CELL & GENE THERAPY INSIGHTS

FULFILLING THE POTENTIAL OF GENE EDITING:
AT THE TIPPING POINT

SPOTLIGHT

INTERVIEW

Developing a multiplex gene editing approach targeting viral infection

David McCall, Senior Editor, *Cell & Gene Therapy Insights*, speaks to **TJ Cradick**, Chief Scientific Officer, Excision BioTherapeutics, reflecting on his 20-year career in gene editing and discussing the cutting edge in multiplexing with CRISPR.

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Q What are you working on right now?

TJC: At Excision, we are developing dual guide RNA (gRNA), CRISPR-based gene therapies to cure chronic viral infectious diseases. We are excited about our first-in-human phase 1/2 clinical trials in HIV for our lead candidate, EBT-101, which is currently enrolling and dosing additional participants. The trial is designed to evaluate safety and biodistribution. We are also undertaking exploratory assay development to help us measure both the gene editing itself and changes in the HIV viral reservoir. The field of HIV assays is an area in which many groups, including our own, are developing cutting-edge technologies to understand the low levels of viruses that are present, and whether or not they are full-length and functional.

Excision is using a multiplex gene editing approach for EBT-101. This unique approach gives us three target sites, which means three chances to cleave and therefore, three different possible large viral excisions. Any of these three excisions could completely inactivate HIV. This means three shots on goal, which is a huge advantage compared to a single guide. These large excisions made by EBT-101, are approximately 1000, 8000, and 9000 nucleotides in size, which are vastly larger than the small number of nucleotides that are deleted when using a single gRNA. Using multiple guides is important to increase viral inactivation and it greatly reduces any chance of viral escape and rebound. We have performed bioinformatic modeling of how the cutting works to understand the best target sites. This software has been applied to EBT-101 to target HIV, in addition to hepatitis B virus (HBV) and herpes simplex virus (HSV).

The R&D team is working on HBV and HSV programs, built upon the technologies demonstrated with HIV. It is hoped that the process can be expedited to other indications moving forward. There are two primary areas of active research: developing guides and nucleases to target HBV and HSV, and employing animal models to characterize the safety and efficacy of these therapeutic strategies.

Q Having amassed two decades of experience at the cutting edge of genome editing, what are your high-level reflections on the field today?

TJC: Excision is one of several organizations developing and clinically testing *in vivo* editing strategies. Early research in the field focused on gene editing cells *ex vivo* and providing them as adoptive cell therapy.

The field has taken decades to evolve from the seminal work of establishing and understanding zinc finger nucleases (ZFNs). When transcription activator-like effector nucleases (TALENs) subsequently came out, numerous labs that provided key reagents allowed the field to expand towards a democratic way of working, so that researchers without expertise in ZFNs could get involved. Those building blocks established a great network of people ready to work on gene editing. Then, when CRISPR came along, the field exploded in size, and we all had the opportunity to capitalize on the wide array of relevant information that helped propel the field forward.

As we have improved our ability to edit precisely, we also have identified a range of other editing options, including different nucleases, base editors, prime editing, and other systems. Through decades of work, we have also established the assays and sequencing methods that will enable those emerging platforms to move forward. Meanwhile, delivery technologies continue to improve. A group hoping to tackle a given disease can look at advancements in editing and choose an applicable system.

Manufacturing remains a challenge that the field continues to address. However, assays and other complementary technologies continue to evolve to improve the technologies. Excision remains committed to developing more active and specific nucleases and assays that will enable us to characterize these advances and measure decreases in viral load.

Q Having been instrumental in the development of several of the foremost genome editing platforms currently used in clinical

application, what made Excision's platform and approach really stand out to you?

TJC: As I mentioned, Excision is using a multi-guide approach. Data initially from the Khalili lab, which has been repeated by many others in subsequent years, has demonstrated an increase in viral inactivation from using multiple nuclease target sites. This is important for activity and to create large deletions between the cut sites. These large deletions between the different target sites excise nucleotide fragments from the HIV genome, effectively preventing the chance for viral escape and rebound.

When we started targeting viruses, we used ZFNs to target HBV. Several papers have described the difficulty in doing this, but we succeeded in creating a pair of ZFNs that bind with correct orientation spacing and cut at a single site. As we showed in our first publication, this led to a single nucleotide but often up to four nucleotides changing. However, people who studied the data later realized that there was a chance for viral escape with error-prone polymerases. This is one of the problems, amongst others, in designing and delivering ZFNs.

One thing to consider is whether using more guides increases the possibility of unintended edits. We take care to pick viral DNA target sites that are vastly different from the human genome. With our HIV multiplexing, we see very few sites nominated by the bioinformatics even when using a greater number of mismatches. As we look towards other viruses, we are using software for sequencing, aligning, selecting the target sites, and modeling the multiplex cutting. This allows us to take this platform and apply it to other viruses.

Q Can you tell us more about the Excision pipeline and the rationale behind the diseases you are currently targeting?

TJC: At Excision, all of our programs target viruses. In addition to HIV, Excision is targeting HBV and HSV. Demonstrating the safety and tolerability of a potential CRISPR-based cure for HIV signals the promise of multiplex *in vivo* gene editing as a potential cure for other viral infectious diseases such as HBV and HSV, which both have large patient populations and represent a significant unmet need.

Ongoing advancements in editing technologies and viral and non-viral delivery, plus a better understanding of the editing and DNA repair mechanisms, have the potential to accelerate more effective therapeutics and potentially cure chronic viral infectious diseases.

