

Clinical-Scale Production of Natural Killer Cells for Immunotherapy

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PACT Workshop - University of Pittsburgh

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Outline

Part I:

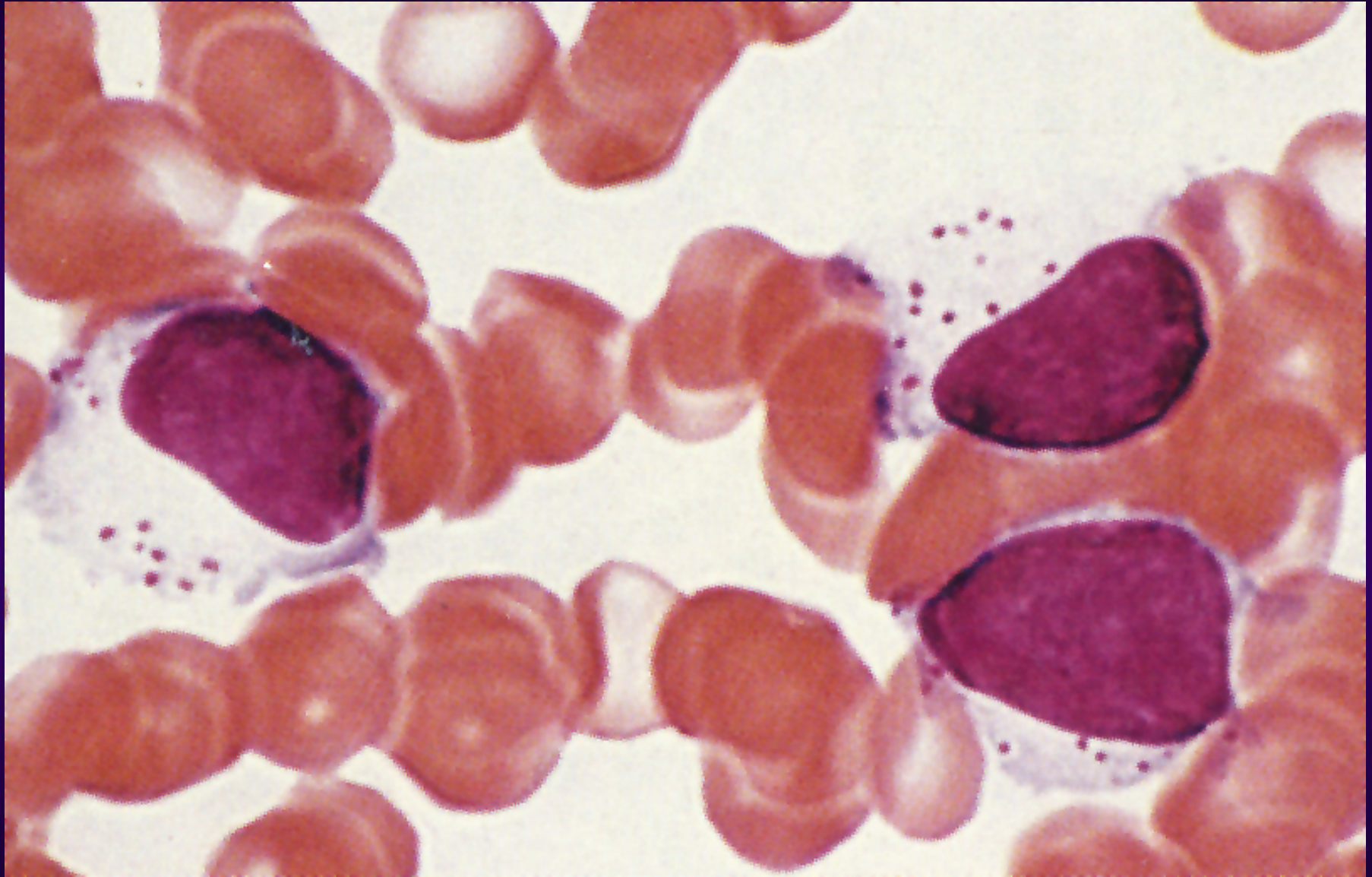
- Background: NK cells
- Clinical-scale processing
- 6-year experience at U of MN
- Conclusions

Part II:

- Stability/shipping of fresh cells
 - The U of MN PACT experience
- Conclusions

Background

- NK cells...
 - Lymph subset (10-20% of circulating)
 - LGL morphology/CD56+/CD3-
 - Innate immune system
 - Defense against viral infection/malignancy
 - Regulation of hematopoiesis



