T cells extracted from a patient's blood must be healthy enough to be modified into CAR T cells and to expand at least five-fold to possibly be effective, explained Barrett. When subsequently infused into the patient, the CAR T cells need to be sufficiently active to attack the cancer. In several patients Barrett's team treated early on, T cells expressed markers of exhaustion when they were collected and either died in the lab or were too worn out for the therapy to work.

"What was the difference between the cells that were successful and those that weren't? It was the starting material," Barrett said. "I think everybody knows that chemotherapy is really bad for your T cells, and the more chemo you get, the less likely you are to have healthy T cells. But I really wanted to know what is the potential [for success] at diagnosis, before these patients have ever seen therapy."

The researchers collected blood from 157 children with ALL, chronic myelogenous leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma, neuroblastoma, osteosarcoma, rhabdomyosarcoma, Wilms tumor, or Ewing sarcoma upon diagnosis and after each cycle of chemotherapy. In the prechemotherapy samples, Barrett reported, they found that the CAR T-cell potential was poor in all of the malignancies except ALL and Wilms tumor. RNA profiling of metabolic pathways revealed that the poorly performing T cells relied on glycolysis for fuel instead of fatty acids like normal cells.

Further, the researchers noted a decline in T-cell potential with successive chemotherapy cycles in all of the cancer types. In one patient with ALL, T cells examined prior to and after the first and second cycles looked relatively normal, but by the sixth cycle, the cells had little potential of success as CAR therapies.

"Cumulative chemotherapy is altering the metabolic profile of the T cells," said Barrett, who outlined his team's findings at a press conference in advance of the American Association for Cancer Research Annual Meeting 2018, which was held April 14–18 in Chicago, IL.

In later preliminary experiments, Barrett said researchers demonstrated that "force-feeding" fatty acids to T cells could restore their spare respiratory capacity, a measure of their energy reserve.

The goal now, said Michael Caligiuri, MD, president and physician-in-chief at City of Hope National Medical Center in Duarte, CA, and moderator of the press conference, is to develop "alternate strategies—different chemotherapies, different preparatory regimens—that really decrease the injury to the T cells' metabolic pathway and, if not, reversing that metabolic pathway."

Some changes in clinical care have already occurred. "Based on this data, we have altered our practice for T-cell collection for children with leukemia, for children with high-risk disease," noted Barrett. "We will collect T cells early, even if that patient is not currently eligible for a CAR T trial, simply because we know that cumulative chemotherapy is going to progressively deteriorate the likelihood that those cells will make a functional product, and we've been recommending that to other centers." –*Suzanne Rose* ■

Machine Learning Improves Diagnosis of CNS Cancers

Artificial intelligence can recognize molecular patterns in DNA methylation data to classify tumors of the central nervous system (CNS) with greater diagnostic precision than a human pathologist.

The finding has led to the creation of a free online diagnostic tool that allows pathologists to upload their raw microarray data on the methylation status of hundreds of thousands of single nucleotides in the genome and receive a report classifying a patient's tumor within minutes (Nature 2018;555:469–74; www.molecularneuropathology.org).

This machine-learning method will not supplant standard protocols in neuropathology anytime soon, experts say, but it could be a valuable aid when existing techniques yield inconclusive results. "It's a landmark article," says David Louis, MD, of Massachusetts General Hospital in Boston, MA, who was not involved in the new study. "It will most likely augment the pathologist's toolbox."

Next-generation sequencing and epigenetic analyses remain uncommon outside a research setting, but the case for these more advanced molecular tools is growing, say Stefan Pfister, MD, and Andreas von Deimling, MD, from the German Cancer Research Center (DKFZ) and Heidelberg University Hospital, the lead authors of the new study.

To develop their diagnostic tool, the researchers trained a computer algorithm to find methylation patterns in pathologist-classified samples of CNS tumors. The algorithm assigned the tumors to 82 classes, only one third of which exactly matched those defined by the World Health Organization (WHO) classification system. The remainder largely represented subclasses or combinations of established categories, although some were tumor types that the WHO has not previously recognized.

The DKFZ team then pitted their algorithm against a pathologist in 1,104 test cases. For 838 of those, the human and the computer agreed, although the computer sometimes also assigned tumors to a subcategory. In 139 cases, there was a mismatch, and further molecular analyses showed that the computer was correct 93% of the time. In the remaining 127 cases, the algorithm proved inconclusive, often because the samples came from rare pediatric tumors that are not yet part of the reference taxonomy.

Independent groups at five clinics across Europe and the United States validated the tool on another 401 cases and came to similar conclusions about its diagnostic accuracy. The DKFZ team is now prospectively testing the platform in a study called Molecular Neuropathology 2.0, which is enrolling about 80% of children diagnosed with brain tumors in Germany.

Stephen Yip, MD, PhD, of the University of British Columbia in Vancouver, Canada, who wrote an accompanying commentary, expects the tool to gain popularity for thorny cases (Nature 2018;555:446–7). "Simple cases that are immediately recognizable by unique microscopic features, or in which we have simple and cheap adjunct diagnostic tools, will not gain too much from the methylation array at present," he says. "It's really the histologically ambiguous cases or those

that present in an unusual manner that would benefit."

