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Circularization of Non-Viral Single-Strand DNA Template for Gene Correction and Gene Insertion Improves Editing Outcomes in HSPCs

Alex Boyne[1], Arianna Moiani[2]*, Gil Letort[2], Diane Le Clerre[2], Isabelle Chion-Sotinel[2], Margaux Sevin[2], Cosimo Ducani[3], Roger Salvatori[3], Alexandre Juillerat[1], Aymeric Duclert[2], Philippe Duchateau[2], and Julien Valton[2]*

[1] Cellectis Inc, New York, NY; [2] Cellectis SA, Paris, France; [3] Moligo Technologies, Solna, Sweden

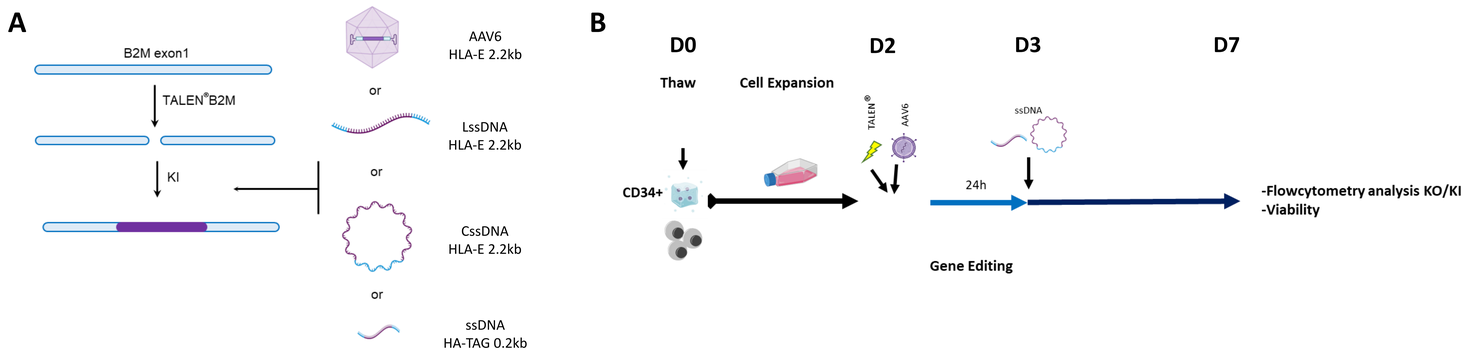
Abstract

Leveraging the TALEN® technology, we developed a gene editing process leading to highly efficient gene correction and gene insertion via homology directed repair, in hematopoietic stem and progenitor cells (HSPCs). We first assessed the potential of non-viral linear single-stranded DNA (LssDNA) donor template delivery strategies versus the more commonly used viral (AAV) delivery. Both strategies led to gene insertion in HSPCs *in vitro*. We then compared the use of LssDNA versus circularized single stranded DNA (CssDNA). We found that circularization markedly increased the knock-in (KI) efficiency with respect to its linear counterparts. Interestingly, this increase of KI was correlated to higher viability and a lower knock-out (KO) in circular versus linear ssDNA edited cells, respectively.

Overall, we showed that non-viral ssDNA delivery associated to TALEN® gene editing allows high levels of gene correction in long-term repopulating hematopoietic stem cells. Circularization of the ssDNA has the potential to further increase the rates of KI without impacting cellular viability and fitness, facilitating the development of next generation cell therapies.

