Boosting the Efficacy of Tumor Infiltrating Lymphocytes via PD-1 Gene Editing

Cell & Gene Therapy

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Background

Adoptive transfer of autologous tumor infiltrating lymphocytes (TIL) has shown clinical success as a treatment for metastatic melanoma with 20% of patients achieving complete and durable tumor regression and a 50% overall response rate.[1] While promising, avenues to improve TIL therapeutic efficacy are needed.

PD-1, a receptor implicated in suppressing T cell activity, is expressed by the tumor reactive fraction of TIL. Conversely, metastatic melanoma tumor cells express PD-1 ligands, PD-L1 and PD-L2, potentially creating an immunosuppressive tumor micro-environment that would negatively impact therapeutic efficacy.[2,3] Clinical treatment of melanoma patients with anti-PD-1 antibodies has led to modest tumor regression, validating the importance of the PD-1:PD-L1/L2 interaction.[4]

Disrupting the PD-1 gene in autologous TIL prior to adoptive transfer offers an alternative and promising approach to improving the efficacy of TIL, while avoiding the toxicities and cost associated with long-term anti-PD-1 antibody treatment.

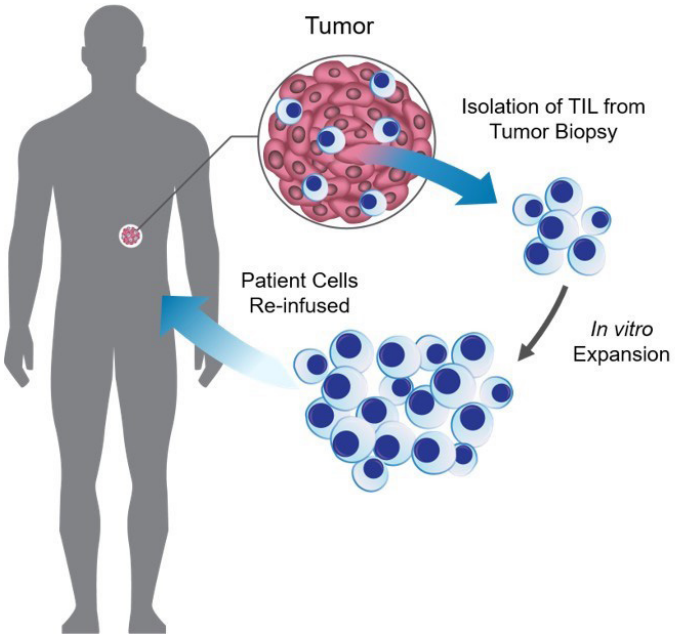
Aim

Determine the feasibility and safety of disrupting the PD-1 gene in human TIL via zinc finger nuclease (ZFN)-mediated gene editing using the MaxCyte GT, a clinically-approved platform for scalable T cell engineering.

TIL Electroporation

- TIL isolated from three melanoma patients were induced to rapidly expand on day 0 using a previously reported expansion protocol.[5]
- On day 7 of the rapid expansion protocol, TIL were harvested, washed, and resuspended in MaxCyte electroporation buffer at a concentration of 1 × 10^8/ml. Cells were mixed with 120 µg/mL of PD-1 ZFN RNA and transferred to a 3 mL CL1.1 (small-scale) or 100 mL CL-2 (large-scale) processing assembly for electroporation on the MaxCyte GT.
- Electroporation was performed using MaxCyte recommended protocol. Up to 3x10^9 were electroporated per donor.
- Post electroporation, cells were resuspended in AIM-V media and incubated overnight at 30°C followed by two days at 37°C.[6]

Full methods for *in vitro* assays and *in vivo* safety studies are detailed in *Mol. Ther.*, 23(8), 1380-1390, 2015.



Results

75% PD-1 gene disruption

Electroporation of TIL from three human melanoma patient donors with mRNA encoding a PD-1-targeted ZFN resulted in 75% PD-1 gene disruption as determined by deep sequencing using the Illumina platform with a 44% bi-allelic disruption frequency (Table 1).Cells electroporated using small-scale versus large-scale MaxCyte electroporation had similar gene disruption frequencies.[7] Additionally, no negative impacts on cell proliferative capacity or changes to T cell phenotype were observed.[7]

PD-1 Knock Out TIL Exhibit Enhanced *In Vitro* Effector Functions

PD-1 gene disruption resulted in a 76% reduction (electroporated vs mock electroporated) of PD-1 expression on CD3+ TIL following anti-CD3/CD28 bead stimulation (Figure 1). These edited TIL had significantly enhanced *in vitro* T-cell effector functions such as secretion of TNFα, GM-CSF, and IFN-γ following co-culture with antigen-specific tumor target cells. Importantly, enhanced cytokine secretion was observed even when the target cells expressed PD-L1, suggesting that PD-1 knock out allows TIL to overcome immunosuppression.[7]

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In Vivo Toxicity Studies Establish Safety

PD-1 edited or mock electroporated TIL were administered to NOD SCID gamma (NSG) mice to assess whether PD-1 gene knock out results in proliferative abnormalities or tumors upon engraftment. No carcinogenic effects were noted supporting the safety of PD-1-ZFN-mediated engineering of T cells prior to adoptive transfer.[7]

Conclusion

Combining high efficiency gene disruption and high primary T cell viability post engineering with consistent performance enabled by MaxCyte's regulatory-compliant GT electroporation platform resulted in extremely effective gene editing at clinical-scale. Using this approach, the efficacy of TIL and other T cell adoptive cell therapies against a wide variety of cancers can be enhanced, enabling developers to attain the required therapeutic index and safety profiles while simplifying manufacturing.

PD-1 Gene Disruption Determined Using Deep Sequencing

	% PD-1 Indels[a]	Bi-allelic PD-1 Disruption
Donor 1	84.1	48%
Donor 2	69.9	N.D.
Donor 3	70.4	40%
Mean	**74.8**	**44%**

Table 1: *TIL from three donors were electroporated with mRNA encoding PD-1-specific ZFN.*

[a] *Percent indels was calculated by dividing the indel containing sequence count by the total sequence count.*

Surface Expression of PD-1 on CD3+ Cells

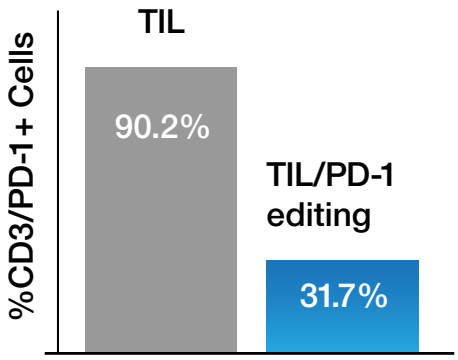

Figure 1: *7 days post electroporation, TIL were restimulated with anti-CD3/CD28 beads for 48 hours. Cells were then assessed for surface expression of CD3+ and PD-1 via FACS.*

References

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