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Faster, Cheaper, Safer, T cell engineering

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In this issue of the Journal of Immunotherapy, Nakazawa et al, present their results on the analysis of the long-term gene expression from human T cells genetically engineered with the *piggyBac* transposon (PBT). Transposons are ubiquitous mobile genetic elements found in both vertebrate and non-vertebrate genomes that in the last decade have been adapted as gene transfer vectors^{1, 2}. A transposon consists of a minimal number of elements necessary moving about in the genome, the transposase enzyme that mediates the transposition and the surrounding DNA elements on which the enzyme acts, these are usually inverted repeats (IR). The transposase recognize the specific sequence of the IR and using a cut-and-paste mechanism, excises the transposon and inserts it into a another region of the genome that has minimal sequence specificity, for *piggyBac*, the target sequence is TTAA. The procedure for turning a transposon into a gene delivery system is simple; replace the transposase coding sequence with your gene expression cassette (bound by the IRs) and introduce this DNA along with the transposase enzyme into you target cell. The transposase enzyme can be delivery as a protein, RNA, or separate DNA express cassette. The result of the co-delivery of transposon and transposase is a highly efficient stable gene transfer event without the use of viral vectors.

In the specific work presented by Nakazawa et al., the investigators were mainly concerned with what happens to the PBT expression cassette (in this case a GFP reporter gene driven by a CMV promoter) in long-term (out to six months) cultured T cell clones. If the PBT were to have been silenced, this would not bode well for its use in potential clinical applications. The authors found no evidence for silencing and additionally demonstrated that the PBT responded to TCR activation, epigenetic modulation with 5-azacytidine or trichostatin-A, and was not significantly influenced by immunomodulatory cytokines. This type of information is critical knowledge to be added to the database of transposon behavior in primary human cells. In addition to these findings, data in Nakazawa et al also suggest that it may be possible to modulate the transgene copy number in engineered cells by varying the size of the transposon, presumptively this has an influence on the efficiency of transposition.

Previous work using transposons in the context of immunotherapy have shown that fairly simple electroporation systems can be used to delivery transposons expressing chimeric antigen receptors (CAR) targeting CD19 or T cell receptors (TCR) recognizing tumor-associated antigens^{3, 4}. Although there have been few comparison studies looking at how transposons compare to other viral vector systems, in the case of transposon-mediated TCR expression, effector cytokine production and cell lysis was similar between T cells engineered by the *sleeping beauty* transposon or gamma-retroviral vectors⁴. Given the encouraging clinical results treating B cell malignancies using viral vectors to transfer anti-CD19 CARs^{5–7} there is now a proposed clinical trial to use transposons as the delivery platform for this CAR (see link to trial, <http://clinicaltrials.gov/ct2/show/NCT01497184?term=CD19-specific+T+Cell+Infusion+B-Lineage+Lymphoid>).

In comparison to viral vector gene transfer platforms, the use of transposons may have significant advantages. The first is speed. Current gamma-retroviral vectors and lentiviral vector gene transfer systems require large-scale cell culture facilities to produce significant volumes of vector supernatant that must then be processed, bagged, then subjected to numerous biological safety testing schemes. The time from having a plasmid DNA containing your viral vector and the actual ready-to-use vector supernatant is a minimum of six months, and most times at least one year. In the case of the transposons, the plasmid DNA is the clinical reagent and there are numerous vendors that produce this reagent for clinical application in times as short as one month. If the field of immunotherapy is ever to seriously consider personal therapies targeted to patient-specific mutations, it is unlikely that viral vectors will be able to be produced in a short enough time to be a useful clinical reagents for treatment of patients with advanced cancers. Second, transposons are less expensive to produce. Again this advantage derives from the differences in production and safety testing, where large-scale cell culture facilities and expensive biological safety testing are much more expensive for the viral vectors than for plasmid DNA (roughly, one quarter the cost). In the case of safety, there will never be a concern for contamination with replication competent viruses or other adventitious viral agents in plasmid DNA preparations. Since both transposons and retroviral vector systems are integrating gene transfer platforms, there will never be zero risk of insertional mutagenesis, although the transposons may have a more random pattern of integration than viral vectors⁸. The main disadvantage of the transposon system is the method of introducing the plasmid DNA into target cells, electroporation, which is associated with toxicity and can be difficult to scale-up. Recent insights into novel T cell subsets, suggest that far fewer engineered T cells may be required for therapeutic applications than previously thought lending itself to applications such as transposon-mediated gene transfer⁹.

Work such as that reported herein by Nakazawa et al., are essential to making transposons a realistic alternative to current viral vectors and may eventually lead to wide-spread, cost-effective, and simple gene therapy.

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