NEWS · 15 MAY 2020

Gene-editing pipeline takes off

Clinical trials of genome-editing agents — including CRISPR–Cas9 editors, zinc finger nucleases and TALENs — are pushing ex vivo, immuno-oncology and in vivo treatment frontiers.

Asher Mullard

Credit: S.Harris/Springer Nature Limited

Gene-editing programmes have been slowly trickling into the clinic since 2005. But the pace is picking up. In the past year and a half alone, at least 11 such programmes entered the clinic in

“It's just such an exciting time right now,” says UC Berkeley’s Jennifer Doudna, a pioneer of the CRISPR–Cas technology and co-founder of multiple CRISPR companies. After years spent imagining how this emerging technology could transform the treatment of disease, key clinical read outs are at last around the corner.

Upcoming safety and efficacy data will showcase the potential of this DNA-cutting modality, and could define the short-term future for these medicines. So far, the majority of gene-editing programmes focus on monogenic rare diseases and immuno-oncology applications. But proponents of gene editing are already looking at broader applications.

“As with any new technology, the typical way that that things get rolled out is that drug developers begin with rare and or extremely severe diseases where there aren't really other options. And the outcomes of the first applications have a very big effect on whether the technology is perceived to be safe or appropriate to be used more widely,” explains Doudna. “I think that's kind of where we are right now with CRISPR.”

Other gene-editing technologies are not to be overlooked, either (Fig. 1; Box 1). Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and meganucleases are also making progress. “It is a pet peeve of mine that people read some of these CRISPR articles and don't actually understand the breadth or history of this field,” says Charles Gersbach, an expert on gene editing and biomedical engineer at Duke University. Sangamo was founded 25 years ago to advance ZFNs, he points out, and has since run landmark in vivo and ex vivo clinical trials.

