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Keep calm and edit on

Despite spooking investors, new insights into DNA repair and the CRISPR gene-editing system are part and parcel of its progress from research tool to human therapy.

It's been a rollercoaster year for companies developing CRISPR gene-editing therapies. In January, a study posted on the preprint server *bioRxiv* raised concerns about the potential immunogenicity of CRISPR–Cas9. In March, *Nature Methods* retracted a study that had suggested unexpected extensive off-target mutations arising from Cas9 activity in mice. In June, two studies in *Nature Medicine* revealed a role for the tumor suppressor protein p53 in antagonizing Cas9 genome editing. Now a study published in our pages by Allan Bradley and colleagues reports that in addition to off-target mutations, Cas9 can sometimes induce extensive on-target DNA damage, including large deletions, inversions and insertions. Although these findings raise concerns that necessitate further study, they also widen the evidence base that will help companies refine and develop safe and effective gene-editing therapies going forward. They also highlight the increasingly volatile investor environment that companies in this space must navigate.

Like most other genome-editing approaches, CRISPR–Cas9 editing first generates a double-strand break (DSB) at a defined position in the genome of a target cell. One of several cellular pathways for genome repair then takes over to mend the break. The most commonly used pathway is non-homologous end-joining, an error-prone mechanism that has been thought to introduce primarily small insertions and deletions in the genome. Alternatively, homology-directed repair faithfully replaces the damaged sequence with a homologous sequence found in another chromosome or externally supplied. These repair pathways have been harnessed to inactivate genes, to correct mutations and to introduce new sequences into the genome.

That Cas9 and other endonucleases may cut the genome at sites other than the target site is a long-recognized problem in the genome-editing field. Such off-target cuts could disrupt the function of other genes or regulatory sequences in the genome.

In recent weeks, however, attention has shifted to an investigation of the potential undesired effects of DSBs induced by CRISPR–Cas activity.

Two papers published online in June by *Nature Medicine* show that Cas9-induced DSBs lead to sufficiently strong activation of the p53 pathway to cause cell-cycle arrest or apoptosis in human embryonic stem (ES) and induced pluripotent stem cells and in an immortalized retinal pigment epithelial (RPE) cell line **(p. 705)**. This raises a hypothetical scenario where selecting for CRISPR-edited cells may enrich for preexisting p53-deficient cells with a propensity for tumorigenesis.

In this issue, Bradley and colleagues describe another underappreciated effect of Cas9 activity and DSBs in p53-competent mouse ES cells and human RPE cells. They detail target-site deletions (up to several thousand base pairs in length) and, in some instances, complex DNA rearrangements at the target site, raising a separate hypothetical scenario where editing could unintentionally affect neighboring genes or regulatory sequences, again with potentially deleterious consequences.

As yet, the implications of these findings for developers of CRISPR–Cas and other gene-editing therapeutics remain unclear. The high dosages of endonuclease used in some of these studies may not be relevant in a therapeutic context. The modality (plasmid, virus, ribonucleoprotein or transposon) used to introduce CRISPR–Cas activity into target cells and the route (e.g., *ex vivo* or *in vivo*) may also affect DNA repair. It is also clear that more work is needed to elucidate any differences in the management of DSBs in different human cell and tissue types (e.g., germ, stem or differentiated cells) and in dividing or non-dividing cells and at different genomic sites.

Many companies are already exploring solutions to these concerns. At UMass' RNA Therapeutics meeting in May, Caribou Biosciences disclosed efforts to address off-target issues by chemical modifications and base substitutions in the guide RNA. Others are improving the specificity of Cas9 through combined rational design, directed evolution and screening for more selective variants. Cell-screening strategies to determine p53 status and chromosomal modifications around the target site are also being introduced. Functional assays are being developed to detect transformation in relevant animal models before clinical testing. Ultimately, if DNA editing proves too challenging in certain indications, DNA or RNA base-editing strategies may offer viable alternatives.

None of which provides much consolation for investors. The *Nature Medicine* reports wiped $664 million from Editas Medicine, Intellia Therapeutics and CRISPR Therapeutics on the day of publication. Although stock had bounced back 70% by the time of the Bradley study, within 20 minutes of online publication they again lost $300 million collectively.

Investor skittishness in biotech is nothing new. We have seen it before with gene therapy, siRNAs and monoclonal antibodies. But as many CRISPR companies publicly floated very early in their technology cycle, the wild ride for CRISPR stocks looks set to continue—particularly in an age where Wall Street algorithms comb hundreds of millions of daily tweets on Twitter, social media posts and news outlets to derive data for trading decisions. In a research setting in which preprints now enter feeds as easily as peer-reviewed papers—and a dwindling number of media outlets have science-literate journalists—CRISPR stock volatility looks here to stay.

Following human testing of Sangamo's ZFN therapies that have proceeded without significant adverse effects, Editas plans to submit an IND for its CRISPR therapy against Leber congenital amaurosis type 10; CRISPR Therapeutics is also awaiting a green light to restart its phase 1/2 trial of a therapy for transfusion-dependent beta thalassemia.

In the meantime, our knowledge of CRISPR–Cas gene editing and DNA repair is progressing. The picture may not be as clear as we would like. But rarely in biology does anything turn out to be as neat and simple as we imagine.