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CRISPR therapies march into clinic, but genotoxicity concerns linger

Following reports of collateral damage caused by CRISPR genome editing, now chromothripsis, a phenomenon associated with cancer, enters the spotlight.

A recent study has identified another potential hazard for developers of genome editing therapies based on CRISPR–Cas9. The double-strand DNA breaks introduced during CRISPR editing could result in chromothripsis, an extremely damaging form of genomic rearrangement that results from the shattering of individual chromosomes and the subsequent rejoining of the pieces in a haphazard order. Although most cells do not remain viable after undergoing such a dramatic alteration, those that do could, in theory, express oncogenic fusion proteins or give rise to dysregulated expression of particular genes that could cause problems.

So far, none of the companies leading the clinical development of CRISPR-based therapies appears to have considered the issue; its clinical implications, if any, remain unclear. However, the study, led by Mitchell Weiss of St. Jude Children's Research Hospital and David Pellman of the Dana–Farber Cancer Institute and Harvard Medical School, adds another layer of complexity to gene-editing's already relatively complicated mechanism of action. "Most importantly, it's an on-target effect. You cannot make this go away by making the cutting more specific," says Pellman.

The study is the latest in a long line of academic analyses that have illuminated possible safety concerns surrounding the development of CRISPR-based gene editing therapies. Other studies have reported CRISPR–Cas9-induced large-scale DNA deletions and chromosomal rearrangements; CRISPR–Cas9-mediated activation of the p53 tumor suppressor protein, which could select for inactivating, cancer-causing mutations; and the induction of large chromosomal truncations. The presence of preexisting antibodies and T cells directed against Cas9 enzymes in large percentages of the general population is not a safety issue but could compromise efficacy. Despite some overblown media reports, none of these issues has so far surfaced during clinical trials, and none has slowed the field's momentum. Intellia Therapeutics and its partner Regeneron recently reported positive interim data from an ongoing phase 1 trial of their in vivo CRSPR editing candidate NTLA-2001 in patients with hereditary transthyretin amyloidosis, a life-threatening disease characterized by accumulation of misfolded transthyretin (TTR) protein, mostly in the nerves and heart. A single dose of the therapy was enough to achieve durable knockdown of the *TTR* gene and significant reductions in serum levels of mutant, misfolded transthyretin protein.

Chromosomes in dividing cells can undergo chromothripsis, which literally means 'chromosome shattering'. Credit: Zoonar GmbH / Alamy Stock Photo

As yet, only a few patients have participated in clinical trials (Table 1), but as the use of CRISPR-based therapies increases, the odds of a genotoxic incident causing a serious adverse event are likely to rise. "As a whole, it's very important to know what's happening in cells. Otherwise, downstream, if there is an adverse event or something nefarious that happens because of this, you don't want to have not been looking for this," says Ben Kleinstiver of Massachusetts General Hospital and Harvard Medical School.

Advances in DNA sequencing and efforts such as The Cancer Genome Atlas revealed the importance of chromothripsis in cancer, which had been an underappreciated phenomenon until a decade ago. Its recognition challenged the generally

Table 1 | Selected CRISPR-based gene editing therapies in development

Developer	Therapy	Indication	Gene target	Description	Clinical stage
CRISPR Therapeutics, Vertex Pharmaceuticals	CTX001	SCD; β-thalassemia	*BCL11A*	Autologous CD34⁺ hematopoietic stem cells and progenitor cells engineered ex vivo with CRISPR–Cas9 to disrupt the *BCL11A* erythroid-specific enhancer and promote fetal hemoglobin production	Phase 1/2
Editas Medicine	EDIT-301	SCD; β-thalassemia	*HGB1/HGB2* promoter region of the β-globin gene	Autologous CD34⁺ hematopoietic stem cells and progenitor cells engineered ex vivo with ribonucleoprotein complex of CRISPR–Cas12a (Cpf1) to express fetal hemoglobin	Phase 1/2 in SCD; β-thalassemia trial due to start in 2021
Editas Medicine, Allergan	EDIT-101	Leber congenital amaurosis	*LCA10*	Subretinal expression of CRISPR–Cas9 delivered via an AAV-5 vector to restore CEP290 expression	Phase 1/2
Intellia Therapeutics, Novartis	OTQ923 and HIX763	SCD	*BCL11A*	Autologous CD34⁺ hematopoietic stem cells and progenitor cells engineered with CRISPR–Cas9 ex vivo to express fetal hemoglobin	Phase 1/2
Intellia Therapeutics, Regeneron	NTLA-2001	Transthyretin amyloidosis	*TTR*	Systemically delivered TTR guide RNA and Cas9 mRNA encapsulated in a biodegradable ester-bridged ionizable LP01 lipid nanoparticle to reduce TTR expression	Phase 1/2
Graphite Bio	GPH101	SCD	β-globin	Autologous CD34⁺ hematopoietic stem cells and progenitor cells engineered ex vivo with CRISPR–Cas9 delivered by AAV-6 to restore expression of adult hemoglobin	Phase 1/2 due to start in 2021
UC San Francisco; UC Berkeley; UC Los Angeles	CRISPR_SCD001	SCD	β-globin	Autologous CD34⁺ hematopoietic stem cells and progenitor cells engineered ex vivo with CRISPR–Cas9 to restore expression of adult hemoglobin	Phase 1/2 due to start in 2021