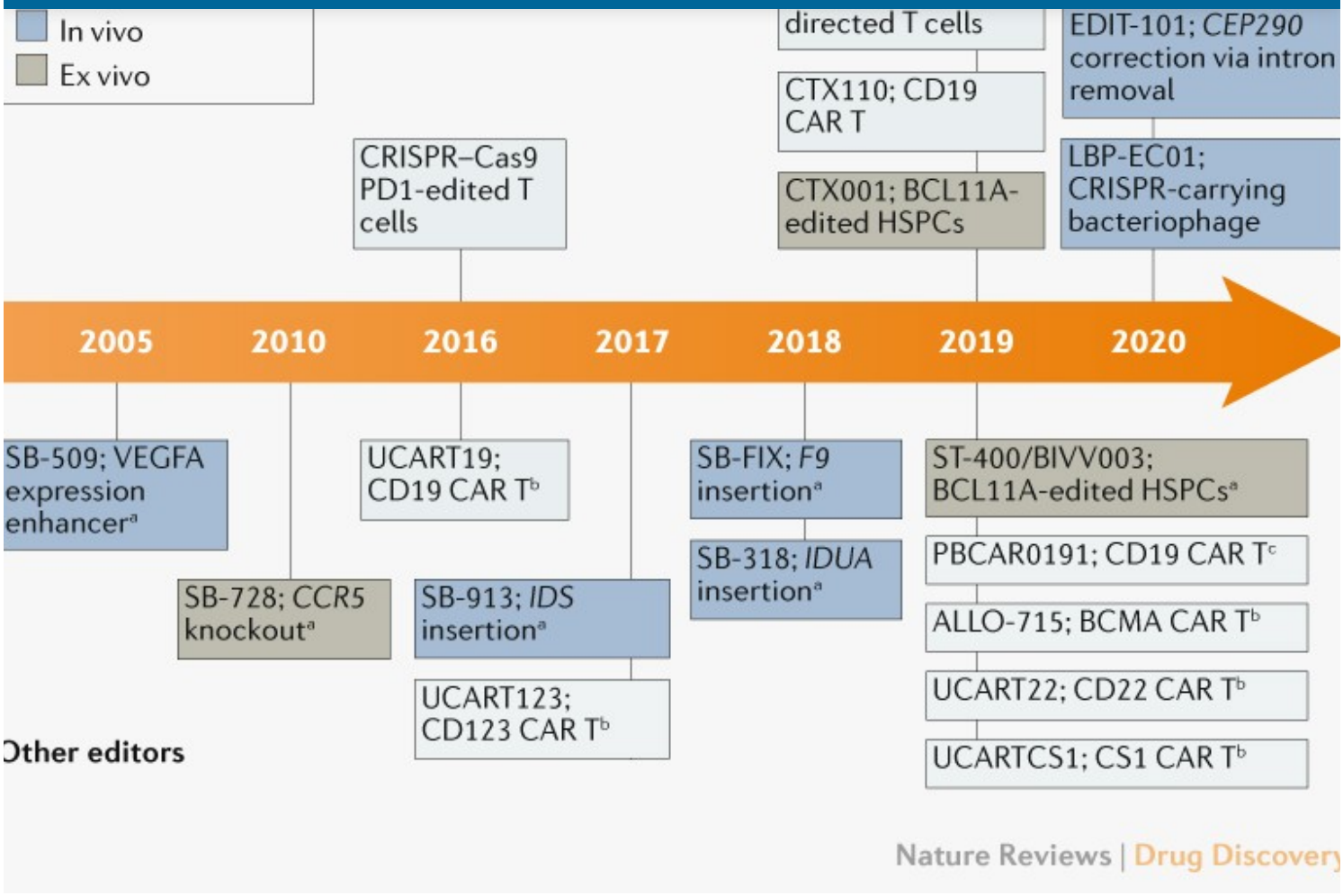

Fig. 1 | **Timeline of selected, industry-supported gene-editing clinical trial starts.** [a]Zinc-finger proteins. [b]Transcription activator-like effector nuclease (TALEN). [c]Meganuclease. CAR, chimeric antigen receptor; HSPCs, haematopoietic stem and progenitor cells.

BOX 1 | THE OTHER EDITORS

CRISPR–Cas systems are often credited with democratizing gene editing. Because they use a guide RNA to bind DNA, they are easily 'programmed' against different targets. The cutting results they provide are also reliable and robust, with little need for tinkering. "It's simple enough to deploy that essentially any starting graduate student, with a little knowledge of molecular biology, can use it to introduce changes in cells of interest," says UC Berkeley's Jennifer Doudna.

Given the simplicity of this system, CRISPR–Cas advocates expect this modality to drive the expansion of the gene-editing pipeline.

find and bind target DNA, these restriction enzymes rely on zinc-finger motifs to recognize regions of interest. Sangamo has freedom to operate in the ZFN space not only by virtue of having secured a solid IP portfolio, but also by having locked down the expertise in the finicky design of these motifs.

"CRISPR is like the Ford car of genome editing: anyone can afford to buy one. We're dealing with the Maserati of gene editing: a technology that very few people can access," says Adrian Woolfson, head of R&D at Sangamo.

Whether there is any clinical difference between ZFNs and CRISPR–Cas systems remains to be determined. But there is rationale to suggest it might, says Woolfson. Because ZFNs are not derived from bacterial systems, for instance, they may be less immunogenic, he argues.

Cellectis is meanwhile using transcription activator-like effector nucleases (TALENs), another restriction enzyme approach, to edit its immuno-oncology candidates. Precision Biosciences is working with a gene-editing meganuclease platform called ARCUS. And Bluebird Bio is advancing with megaTALs, a hybrid of the TALEN and meganuclease machinery.

"They all have different pros and cons, and they'll all find different places where their different advantages are more applicable," predicts John Evans, CEO of Beam Therapeutics.

Gene therapy frontrunners like Bluebird bio and Spark Therapeutics have also been laying critical groundwork, exploring delivery vectors and establishing indications or interest. Immuno-oncology cell therapy trailblazers like Novartis and Kite Pharma and oligonucleotide pioneers such as Ionis and Alnylam have also been paving the way. The gene-editing foundations are now in place. "The whole field is really hitting its stride," says John Evans, CEO of Beam Therapeutics, a base-editing CRISPR company. "I think things are only going to accelerate across the board."

