ASGCT ANNUAL MEETING | POSTER PRESENTATION | ABSTRACT #427

Bridging NK cell expansion methods towards a feeder-cell free scalable GMP production of hyperfunctional NK cells

Jeremiah Oyer[1], Alicja Copik[1], Jon Pileggi[2], Mathieu Streefland[3], Dean Lee[4], Robert Igarashi[3]

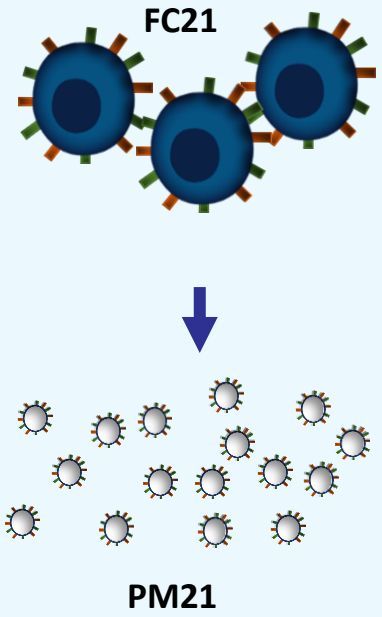
[1]Burnett School of Biomedical Sciences, University of Central Florida, Orlando, FL, [2]KBI Biopharma, The Woodlands, TX, [3]Kiadis Pharma, Amsterdam, Netherlands, [4]Nationwide Children's Hospital, Columbus, OH

Foundation for a new cell therapy platform: “K-NK cells”

- The Kiadis NK (K-NK) cell platform is a scalable GMP compliant feeder-cell-free NK cell expansion process.
- K-NK technology harnesses membrane particles that express IL-21 and 41BB ligand (PM21) to expand high numbers of hyperfunctional NK cells.
- K-NK cells present a unique hyperfunctional phenotype that is highly reactive upon tumor recognition, produces large amounts of anti-tumor cytokines and exhibits potent cytotoxicity.
- K-NK technology is a cell-free evolution of the FC21 platform which used K562 cells genetically modified to express membrane bound IL-21 (mbIL21) and 41BB ligand as feeder cells to expand an NK cell product from peripheral blood, cord blood and iPSCs.
- To date, FC21-NK cells have shown encouraging clinical activity in >45 patients treated in Phase I/ II studies.[1-3]

Here we show that K-NK cells expanded at scale with proprietary PM21 particles have a comparable phenotype and function to lab generated FC21-NK and PM21-NK cells.

Some definitions as we go through this presentation



Platform	Expansion method	Resulting NK cells
FC21	Feeder Cell line K562 modified to express mbIL-21 and 41BBL	FC21-NK = NK cells expanded with FC21, at academic lab scale
PM21	PM21: Lab-generated membrane particle derived from FC21, maintaining mbIL-21 and mb41BBL	PM21-NK = NK cells expanded with PM21, at academic lab scale
Kiadis NK	Industrially manufactured PM21 particles	K-NK = NK cells expanded with PM21, in an industrial GMP process

Advantages of the feeder-cell free particle membrane (PM21) based approach

PM21 Characteristics	K-NK Operational Advantages
Large scale manufacturing with long shelf life	Improved control over NK cell culture conditions
Quality controlled including quantification and standardization of protein- and IL21 content	Improved product risk profile
Removal of feeder cells and reduction of feeder cell related impurities	
Terminally sterilized	Simplified and more robust supply chain

K-NK GMP manufacturing on standard industry platforms

Day -1

Receipt of donor starting material from clinical site

Day 0

NK Cell enrichment by CD3 depletion

Prodigy

Day 1 - 7

Stimulation and initial expansion of NK Cells using PM21 particles

Prodigy CCU

PM21 - Proprietary membrane particles presenting mbIL21 and 41bbL; No feeder cells used in NK cell culture

Day 7 - 13

NK Cell expansion in a 10L perfusion bioreactor

Xuri

< Day 14

Harvest and concentration of NK Cells

Formulation of NK Cells in a cryo-preservative

Cryopreservation

Lovo

Custom Biogenics System 2101

Day 14-30

Quality Control testing and release

PM21-based stimulation of NK cells yields robust 1000-fold expansion of peripheral blood NK cells in less than 2 weeks