SCD, sickle cell disease; AAV-5, adeno-associated virus serotype 5; AAV-6, serotype 6; UC, University of California. Sources: ClinicalTrials.gov; PubMed; company and university websites.

prevailing view that all cancers resulted primarily from a gradual accumulation of somatic mutations and chromosomal rearrangements. Chromothripsis, in contrast, occurs as a single catastrophic event. "The whole process can happen within one cell division cycle," says Pellman.

The term—which means 'chromosome shattering'—was coined by a group led by Peter Campbell, of the Cambridge, UK-based Wellcome Trust Sanger Institute, after observing highly anomalous genomic rearrangements in the DNA of a patient with chronic lymphocytic leukemia. The rearrangements were unusual: instead of being scattered genome-wide or highly localized to a single region, as is the case with gene amplification, they were only evident on a small number of chromosomes, affected one or two regions and occurred in all possible orientations. The group subsequently detected similar events in 2–3% of all cancers, but in about 25% of bone cancers.

Chromothripsis occurs as a result of errors that take place during mitosis. If the cell fails to repair a double-strand DNA break before the nucleus divides and the chromosomes segregate, the two resulting chromosomal fragments can each contribute to the process. "If the break is not repaired, one end of the break can form a micronucleus, and the other end can form a chromosome bridge," says Pellman. Each of these anomalous structures contributes to the mutational process. 'Dicentric' chromosomal bridges can result from the ligation of two sister chromatids, each of which contains a centromere. This structure can then enter the chromosome breakage–fusion–bridge cycle, in which the two chromatids are pulled apart and then fused back together during successive cell divisions. The breakage can occur at any point, and genetic reshuffling continues at every cycle.

The other 'acentric' chromosomal fragments, which lack a centromere, will not segregate normally either but can become incorporated into a micronucleus—a small extranuclear structure enclosed by a section of nuclear membrane. Pellman and colleagues previously used a combination of live-cell imaging and single-cell whole-genome sequencing to detect chromothriptic alterations after the formation of micronuclei. Micronuclei have fragile envelopes that easily rupture and spill their DNA contents into the cytoplasm. "Somehow, this leads to extensive DNA damage," Pellman says. That DNA, moreover, can become reincorporated into the primary genome and further add to the genomic chaos within progeny cells.

In the present study, Pellman and colleagues report that "in actively dividing cells, genome editing with Cas9 causes up to a 20-fold increase in the formation of micronuclei and/or chromosome bridges." In percentage terms, the numbers are small—the rates of micronucleus formation associated with genome editing at different target sites ranged from 4.0 to7.5%. "There is an increase, but what is the clinical risk?" Pellman asks. "Every cut is different. They're all going to have different effects on different genes, depending on the localization of the cut." Cells are constantly being bombarded with double-strand DNA breaks in any case. "The difference here is we're intentionally making double-strand breaks in cells," says Kleinstiver, who sat on the PhD thesis defense committee of Mitchell Leibowitz, first author on the current study. "This is something we're actively doing with the genome-editing technologies, so I think it's really incumbent on the scientific community to monitor and understand what are the genotoxic side effects of doing this."

Of course, not every form of genome editing involves the introduction of double-strand DNA breaks in actively dividing cells. "I think it's important to note this observation may only be relevant to a subset of eventual clinical genome editing programs," says Kleinstiver. "With in vivo

editing in cell types that are postmitotic—they're not dividing—this is much less of a concern." Therapies targeting ocular, muscle or liver tissues, for example, are less likely to give rise to the problem. In addition, base-editing and prime-editing approaches, which employ single-strand nicks rather than double-strand breaks to introduce an edit, are also far less likely to cause chromothripsis.

