Innovators want pills to treat sickle cell disease. Can they match gene therapy?

Although CRISPR-based gene therapy for sickle cell disease offers transformative outcomes, drugmakers are striving to develop treatments that are easy to manufacture and can reach much larger numbers of patients.

By Cormac Sheridan

A red blood cell affected by sickle cell disease.

Just as the world's first CRISPR-based gene editing therapy gained approval for treating sickle cell disease late last year, Pfizer was unveiling its progress with an investigational oral drug for the same condition. Regulatory backing for Casgevy (exagamglogene autotemcel) received far more attention, given its historic significance and dramatic effects on relieving acute symptoms of the condition. But for many people living with sickle cell disease an oral drug such as GBT021601 might be more important. GBT021601 (also called osivelotor) is a small-molecule drug and is not hard to manufacture in large quantities. In contrast, both Casgevy, which Vertex and CRISPR Therapeutics co-developed, and Lyfgenia (lovotibeglogene autotemcel), the ex vivo-engineered cell therapy from Bluebird Bio that gained approval at the same time, are difficult to scale and will only have a limited reach. Oral and injected drugs (Table 1) may not offer the profound effects on acute sickle cell symptoms observed with Casgevy and Lyfgenia, but their greater accessibility means they will have a bigger impact on the global disease burden.

The drug GBT021601 came into Pfizer's hands through its $5.4-billion acquisition of Global Blood Therapeutics in 2022. It is a more potent successor to another drug from Global Blood Therapeutics, Oxbryta (voxelotor), which gained approval in 2019. Both act by preventing the mutant, sickling form of hemoglobin, known as HbS, from polymerizing, which gives rise to rigid, sickle-shaped red blood cells. These misshapen erythrocytes, which are the hallmark of the condition, are unable to move through blood vessels as readily as their healthy counterparts, resulting in blockages in blood vessels, impaired blood flow to tissues, red blood cell destruction by hemolysis and, in the long-term, organ damage, stroke and other problems.

In people with sickle cell disease, hemoglobin adopts the 'sickling' conformation only in hypoxic conditions, when the molecule is deoxygenated. Oxbryta and GBT021601 counter these processes by increasing the affinity of HbS for oxygen and lengthening the time patients' hemoglobin remains functional.

Oxbryta gained approval based on its ability to boost functioning hemoglobin by at least 1 gram per deciliter of blood — known as a 'hemoglobin response' — after 24 weeks of daily dosing. In a pivotal trial, Oxbryta enabled a hemoglobin response in just over 51% of patients, compared with 6.5% of patients on placebo. "We know that every gram increase in hemoglobin is associated in retrospective studies with improvements in long-term outcomes," says Kim Smith-Whitley, a pediatric hematologist, site head and advisor of scientific & clinical affairs at Pfizer. The drug's ability to reduce vaso-occlusive crises (VOCs) — a key efficacy measure — was variable, however. These severe acute episodes of inflammatory pain arise from blockages in blood vessels and often require hospitalization. In a real-world study of Oxbryta, the number of VOCs patients experienced in a year dropped 23% from baseline. In contrast, in the phase 3 trial that secured its approval, the drug had only a limited impact on this parameter. That may reflect the variability of the condition, between different patients and across the lifetime of individual patients.

Pfizer hopes that GBT021601's higher affinity for oxygen will translate into a superior safety and efficacy profile over that of Oxbryta. "I do think '601 has the potential to be more efficacious at a lower dose," says Smith-Whitley.

Jefferies, an investment bank, has set out a bullish investment case for GBT021601, based on interim data Pfizer unveiled at the American Society of Hematology's 2023 annual meeting. After 12 weeks' therapy, patients in the high-dose and low-dose arms of an ongoing phase 2/3 study achieved mean increases in hemoglobin of 3.17 g/dL and 2.67 g/dL, respectively. (Their baseline levels ranged from 5.5 to 10.5 g/dL). That boost is "comparable" to that of gene therapy, according to an investor note from Jefferies analysts, who have forecast peak sales of $2.2 billion for the product. That improved efficacy is also reflected in an effect on VOCs: the annualized rate of such