The haemoglobinopathy herd

Ex vivo programmes — in which cells are harvested from patients, edited and then re-infused into patients as a therapeutic — offer a relatively low-risk starting point for gene-editing pioneers.

First, it is simpler to get gene-editing machinery into cells in a laboratory setting than into cells in the body. In the case of in vivo CRISPR applications, this machinery consists of a guide RNA

these cells, holes in the cell membranes open up, and ZFNs and CRISPR–Cas constructs alike can traffic into the nucleus.

Ex vivo applications also bring safety benefits. The risks of off-target and off-tissue editing with these programmes are bounded. Gene-editing machinery is unlikely to trigger immune responses when used in autologous harvested cells. And investigators can quantify editing rates before they re-infuse the cells into patients, carefully controlling dosing.

There are also two well-validated ex vivo use cases, with considerable unmet need. Sickle cell disease — which affects up to 80,000 people in the US alone — and β-thalassaemia are both inherited red blood cell diseases that are caused by dysfunctional or insufficient levels of haemoglobin, the protein that carries oxygen throughout the body.

All of the first ex vivo gene-editing programmes focus on these two diseases (Table 1). “Why did we all start here? Because it’s compelling,” says Laura Sepp-Lorenzino, CSO at Intellia.

TABLE 1 | SELECT LIST OF EX VIVO GENE-EDITING AGENTS IN OR APPROACHING THE CLINIC

Drug	Sponsors	Editor	Properties	Indication	Status
ST-400/BIVV003	Sangamo/Sanofi	ZFN	*BCL11A*-edited HSPCs	SCD and β-thalassaemia	Phase I/II
CTX001	CRISPR Therapeutics/Vertex	CRISPR–Cas9	*BCL11A*-edited HSPCs	SCD and β-thalassaemia	Phase I
OTQ923	Intellia/Novartis	CRISPR–Cas9	*BCL11A*-edited HSPCs	SCD and β-thalassaemia	IND approved
EDIT-301	Editas	CRISPR–Cas12a	*HBG1/2*-edited HSPCs	SCD and β-thalassaemia	IND filing in 2020

HSPC, haematopoietic stem and progenitor cell; IND, Investigational New Drug application; SCD, sickle cell disease; ZFN, zinc finger nuclease.

The first crop of gene-editing technologies doesn't aim at directly correcting the mutations that lead to these diseases, however. Instead, these cutting-based approaches modulate the expression of compensatory fetal haemoglobin. Fetal haemoglobin is typically only expressed for 2–4 months after birth. But when expressed into adulthood, in a rare benign genetic condition called hereditary persistence of fetal haemoglobin, it provides protection against both sickle cell disease and β-thalassaemia.

Like most others in the ex vivo race, Sangamo's proof-of-concept ST-400 and BIVV003 attempt to similarly boost fetal haemoglobin by editing *BCL11A*. This regulatory gene encodes a zinc finger transcription factor that normally turns fetal haemoglobin expression off. Variants in this gene can boost fetal haemoglobin levels, however, and inactivation of *BCL11A* can correct sickle cell disease in mice.

Sangamo started clinical trials of these agents in May 2018. It expects a primary completion date later this year, according to ClinicalTrials.gov.

CRISPR Therapeutics and Vertex entered the clinic in February 2019 with the CRISPR–Cas candidate CTX001. The partners reported preliminary safety and efficacy data from 2 patients last year, and list an early 2021 completion date.

But competition in this space could be stiff. Beyond recent small-molecule and antibody success in sickle cell disease, Bluebird bio secured approval in the EU in 2019 for its betibeglogene autotemcel — a one-off ex vivo gene therapy that uses a lentiviral vector to introduce a functional form of the globin gene into red blood cells, for β-thalassaemia. In two pooled trials that supported this approval, nearly 80% of β-thalassaemia patients no longer needed blood transfusion for at least 12 months after treatment. The company has started a rolling NDA for approval in the US, and development in sickle cell disease is ongoing.