Background

- NK cells...
 - Complex mechanism of target recognition
 - Activation → proliferation → killing
 - Kill via perforin/granzyme and Fas ligand
 - Renewed interest in NK cells

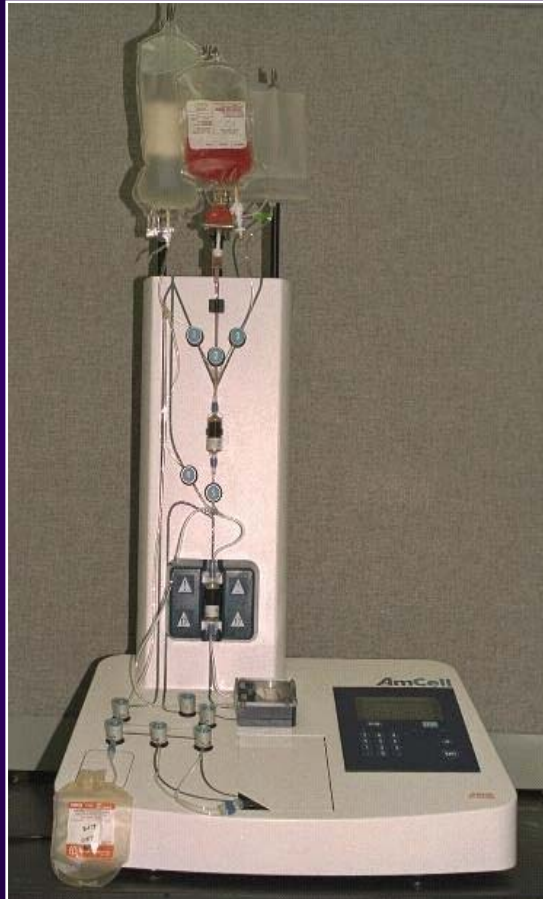
Clinical-Scale Processing

- 15 L MNC apheresis procedure
- Cell selection (CliniMACS, Miltenyi Biotec)
- NK Product 1 (CD3-depleted only)
- NK Product 2 (CD3-depleted/CD56-enriched)
- Re-suspended at 2 million cells/mL
 - X-VIVO 15 (Cambrex)
 - 10% AB serum (Valley Biomedical)
 - 1000 U/mL IL-2 (Chiron)
 - VueLife teflon bags (American Fluoroseal)

Clinical-Scale Processing

- Overnight incubation at 37°C/5% CO₂
- Washed x 2 with 5% HSA (Baxter)
- Re-suspended at 5-25 million cells/mL

