MaxCyte®

One Unifying Technology from Concept to the Clinic

Using a Strong Technology Platform to Build Successful Outcomes from Bench to Clinic

The development of novel and efficacious therapies is dependent on a strong technological platform from which to bring your ideas from bench to bedside. For both academic and corporate preclinical and clinical research, as well as the scaling of drug compounds and cell therapy platforms for FDA approval and treatment of patients, the ability to quickly optimize experimental or manufacturing parameters could enable the rapid and cost-effective development of therapies to use safely in clinical trials.

As the COVID-19 pandemic has drastically changed society, science and progress is no exception. Researchers and physicians must carry on critical work required to support the development of novel therapeutics that patients desperately need for a wide variety of indications. Whether you have just developed a new technology in the preclinical stages of testing, are aiming to scale up production according to good laboratory practices, or have an established technology in clinical trial, embarking on the journey to provide patients with care and improved quality of life can benefit by recent scientific advances. We have compiled a booklet providing examples of novel insights with the potential to move your research forward and help you reach your goals.

Novel Delivery System Increases the Precision of Gene Editing Delivery

CRISPR-Cas9 has tremendous potential as a therapeutic tool for treating human diseases, but it's not perfect. For example, as Dr. Peter Gee pointed out during MaxCyte's September 17 webinar, prolonged expression of the nuclease and gRNA from viral vectors in an in vivo context may cause unwanted off-target activity and immunogenicity.

Dr. Gee, a MaxCyte Field Application Scientist in Southeast Asia, has a solution. Nanomembrane-derived extracellular vesicles could deliver macromolecular cargo such as muscle myoblasts, T cells, human induced pluripotent stem cells, or monocytes in a more efficient manner. The vesicles are part of the NanoMEDIC system, which demonstrated efficient genome editing in various hard-to-transfect cell types in a recent study published by Gee et al. in Nature Communications.

The system is designed to overcome some CRISPR-Cas9 safety issues. NanoMEDIC offers transient delivery of CRISPR-Cas9 ribonucleoprotein. The strategy recruits Cas9 protein by chemically-induced dimerization and sgRNA via a viral RNA packaging signal into extracellular nanovesicles.

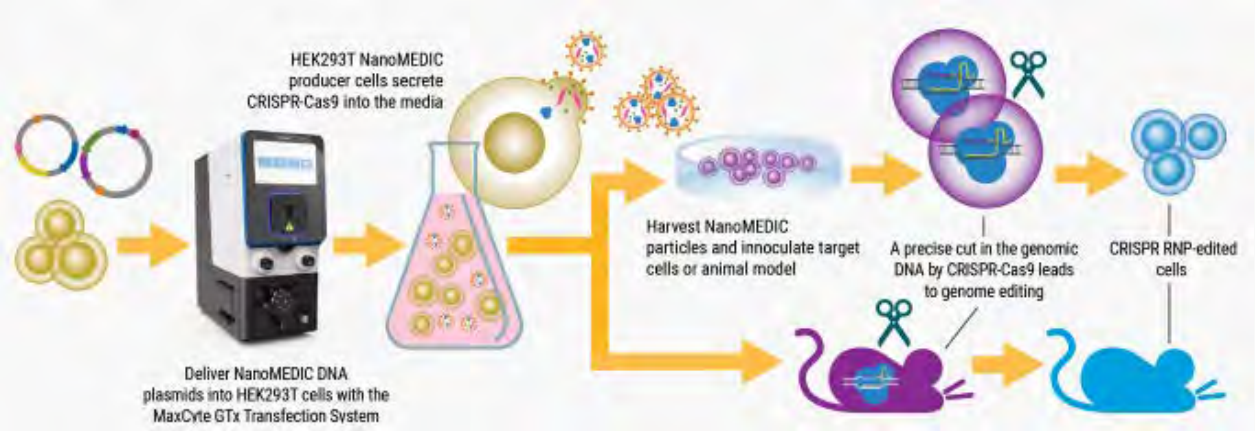
Peter Gee, Ph.D
Field Application Scientist

For patients to realize the full benefit of recent developments in genomics, clinicians need an alternate delivery system, he points out. The system should transport CRISPR-Cas9 components, transiently, allowing CRISPR-Cas9 to act on a target DNA sequence, make the therapeutic change, and then degrade quickly afterward to limit the chance of adverse events.

