Ex-vivo Activation and Expansion of NK Cells to Generate "Memory-Like" NK Cells for the Treatment of Relapsed or Refractory Neuroblastoma

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Background:

We previously characterized NK cells that were activated/expanded, *ex-vivo*, utilizing irradiated K562 cells modified to express transmembrane IL-15 and 41BBL, for use in a clinical trial for relapsed/refractory neuroblastoma (NCT03209869). Given the substantial cost and complexity using feeder cells to generate a GMP grade cellular product, we investigated a cytokine-only culture system to manufacture "memory-like" NK cells for clinical use.

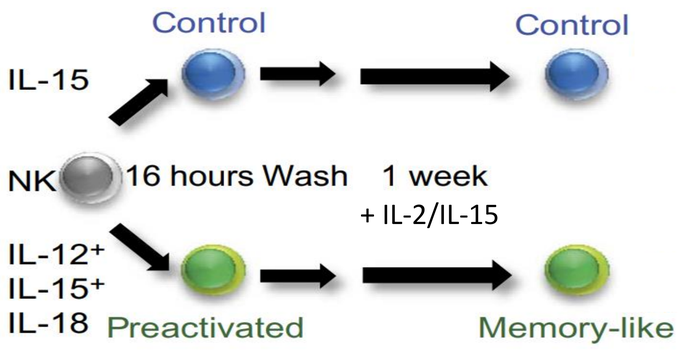
Methods:

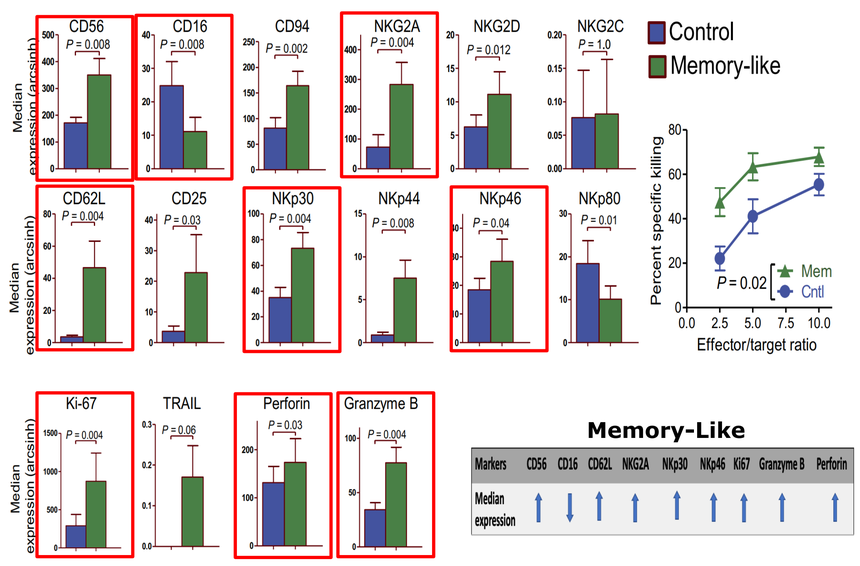
NK cells were isolated from PBMCs using the Miltenyi Biotec NK Cell Isolation Kit and autoMACS ® Pro Separator. Cells were incubated with IL-15 alone (1 ng/ml, control), or preactivated with IL-12 (10ng/ml) + IL-15 (50ng/ml) + IL-18 (50ng/ml) for 16 hours. Cells were then incubated for 7 days with half the media replaced every 2 days with addition of IL-2(100 IU/ml) and IL-15(10 ng/ml). Control and preactivated cells were then compared for expression of different cell surface markers. In a second set of experiments, control or preactivated NK cells were cultured for 14 - 21 days in NK cell optimized Enable media with addition of either IL-15 (10 ng/ml) alone, or with IL-15 combined with IL-2 (100 IU/ml). NK cells generated under these different conditions were then compared for their level of expansion, and ability to mediate cytotoxicity against neuroblastoma cell line (CHLA-20) and osteogenic sarcoma (MG63) cell lines, with or without addition of anti-GD2 antibody (ch14.18), using the IncuCyte Live Cell Analysis system.

Conclusions:

- We have identified a culture system achieving > 50-fold expansion of NK cells without utilizing a feeder cell line.
- Optimal NK cell expansion was achieved using activated NK cells incubated with IL-15 alone in Enable media for 21 days
- These NK cells express a "memory-like" phenotype and demonstrated potent cytotoxicity against a neuroblastoma and osteosarcoma cell line.
- Maximum cytotoxicity was seen using activated NK cells expanded with IL-15 in Enable media. Killing was enhanced by the addition of an anti-GD2 mAb.
- These memory-like NK cells may be ideally suited for clinical use in combination with an anti-GD2 immunocytokine or mAb.
- Significant retention of NK cell mediated cytotoxicity was retained after cryopreservation (data not shown).
- We are in the process of scaling up this methodology in our Program for Advanced Cellular Therapy (GMP) facility utilizing a TCR-αB+ and CD19+ depleted apheresis product