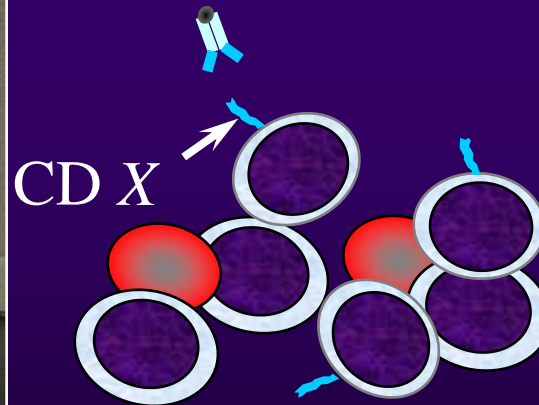
Cell Selection Systems



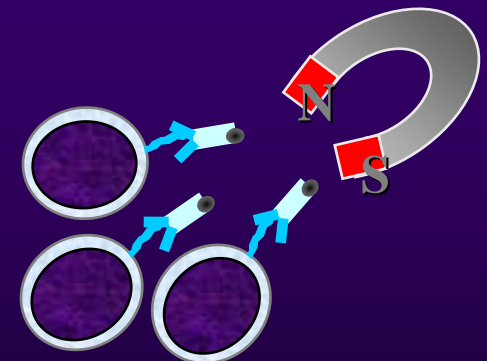
Miltenyi Biotec

Miltenyi CliniMACS:
Mouse Anti-Human CD X/ 50 nM ferro-
magnetic particle

Bind



Capture



PBMC Apheresis



CD3+ cell depletion

Activation/Expansion



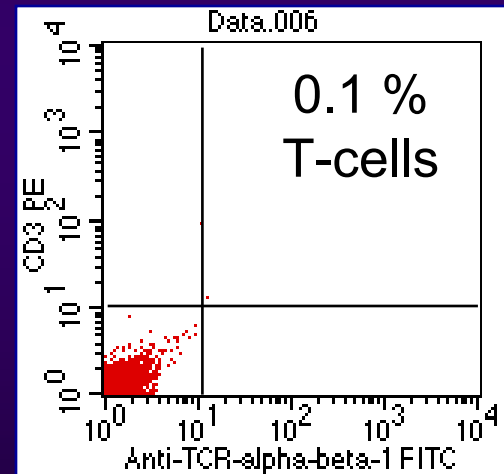
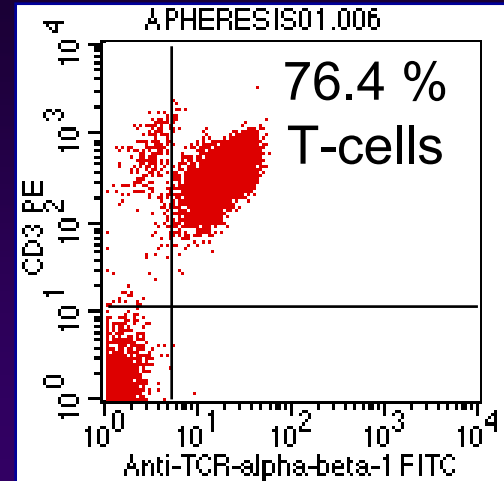
IL-2/X-VIVO-15

Harvest



Suspend in NS/
5% HSA

Infuse



38% NK cells

PBMC Apheresis



CD3+ cell depletion/
CD56+ cell enrichment

Activation/Expansion



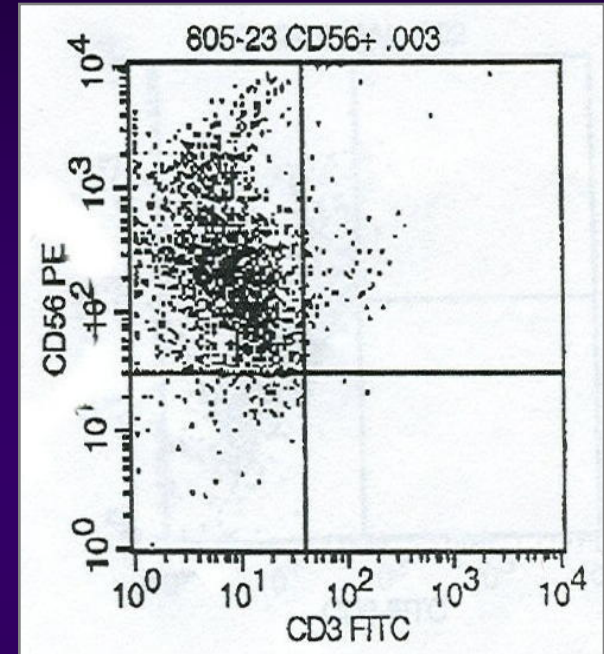
IL-2/X-VIVO-15

Harvest



Suspend in NS/
5% HSA

Infuse



90% NK cells

Clinical-Scale Processing

QC Testing

- NC counts (MDII, Beckman Coulter)
- Viability (AO/PI, 7-AAD)
- Gram stain
- Culture (BacTec System, BD Diagnostics)
- Endotoxin (chromogenic LAL, Cambrex)
- Flow cytometry (FACSCalibur, BD Biosciences)
 - CD3, CD14, CD19, CD45, CD56
- Cytotoxicity (4 hr Cr⁵¹ release assay)

Clinical-Scale Processing

Lot Release

Assay	Test Method	Specification
Viability	AO/PI or 7-AAD	$\geq 70\%$
NK Cell (CD56+/CD3-) Enumeration	Flow Cytometry	$\geq 20\%$, $\geq 70\%$
T Cell (CD3+) Enumeration	Flow Cytometry	<u>Protocol dependent</u>
Endotoxin	LAL Method	< 5 EU/kg
Gram Stain	Clinical Microbiology Lab	No organisms