Accuracy is important, but so is scalability. While the production of NanoMEDIC was feasible at small-scale for basic research, the researchers also demonstrated a scalable method to produce NanoMEDIC using a cGMP compatible, Flow Electroporation® system (MaxCyte) which could be applicable for industrial manufacturing.

Download our Research Spotlight

Watch the Webinar Recording



Shigeki Yagyu, MD, PhD
Pediatric Oncologist and Principal Investigator

Spotlight: Staying the Course: Delivering Non-Viral CAR-T Therapy to Neuroblastoma Patients in Need

Shigeki Yagyu, MD, PhD, faced challenges in making GD-2 CAR-T cells without a universal engineering system. Existing protocols worked for CD19 CAR-T cells, but not for other types of CAR-T cells.

"I struggled for one year trying to establish non-viral GD-2 CAR-T cells using published protocols," said Dr. Yagyu, a pediatric oncologist and Principal Investigator at Kyoto Prefectural University of Medicine (KPUM) in Japan. "Most of the cells died after electroporation and, at most, I got maybe 5% to 10% CAR-T cells, which was barely enough cells to conduct basic research."

Realizing he could no longer go it alone, Dr. Yagyu started looking to collaborate with a company offering an optimal culturing method and electroporation instrument. However, many electroporation companies were not interested in helping. "They were not interested in helping me to optimize resting T cell conditions," he said.

The MaxCyte difference included customized protocols and scientific guidance that helped Dr. Yagyu achieve improved efficiency and viability that he was unable to obtain with other electroporation instruments.

"MaxCyte's electroporation technology and support helped to speed up the process. Cell viability is always higher than other electroporation platforms and they created new protocols for me to meet my experimental needs," Dr. Yagyu said. "Now I can get 50% to 80% CAR positive T cells in a sufficient number."

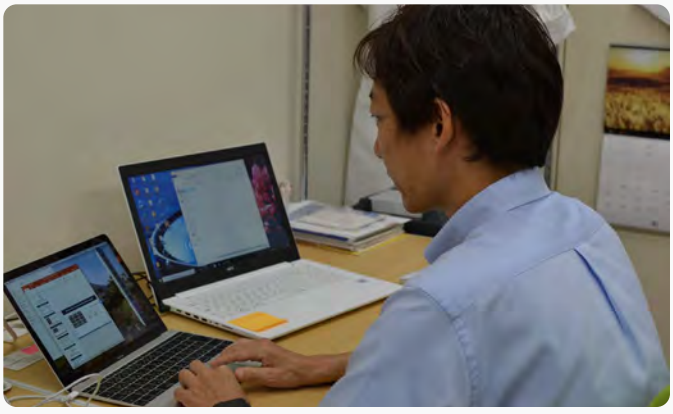
These CAR-T cells, immune cells engineered to express a chimeric antigen receptor (CAR), can seek and destroy cancer cells.

The first step in his scientific method is to draw blood from a patient and isolate T cells. Then, using electroporation, the goal is to introduce a DNA transposon element containing a CAR molecule into resting T cells from peripheral blood mononuclear cells. The transposon permanently inserts the CAR element into the genome of T cells for long-term expression, reprogramming the T cells to fight cancer.

Dr. Yagyu's motivation for developing these living cells is based in a desire to help children with cancer. The first patient Dr. Yagyu treated in 2000 was a two-year-old boy with relapsed neuroblastoma. Unfortunately, with only standard chemotherapy and radiation treatment available, his young patient succumbed to this rare cancer after a valiant year-long fight.

Feeling helpless with few treatment options to treat his patients, Dr. Yagyu enrolled in postgraduate studies focused on novel therapies for pediatric cancers. After earning his PhD degree, his quest to find new effective therapies continued. Dr. Yagyu's frustration peaked when he was unable to recommend an effective treatment for an 8-year-old girl with relapsed neuroblastoma who also sought his help.

Dr. Yagyu continued his search for answers and came across a paper published by Dr. Malcom Brenner's group at Baylor College of Medicine in 2011. The authors used virally engineered CAR-T cells to target an antigen, GD-2, found specifically on neuroblastoma cells. To Dr. Yagyu's surprise, the CAR-T cells successfully led to complete remission in several patients with high-risk neuroblastoma.

