The outlook for gene editing in treating disease

Gene editing (GE) utilising bacterial nucleases has been a feature of genome research for many decades. However, a breakthrough of the technology in drug development was achieved much later, in 2012, when a novel method using clustered regularly interspersed short palindromic repeats (CRISPR), together with Cas9-endonuclease to cut genomic DNA, was discovered.[1] Other gene editing platforms include Zinc Finger Nucleases (ZFN) and transcription activator-like effector nucleases (TALEN). All of these technologies are based on the generation of nuclease-induced double-stranded breaks (DSBs) in DNA, which results in the efficient repair processes of cellular DNA in eucaryotic cells.[2]

The breaks in DNA can be repaired by homology-directed repair (HDR) or non-homologous end-joining (NHEJ), resulting in targeted integration or gene disruptions, respectively. Base editing (BE) is a novel approach for targeted gene editing with CRISPR-Cas9, where point mutations are introduced into DNA without generating DSBs.[3]

In recent years, the gene editing technologies have passed non-clinical tests and moved to first in human clinical studies. Searches of clinicaltrials.gov using "gene editing," "Crispr Cas9," "Zinc Finger Nuclease," and "Talen" gave 51 results of ongoing, recruiting and completed trials (see Table 1). The first approach tested was *ex vivo* editing of patient cells, where the edited and quality-controlled cells were given back to the patient. In this case, the cells are usually autologous hematopoietic CD34+ cells, which can engraft back to the bone marrow and generate a new population of edited cells and thus provide a therapeutic effect.

This approach is used e.g. by Vertex Pharmaceuticals, Crispr Therapeutics, Editas and Graphite Bio for the treatment of hemoglobinopathies (Sickle Cell Disease and Thalassemias). *Ex vivo* gene editing can be also used to edit different immune cells to generate e.g., allogeneic chimeric antigen receptor (CAR) T or natural killer (NK) cells. The *ex vivo* Crisp/Cas9 system is utilised by Intellia Therapeutics for its immuno-oncology and auto-immune programmes. Cellectis, on the other hand, is using TALEN technology for the UCART gene-editing (allogenic CAR T) programme.

A more recent approach has been to utilise *in vivo* gene editing, which means direct editing of the genome inside the patient; the necessary components (guide RNAs, nuclease or vectors) are administered systemically to the patients. Such first in human studies have been initiated by Sangamo Therapeutics using AAV vectors for targeted delivery of the ZFN and guide RNAs into liver cells. Intellia Therapeutics is utilising *in vivo* GE for the treatment of transthyretin amyloidosis and Editas for the treatment of ocular disorders like Leber´s Congenital Amaurosis and Retinitis Pigmentosa.

Most of the *in vivo* first in human GE trials have involved adult patients. LogicBio Therapeutics announced recently early clinical trial results demonstrating the first-ever *in vivo* genome editing in children.[4]

Risks and challenges of GE

There is a known risk for tumour-formation upon the clinical use of gene therapy medicinal products. This relates to the risk for genetic disturbance of proto-oncogenes. Given the mode of action of all GE technologies, this risk is also valid for these products regardless of whether the gene editing is

Table 1. Status of clinical trials using gene editing or base editing technologies (based on information available at clinicaltrials.gov on 4 November, 2021)