Q What might be some future application areas of interest?

TJC: The bioinformatics and strategies demonstrated with HIV, HBV, and HSV can be similarly applied to a range of other viral targets, which is an exciting aspect of this technology. Establishing that we can do this effectively and safely allows us to think about what we can do to similar or even completely different viruses with small or large patient populations. We are actively discussing additional targets but have yet to disclose these internal or partnered projects.

Q The range of ***in vivo*** delivery platform options is growing for genome editing therapeutic developers—how would you profile this technology space currently?

TJC: Advances are coming both in viral delivery and non-viral delivery that permit effective targeting of the necessary tissues and cells.

The central idea is that we are developing better manufacturing and better targeting. Manufacturing advances in the wider field are useful, such as how advances in AAV can allow us to achieve higher, more homogeneous titer. Similarly, there have been many improvements in the development of lipid nanoparticles. A challenge we have had as a field is understanding how to harness these advancements to get to the relevant tissues for our viral targets. We successfully demonstrated that we get to the reservoirs for HIV in a recent publication looking at rhesus macaques.

Q In an area where regulatory guidance and opinion are still being formed, what are the keys to ensuring industry best practices align with regulators' expectations?

TJC: We want to weigh up the risk–benefit balance and make the safest but most effective medicines that we can. Just as the editing technologies are quickly advancing, so are the sequencing and detection methods. It remains important to evaluate the new developments and employ them once they are characterized and established. Advances in sequencing have permitted assaying for rarer events than in past years. We also know more about the noise and possible artifacts that might be present around these low levels. It is important, then, to weigh the output of these assays as part of the bigger risk–benefit calculation to ensure that we are providing an effective therapeutic strategy.

We are continuing to have discussions with the regulators and the wider field about learning from previous studies to enable us to move through this process quicker. While we all appreciate the opportunity to deliver for patients faster, we do not want to take any shortcuts in the safety regard, so understanding the process is important.

Q Where next for innovation in gene editing platforms?

TJC: The development of assays is key as once we can measure things, we know how to improve them. We are also continuing to embrace our ability to computationally model in order to help us design our targeting strategies.

Other groups that are editing a range of disease-causing mutations face a challenging task, but it is exciting to see several different technologies being developed that offer alternative means to correct the range of mutations causing some of these diseases. As these technologies develop, they will enable the field to target new indications or allow more effective targeting of diseases currently being investigated.

Similarly, advances are coming both in viral delivery and non-viral delivery that permit effective targeting of the necessary tissues and cells. Decades of work on AAV have produced a

range of improvements in the technology, including novel serotypes with improved specificity and manufacturing scalability. Similarly, the field of non-viral delivery has seen dramatic improvements in recent years, best demonstrated by lipid nanoparticles in their application with COVID-19 vaccines.

Q What for you are the most pressing next steps for the field in making genome editing-based therapeutics more 'commercializable'?

TJC: There are several challenges in making these therapies more accessible to patients. As mentioned earlier, one of the biggest priorities for the field is addressing manufacturing challenges. As we have more gene editing indications taken to the clinic and we get going on manufacturing, this will lead to improvements, and each improvement will drive down costs.

To expedite the regulatory process and get the data to the agencies quicker, we need better assays and manufacturing. This will lead to an increase in both the number of approved products and the speed to commercialization.

We are excited about how quickly we are approaching commercialization with this technology. We have learned a lot already that can be applied to help the whole field of *in vivo* gene editing. The more people doing this, the easier it is for others to follow along, file clinical trials, and drive towards commercialization.

Q Finally, can you sum up one or two key goals or priorities that you have for your work over the foreseeable future?

TJC: Firstly, our team at Excision is excited to continue to enroll for the first-in-human EBT-101 clinical study for HIV, continuing the ascending dose trial. Secondly, we are excited about our advances in targeting HSV and HBV and evaluating the efficacy of our approach in animal models. Our goal is to advance both forward.

It has been exciting to see a number of platforms we have worked on, such as ZFNs, TALENs, and CRISPR, moving forward now with great data. However, there are also technologies coming along that will be more applicable to other disease indications, which will be exciting to see. Having been in the field for a while, it is amazing to watch the advancements in disease targets we have been chasing for decades.

BIOGRAPHY

TJ CRADICK has more than two decades of experience in gene editing, working on therapeutics, bioinformatics and assays as the field progressed from Zinc Finger Nucleases (ZFNs) and TAL Effector Nucleases (TALENs) to CRISPR-Cas9. This research included academic work at the University of Iowa and as faculty at the Georgia Institute of Technology. Cradick has held positions at Sangamo Therapeutics and as the Head of Genome Editing at CRISPR Therapeutics. He is Chief Scientific Officer at Excision BioTherapeutics, where he leads the company's research and development functions, including gene editing, bioinformatics, development of the viral-targeting platform and collaborations with academic and industry partners.

Cradick led the first work on the use of engineered nucleases as a therapeutic strategy for targeting virus, ZFNs that specifically cleaved hepatitis B virus DNAs, and co-authored the first publication on the topic. He has co-authored manuscripts on ZFNs, TALENs and CRISPR and on developing bioinformatics web tools, including ZFN-Site, PROGNOS, SAPTA, and COSMID. At the Georgia Institute of Technology, Cradick was a member of the faculty and director of the protein engineering facility, where his research included developing assays and bioinformatics for CRISPR/Cas9 specificity, which have been applied across a range of gene therapy targets.

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AUTHORSHIP & CONFLICT OF INTEREST

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