But Bluebird bio's success here validates the approach, says Sepp-Lorenzino. And there is plenty of room for improvement, she adds. Harvested cells don't like to be messed with, so the ability

different modalities can only be determined with long-term follow up.

“I think that there will be differentiators,” she adds.

Tweaking T cells

T cells also offer accessible and attractive ex vivo gene-editing opportunities.

Sangamo first tested this approach in 2010, with its second programme to the clinic. Before immuno-oncology was all the rage, Sangamo saw a way to tune T cells to take on infectious diseases. With its ex vivo SB-728 programme, the company used a ZFN to disrupt the expression of the CCR5 receptor in harvested T cells. HIV co-opts the CCR5 receptor to gain entry into T cells, so Sangamo theorized that this approach would protect T cells from HIV infection. In 2014, primary investigator Carl June, of the University of Pennsylvania, and colleagues reported in the *New England Journal of Medicine* that Sangamo’s gene editor could modify CCR5 and was safe, proving the feasibility of using ZFNs to make designer T cells.

SB-728 has since fallen by the wayside. But the potential for engineered T cells in immuno-oncology has taken off (Table 2).

TABLE 2 | SELECT LIST OF IMMUNO-ONCOLOGY GENE-EDITED CANDIDATES IN OR APPROACHING THE CLINIC

Drug	Sponsors	Editor	Properties	Indication	Status
UCART19/ALLO-501	Allogene/Cellectis/Servier	TALEN	CD19 CAR T, allogeneic	$CD19^+$ cancers	Phase I
PBCAR0191	Precision Biosciences/Servier	Meganuclease	CD19 CAR T, allogeneic	NHL and ALL	Phase I
CTX110	CRISPR Therapeutics	CRISPR–Cas9	CD19 CAR T, allogeneic	$CD19^+$ cancers	Phase I/II

ALLO-715	Allogene	TALEN	BCMA CAR T, allogeneic	MM	Phase I
CTX120	CRISPR Therapeutics	CRISPR–Cas9	BCMA CAR T, allogeneic	MM	Phase I/II
UCART123	Cellectis	TALEN	CD123 CAR T, allogeneic	AML	Phase I
UCART22	Cellectis	TALEN	CD22 CAR T, allogeneic	B-ALL	Phase I
UCARTCS1	Cellectis	TALEN	CS1 CAR T, allogeneic	MM	Phase I
NTLA-5001	Intellia	CRISPR–Cas9	WT1 TCR, allogeneic	AML	IND in 2021
Undisclosed	Refuge Biotech	CRISPR–dCas9	HER2 CAR T	Cancer	IND in 2021
EDIT-201	Editas	CRISPR–Cas	NK cells, allogeneic	Solid cancers	IND-enabling studies

AML, acute myeloid leukaemia; B-ALL, B cell acute lymphoblastic leukaemia; CAR, chimeric antigen receptor; IND, Investigational New Drug application; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NK, natural killer; TALEN, transcription activator-like effector nuclease; TCR, T cell receptor.

Novartis's 2017 FDA approval of tisagenlecleucel, developed initially by June and colleagues, highlights how reprogrammed T cells can be used to hunt down cancer cells. With tisagenlecleucel, researchers harvest T cells and treat them ex vivo with a lentiviral vector encoding a CD19-recognizing chimeric antigen receptor (CAR). When these cells are re-infused into patients, they carry a CD19 CAR that can seek out and destroy CD19-expressing cancer cells. This has driven long and lasting remissions in B cell acute lymphoblastic leukaemia and in diffuse large B cell lymphoma.

dysfunction and exhaustion. And CD19-targeted cell therapies have become one of the most crowded areas of the drug discovery pipeline, as researchers attempt to troubleshoot these issues.