GE Type	Status	Number	Country	Technology	Phase
Ex vivo GE	Unknown	3	China	Crispr Cas 9	FIH
Ex vivo GE	Active, not recruiting	2	Egypt, US, China	Crispr Cas 9	FIH
Ex vivo GE	Recruiting	10	US, China, UK	Crispr Cas 9	FIH
Ex vivo GE	Completed	2	China	Crispr Cas 9	FIH
Ex vivo GE	Active, not recruiting	2	China, US	Crispr Cas 9	Ph1/2
Ex vivo GE	Recruiting	9	China, US	Crispr Cas 9	Ph1/2
Ex vivo GE	Active	1	US	Crispr Cas 9	Follow-up
In vivo GE	Recruiting	1	NZ/Sweden/UK	Crispr Cas 9	FIH
In vivo GE	Active, not recruiting	1	China	Crispr Cas 9	Ph 1/2
Ex vivo GE	Unknown	1	China	Talen and Crispr Cas9	FIH
Ex vivo GE	Active, not recruiting	1	US	Zinc Finger Nuclease	FIH
Ex vivo GE	Completed	2	US	Zinc Finger Nuclease	FIH
Ex vivo GE	Recruiting	1	US	Zinc Finger Nuclease	FIH
Ex vivo GE	Active, not recruiting	1	US	Zinc Finger Nuclease	Ph1/2
Ex vivo GE	Recruiting	2	US	Zinc Finger Nuclease	Ph1/2
Ex vivo GE	Completed	3	US	Zinc Finger Nuclease	Ph1/2
Ex vivo GE	Active	1	US	Zinc Finger Nuclease	Follow-up
In vivo GE	Unknown	1	China	Zinc Finger Nuclease	FIH
In vivo GE	Terminated	1	US	Zinc Finger Nuclease	FIH
In vivo GE	Terminated	1	US	Zinc Finger Nuclease	Ph1/2
In vivo GE	Active, not recruiting	1	US	Zinc Finger Nuclease	Ph1/2
In vivo GE	Recruiting	4	China, US	Talen	FIH

conducted *ex vivo* or *in vivo*.[5] A substantial risk for tumour formation is in many ways unacceptable for all regulatory authorities. Hence, developers are required to address this risk before first in human administration. Normally this is accomplished by extensive *in vitro* analysis complemented with *in vivo* safety data. *In vitro* tests normally include various genetic analyses of insertion mapping, karyotyping, assessment of telomers, genomic hybridisations and expression analysis of various oncogenes.

This is complemented with culture-based assays like the ability to grow cells without plastic support and extended culturing to senescence. *In vivo* testing normally implies extended studies in immuno-deficient mice accompanied by a direct analysis of tumour-formation due to insertional oncogenesis. There are still many questions as to the human relevance of the data generated by these methods. For instance, the resolution of the genetic analysis might not be sensitive enough to detect the tumour-causing mutations. In relation to the *in vivo* studies, it is well known that many genetically altered mouse strains are prone to tumour formation, which in turn might increase the likelihood for a positive tumour signal following insertional mutagenesis.

Consequently, the *in vivo* data will be difficult to confidently use to address the risk for human tumour formation. In addition, since only a sample of the gene-edited cell-based product is analysed by any method, there is an inherent risk that the transformed cells are missed altogether by any of the assays employed. When the product is comprised of autologous individually cultured gene-edited cells, the risk is increased even further due to minor or major differences in the starting material and culturing conditions for every individual cell preparation. In addition, since many of these technologies, especially for *in vivo* transfer, depend on a viral vector delivery system, the risk of off-target editing is increased further by the integrational abilities associated with the various viral vector systems (retro, lenti or adeno-associated vectors).[6]

On top of the risks associated with off-target genetic disturbance and tumorgenicity, there are more straightforward risks associated with the complete removal of targeted genes. However, given the novel status of these technologies in human drug development, very little is known about how to mitigate these potential risks from the start of non-clinical to clinical development, especially in relation to the long-term safety for patients.

Clinical experience

It is still early days for the clinical development of gene editing technologies although many single case reports are optimistic. A substantial number of early-stage clinical trials are ongoing (Table 1), but interim results have so far only been published from six. Outcomes from *ex vivo* gene editing using CRISPR-Cas9 have been reported in sickle cell disease and beta-thalassemia[7], refractory non-small-cell lung cancer[8], and refractory advanced myeloma and refractory metastatic sarcoma.[9] By comparison, only one publication has reported the outcome of *in vivo* CRISPR-Cas9 gene editing in patients with transthyretin amyloidosis.[10] One trial sponsor has reported the outcome of CCR5-edited CD4-positive T-cells to augment HIV immunity using zinc finger nuclease *ex vivo*[11] and one has reported the use of TALEN gene edited allogenic anti-CD-19 CAR-T cells in patients with acute B-cell lymphoblastic leukaemia.[12]