At the same time, some of the initial therapies now in clinical trials could, at least theoretically, be affected. On the basis of published protocols for editing $CD34^+$ hematopoietic stem cells to treat inherited hemoglobin disorders, Pellman and colleagues estimate that up to one million cells containing micronuclei could be infused into a single patient. In their paper, they report a 16-fold increase in micronucleus formation in genome-edited hematopoietic stem cells at the targeted locus.

Even so, Pellman remains "excited" about the potential of genome editing in conditions such as sickle cell disease, given the effects of the condition. The purpose of the study is to draw attention to the phenomenon, not to slow the development of CRISPR-based therapies. "We think it's a serious enough consequence—blowing up a chromosome and making a new one—for people to know about it," he says.

Fyodor Urnov, of the University of California, Berkeley, calls the study "a call to action" that should spur the development of assays, methods and protocols that can help detect and reduce the frequency of chromothripsis, even if it may be impossible to eliminate it completely. "The notion that we will be able to take 200 million cells and successfully identify one of them which has chromothripsis is, at this point, outside of the realm of reality," he says. But it should be possible to manage the risk. "This is one of those rare cases where looking for keys under the lamppost is appropriate," he says. Computational and wet-lab approaches can help assess the risks associated with introducing double-strand breaks at particular target loci.

At the same time, Urnov warns against any tendency to become frozen by risk, given the gravity of the illnesses that developers of CRISPR-based therapies seek to treat and our lack of knowledge concerning background levels of chromothripsis in the body over a lifetime. "Our challenge as a field is not to succumb to epistemic paralysis in the face of these first-principle concerns," he says. Theoretical safety problems uncovered by academic research often bear no relation to the actual adverse events that arise during clinical trials. "You could publish a thousand *Nature* papers about preclinical concerns—not one of them will predict the real safety concern that will happen," he argues.

At this point in the clinical development process, the study does not appear to have unnerved developers of CRISPR-based therapies unduly. In characterizing the genetic safety of its therapies, Intellia, says spokeswoman Julie Ferguson, is using "various sophisticated molecular assessments designed to sensitively detect chromosomal structural variants" and has so far not observed any editing-related effects that would limit their use. Editas Medicine, says spokeswoman Cristi Barnett, does not believe that the issue "is specifically problematic in our work to make CRISPR-based medicines" and expects that preclinical experiments will answer the questions raised by the chromothripsis study.

Clinical development of these highly promising therapies remains fraught with uncertainty, however, given their novelty and complexity. "We as a community understand we're one serious adverse event away from a clinical hold on everyone," says Urnov. But the field will innovate its way around whatever unforeseen problems that do arise, he says, just as it has done previously in other areas of genomic medicine. "We will have to mitigate them retrospectively, in reactive mode." Their unpredictability imposes that restraint. ❐

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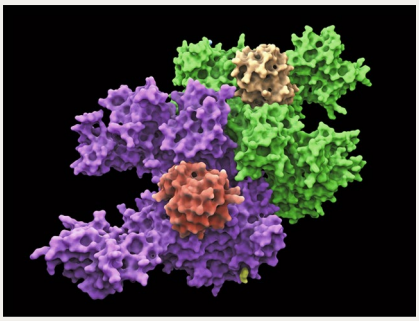
The 3D protein deluge



Protein structure predictions will turbocharge drug discovery. Credit: Science Photo Library / Alamy Stock Photo

Two newly published machine-learning algorithms that predict protein three-dimensional structure from amino acid sequence will have far-reaching implications for biological research and medicine. On 15 July, DeepMind, a London-based company owned by Google, published its deep-learning neural network tool AlphaFold in *Nature*; on the same day, a rival academic research group led by David Baker published its protein prediction tool RoseTTAFold in *Science*. Both software tools are unprecedented in their speed at predicting how an amino acid sequence will fold, and both will be open source and freely available to scientists.

Traditional methods to solve protein structures use X-ray crystallography, nuclear magnetic resonance and cryo-electron microscopy, but these are cumbersome and expensive to run. The speed and accuracy of these AI-driven predictions is unprecedented. Each tool has different merits: RoseTTAFold is slightly less accurate, but it can predict complexes, whereas AlphaFold predicts only structures of single proteins. Also, RoseTTAFold's web server, which allows anyone to submit a sequence and get a structure prediction back, is accessible for people with little machine learning experience.

These AI-driven methods will help scientists to interpret genomic data and add to their understanding of protein function. Structure prediction will also usher in the possibility of designing new features into proteins: enhanced binding for antibodies, scaffolds for better stability, engineered protein switches, ligand- or light activated controls to design 'smart' drugs, and other exciting new applications.

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