Selection of Engineered K562 Feeder Cell Clone for CAR-NK Drug Product Manufacturing Process

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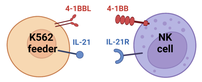
INTRODUCTION

Engineered Natural Killer (NK) cells are an attractive allogeneic off-the-shelf therapy for cancers, including solid tumors. In our drug product process, CAR-NK cells generated via electroporation with TcBuster™ transposase mRNA and CAR-containing plasmids are expanded with irradiated, engineered K562 feeder cells. To select a feeder cell clone for GMP manufacturing, K562 cells were transduced at different multiplicities of infection (MOIs) with a lentiviral vector encoding 4-1BBL and membrane-bound (mb)-IL-21. Candidate K562 clones were first assessed for growth rates and transgene expression levels. Next, K562 feeder candidates were co-cultured with HER2-CAR-engineered NK cells to determine the optimal feeder clone for effective CAR-NK cell expansion, as well as *in vitro* cytotoxicity against HER2-expressing tumor targets. From these outputs, **we determined the optimal 4-1BBL and mb-IL-21-engineered K562 feeder clone from which to derive our GMP master cell bank.**

1 Overview of K562 engineering and candidate feeder clone selection

- K562 erythroleukemia cells stimulated NK cells through endogenous activating receptors
- Coordinated stimulation by 4-1BBL (CD137 ligand) and membrane-bound (mb)-IL-21 ligands enables robust NK cell activation and expansion by engineered K562 "feeder" cells



Gurney M. et al. *Front Immunol*. (2022). PMID: 35222382.

A. Engineering and selection of candidate K562 feeder clones

Parental K562 engineering
- Lentiviral engineering of K562 cell line with 4-1BBL and membrane-bound IL-21 at varied multiplicities of infection (MOI)

Clonal plating and expansion
- Clonal plating and selection of engineered cells
- Expand clonal sublines and select for clones with stable transgene co-expression

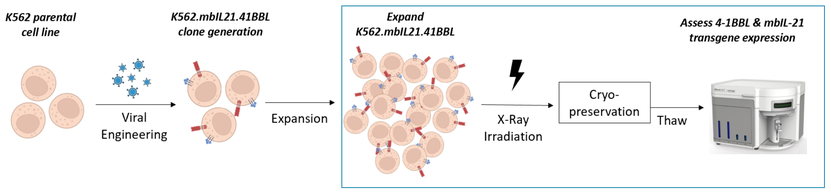
Clonal evaluation
- Assess 4-1BBL and mb-IL-21 transgene expression by flow cytometry and ELISA
- **Select 6 clones with range of expression and MOIs for internal characterization and functional analysis in Catamaran drug product process**

B. Attributes of selected candidate K562 feeder clones

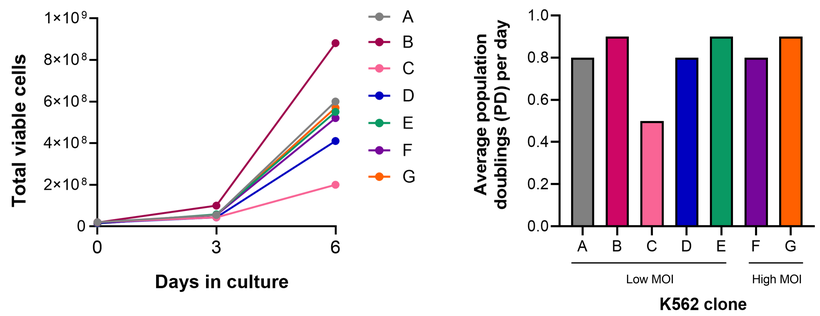
Clone ID	MOI	Transgene expression
A	Low	Negative
B	Low	Low
C	Low	Low
D	Low	Medium
E	Low	Medium
F	High	High
G	High	High

2 Characterization of candidate K562 clone growth kinetics and post-irradiation transgene expression levels

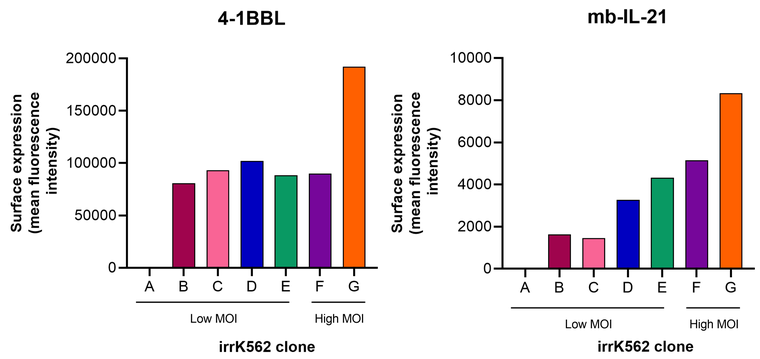
A. Workflow to evaluate candidate engineered K562 clone attributes



B. Growth kinetics of engineered K562 clones



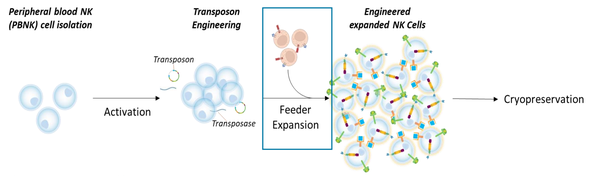
C. 4-1BBL and mb-IL-21 transgene expression on thawed, irradiated K562 feeder clones