Table 1 | Selected drugs in development for sickle cell disease

Developer(s)	Agent	Mechanism	Clinical data	Status
Novartis	Adakveo (crizanlizumab)	Binds the adhesion molecule P-selectin, which is expressed on the surface of activated endothelium and platelets, and blocks their interaction with red blood cells, leukocytes and endothelial cells	In a phase 3 trial, those on Adakveo had a median annual rate of vaso-occlusive crisis of 1.63, versus 2.98 for those on placebo	FDA approval 15 November 2019; the EMA revoked a European approval in 2023
Pfizer	Oxbryta (voxelotor)	HbS polymerization inhibitor	In a phase 3 trial, the 24-week hemoglobin (Hb) response rate (an increase in Hb >1 g/dL) for those on Oxbryta was 51.1%, versus 6.5% for those on placebo.	FDA approval 25 November 2019
Pfizer	Inclaclumab	P-selectin inhibitor administered once a quarter	No dose-limiting toxicities observed in a phase 1 trial in healthy volunteers	Phase 3
Agios Pharmaceuticals	Pyrukynd (mitapivat)	Allosteric activator of pyruvate kinase, which extends red blood cell lifespan by increasing ATP levels and decreasing 2,3-disphosphoglycerate levels	In a phase 2 trial, the 12-week Hb response rates for patients on low-dose mitapivat, high-dose mitapivat or placebo were 46.2%, 50.0% and 3.7%, respectively	Phase 2/3 (gained FDA approval for treating hemolytic anemia due to pyruvate kinase deficiency on 17 February 2022)
Forma Therapeutics (Novo Nordisk subsidiary)	Etavopivat	Allosteric activator of pyruvate kinase	After 12 weeks of treatment in an open-label extension portion of a phase 1 trial, mean maximal Hb increase was 1.5 g/dL; 11 of 15 patients (73%) attained an Hb response	Phase 2/3
Pfizer	GBT021601 (osivelotor)	HbS polymerization inhibitor	After 12 weeks of treatment in an open-label, dose-finding portion of a phase 2/3 study, Hb levels increased by a mean of 2.67 g/dL in the 11 patients on 100 mg per day and by a mean of 3.17 g/dL in the 12 patients on 150 mg per day over baseline levels of 5.5–10.5 g/dL	Phase 2/3
Novo Nordisk	NDec	Combination of decitabine, a DNMT1 inhibitor that boosts HbF production, and tetrahydrouridine, which prolongs decitabine's half-life by inhibiting the inactivating enzyme cytidine deaminase	In an investigator-initiated study, the combination increased total Hb, HbF, and the percentage of HbF-enriched red blood cells	Phase 2
Agios Pharmaceuticals	AG-946	Allosteric activator of pyruvate kinase	NA	Phase 1
Fulcrum Therapeutics	Pociredir (FTX-6058)	Inhibitor of EED, part of PRC2, which boosts expression of HbF by downregulating expression of BCL11A	In the first three patients to receive FTX-6058 for at least 42 days of therapy, HbF levels rose from baseline by 2.1%, 5.2% and 6.3%; maximal levels may not have been reached	[a]Phase 1
GSK (Brentford, UK)	GSK4172239D (oral prodrug of GSK4106401)	DNMT1 inhibitor, which de-represses expression of the γ-globin genes *HGB1* and *HGB2* and thus boosts HbF production	NA	Phase 1

[a]The FDA placed a clinical hold on the study on 23 February 2023, which it lifted on 22 August 2023. DNMT1, DNA cytosine 5 methyltransferase 1; EED, embryonic ectoderm development protein; Hb, hemoglobin; HbF, fetal hemoglobin; HbS, sickling hemoglobin; NA, not available; PRC2, Polycomb repressive complex 2.

events dropped from the baseline level of 2.3 episodes per year to 1.2. (The two approved genetic therapies eliminated VOCs in the vast majority of patients.) GBT021601 also appears to be more tolerable than Oxbryta, which is administered at a high dose. During the trial, proportionally fewer patients stopped taking the drug because of gastrointestinal side effects than is historically the case with Oxbryta.

Several other drug developers, including Fulcrum Therapeutics, are attempting to induce fetal hemoglobin (HbF) expression, a strategy similar to that pursued by gene therapy and gene editing firms. HbF, the main oxygen transport protein during pregnancy and early infancy, comprises two α-globin and two γ-globin chains and is therefore unaffected by sickle cell disease, which arises from a single amino acid substitution in the β-globin chain of adult hemoglobin. Hereditary persistence of HbF, which can arise through mutations in a number of genes, has long been recognized to protect against severe symptoms of sickle cell disease.