Gene-editing technologies offer one way forward. In 2019, June set another landmark, with colleagues in academia and at Tmunity, by treating a first patient in the US with a CRISPR-engineered candidate called NYCE T cells. The team first used CRISPR–Cas9 to knock out two genes encoding endogenous T cell receptors (TCRs) and the gene encoding PD1, a regulatory checkpoint. They then used a lentiviral vector to insert a transgene for a TCR that could recognize the NY-ESO-1 cancer antigen. (Whereas CARs only recognize antigens that are expressed on the surface of cancer cells, TCRs can recognize a broader array of antigens.)

The team published first results in February in *Science*, showcasing the safety and feasibility of this approach in three patients. Efficacy data are still being collected.

Others are taking similar approaches. Advanced editing techniques could also be key to off-the-shelf allogeneic cell therapies that would be easier to manufacture than autologous approaches.

The primary focus, for now, is on developing better CD19-targeted cells. “CD19 allows you to benchmark your technology against what other people are doing,” explains Adrian Woolfson, head of R&D at Sangamo and a former Global Clinical Leader of Immuno-Oncology and Hematology at Pfizer. There is also big commercial market for CD19-targeted therapies, further boosting the appeal. “It’s a very intuitive kind of indication to go for, and there's a well-trodden path. People probably just feel comfortable with it,” says Woolfson.

The same is true for BCMA, another heavily researched immuno-oncology target.

In vivo opportunities

The in vivo pipeline — in which the editors are the drugs — consists of a more diverse set of programmes (Table 3).

Drug	Sponsors	Editor	Properties	Indication	Status
SB-913	Sangamo	ZFN	*IDS* insertion	MPS II	Phase I/II failed
EDIT-101	Allergan/Editas Medicine	CRISPR–Cas9	*CEP290* correction, via intron removal	LCA10	Phase I/II
LBP-EC01	Locus Biosciences	CRISPR–Cas3	CRISPR-carrying bacteriophage	UTI	Phase II
EBT-101	Excision BioTherapeutics	CRISPR–Cas9	HIV DNA excision	HIV	Phase I in 2020
NTLA-2001	Intellia/Regeneron	CRISPR–Cas9	*TTR* knockout	ATTR	Phase I in 2020
Undisclosed	Navega Therapeutics	CRISPR–dCas9	Nav1.7 epigenetic silencing	Pain	Phase I by 2021
Undisclosed	Precision Biosciences/Gilead	Meganuclease	HBV DNA	Hepatitis B	IND 2021

ATTR, transthyretin amyloidosis; HAE, hereditary angioedema; HBV, hepatitis B virus; IND, Investigational New Drug application; LCA10, Leber's congenital amaurosis 10; MPS II, mucopolysaccharidosis type II; UTI, urinary tract infection; ZFN, zinc finger nuclease.

Sangamo broke ground here as well, with its SB-913 for a rare genetic disorder called mucopolysaccharidosis type II (MPS II). With this programme, Sangamo sought to move a step beyond what in vivo gene therapy products like Spark's voretigene neparvovec and Avexis's onasemnogene abeparvovec had achieved. These programmes showed that adeno-associated virus (AAV)-based approaches can smuggle new genes into the nucleus, when used in in vivo applications. But the transgenes they carry are primarily transcribed from free-floating circularized DNA, with minimal integration into the genome. With SB-913, Sangamo wanted to

To achieve this effect, Sangamo's SB-913 was comprised of three different AAV vectors. The first carried the required mRNA to transcribe a ZFN that could make an upstream cut in the human genome, the second carried the code for a ZFN to make a downstream cut, and the third carried a genetic template of the *IDS* gene that they wanted to insert between the two cuts.

Sangamo started the SB-913 trial in November 2017. By February 2019, it was clear patients weren't experiencing enough benefit. *IDS* levels barely seemed to move.

But there was nevertheless preliminary evidence of *IDS* transgene integration, validated by PCR-testing of liver biopsy, the company reported. There was also evidence of substantial enzyme synthesis in one patient treated at the highest dose, adds Woolfson. "That, in our view, suggested that we'd edited the human genome in vivo for the first time in the history of mankind," he adds. "In that sense it appears we were the first people to take off … the Wright brothers of genome editing. And now we are fixing up our plane."