Despite this diverse groups of conditions, preliminary clinical trial results are in general encouraging, demonstrating proof-of-concept. Adverse events have either been reported as minimal or in more severe cases, as potentially related to the underlying condition and/or concomitant medication. Signs of off-target gene editing has unfortunately not been consistently reported in these publications and if so, reported as minimal. Moreover, the duration of these early-stage studies would in any case, not have allowed for a sufficient assessment of longer-term effect resulting from off-target activity. The US Food and Drug Administration recently issued a clinical hold for the investigation of an allogenic anti-CD19 CAR-T therapy using TALEN gene editing[13] due to the detection of a chromosomal abnormality in a patient experiencing pancytopenia. The root cause of this and its impact on the trial in question needs further assessment, but it points to the fact that the clinical safety of gene editing technologies has not yet been fully established.

Regulatory aspects

Although there are multiple first in human trials ongoing/completed both with *ex vivo* and *in vivo* approaches, very little information and guidance from the regulatory authorities is currently available. In February 2021 the European Medicines Agency (EMA), together with EU Heads of Medicines Agencies (HMA), published an EU-IN Horizon Scanning Report for Genome Editing[14], which discusses the challenges of GE, regulatory preparedness, and how the authorities can collaborate to support oversight of GE/BE development.

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News Briefs

Arbor Biotechnologies closes Series B round

Arbor Biotechnologies Inc, a company co-founded by Feng Zhang, one of the original developers of the Crispr-Cas9 genome editing tool, has raised $215 million in an over-subscribed Series B financing to advance its pipeline of genetic medicines. The round was led by Temasek, Ally Bridge Group and TCG Crossover and included more than 12 other venture capital groups. As part of the financing, Chen Yu, managing partner at TCG Crossover, will join the company's board of directors. Based in Cambridge, US, the company uses artificial intelligence to identify Crispr genomic editors. Cispr-Cas9 was discovered in 2012 and has since become a widely used tool in pharmaceutical research and development. Arbor has built a platform for discovering novel editing enzymes and effectors that can then be directed against diseases. The company has a preclinical pipeline with an initial focus on diseases of the central nervous system and liver. The company has also partnered with Vertex Pharmaceuticals Inc on several gene editing and ex vivo cell therapy programmes. "While our primary focus has been on developing our bespoke Crispr nucleases, we are also looking to progress our other precision editing innovations such as Crispr transposases," said Devyn Smith, the company's chief executive.

Novo Holdings A/S co-leads Asgard financing

Novo Holdings A/S, which manages the assets of the Novo Nordisk Foundation, has co-led a €6 million seed financing for Asgard Therapeutics AB, a biotech company working in the field of *in vivo* direct cell reprogramming. The company's technology is being used to develop therapies for cancer. The lead programme is an allogeneic gene therapy that is said to induce a personalised immune response. Besides Novo Holdings, the financing was co-led by the Boehringer Ingelheim Venture Fund and Industrifonden. As part of the transaction, Søren Møller from Novo, Philipp Müller of Boehringer, and Jonas Jendi from Industrifonden will join the board of directors. Asgard is a spin-out from Lund University in Sweden.

Bluebird bio completes business separation

Bluebird bio Inc has completed the spin-out of its oncology assets into an independent, publicly listed company in order to focus on new medicines for genetic diseases. The new company is called 2seventy bio Inc. In September, bluebird disclosed that it had submitted a regulatory application to the US Food and Drug Administration for a treatment for beta-thalassaemia. A filing for a second product for cerebral adrenoleukodystrophy is on track for the end of 2021, the company said on 4 November. Founded in 2010, bluebird says it has one of the largest *ex-vivo* gene therapy data sets in the world.

Kathryn Corzo becomes COO at bit.bio

The UK-based cell coding company bit.bio has appointed Kathryn Corzo as chief operating officer to oversee the company's global operations. Ms Corzo was most recently a partner at Takeda Ventures Inc and before that, head of oncology cell therapy development at Takeda Pharmaceutical Company Ltd.