editing could repair pathogenic alterations in rare monogenetic diseases. Indeed, in cultured fibroblasts isolated from patients with Hermansky–Pudlak syndrome or Menkes disease, Cas9 and a guide RNA specific for the microduplication allele restored 88% and 94% of the alleles to wild type for the two diseases, respectively. The cells were not used to treat patients.

Despite this in vitro success with the inDelphi tool in designing guides RNAs for reducing microduplication, the clinical utility of the two prediction tools may be limited, argues Fyodor Urnov, a pioneer in the field and now deputy director of a nonprofit research organization, the Altius Institute for Biomedical Sciences.

Urnov says he also would have liked to see the predictive algorithms developed in "cell types that actually matter." inDelphi was trained on data from three human cancer cell lines, on mouse embryonic stem cells, and on an immortalized line of human embryonic kidney cells. "We've known for 30 years," says Urnov, "that cancer cells or stem cell differ in the biology of DNA double-strand break repair from normal cells, and that dividing cells differ from stationary cells." He singles out the human cancer cell line K562 used as the main workhorse in the Wellcome Sanger study: "I assure you they are not normal cells."

Sherwood acknowledges this limitation: "Everything we have done has been in culture, with rapidly dividing cells. Going forward, we'd like to try this in animals, or in cells that grow slowly, or not at all."

The Wellcome Sanger work also explored the influence of cell context, using a subset of 3,777 guide RNAs in five more cell types. Allen says that the repair outcomes were still predictable. But Urnov wants predictions made in different brain and CNS cell types and in those tissues already playing host to gene editing modalities in the clinic.

Systematic exploration of repair outcomes in other host environments might help build bridges between laboratory and clinical studies. However, as Dennis Eastburn, CSO and cofounder of Mission Bio points out, variability in cell type is not the only cellular factor that would need to be taken into account. "On-target and off-target edits can be influenced by the cell line," he says, "but also potentially by the cell state, rate of growth, cell signaling effects and other factors."

Mission Bio is an instrument and reagent provider rather than a gene editing company. But since 2017, when it launched a product to track somatic mutations in oncology at the single cell level, pharma, biotech and academic investigators have approached the company asking for help resolving gene editing outcomes. Mission Bio has recently joined the National Institute of Standards and Technology (NIST) Genome Editing Consortium, a group formed in January 2018 to develop common standards in the gene editing field, including techniques that are bound for the clinic.

Homology Medicines, for instance, uses homology-directed repair, a technique that can, in theory at least, deliver a more precisely controlled form of gene editing. CSO Albert Seymour believes the key for CRISPR tools to progress to the clinical will be experimentation, coupled with prediction. Homology's nuclease-free gene-editing system has worked in mouse models of phenylketonuria, he says. "We can restore phenylalanine hydroxylase to 10% of wild type," sufficient to address the disease. The company is continuing to track down any off-target editing.

Much further ahead is Sangamo Therapeutics, whose zinc-finger nuclease (ZFN) gene editing products had reached the clinic before anyone had even heard of CRISPR–Cas9. Sangamo developed a ZFN system to disrupt the gene encoding the HIV co-receptor, *CCR5*, in primary human $CD4^+$ T cells in 2008 (*Nat. Biotechnol.* **26**, 808–816, 2008). Its current gene editing pipeline includes two ZFN products to treat inherited metabolic diseases and a third for hemophilia B; in February, Sangamo announced that its ZFN SB-318 had failed to change baseline leukocyte α-L-iduronidase in interim results from three patients in a phase 1/2 trial of mild mucopolysaccharidosis type 1. In terms of targeting fidelity, says Ed Rebar, Sangamo's chief technology officer, the ZFN target sites are longer (33–36 base pairs) than those used by the Cas9 enzyme (22 base pairs), and that is important for specificity. While 22-mer sites may be unique within the human genome, highly similar sites (i.e., single or double mismatches) will typically be present. "The longer zinc finger sites can tolerate 6–8 mismatches and still be unique," explains Rebar. In turn, the high specificity of the interaction between the ZFN and its target sequence within the genome means that the activity of the enzyme can be 'titrated up' to clinically appropriate levels. ❐

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Vertex ramps up CRISPR repair

Vertex Pharmaceuticals signed two CRISPR-based licensing deals in January that boost the company's gene editing portfolio. In one deal, Vertex regains rights to two DNA-dependent protein kinase (DNA-PK) inhibitors that it had originally licensed to Merck KGaA in 2017. Under the new agreement, Merck KGaA, of Darmstadt, Germany, retains rights to use the compounds in oncology and other therapeutic areas whereas Vertex will pursue these molecules in gene editing applications.

DNA-PKs are enzymes that repair double-stranded breaks in DNA. Following CRISPR–Cas gene editing, which creates such double-stranded breaks, DNA-PKs repair them using non-homologous end joining, which directly joins the two ends but may lead to insertions or deletions at the break site. Vertex intends to use the DNA-PK inhibitors to skew the DNA repair in Cas gene editing toward an alternative and more precise repair mechanism that uses a homologous template to restore the DNA sequences.

In cancer, DNA-PK inhibitors could make DNA-damaging agents such as radiotherapy and chemotherapy more effective. One compound that formed part of the deal—M9831 (also known as VX-984)—has completed a phase 1 trial, in combination with doxorubicin chemotherapy in patients with advanced solid tumors.

Vertex's second January deal focuses on the development of DNA endonucleases as alternatives to the commonly used Cas9 subtype. The partnership is with Arbor Biotechnologies, a small biotech that, also in January, published the identification and characterization of several subtypes of the Cas12 endonuclease.

Vertex already has a CRISPR–Cas gene therapy in the clinic, CTX001, through a 2015 deal with CRISPR Therapeutics. This therapy uses CRISPR–Cas9 gene editing to engineer a patient's own stem cells to produce high levels of fetal hemoglobin, and is in phase 1/2 trials for sickle cell disease and β-thalassemia. The latest deals suggest that Vertex continues to see CRISPR–Cas-mediated gene editing as an important part of its pipeline.

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