A key challenge with this programme was that the target cells had to be successfully co-transduced and co-edited by all three vectors to achieve *IDS* integration, he explains. The company has since reformatted its approach to deliver the cutting ZFNs and the *IDS* template in just two vectors, increasing their chances of *IDS* integration.

"If we crack it this time round, it is likely that we will have shown that this method has general utility," says Woolfson.

CRISPR-based companies are also exploring opportunities to use their editors to insert genes, with precision, into the human genome. But their first in vivo programmes into the clinic are taking a more straightforward approach: deleting DNA.

Editas and Allergan's landmark study with EDIT-101, for instance, uses CRISPR–Cas9 to cut out a chunk of intronic DNA. By cutting out a section of mutated CEP290 in patients with Leber congenital amaurosis 10 (LCA10) blindness, they hope to force the production of functional protein, restoring vision.

complications that were holding up the gene therapy field by picking an ophthalmological indication. Because the eye is immunoprivileged, the risks of immune response to foreign vectors and introduced transgenes is lower. Circulation from the eye to the rest of the body is limited, reducing the risk of off-tissue effects. And there is no turnover of the edited cells, boosting the probability of long-term effects.

Editas, co-founded by Doudna, hopes to leverage these same benefits, with the CRISPR–Cas9 system.

Cutting it up

Other leading in vivo editing candidates are similarly using CRISPR–Cas systems to cut DNA up.

Intellia's lead programmes use CRISPR–Cas functionality to knock out genes altogether, for example. With NTLA-2001, Intellia and partner Regeneron are going after transthyretin (TTR). When this transport protein is mutated, it can misfold and aggregate, causing TTR-mediated amyloidosis. By knocking down TTR expression, they hope to provide patients with a life-long treatment.

"With our sentinel indication, we wanted a high degree of validation that the mechanism we want to elicit will work," says Sepp-Lorenzino. "And then we can use this to validate our technology."

Indeed, antisense and RNA interference (RNAi) forerunners have built up a strong case for efficacy in TTR already. In 2018, the FDA approved Alnylam's patisiran for hereditary TTR-mediated amyloidosis, making it the first ever approval for the RNAi modality. The oligonucleotide binds TTR mRNA, silencing its expression. (Sepp-Lorenzino used to work at Alnylam.) Regulators have also approved Akcea Therapeutics and Ionis's inotersen, an antisense oligonucleotide that achieves a similar effect in the same setting.

With the biology derisked, and an established path to approval, Intellia can focus on showcasing the CRISPR–Cas9 edge. Does a gene-editing approach result in lower TTR levels, for example? An improved safety profile? Single-dose efficacy?

BOX 2 | VECTOR VARIATION

Gene-editing drugs are only as good as their delivery vehicles. After all, if the editors can't get into the nuclei of the cells they need to edit, then how can they fix faulty genes? So, following in the footsteps of gene therapy pioneers who have used adeno-associated virus (AAV) vectors to deliver genetic payloads into the nucleus, most gene-editing hopefuls have embraced AAV vectors for their first in vivo programmes.

This risk-limited choice does pose some problems, however. Some patients have pre-existing immunity to some AAV vectors. Patients might also develop immunity to AAV vectors if repeat dosing is required. Combined, these factors could limit the scope of AAV-delivered agents. AAVs also tend to traffic to the liver, muscle and central nervous system, restricting the types of tissue that can be edited most efficiently. They have a carrying capacity of around 5 kb, making for a tight fit for 3–4 kb CRISPR–Cas editors. And they can integrate into the host genome, albeit at low frequency, leading to potential for lasting expression of gene-editing machinery.

Intellia and partner Regeneron are taking a different approach, working instead with lipid nanoparticle (LNP) delivery systems. Regulators in the US and EU have approved nearly a dozen LNP-delivered drugs over the past decades, including Alnylam's RNAi oligonucleotide patisiran. Like AAVs, LNPs also tend to congregate at the liver and can trigger immunogenic responses. But they don't integrate into the host genome, says Intellia CSO Laura Sepp-Lorenzino. And their carrying capacity is much larger than the AAVs. "We can package with no limitation," says Sepp-Lorenzino.

