T-cell stimulation, seems to prime macrophages into an M1 phenotype, O'Neill says, "but it's probably not as good as the inherent mechanism" of macrophage activation.

Carisma is now developing novel co-stimulation domains that are "rationally designed" for macrophage activation, Klichinsky says. "If we find significant improvement, those will be implemented in future iterations" of the therapy. *–Elie Dolgin* ■

Looking to Scorpion Venom for GBM Treatment

When engineered into chimeric antigen receptor (CAR) T cells, chlorotoxin (CLTX)—a peptide component of the deathstalker scorpion's venom—may help pinpoint glioblastoma (GBM) cells for destruction. This concept, developed by researchers at City of Hope Comprehensive Cancer Center in Duarte, CA, has shown preclinical promise and will soon enter a first-in-human study (Sci Transl Med 2020;12:eaaw2672).

CLTX "probably helps scorpions deliver poison into their prey's nervous system, while not being toxic itself—which is loosely reminiscent of how we're applying it now," says Michael Barish, PhD, a co–senior author. "It's evolved through predator–prey relationships to have rather exquisite specificity, with useful therapeutic outcomes for us."

CLTX's binding affinity for GBM and other neuroectodermal tumors, sparing normal tissue, was established a couple of decades ago, says co–senior author Christine Brown, PhD. Since then, it's been developed as ^{131}I-conjugated radiotherapy for high-grade glioma, used to coat nanoparticles for targeted drug delivery, and turned into a fluorescence imaging tool, enabling more precise brain tumor resection. The imaging tool, called "Tumor Paint," was created by James Olson, MD, PhD, of Fred Hutchinson Cancer Research Center in Seattle, WA. "It's his work we're building on," Brown notes. "We wanted to get from tumor binding to tumor killing, by exploiting CLTX in CAR T cells."

Graduate student Dongrui Wang was tasked with achieving this goal, which involved going beyond the familiar terrain of antibody-based constructs (commonly targeting CD19) to ligand–receptor CARs. "We did have some experience" with the latter, he says, but pioneering an optimal design to incorporate a peptide toxin targeting the membrane-associated protein MMP2, a crucial part of CLTX's receptor complex, was challenging.

Interestingly, Wang observes that "our construct worked much better with CD28 as a costimulatory domain," instead of 4-1BB. Possible reasons why remain unknown, but the field "has had this idea for a while now that 4-1BB is a preferable design," Brown notes, "and our data seem to suggest something different."

In patient-derived xenograft models, potent anti-GBM activity and tumor regression were seen with CLTX–CAR T cells, Wang says—even when there was minimal expression of IL13Rα2, HER2, or EGFR, three key GBM antigens. The therapy was also active against glioma stem cells, a subpopulation that often seeds recurrence. Treatment was well tolerated, with no off-target effects or other toxicities.

Encouraging preclinical safety aside, CLTX–CAR T cells' potential immunogenicity in humans "is difficult to model," Brown says, "so it will be an important end point" in the phase I trial. "We're screening patients based on tumor MMP2 expression," she adds, "because that's essential for CAR T recognition and targeting."

Antonio Iavarone, MD, of Columbia University in New York, NY, considers CLTX–CAR T cells "a novel approach

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A peptide component of the deathstalker scorpion's venom may help pinpoint glioblastoma cells.

that should allow more comprehensive targeting of a highly heterogeneous cancer." In general, GBM remains a poor candidate for immunotherapy, but Iavarone and others are gradually unearthing features, in small subsets of patients, that may better predict benefit. He therefore lauds patient stratification, such as Brown's team is doing, as "absolutely key" (Commun Biol 2, 135 [2019]).

"The more molecular profiling up front, the more likely that even if a given trial turns out negative, it will still be informative," Iavarone says. He hopes for clinical efficacy with CLTX–CAR T cells but thinks concurrent immune checkpoint inhibition may well be necessary, based on the researchers having shown that one route of treatment resistance is PD-L1 induction.

"Once we've shown our therapy is safe in people, we do want to start a combination study," Brown agrees. She's encouraged that despite the disease's seeming intractability, "there are more CAR T trials for GBM than any other solid tumor. It should accelerate our understanding of what can be achieved, therapeutically, for this population." *–Alissa Poh* ■

Microbiome Predicts Blood-Cell Transplant Success

A large multicenter international study concludes that the composition of the intestinal microbiome in patients undergoing allogenic hematopoietic-cell transplants (HCT) for leukemia and other blood cancers can predict treatment success. The