Figure 1A

Feeder Cell NK (FC21-NK) vs Particle Membrane NK (PM21-NK) Expansion[4,5]

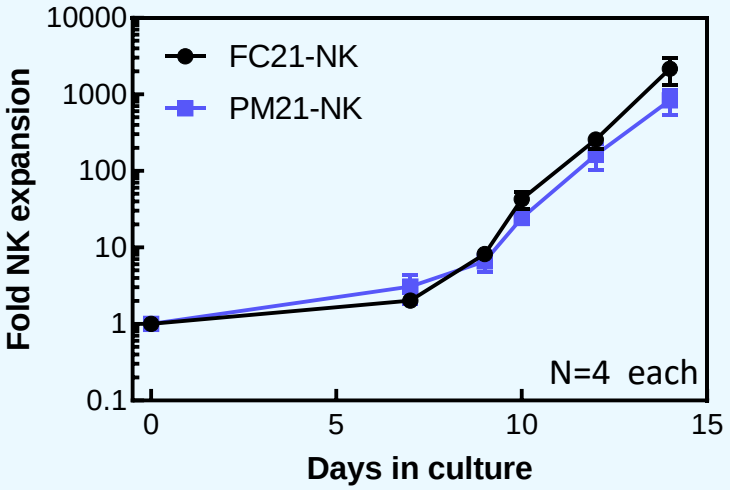
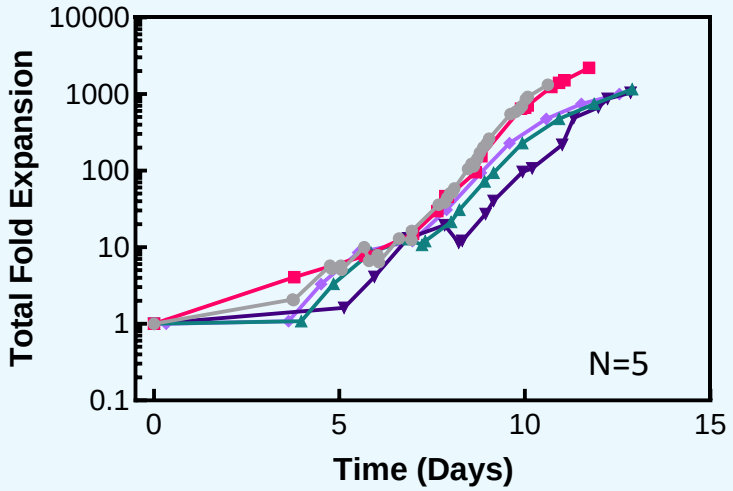