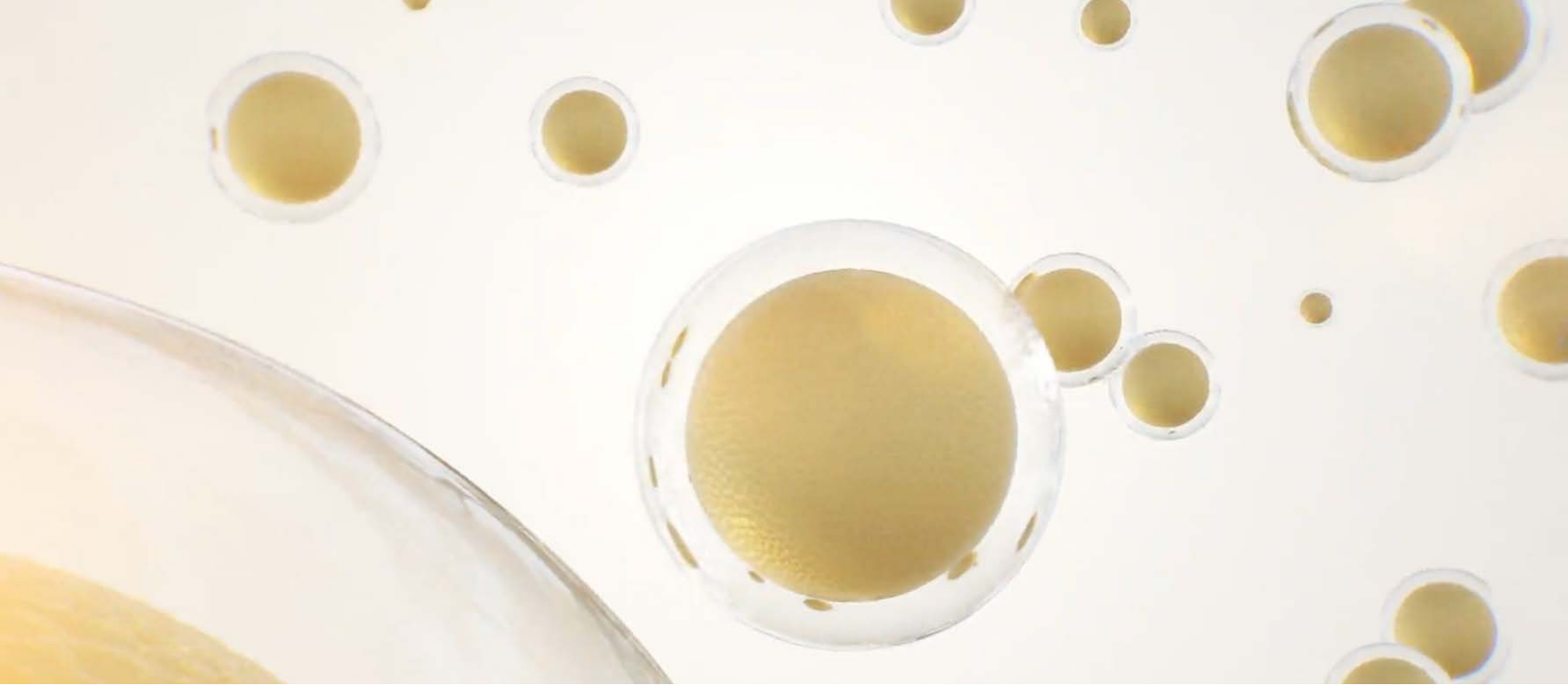


"I sent an email to Dr. Brenner to ask for an opportunity to learn about this therapy," recalls Dr. Yagyu. "I had a dream to establish CAR-T therapy in Japan one day."

In 2015, a chance encounter with Professor Yozo Nakazawa, MD, PhD, a pioneer in piggyBac CAR-T cells at Shinshu University in Matsumoto, Japan, convinced Dr. Yagyu to develop non-viral CAR-T therapies in Japan.