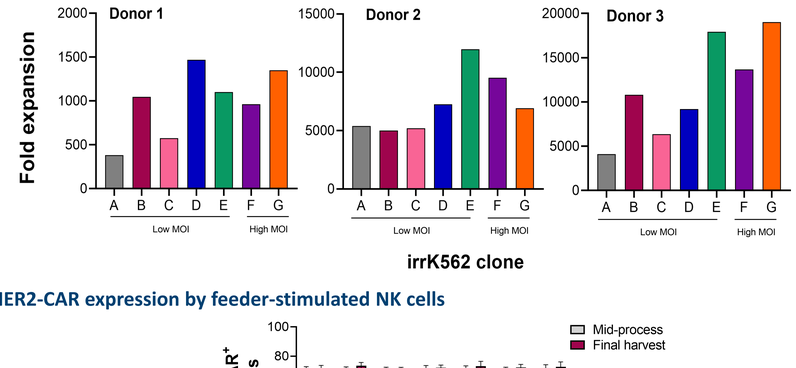
A) Schematic of workflow to select and evaluate candidate K562 feeder clones.
B) Candidate K562 clones from (A) were thawed and cultured for six days. Total viable cell counts shown from days 0 (seeding), 3 (media exchange day), and 6 (final harvest). Total viable cells (left) and average growth rate (right) indicate variable expansion across clones not directly correlated to clone MOI or transgene expression level.
C) Irradiated feeder clones from (B) were analyzed for surface expression of engineered transgenes via flow cytometry. Mean fluorescence intensity (MFI) values for 4-1BBL (left) and mbIL-21 (right) indicated on each graph.

3 Expansion and functional assessment of TcBuster™ transposon-engineered CAR-NK cells with candidate K562 feeder clones

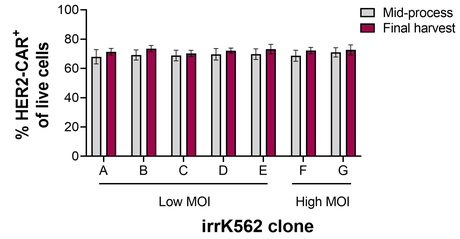
A. CAT-179 (HER2-CAR, TGF-β-DNR, IL-15) manufacturing process includes expansion K562 feeders engineered with 4-1BBL and mb-IL-21



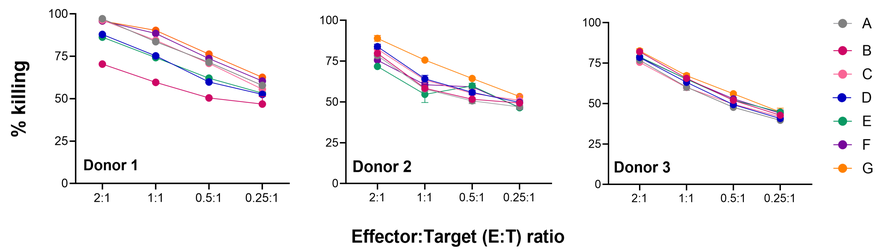
B. Feeder-stimulated CAR-NK cell expansion



C. HER2-CAR expression by feeder-stimulated NK cells



D. *In vitro* cytotoxicity against HER2⁺ tumor cells



A) Overview of CAT-179 manufacturing process.
B) HER2-CAR-engineered peripheral blood-derived NK cells (n=3 HDs) were expanded with candidate irradiated K562 feeder cell clones. Fold expansion from engineering to final harvest calculated.
C) CAT-179 NK cells were stained for surface expression of HER2-CAR via flow cytometry in-process and at final harvest. Graph depicts averages from n=3.
D) CAT-179 cells were thawed and assessed for *in vitro* cytotoxic activity against luciferase-expressing HER2⁺ SKOV3 target cells. Data shows percent of target cell killing at the indicated effector to target ratios after 24 hours of co-culture.

4 CONCLUSIONS

We describe here the assessment of candidate 4-1BBL and membrane-bound IL-21 engineered K562 feeder cell clones for our CAT-179 NK cell expansion process. **We selected Clone D from which to derive our GMP master cell bank for manufacturing**, given that it has **high growth rates, provides high CAR-NK yield, and provides cytotoxic CAR-NK drug product in all three healthy donors evaluated.** In addition, Clone D was generated using a **lower MOI** of the virus encoding the transgenes, thereby reducing the risk of genomic instability from the presence of multiple copies of the viral genome.

- 4-1BBL and mb-IL-21 surface expression is preserved in K562 cells after irradiation and cryopreservation
- Differences in transgene expression and MOI in K562 feeder clones did not determine the relative expansion or cytotoxic activity of transposon-engineered CAT-179 NK cells
- The activation and engineering platform developed for Catamaran products may reduce the need for 4-1BBL and IL-21 and/or feeder cells for effective CAR-NK expansion, pending further functional assessment of CAR-NK drug products.