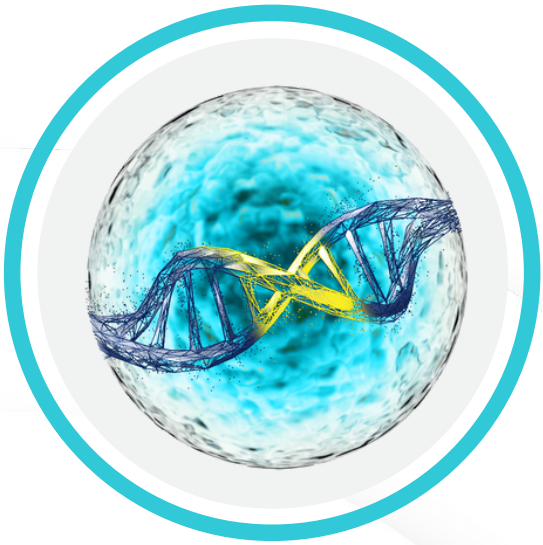
TcBuster™ - Non-Viral Gene Engineering FAQ



Gene engineering of immune cells is a powerful tool for creating advanced and novel cellular therapies. Currently, the critical step of cellular gene editing is primarily performed using virus-based gene delivery systems. Virus-based engineering methods are commonly plagued with long lead times, inconsistent batches, low cargo capacity, and high costs. Conversely, TcBuster is a non-viral gene editing platform aligned for stable high-efficiency integration of a gene of interest (GOI) into the genome as well as large cargo capacity, shorter timelines at lower cost, and consistency of efficiency enabling multiple edits in one step.

The benefits of non-viral gene engineering, when compared with its viral counterpart, offer simplicity, and streamlined capabilities all while lowering risk and cost. TcBuster as part of the ScaleReady portfolio empowers cell therapy programs to move to market faster and with superior scalability ensuring confidence in reproducibility, bench to bedside. TcBuster is produced by R&D Systems and Bio-Techne as part of the ScaleReady joint venture with Fresenius Kabi and Wilson Wolf.

This document addresses some of the commonly asked questions when cell and gene-modified cell therapy programs are evaluating alternatives for lentiviral gene engineering and other non-viral methods.

Is there a GOI size limit for TcBuster, and is the integration site-specific?

The size limit for TcBuster is based on the transfection technologies. It's understood that electroporation generally cannot get a plasmid larger than 10–12kb into the cell.TcBuster itself can transfer large cargoes over 10–12kb. However, practically speaking, when transfecting T cells or NK cells, getting plasmids much larger than 10–12kb starts to become a challenge.

The integration is not site-specific. It is a random integration, though we do offer perspective on how to skew that integration profile and could eventually perhaps make it site-specific.

How does TcBuster compare to other non-viral systems such as Sleeping Beauty or PiggyBac?

Some limited direct comparisons between TcBuster and Sleeping Beauty have been conducted and it was discovered that TcBuster does have higher integration rates than the Sleeping Beauty SB100. A direct comparison to Piggy-Bac has not been completed to date, especially with some of the hyperactive mutants that are being utilized by Poseida Therapeutics.

In comparing it to the literature data of integration profiles, out of the three transposons, Sleeping Beauty is more random than TcBuster. PiggyBac is slightly more preferential to active chromatin. All of them show more random integration than a lentivirus. It is important to note that TcBuster is the only commercially available non-viral system, so as your program thinks about scaling to market, TcBuster uniquely offers this capability.

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How many copies are integrated into the genome and what is the variability in copy number from cell to cell?

A copy number analysis has been completed using droplet digital PCR. For our populations, our copy number is found to be around three–five in T cells, and slightly lower in NK cells at two–three copies per cell range in terms of the entire population. With T cells and NK cells, there has not been analysis in individual cells to evaluate copy numbers, so we do not currently have the data on variability.

How can TcBuster be evaluated by interested parties?

There are two methods to start evaluation. One would be a proof-of-concept study at Bio-Techne, where we test cells using TcBuster, providing the final frozen cells, the protocol, and the test results.

The other is a material transfer agreement, which provides the transposase and either standard transposons such as CD19 or GFP, or custom transposons with the GOI. This can be done quickly and relatively inexpensively for proof-of-concept.

Is there a license fee for the use of TcBuster?