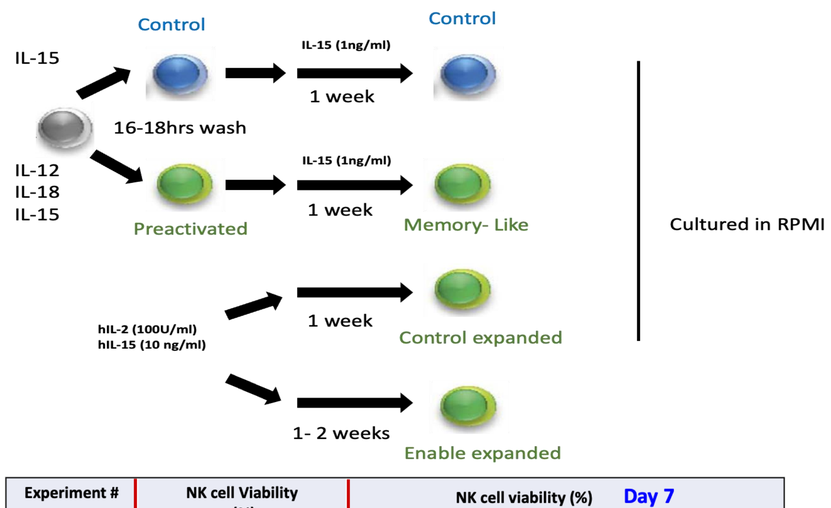
Results:





Memory-Like

Markers	CD56	CD16	CD62L	NKG2A	NKp30	NKp46	Ki67	Granzyme B	Perforin
Median expression	↑	↓	↓	↑	↑	↑	↑	↑	↑

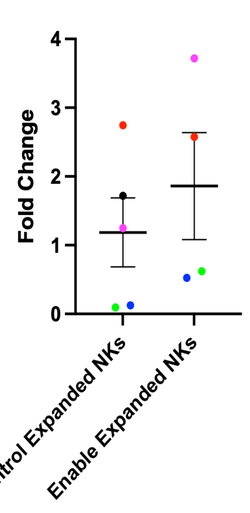


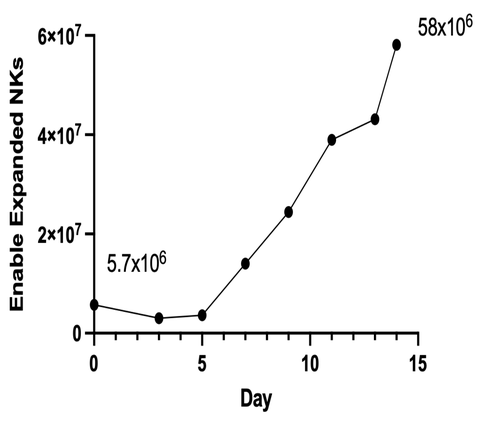
Experiment #	NK cell Viability (%) Day 0	NK cell viability (%) Day 7				
		Control	Memory Like	Control expanded	Enable Expanded 1 week	Enable Expanded 2 weeks
Donor #1	81%	93%	87%			
Donor #2	100%		80%	81%		
Donor #3	99%	81.6%	72.5%	88.6%	87.7%	
Donor #4	99%	-	-	-	-	
Donor #5	99.4 %	-	-	76.5%	90.4%	
Donor #6	99.2%	85%	80.9%	87.2%	86.2%	84.1%

Experiment #	NK cell number Day 0	NK cell number Day 7				
		Control	Memory Like	Control expanded	Enable Expanded 1 week	Enable Expanded 2 weeks
Donor #1	2×10^6	1.58×10^6	0.8×10^6			
Donor #2	1.6×10^6		0.4×10^6	2.75×10^6		
Donor #3	5.79×10^6	2.1×10^6	1.9×10^6	15.9×10^6	14.9×10^6	
Donor #4	1.6×10^6	0.12×10^6	0.12×10^6	0.2×10^6	0.84×10^6	
Donor #5	3.21×10^6	0.03×10^6	0.15×10^6	0.3×10^6	2×10^6	
Donor #6	5.7×10^6	4.1×10^6	3.3×10^6	7.12×10^6	21.2×10^6	58.1×10^6

Expression in Enable expanded condition compared to control expanded condition

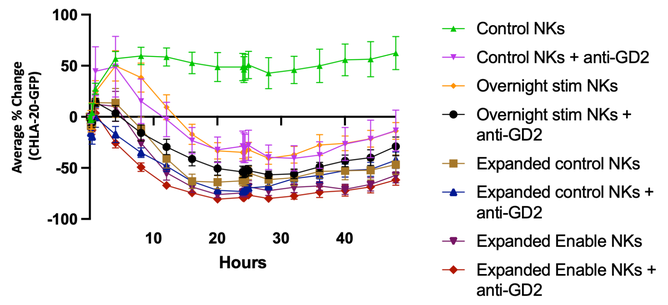
CD56	CD16	CD62L	NKG2A	NKp30	NKp46	Ki67	Granzyme B	Perforin
↑	↓	↓	↓	↓	↓	↑	↑	↑