For the first cutting-based gene-editing programmes, carrying capacity isn't an issue. But as editing applications become more sophisticated, and drug developers start thinking about the benefits of making multiple cuts at a time, larger vectors that can carry more than one editor might make a difference, she adds.

Many of the most advanced in vivo programmes focus on rare genetic diseases. Genes that are expressed in the liver, the muscle and the CNS are particularly high priority targets, in part because of where AAV vectors tend to traffic to. But there are also a few infectious disease standouts.

At Locus Biosciences, co-founded by Gersbach, researchers have subverted the original purpose of CRISPR–Cas systems. Bacteria first evolved these gene-cutting scissors as a layer of defence

its bacteriophage more bacteria-killing activity.

Locus advanced its first CRISPR–Cas product — a cocktail of bacteriophage, carrying CRISPR–Cas3 that targets *Escherichia coli* DNA — into the clinic in January 2020. "What I think this might do for the field is increase the amount of creativity, and the view of what types of things can be done with CRISPR," says Joseph Nixon, vice president of business development at Locus.

Viruses are on the table too. Excision BioTherapeutics is approaching the clinic with a CRISPR–Cas9 candidate that cuts integrated HIV DNA out of patient genomes, potentially enabling HIV-infected patients to stop taking antiretroviral therapies. A first trial is set to start later this year. Precision Biosciences and partner Gilead have a meganuclease-based hepatitis B virus programme headed to Investigational New Drug (IND) submission in 2021. And some researchers are thinking about how to use DNA-cutting modalities for the treatment of COVID-19.

Critical questions

As gene-editing trials start to read out, a few key clinical findings are likely to have outsized implications.

For one, how immunogenic are the various different editing technologies? Both the editors and the vectors that are needed to get them into cells for in vivo programmes are foreign, and as such have the capacity to trigger immune responses that can reduce efficacy — by destroying the gene-editing machinery — or cause harm. AAV vectors have been derisked considerably by gene therapy applications. But CRISPR–Cas systems are bacterially derived, and it is unclear how patients' immune systems will react to these.

Preliminary immunogenicity screening data are mixed. One analysis of human immunity to Cas9 proteins found that as many 58–78% of probed individuals had pre-existing immunity to different Cas9 variants. Another study, using a different type of measurement, found more modest predictions of 2.5–10%.

Pre-existing immunity to Cas9 led to the elimination of genome-edited cells in mice, a recent study also showed. But it remains to be seen if the same is true in humans. "This is something

“Right now I don’t see [immunogenicity] as a showstopper,” says Doudna. Researchers have already identified multiple CRISPR–Cas editors from different bacterial originators, she explains, and they are constantly finding and developing more. If some are too immunogenic, or pre-existing exposure levels are too high, others will be swapped in, she speculates. “But it is certainly something to keep an eye on,” she adds.

Off-target editing is also an ongoing concern. Gene editors are specific, but they can nevertheless cut DNA at unintended locations. The extent to which this happens is still up for debate, and better tools are still needed to assess the magnitude and effects of off-target editing.

Additionally, there are questions around the longer-term effects of gene editing. “Are these genetic manipulations stable? And, are there unexpected or unintended consequences of these kinds of manipulations?” asks Doudna. “I think these are questions that remain to be addressed, and will probably be very important in these initial studies. They will set the stage for future downstream applications of CRISPR.”

The first gene-editing programmes into the clinic — and therefore to read out — are unlikely to provide much across the board insight into these broader questions. Ex vivo and immuno-oncology programmes that rely on harvested cells will provide little insight into the effects of different vectors and editors on the host immune system, for example.

The read-through from ZFN-mediated immune responses to CRISPR–Cas ones is similarly limited. And even Editas’s first in vivo trial with EDIT-101 will shed little light on immunogenicity or off-target editing, because the retina is so well firewalled from the rest of the body.