There is a fee. The business model has a few different components, and is milestone-based, as the clients progress through the clinic. There is a royalty associated with the use of a commercial basis. This is a reasonable upfront cost until customers will be successful commercially. Please reach out to a ScaleReady expert to discuss this further.

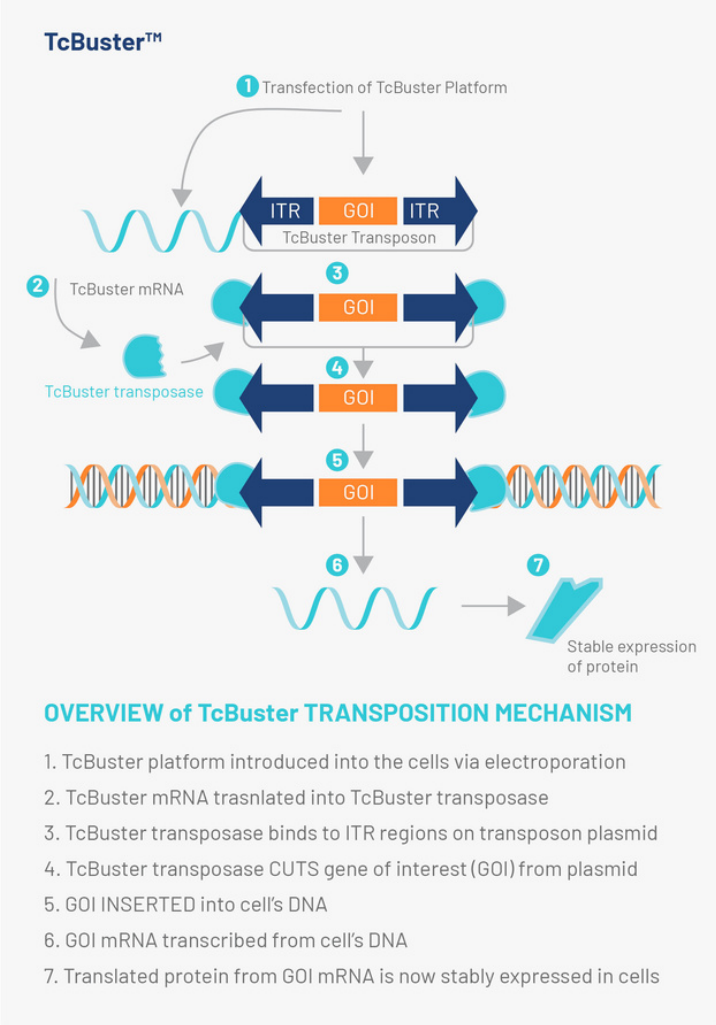

Figure 1. Overview of TcBuster transposition mechanismto produce stable expression of protein.

Has TcBuster been used in the clinic?

The first clinical use is imminent, the IND has been approved.

Have you tried any transfection technologies other than electroporation?

Our team is always interested in evaluating the squeeze technologies. To date, the investigation of some of the other technologies such as the SE cell Line or Tito Pen, as well as lipid nanoparticles has been completed. ...continued on the next page

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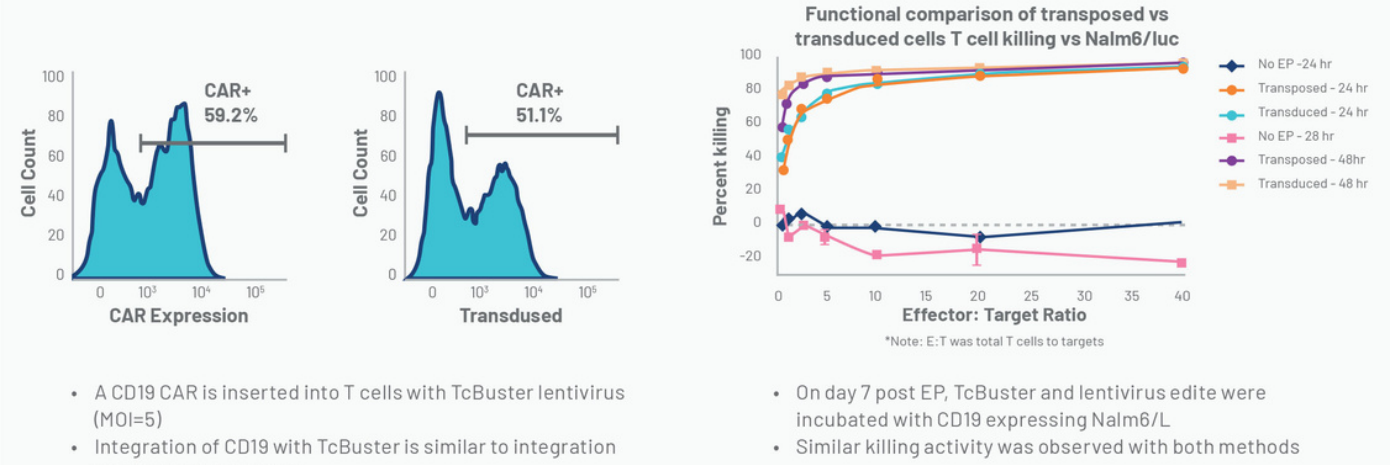
However, there is not any concrete data that can be shared at this time as this is something our team is actively pursuing. One of the major limitations in terms of getting the cargo in and the size of the cargo comes down to the transfection technology. If we can move away from electroporation into another transfection modality that allows for larger plasmids, then the ability to transfer even larger cargoes will improve.