> ***"MaxCyte's electroporation technology and support helped to speed up the process. Now I can get 50% to 80% CAR positive T cells in a sufficient number."***
> ***– Dr. Shigeki Yagyu***

Dr. Yagyu's perseverance despite multiple scientific setbacks is starting to pay off. Recently, Dr. Yagyu established a robust CAR-T protocol applicable to at least five distinct types of CAR-T cells. This success moves him closer to realizing his dream of bringing non-viral CAR-T therapy to patients in Japan. He is working with BrightPath Biotherapeutics to manufacture CAR-T cells and is hopeful clinical trials will start within the next 2 to 3 years.



Case Study: CRISPR-Engineered T Cells in Patients with Refractory Cancer

Translating basic science advances to improved patient therapies and outcomes. A case study presented in this video highlights how electroporation technology can bolster the potency and persistence of engineered immune system T cells in patients.

CRISPR-engineered T cells in patients with refractory cancer
Science 28 Feb 2020: Vol. 367, Issue 6481, eaba7365

The video outlines the manufacturing process for these T cells, which spans 13 days from preparation by electroporation using MaxCyte's GTx system to harvesting and cryopreservation. Furthermore, over an 8 to 10 day expansion phase using the same device, researchers created more than 10 billion engineered T cells.

These engineered cells demonstrated potent antigen specific cytotoxicity.

This multiplex CRISPR Cas9 editing was shown to be safe, feasible at a clinical scale and free of concerns regarding a pre-existing immune response to the Cas9 protein.

These and other findings of a Phase 1 study were published February 28, 2020 in the journal *Science*.

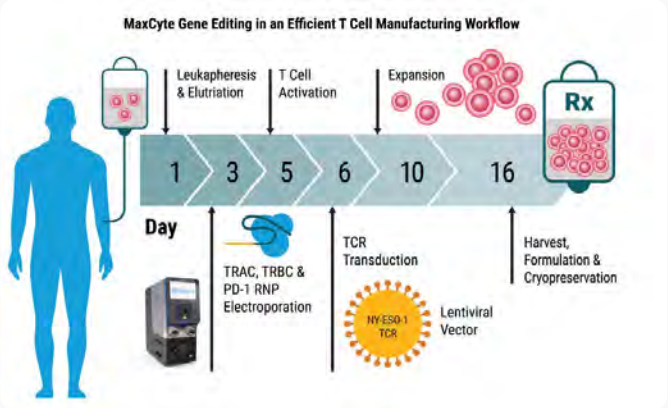
To learn more, watch the video, "Case Study: CRISPR-Engineered T Cell in Patients with Refractory Cancer."

Boost Your Business Viability by Considering Non-Viral Gene Editing Strategies

A growing number of successful laboratories are expanding their options to consider non-viral delivery of precise gene editing.

A clinical approach garnering attention involves bolstering a person's T cells to specifically target tumor cells using electroporation technology and CRISPR ribonucleoproteins. This form of cancer immunotherapy aims to help a person fight cancer in the near term and has the potential to afford long-term protection against recurrence.

If your lab values speed and flexibility, MaxCyte's ExPERT GTx can be used for efficient T cell manufacturing. The workflow, as shown in this infographic, can be accomplished in as little as 16 days:



For more information, download our application note on: CRISPR Electroporation of T Cells Improves Treatment in Patients with Refractory Cancer

Gene Editing Beyond CRISPR-Cas9: Base Editors

Point mutations are changes to one or very few nucleotides in a DNA strand. Usually they are harmless, but now and then they could disrupt cellular functions, leading to grievous consequences. For example, children born with the disease Progeria have a point mutation where a single Cytosine (C) base is swapped for a Thymine (T). These unfortunate children age rapidly with an average life span of about 13 years. In patients with Sickle Cell Anemia, an Adenine (A) base is changed to a T base, causing the hemoglobin chain to fold resulting in red blood cells acquiring a sickle, or crescent, shape. This alteration leads to interrupted blood flow due to clumping of cells and clogging in the blood vessels.

We know of more than 30,000 such mutations that cause genetic diseases in humans. Given today's gene editing technologies, can they be corrected? Unfortunately, the popular and versatile gene editing tool, CRISPR-Cas9, is not particularly efficient in correcting these single base substitutions. Imagine a world where these point mutations can be edited efficiently, precisely, and without completely breaking the DNA backbone. A coterie of determined researchers at Harvard University, Dr. David Liu and his post-doctoral fellows, Drs. Alexis Komor and Nicole Gaudelli have done just that. Their fortuitous discovery of base editors has expanded and armed the CRISPR toolbox with a pair of nifty molecular machines that can change one base to another without the introduction of a double-stranded break, and without the need for a donor template (Ravindran S., Nature 575, 573-555(2019)).