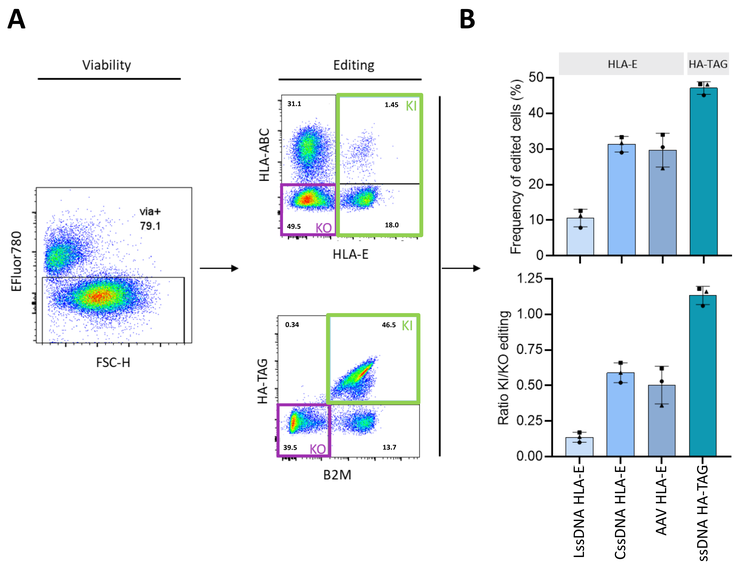
#1 Background

Several viral and nonviral approaches for the delivery of donor DNAs into mammalian cells have been explored. While viral vectors, with Adeno-Associated Virus (AAVs) being the most prominent example of a donor template carrier, remain the mainstay in many applications, non-viral templates such as linear single-stranded DNA (LssDNA), or the more recent circularized single stranded DNA (CssDNA), represent a promising alternative. Here we describe a comparison of four different strategies for delivery and editing in human HSPCs. (**A.** Schematic representation of a TALEN® driven targeted KI utilizing four different strategies for repair template delivery. **B.** Schematic representation of basic transfection strategy of viral or nonviral DNA.)



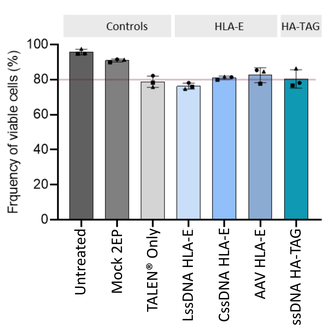
#2 Circular ssDNA shows higher editing than linear ssDNA

A. Gating strategy, first gated on viable cells then gated on B2M/HLA-ABC and knocked in (KI) expression Tag or HLA-E to assess editing. **B.** Frequencies of edited cells and Ratio KI/KO obtained at D7. (N=3).



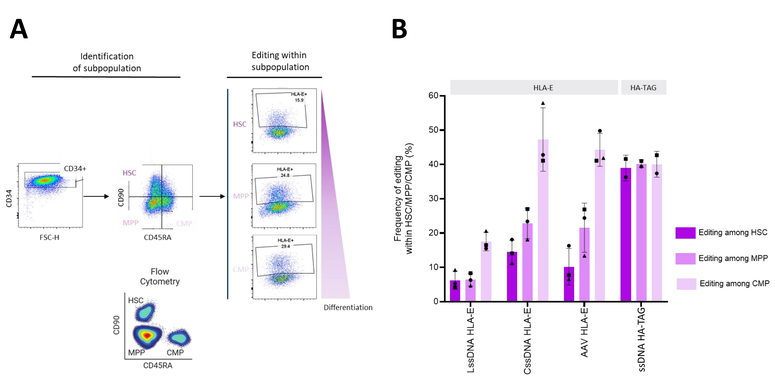
#3 Donor template impact on viability

Cell viability following targeted gene insertion was assessed for each donor template delivery strategy along with TALEN® only, electroporation control, and untreated cells. Viability was similar across all four delivery methods with a slight, but consistent drop for linear ssDNA relative to other delivery methods (D7, N=3).



#4 Frequency of editing within HSPC subpopulations

A. Gating strategy, first gated on CD34+/forward scatter, then gated on CD90/CD45RA to determine subpopulations, finally assessing frequency of KI of marker gene **B.** Frequency of edited cells by subpopulation (N=3).



#5 Conclusions

We show that:

- Circularized ssDNA increases editing frequency over linearized ssDNA, achieving editing levels at least as high as AAV.
- Cell viability levels are similar amongst all four template delivery methods
- Editing levels are markedly higher amongst HSPC subpopulations with circularized ssDNA relative to linearized ssDNA, achieving levels similar to AAV