A Rapid and Scalable 96-Well Platform to Screen CARs and TCRs

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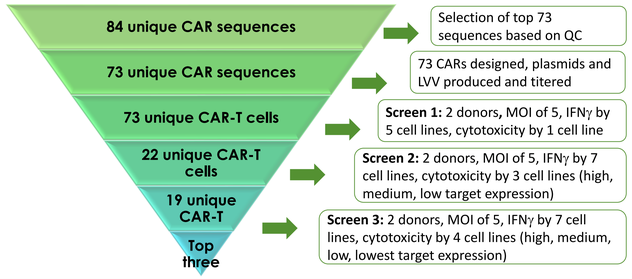
Abstract

- Identification of a binder sequence that will give rise to a chimeric antigen receptor (CAR) or T cell receptor (TCR) construct is a necessary first step in the development of engineered cell therapies.
- Screening platforms for CAR and TCR construct nomination in primary T cells are seldom scalable, utilize non-MOI normalized lentiviral vector preparations and rely on limited phenotypical or functional assessments.
- Because of these limitations, the performance of top early candidates may not translate to larger-scale preparations, thus requiring time consuming repeat experiments to achieve reproducible data at-scale for *in vivo* work.
- Here we report a **rapid, mid-throughput, comprehensive, scalable CAR/TCR screening platform for the selection of candidate CARs and TCRs in primary T cells.**
- The platform relies on a **scalable mid-throughput lentiviral vector production platform**, and a **96-well scalable T cell expansion that produces enough cells for characterization and functional assays** including vector copy number (VCN), surface binder expression and function.
- The platform was utilized to **screen candidate CARs by surface expression and VCN, tonic signaling, functional activation by engineered cell lines as well as cell lines expressing endogenous high, medium and low levels of target.**
- Within **three rounds of screening, we were able to nominate top candidates ready for *in vivo* testing**.
- Our **proprietary CAR/TCR screening platform thus allows acceleration of nomination of binders for faster development of CAR and TCR therapies.**

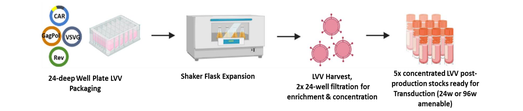
Summary of Screening Workflow

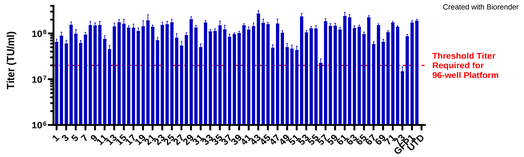
Criteria for Candidate Selection:

1. **CAR Expression at equal or higher than 30%**
2. **IFNγ production and cytotoxic function equal or higher than control, lack of tonic signaling**
3. **Diversity in B-Cell lineage for top clones**
4. **Sensitivity to target level expression in cell lines**

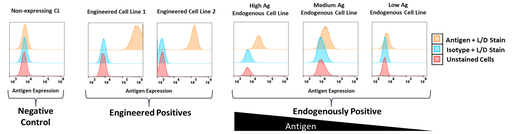


Lentiviral Platform and Target Cell Lines



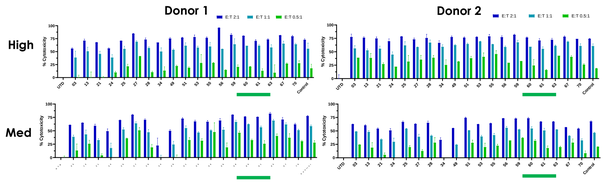


Target Cell Lines for Cytotoxicity Assays

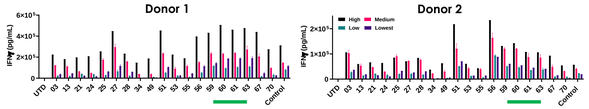


Screens 2&3: Cell Lines Expressing Varying Levels of Target Enable Top CAR Sequence Selection

Cytotoxicity for High and Medium Target Expressing Cell Lines

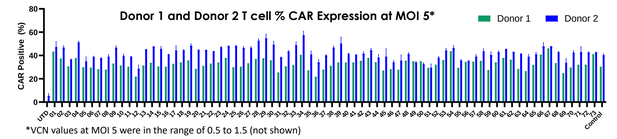


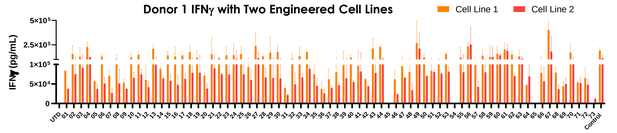
IFNγ for High, Medium, Low and Lowest Target Expression

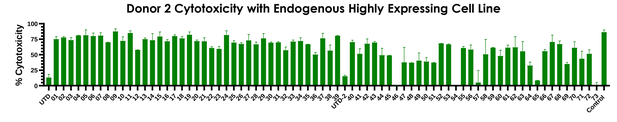


Based on These Data, CARs 60, 61 and 63 were chosen as top candidates

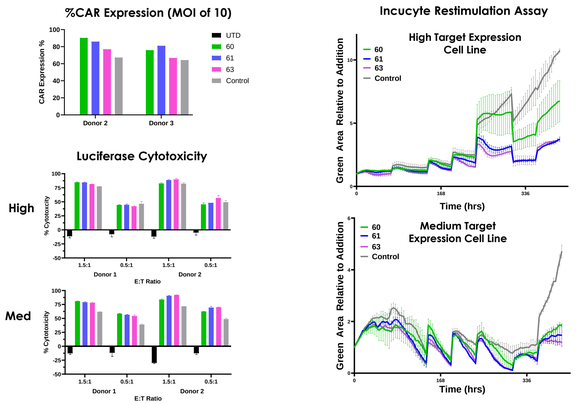
Screen 1: CAR Expression, IFNγ and Cytotoxicity Data for 73 CAR Sequences





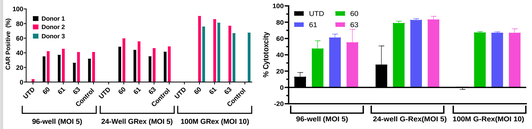


Screen 4: *in vitro* Validation of Top Three Candidates in Large Scale 100M G-Rex Shows Scalability, and Superior CAR Activity Compared to Control



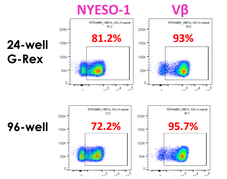
Small to Large Scale Activity Comparison of Top Three CAR Candidates

% CAR and % Cytotoxicity is Consistent Across Scales



Platform Validation for NYESO TCR

Transduction and Expansion Scalability was demonstrated with NY-ESO TCR as Benchmark



	24-well GRex	96-well*
Total Cells	4.08e7	3.24e7

* Cell number adjusted to theoretical yield for 24-well scale

Conclusion

- Our 96-well screening platform was validated for CARs & TCRs, and enabled **selection of candidate CARs within three rounds of screening.**
- The throughput and cell yield were sufficient to perform an extensive array of assays: **surface expression and VCN, tonic signaling, functional activation by engineered cell lines as well as endogenously expressing cell lines.**
- Inclusion of cell lines **expressing endogenous high, medium and low levels of target enabled higher stringency to select top candidates.**
- The **platform data is reproducible, and the platform is scalable.**
- Our **proprietary CAR/TCR platform thus allows acceleration of nomination of binders for faster development of CAR and TCR therapies.**

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