Is there an off-the-shelf TcBuster GFP to test or use as a control for initial feasibility studies?

Bio-Techne offers CD19 GFP constructs that can be provided quickly.



- A CD19 CAR is inserted into T cells with TcBuster lentivirus (MOI=5)
- Integration of CD19 with TcBuster is similar to integration
- On day 7 post EP, TcBuster and lentivirus edite were incubated with CD19 expressing Nalm6/L
- Similar killing activity was observed with both methods

How do you assess days post-NK activation for best transposition?

Normally, the transposition rates in NK cells are most dependent on the method of growth. The development team thinks up the transgene expression with the activation. For example, if K562s are being used as a feeder line, and being stimulated every 7 days, typically, looking at the transgene expression one-two days after stimulation there will be some variability seven days after stimulation, and the transgene expression percentage will be lower than two-three days after stimulation. That holds true through multiple rounds of stimulation. There is part of the cell cycle that comes into play in terms of detecting it.

How would you say this approach compares with the latest advances in the lentiviral space for CAR T cell generation?

In terms of the phenotype for transposed cells versus transduced cells, in general, transposon systems lead to a higher percentage of memory cells. This is related to how the mechanism of transposons performs better in naïve cells than in the effector populations from the apheresis product.

Have you compared with CRISPR/Cas9, and if so, are there any advantages with TcBuster?

A head-to-head comparison has not been completed, though we have worked with customers who are evaluating them side by side. The integration rates tend to be higher using TcBuster than with a CRISPR/Cas9 directed site integration. The CRISPR/Cas9 mechanisms work acceptably for relatively small cargo. However, as clients move to larger cargo, the rates have been known to fall off significantly.

Does copy number increase with cell propagation, or is it limited by turnover of transposase?

Transposition occurs in the first 48–72 hours after electroporation. After that point, the transposase mRNA as starts to become undetectable by a qRT-PCR reaction. After the first 72 hours, there is no more transposition occurring, so the copy number does not continue to increase.

The integrations are also stable long-term. T cells have continued to be activated through CD3/CD28 bead activation to the point where the T cells no longer expand ex vivo; and still maintain the same level of integration throughout. There is no difference in copy number whether there is a run in 7 days post-transposition or after three to four rounds of stimulation.

Can you clarify why GFP affects Sleeping Beauty but not TcBuster mechanisms?

Some people have tried to fuse GFP onto both the N terminal and C terminal of Sleeping Beauty, and it has been shown to prevent all transposition activity. Our team has been able to fuse GFP to TcBuster and still get equal transposition efficiency. It seems to be an inherent property of the enzymes.

What is the cost of transposition versus LV transduction per sample?

In a clinical-scale manufacturing run, the cost of lentivirus can be anywhere from about $15,000–$25,000 depending on lentivirus run efficiency. Our target is <$10,000 per patient cost of goods for both the transposon and transposase.

Simplify, streamline and scale your gene engineering with TcBuster™

Achieve:

- Stable, high-efficiency integration
- Shorter timelines at lower cost
- Large cargo capacity
- Multiple edits in one step

Learn More

scaleready.com

Contact us: info@scaleready.com

ScaleReady is a Joint Venture formed by Bio-Techne, Fresenius Kabi, and Wilson Wolf. Combining selected offerings from the three partners, the ScaleReady manufacturing platform combines tools and technologies for cell culture, cell activation and expansion, gene editing, and cell processing.

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