Approved gene therapy Casgevy — as well as a number of other gene editing therapies in development, including Beam Therapeutics' ex vivo base editing therapy BEAM-101 and Editas Medicine's renizgamglogene

autogedtemcel (formerly EDIT-301) – induces HbF production. Fulcrum set out to identify small-molecule targets that could also modulate HbF gene expression. Using a CRISPR-based screening system, it identified EED (embryonic ectoderm development), a component of the Polycomb repressive complex 2 (PRC2), as an epigenetic regulator of HbF expression. From this effort emerged pociredir, a small molecule that binds EED, resulting in PRC2 inhibition and increased HbF production. "After only six weeks of dosing, we were able to get patients to around 25%," says CEO Alex Sapir. "We could potentially get up to fetal hemoglobin levels that are comparable with what is being seen with cell and gene therapy."

Progress with pociredir was temporarily halted, however, when the US Food and Drug Administration placed a clinical hold on the program in February 2023, following the emergence of secondary malignancies associated with a third-party cancer drug that also targets PRC2. "We believe it's tazemetostat," says Paul Bruno, executive vice president, corporate and business development at Fulcrum, referencing a drug for lymphoma and sarcoma that Paris-based Ipsen markets as Tazverik. Although the agency lifted the hold after six months, it has imposed restrictive inclusion criteria, which limits the resumed study to patients with severe sickle cell disease.

Several other targets linked to HbF expression have recently emerged. A team of scientists at Basel, Switzerland-based Novartis has used phenotypic screening to identify compounds that degrade a transcription factor, Wiz, thereby de-repressing γ-globin expression and boosting HbF production in patient-derived cells. Others are attempting to drug BCL11A directly. Scientists at the lab of Stuart Orkin, of the Dana-Farber Cancer Institute in Boston, reported at the American Society of Hematology annual meeting last year that BCL11A functions as a tetramer and that its stability depends on a particular zinc finger domain, an unexpected finding that means it has a stable structure that can be targeted with small molecules.

At the other end of the development cycle, Agios Pharmaceuticals is seeking to extend the use to sickle cell disease of Pyrukynd (mitapivat), a small molecule already approved for patients with hemolytic anemia due to pyruvate kinase deficiency. In either indication the goal is to reduce hemolysis and extend the lifespan of patients' red blood cells. "The hemolytic anemias create profound metabolic oxidative stress in the cell," says CEO Brian Goff. Moreover, the large-scale release of the contents of lysed red blood cells into the circulation is toxic. The enzyme pyruvate kinase catalyzes the conversion of phosphoenol pyruvate to pyruvate while generating adenosine triphosphate. Increasing the enzyme's activity improves the cells' energy status, but it also depletes levels of the glycolysis pathway intermediate 2,3-diphosphoglycerate, which is a negative allosteric regulator of hemoglobin's oxygen affinity. In sickle cell disease, this additional effect could lower hemoglobin polymerization. Agios has a second pyruvate kinase activator in the clinic, which has enhanced efficacy and may allow a once-daily dose regimen. Also pursuing a pyruvate kinase activator is Novo Nordisk, of Bagsværd, Denmark. The pharma paid $1.1 billion to move into this space in 2022, when it acquired Forma Therapeutics, the biotech developing etavopivat.

At present, neither drug therapies nor gene or gene editing therapies are a panacea. Casgevy and Lyfgenia appear to be remarkably effective, but only a small number of patients will benefit from these therapies. Small molecules are inherently scalable, but they do not offer the transformative outcomes available with ex vivo gene or gene editing therapies.

In vivo gene or gene editing therapy would bypass these problems. That may seem a remote prospect at this point, but it is the long-term ambition of a partnership between the Innovative Genomics Institute (IGI) at the University of California, Berkeley, led by Jennifer Doudna, and Pioneer Science, a Brazilian philanthropic organization based in Rio de Janeiro.

The initial plan is to develop an ex vivo CRISPR-based therapy, which will be available in Brazil on a not-for-profit basis. "The special thing about the way that we're delivering the CRISPR enzyme is that it can be modified for in vivo use," says Ross Wilson, of UC Berkeley and the IGI, who is principal investigator on the US side of the alliance.

A new delivery technology could make this possible. Wilson's group has previously reported on an amphiphilic peptide they identified by screening. The team succeeded in editing T cells, B cells and natural killer cells simply by simply mixing the peptide with a CRISPR ribonucleoprotein. It does not require any dedicated hardware and, because the approach avoids electroporation, which is damaging to cells, it may lead to an improved product profile. "Our current data suggest that the cells are doing just fine. That's one of

News in brief

World's first TIL therapy approved

Iovance Biotherapeutics has received a long-awaited go-ahead from the US Food and Drug Administration for its tumor-infiltrating lymphocyte (TIL) cancer therapy. The approval for Amtagvi (lifileucel) for treating patients with unresectable or metastatic melanoma is a milestone: it marks the first immune cell therapy approved for solid tumors, and it is also the first made from TILs.