Figure 1B

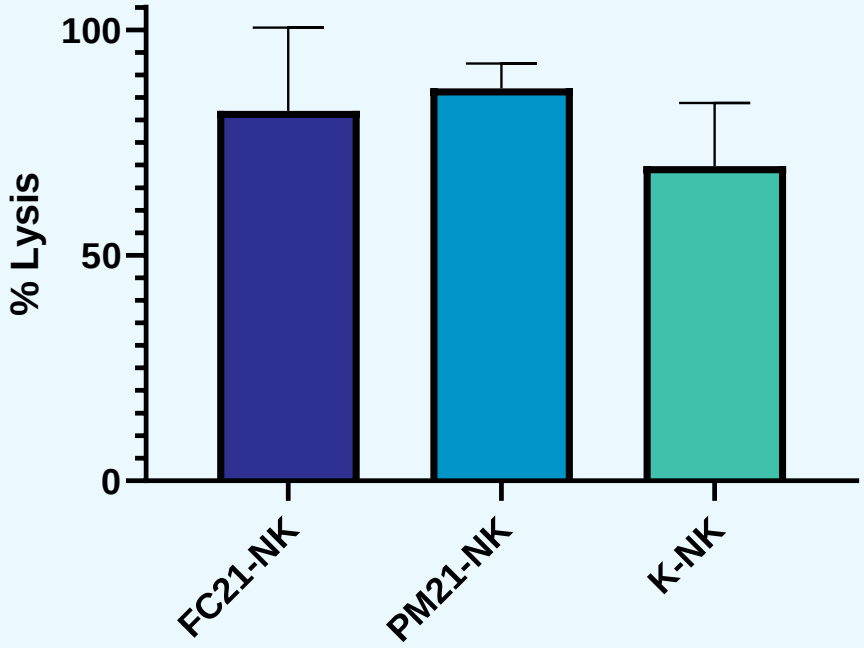
K-NK process in commercial GMP facility



Existing K-NK platform allows for robust expansion of unique K-NK cells allowing for multiple dosing at 1x10^8 cells/kg.

K-NK cells exhibit similar potent cytotoxicity to lab-expanded NK cells

Figure 2: Cytotoxicity (1:1 E:T Ratio)

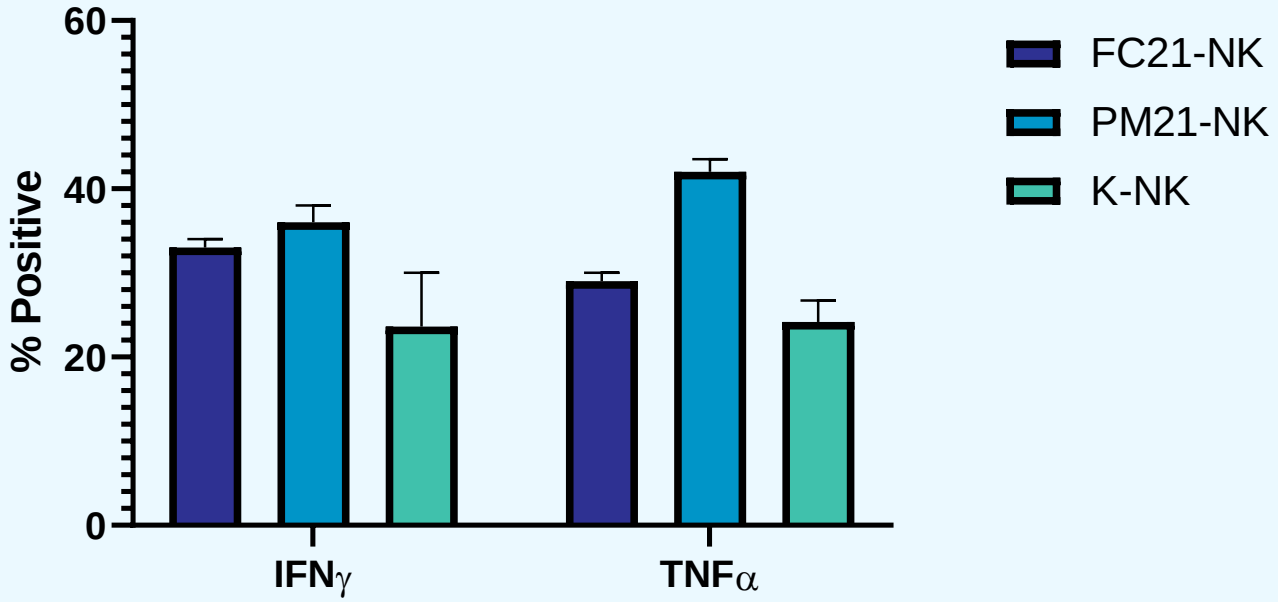


FC21-NK, PM21-NK and K-NK cells kill at least 70% of leukemia target cells at a low 1:1 effector target ratio in 90 minutes, which is ~10-20-fold higher than IL-2 stimulated NK cells[6]

Measured by Annexin V detection with K562 leukemia target cells at a 1:1 E:T ratio for 90 minutes. FC21-NK and PM21-NK N=4 each; K-NK N=5.
This is an indirect comparison of similar assays across laboratories.