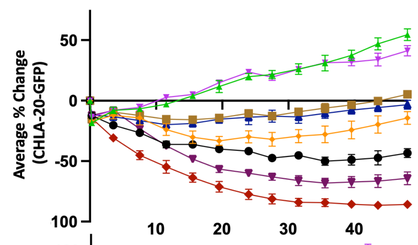




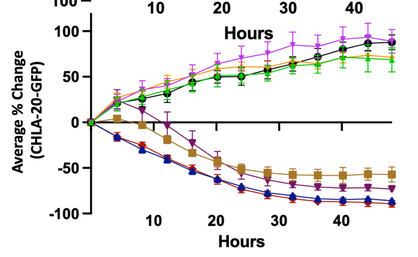
IncuCyte Cytotoxicity Experiments

- Target Cells: CHLA-20-GFP
- E:T Ratio = 1:1
- 4 NK Conditions
  - Control NKs
  - Overnight stim NKs (memory like)
  - Expanded control NKs (memory like)
  - Expanded Enable NKs (memory like)





Donor 2



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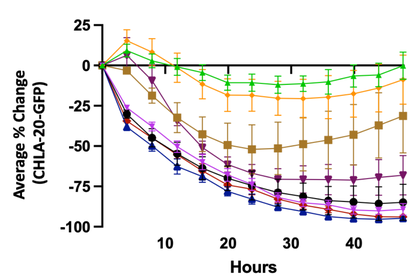
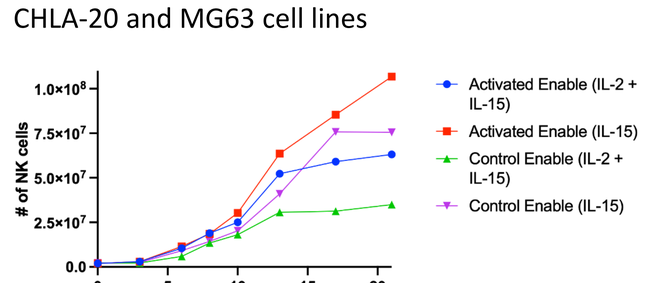


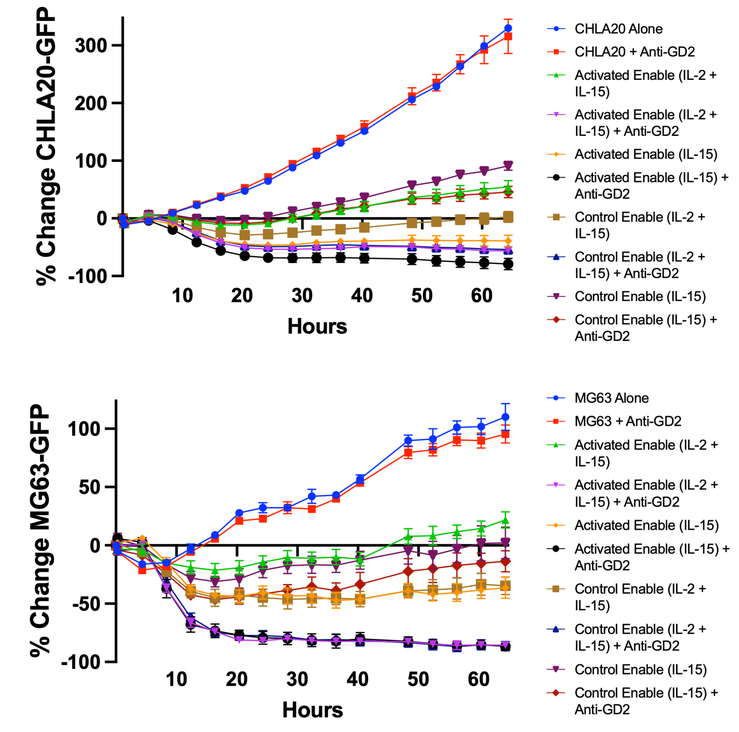
Donor 4

To determine if IL-2 is required during the NK cell expansion, control and activated NK cells were cultured in phase 1 Enable media with IL-15 alone or with a combination of IL-15 and IL-2. Fresh phase 1 media and cytokines were added to all flasks on days 3, 6, 8, 10, 14. Phase 2 enable media was added on day 17 and cells were maintained in culture for 21 days.

IncuCyte cytotoxicity assays were performed on day 17 of expansion with or without an anti-GD2 mAb on the CHLA-20 and MG63 cell lines



	Activated Enable (IL-2 + IL-15)	Activated Enable (IL-15)	Control Enable (IL-2 + IL-15)	Control Enable (IL-15)
Day 0	2.0×10^6	2.0×10^6	2.25×10^6	2.25×10^6
Day 14	5.23×10^7	6.36×10^7	3.07×10^7	4.1×10^7
	26.1 fold increase	31.8 fold increase	13.6 fold increase	18.2 fold increase
Day 21	6.31×10^7	1.07×10^8	3.50×10^7	7.55×10^7
	31.55 fold increase	53.9 fold increase	15.5 fold increase	33.5 fold increase



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