Amtagvi is a living therapy. It involves harvesting T cells from within a patient's tumors, expanding them in bioreactors and reinfusing them. Those T cells seek out and recognize cancer neoantigens and, when re-infused, react to these antigenic epitopes and destroy the cancer cells. Steven Rosenberg, a surgeon at the US National Cancer Institute, first began treating patients with melanoma with TILs in 1988, later licensing the technology to Iovance.

Amtagvi's approval was based on an open-label phase 2 trial in patients with advanced melanoma. The overall response rate in 73 patients was 31.4%, with three (4.1%) complete responses and 43.5% responses lasting 12 months. Side effects were mostly from the lymphodepletion given before TILs are infused and include low blood count, severe infections and cardiopulmonary and renal risks. An ongoing phase 3 study will provide confirmatory evidence of benefit.

TIL therapies have the potential to be curative for solid cancers, but over the years, manufacturing has been a hurdle. TILs, unlike CAR-T cells, are not genetically engineered, and boosting their proliferation involves adding growth factors and cytokines such as interleukin-2, in a process that takes 22 days. Another disadvantage is that TILs vary from patient to patient, making quality control difficult. The company will produce Amtagvi at its own facilities in Philadelphia and says it has capacity to treat up to several thousand patients a year. The wholesale cost will be $515,000, slightly above that of CAR-T cell therapies. Iovance hopes to expand to other indications and is in phase 2 testing for cervical cancer.

the goals, to put the cells in a healthier state, that is less impacted by electroporation, to improve engraftment and minimize risks of adverse events," he says.

The team is now refining a protocol for editing hematopoietic stem and precursor cells of patients with sickle cell disease before transferring the technology and know-how to Brazil. Bruno Solano, a physician-scientist who has posts at the Instituto D'Or de Pesquisa e Ensino and Hospital São Rafael in Salvador, will lead the clinical development and rollout of the therapy in the country. A cell therapy specialist, he already has experience in administering chimeric antigen receptor T cell therapy to patients with cancer. Brazil has close to 100,000 people with sickle cell disease, which is similar in scale to the US patient population, but, says Solano, the prevalence is particularly concentrated in the Bahia region in the northeast, where about one in every 650 people has the condition. "It's the highest incidence in the country."

Successfully delivering a CRISPR-based therapy to Brazilian patients would represent an important step in globalizing this transformative approach. There is a still a long way to go before all patients can receive effective therapies. Sub-Saharan Africa is where the need is greatest and is growing most rapidly: according to a study published last year, the region accounted for 79% of all new cases in 2021. Tackling this huge burden of disease remains a critically important task.

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Food security

Cell-based coffee future-proofs world's favorite brew

Your next caffeine jolt could come from a lab. Scientists from Pluri Biotech, a regenerative medicine and cultivated meat company based in Haifa, Israel, propose to make coffee without the need to grow the entire plant. Coffee crops are expected to be hit hard by climate change. Arabica, *Coffea arabica*, which is the world's most popular coffee and has been harvested for millennia, is threatened with extinction from climate change and the spread of fungal pathogens. Cell-based coffee could prove the solution to a shrinking supply of coffee and an ever-increasing number of coffee drinkers.

CREDIT: MICHAEL BRIKMAN

Lior Raviv, Pluri's chief technical officer, says: "We hypothesized we could take the cells from the plant and put them in a bioreactor [to grow coffee]." Through their work in cell therapy and cultivated meat, the Pluri team knew that not all cells like the same growing conditions. Taking plant cell samples, they made cell lines and, instead of growing them swirling around in suspension culture, they used a packed-bed bioreactor. The coffee cells slowly flow through, taking on a tissue-like structure. The cells are fed salts and vitamins, and their natural metabolism then takes over to produce secondary metabolites such as caffeine. The resulting biomass, which forms as small clumps, is dried and gently roasted. The final product looks and tastes like ground coffee. Pluri is now focusing on scaling up and seeking regulatory approvals.

Stem, a Paris-based company, is also propagating and fermenting coffee cells lines derived from coffee plants in bioreactors. The company adds natural flavors taken from coffee byproducts to the resulting green coffee powder, which they dry and roast like coffee beans. Another startup, California Cultured, is applying plant cell culture technology to both coffee and cocoa beans.

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