K-NK cells exhibit similar & consistently high intracellular cytokine production to lab-expanded NK cells

Figure 3: IFNγ and TNFα Production

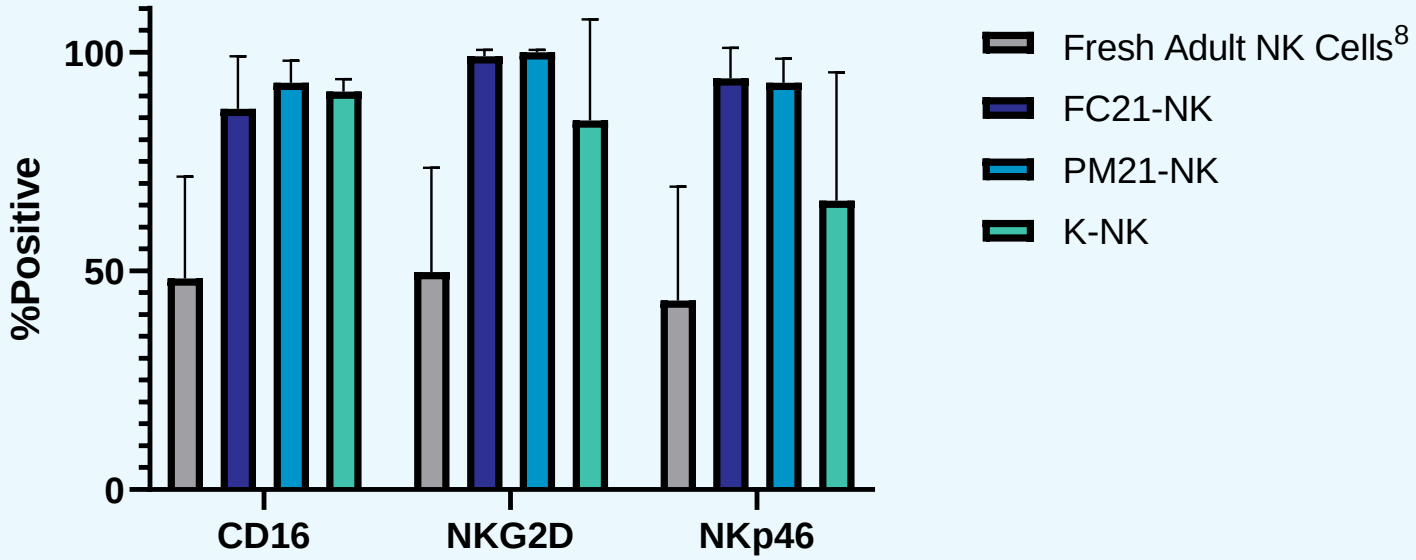


While maintaining potent cytotoxicity, K-NK cells also produce IFNγ and TNFα, unlike fresh peripheral blood NK cells[7]

NK cells were analyzed by flow cytometry for intracellular expression of IFNγ and TNFα.
This is an indirect comparison of similar assays across laboratories.