“The answers are going to be highly dependent on all the details. It's going to depend on what you're delivering, how much you're delivering, where you're delivering, how long it's around, and what the underlying disease looks like,” says Gersbach.

CRISPR–Cas is an umbrella term, after all. “When you use the word CRISPR, you're capturing this huge bucket of lots of different technologies,” says Gersbach. “And I think that’s just going to continue to evolve.”

Beam Therapeutics, for example, hope to use a base-editing approach licensed from Harvard's David Liu and others to take CRISPR–Cas in a new direction. Rather than cutting out unwanted DNA, Beam's platform swaps bases in the DNA chain. "The punch line is that we can now not only precisely target a certain area of the genome, but we can precisely control the sequence that will result," says Evans.

More than half of the genetic errors associated with disease are caused by single-letter changes, adds Evans.

Some of Beam's programmes may end up in the ultra-orphan space. "There's no question that eventually you do run into that long tail of diseases with very small populations, which might ultimately require a different regulatory regime. I think that's something we'll be active in," says Evans.

But there will be bigger opportunities as well, adds Evans. All 80,000 sickle cell disease patients in the US have the same E6V point mutation in at least one allele of a haemoglobin gene, for example. And Beam can consider editing the E6V variant directly — to an asymptomatic variant called E6A. The company is also testing base-editing approaches to increase the expression of fetal haemoglobin.

"We view these two different approaches as complementary, and we're planning to move both of them forward in parallel until some data set declares which one is going to be preferred," says Evans. The company plans to have a first IND filing in 2021, from its ex vivo pipeline.

"There's no question that this is a crowded space. But it's so early days in these technologies that we're not afraid of going into this space," Evans.

Back to the future

Other editors are also gaining momentum. In 2013, for example, Doudna showed with colleagues in *Cell* that CRISPR could be coupled with a catalytically inactive dCas protein. Rather than

“We're seeing just an explosion of opportunities with CRISPR effectors,” says Doudna.

For Gersbach, these types of editor could be game changing. “I'm a big believer that these effector functions are much more flexible, powerful and adaptable way to treat diseases,” says Gersbach.

For one, the off-target editing effects of these approaches may be more manageable. A transcriptional candidate might bump into the wrong gene and result in an mRNA transcript or two. But this is unlikely to have as deep or lasting an effect as an inadvertent cut.

These next-generation gene editors could also offer transient regulatory editing that reverses over time. While one-and-done gene-editing therapies are exciting for rare monogenic diseases, drug developers might want the option to continuously dose and adjust target activity over time in other diseases — especially if this helps to alleviate patient, doctor and regulatory concerns about possible long-term effects of permanent editing.

A third benefit is that many common diseases are caused by imbalances in gene expression. As such, these might be more amenable to correction by regulatory editing.

“I think we're about to see a new wave of companies that are taking advantage of these approaches,” says Gersbach.

Notably, Sangamo’s first programme into the clinic — predating their early efforts with CCR5 — was a zinc finger DNA-binding protein transcription factor (ZFP-TF) that was designed to upregulate the expression of VEGFA. The drug, SB-509, ultimately failed in a phase II trial in diabetic neuropathy. The company believes a combination of factors contributed to this failure, including insufficient VEGFA activation and the complexity of disease biology. But it has persisted, and innovated beyond this legacy technology. Nearly half of the company’s disclosed preclinical programmes now consist of regulatory editors. Sangamo’s development deal with Biogen earlier this year — for US$350 million upfront and up to $2.4 billion in potential milestone payments — also underscores the opportunity here in neurological diseases, including Alzheimer and Parkinson disease.

component of the pain pathway that has stymied small-molecule and antibody drug developers. CEO Ana Moreno hopes to have this candidate in the clinic next year.

“We're just getting started,” says Gersbach. “There's still a lot to learn.”

doi: 10.1038/d41573-020-00096-y

Nature Reviews Drug Discovery ISSN 1474-1784 (online)