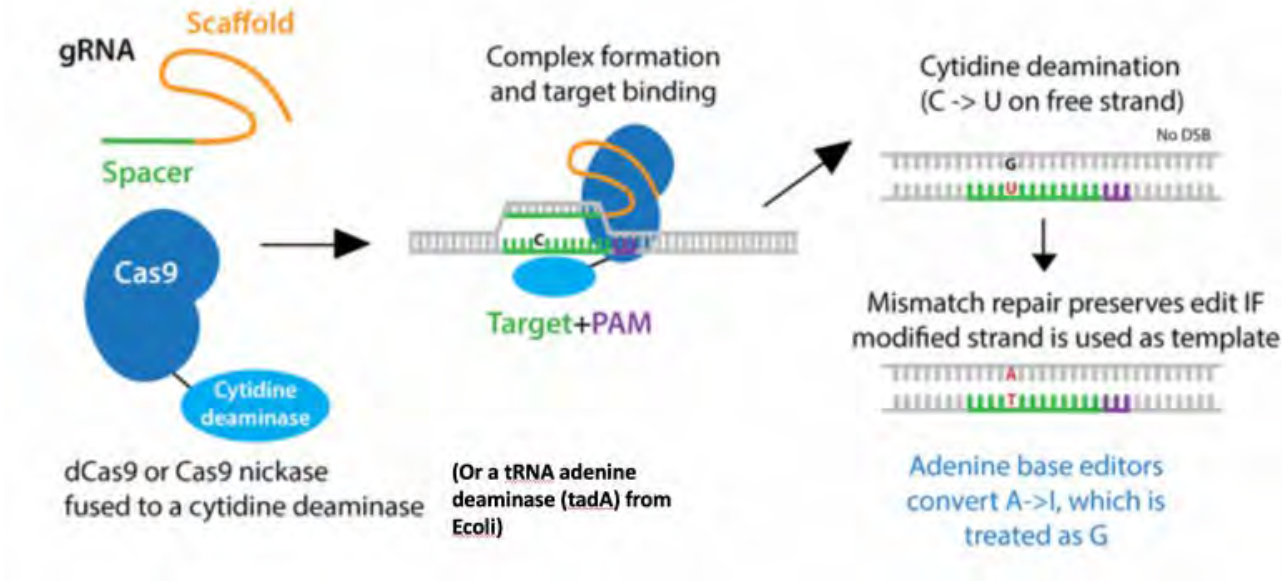


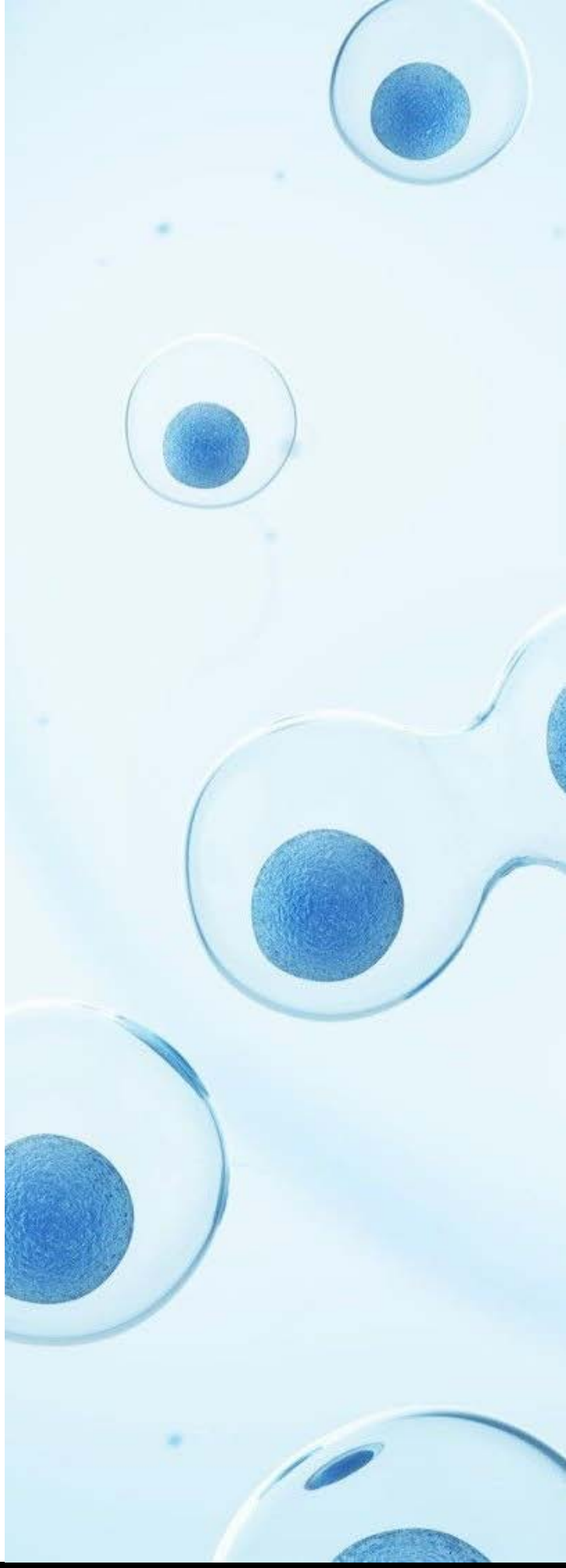
Image source: https://www.addgene.org/crispr/base-edit