The methylation-based classification tool is not restricted to CNS tumors. In another paper, the DKFZ team showed that methylation profiling provides valuable diagnostic information for sarcomas, and they have developed a machine-learning algorithm to classify those cancers as well (Mod Pathol 2018 March 23 [Epub ahead of print]).

"This principle of using methylation patterns to determine cell of origin will be useful for any tumor for which there is diagnostic uncertainty," Pfister says. *–Elie Dolgin* ■

Organoids May Point to Best Therapy

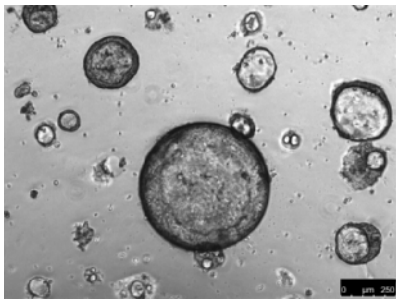
Organoids—miniature, three-dimensional tumors grown from a patient's cancer cells—accurately predict response to treatment in patients with gastrointestinal cancer, according to a recent study (Science 2018;359:920–6).

Technology for creating patient-derived organoids was pioneered by Hans Clevers, MD, PhD, a scientist at the Hubrecht Institute and research director of the Princess Máxima Center in the Netherlands, who showed that organoids have biologic properties similar to the original tumors.

Nicola Valeri, MD, PhD, of the Institute of Cancer Research in London, UK, the new study's senior author, and his team wanted to investigate whether organoids created from gastrointestinal cancers would respond to treatment like the original tumors did.

"Very little was available when we started, and even now, on whether *ex vivo* responses in organoids could inform us as to what's going to happen in the patient," he says. "But these are cancers where there are options, and you want to find the right option for the right patient."

Valeri and his team grew organoids from 110 metastatic tumor samples from 71 patients with chemorefractory colorectal or gastroesophageal cancer who were enrolled in phase I/II clinical trials. In 21 cases, researchers exposed organoids to the same treatments that patients received, finding that drugs that were ineffective in organoids were ineffective in patients 100% of the time, whereas drugs that yielded a response in organoids yielded a response in 88% to 90% of the patients.



George Vlachogiannis

Organoid cultures established from a patient with metastatic colorectal cancer.

The team also profiled 151 cancer-related genes, establishing a 96% match between the mutational spectrum of the organoids and the original tumors, and tested a library of 55 drugs on the organoids.

"This is one of the first studies where we managed to compare what happens in the clinical model, in this case organoids, and the clinical response," Valeri explains, adding that he expects organoids to be used with sequencing to determine whether a patient is likely to respond to a treatment. In addition, organoids will be valuable for studying tumors that don't respond to treatment, he says.

Valeri and his team now plan to grow organoids from circulating tumor cells rather than tumor tissue to better capture the heterogeneity of a patient's cancer. They are also attempting to grow organoids in a microenvironment like the tumor's.

"This is the first formal demonstration for cancer that tumor organoids predict patient responses," Clevers says, "so I think it's a big breakthrough."

For Clevers, who was not involved in the study, the next advance would be a prospective trial that compares patients who receive treatment based on their organoid response with patients who receive treatment based on tumor pathology or DNA analysis, "and shows that decision-making based on organoids is better."

Clevers points out, however, that until organoids become less expensive and less complicated to produce, they will likely be used only in specific contexts, such as for improving the utility of DNA sequencing and for drug development.

"Using organoids, you can gather information on drug resistance and drug sensitivity with much higher throughput than in clinical trials, and you can match that information to DNA profiles and learn what exactly the DNA sequence can tell you about whether a patient will or will not respond," he says. "So, organoids would help to break the resistance/sensitivity code." *–Catherine Caruso* ■

CSPG4 Shows Promise for Glioblastoma CAR T Therapy

A new target for chimeric antigen receptor (CAR) T-cell therapies for glioblastoma could help overcome some of the problems of tumor escape and heterogeneous expression that have limited the effectiveness of other tumor-associated antigens included in clinical CAR T-cell candidates for this deadly brain cancer.

According to a preclinical study, T cells transduced to express a transmembrane signaling protein called chondroitin sulfate proteoglycan 4 (CSPG4) can successfully blunt the growth of brain tumors in cultured neurospheres and in glioma xenograft models, with no signs of immune evasion owing to loss of antigen expression (Sci Transl Med 2018;10:eaao2731).

Experts who study CAR T-cell therapies for glioblastoma have been buoyed by the findings. "This antigen definitely could have great potential," says Irina Balyasnikova, PhD, of Northwestern University Feinberg School of Medicine in Chicago, IL. However, as Donald O'Rourke, MD, of the University of Pennsylvania in Philadelphia, points out: "There's just not a lot known about this molecule in glioblastoma."

Plus, adds O'Rourke, who has tested an EGFRvIII-directed CAR T-cell therapy for brain cancer and is planning another trial of the treatment in combination with a checkpoint inhibitor, "extrapolating from mouse CAR T data to humans is a big leap" (Sci Transl Med 2017;9:eaaa0984).