K-NK cells exhibit a unique phenotype distinct from non-expanded NK cells

Figure 4: NK Cell Phenotype

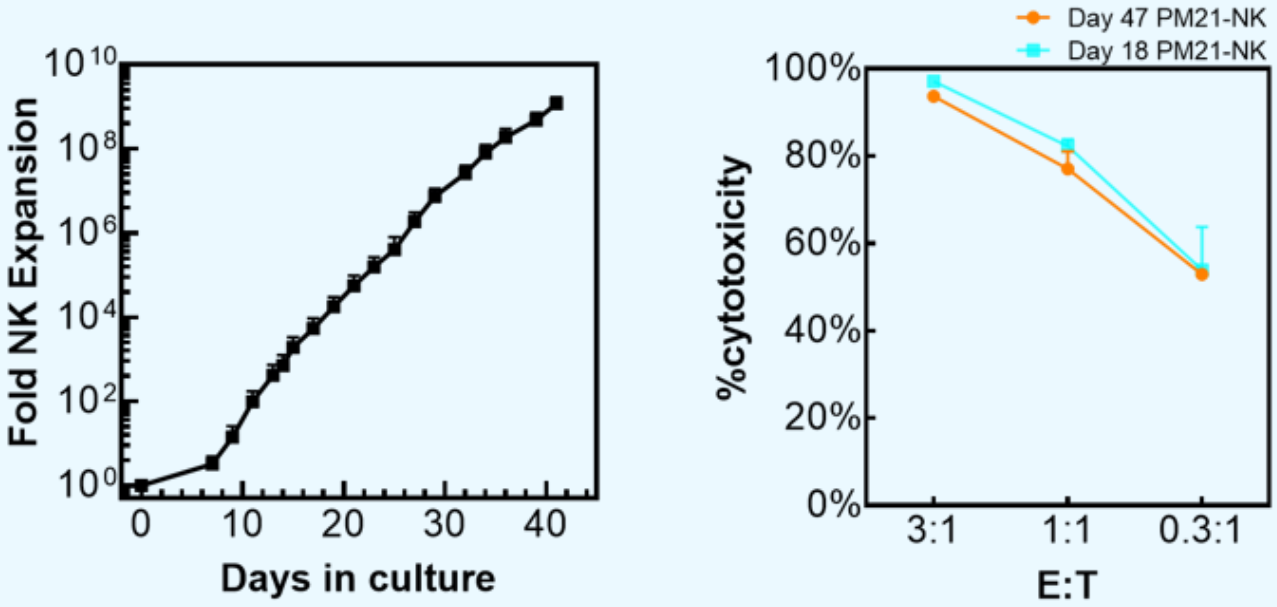


K-NK cells have a unique hyperfunctional phenotype, with high expression of activating receptors: CD16 for effective ADCC, NKG2D for tumor recognition, and high NKp46 among the natural cytotoxicity receptors (NCRs).

NK cells were analyzed by flow cytometry for the expression of CD16, NKG2D and NKp46.
This is an indirect comparison of similar assays across laboratories.

Further scale up of Kiadis K-NK platform will extend NK cell expansion, maximizing yield while preserving potency

Figure 5: Expansion and cytotoxicity with longer culture time



PM21 expansion of K-NK over 40 days yields 10^8+ fold-expansion while maintaining cytotoxic potency.

The Kiadis K-NK platform can be scaled to deliver hundreds of K-NK doses per batch

PM21-NK expanded for 47 days maintains cytotoxicity achieved during 18-day PM21-NK expansion

K-NK Platform: Advantages and Conclusions

- The Kiadis NK (K-NK) platform is a robust GMP expansion platform already capable of yielding more than 50bn activated K-NK cells per batch.
- K-NK cells present a unique, hyperfunctional phenotype with potent cytotoxicity and potential for anti-leukemic clinical activity, as previously established for the FC21-NK platform.
- K-NK cells offer the potential for broad application in oncology and infectious diseases.
- Further evolution of Kiadis' proprietary *universal donor platform* will offer large scale manufacturing of off-the-shelf K-NK cells with direct availability to patients in urgent need of novel therapeutic options.

Disclosures & Acknowledgments

- Jeremiah Oyer: IP licensed to, consultancy and equity in Kiadis Pharma
- Alicja Copik: equity in, IP licensed to, consultancy and research support from Kiadis Pharma
- Jon Pileggi: no disclosure
- Mathieu Streefland: options and employee of Kiadis Pharma
- Dean Lee: equity in, consultancy and research support from Kiadis Pharma
- Robert Igarashi: equity in and employee of Kiadis Pharma

Authors would like to acknowledge Laura Witkam, Jolie Schafer and Juan Pablo Labbrozzi for their support in the analyses and preparation of this poster. All are employees of Kiadis Pharma.

References

1. Ciurea et al. Blood 2017 19;130(16):1857-1868
2. Ciurea et al. Presented at ASH 2019. Blood (2019) 134 (Supplement_1): 700
3. Ciurea et al. Presented at ASCO 2018. Journal of Clinical Oncology 36, no. 15_suppl 7008
4. UCF Copik Laboratory Report #005
5. Oyer et al. Cytotherapy 2016 18(5):653-663
6. Oyer et al. OncoImmunology 2018;7:11
7. Poli et al. Immunology 2009 126(4):458-65
8. Angelo et al. Immunol Res 2015;62(3):341-56