Construction of a Base Editor

The CRISPR-Cas9 components are the foundation on which base editors are built. A catalytically dead Cas9 (dCas9) or Cas9 nickase (Cas9n) is joined to a nucleobase deaminase enzyme to form a fusion protein. Guide RNAs are used to search the cellular DNA for the desired target sequence; the fusion protein complex then binds the target upstream of the protospacer adjacent motif (PAM) sequence. A nick is generated in the non-edited DNA strand. Once the bases are swapped in the edited strand, the cells repair the non-edited strand using the edited strand as a template (Rees, H. et al, Nature Reviews Genetics 19, 770-788(2018)).

There are two kinds of base editors. In 2016, Dr. Komor created the first Cytosine Base Editor (CBE) by fusing a naturally occurring cytidine deaminase enzyme called APOBEC1 to a dCAs9 or Cas9n. When guided to a target site by a gRNA, this fusion protein was able to convert a cytidine (C) base to an intermediate base commonly found in RNA called Uridine (U). The U was then converted to a T through base excision repair, thus creating a C->T change or a G->A change in the opposite strand (Komor, A. et al Nature 533, 420-424(2016)).

The second Adenine Base Editor (ABE) was much harder to create. There were no naturally occurring enzymes that could chemically convert A->G in DNA. Dr. Gaudelli persisted and after several rounds of protein engineering, finally harvested a tRNA adenine deaminase (tadA) from E.coli, a year later, in 2017. When target-bound, this ABE converts an adenine base (A) to an intermediary inosine (I) which is treated like Guanosine (G) by the cell, thereby instituting an A->G change or a T->C change in the opposite strand (Gaudelli, N.M. et al Nature 551, 461-471(2017)).



A Single Unifying Technology - from Concept to the Clinic

The ExPERT instrument family represents the next generation of the industry's leading, clinically validated, electroporation technology for complex and scalable cellular engineering. By delivering high transfection efficiency with enhanced functionality, the ExPERT platform delivers the high-end performance essential to enabling the next wave of biological and cellular therapeutics.

When you partner with MaxCyte, you can take advantage of a seamless transition between phases of your research, propelling you forward in your work and shortening your time to clinical applications.

EFFICIENCY PRODUCTIVITY REPRODUCIBILITY

expert®

EFFICACY THERAPY

Previously Unfeasible Cell-Engineering Applications

- Cell and gene therapies
- Functional cell-based assay
- Gene Editing
- Protein and antibody expression
- Stable cell line development

Meet GTx

Your "Go To" System for a Wide Range of Cell Engineering Needs

- Save time and cost
- Shorten timelines
- Easy to use
- Interactive controls
- Expand beyond virus-only delivery strategies
- Gain from a universal platform enables scalability, high efficiency and reproducibility
- 21CFR Part 11 enabled software
- Closed, cGMP-compliant, ISO-certified and CE-marked

Don't just take our word for it!

Request a demo today