Results – NK Product 1

Donor	TNC Pre-Processing (10 ⁶)	% CD56+/CD3 (Pre-Processing)	TNC Post-Depletion (10 ⁶)	% CD56+/CD3- (Post-Depletion)	CD56+/CD3- Cell Recovery (%)	Log Depletion (T cell)	Total CD56+/CD3- Cells Infused (10 ⁷)
1	2.54	12.37%	3.38	37.64%	40.49%	2.9	553.26
2	2.05	8.84%	4.95	18.22%	49.78%	1.8	374.14
3	1.75	18.89%	4.71	51.34%	73.10%	2.7	930.63
4	1.00	13.76%	2.83	34.78%	71.23%	2.6	516.24
5	4.64	18.60%	14.34	44.09%	73.26%	3.1	844.46
6	1.16	10.97%	1.93	33.50%	50.80%	2.7	847.56
7	1.68	6.75%	3.17	27.62%	77.20%	2.5	912.49
8	1.64	12.72%	3.89	38.60%	72.10%	2.2	678.86
9	1.42	9.59%	1.90	37.54%	52.38%	2.8	620.55
10	2.03	7.79%	7.03	17.53%	77.99%	2.3	243.22
11	0.95	6.43%	1.62	28.04%	74.28%	2.6	237.65
12	0.60	8.52%	0.70	44.88%	60.66%	2.5	245.75
13	1.93	12.05%	4.86	45.73%	95.34%	2.7	1274.18
14	2.58	6.03%	4.05	34.13%	88.79%	3.1	417.38
15	1.81	15.63%	5.51	46.74%	90.84%	2.4	1139.71
16	2.03	15.98%	6.19	68.25%	130.29% (100%)*	2.6	963.28
17	1.75	9.54%	4.64	62.18%	173.20% (100%)*	2.5	519.87
18	2.22	5.56%	5.01	25.88%	105.23% (100%)*	2.6	353.61
19	1.17	11.41%	2.38	52.26%	93.36%	2.7	771.32
20	2.89	5.26%	5.42	21.50%	76.76%	2.7	540.50
21	1.64	9.22%	4.38	47.24%	136.87% (100%)*	3.2	592.72
22	2.25	16.45%	6.92	48.18%	90.20%	2.3	860.10
23	1.52	7.75%	3.17	28.21%	76.02%	2.9	442.63
24	1.58	19.40%	6.82	35.90%	80.00%	2.6	870.08
25	2.19	9.29%	7.07	24.48%	85.06%	2.8	487.19
26	2.39	9.43%	4.66	75.15%	155.78% (100%)*	3.2	800.25
27	2.53	12.79%	7.96	36.62%	90.00%	2.6	984.26
28	1.81	6.92%	2.84	33.03%	75.06%	2.3	889.70
29	1.59	16.69%	5.16	43.55%	84.83%	2.7	916.77
30	1.09	9.14%	2.83	27.18%	77.11%	2.6	721.05
31	1.86	5.64%	2.87	22.76%	62.21%	2.9	519.99
32	1.41	15.12%	4.08	45.29%	86.55%	2.8	655.66
33	2.25	6.53%	4.21	25.74%	73.74%	2.9	850.65
34	2.39	7.05%	5.08	28.19%	85.03%	3.4	875.85
35	4.26	8.58%	6.87	34.72%	65.29%	3.4	308.07
36	2.20	8.28%	5.25	30.30%	87.35%	3.5	960.47
N	36	36	36	36	36	36	36
Mean	1.97	10.69%	4.69	37.69%	78.80%	2.7	686.67
SD	0.80	4.13%	2.40	13.32%	15.40%	0.4	264.47
2 SD	1.60	8.26%	4.80	26.64%	30.80%	0.7	528.94

Table 1. Efficiency of *NK Cell Processing I* (CD3 cell-depleted product).

Results – NK Product 1

	TNC Pre-Processing (10 ¹⁰)	% CD56+/CD3 (Pre-Processing)	TNC Post-Depletion (10 ⁹)	% CD56+/CD3- (Post-Depletion)	CD56+/CD3- Cell Recovery (%)	Log Depletion (T cell)	Total CD56+/CD3- Cells Infused (10 ⁶)
N	36	36	36	36	36	36	36
Mean	1.97	10.69%	4.69	37.69%	78.80%	2.7	686.67
SD	0.80	4.13%	2.40	13.32%	15.40%	0.4	264.47
2 SD	1.60	8.26%	4.80	26.64%	30.80%	0.7	528.94

Results – NK Product 2

Donor	TNC Pre-Processing (10 ¹⁰)	% CD56+/CD3- (Pre-Processing)	TNC Post-Depletion (10 ⁹)	% CD56+/CD3- (Post-Depletion)	TNC Post-Enrichment (10 ⁸)	% CD56+/CD3- Cells (Post-Enrichment)	CD56+/CD3- Cell Recovery (%)	Log Depletion (T Cell)	Total CD56+/CD3- Cell Infused (10 ⁶)
1	3.07	17.70%	8.51	26.86%	17.10	89.09%	28.04%	3.8	141.61
2	2.48	7.97%	5.81	26.44%	4.68	84.53%	20.05%	4.2	227.76
3	2.19	9.09%	4.79	34.03%	8.18	91.97%	37.83%	3.7	442.01
4	1.18	17.20%	3.29	45.23%	8.36	89.91%	37.18%	3.9	465.72
5	2.03	7.71%	2.96	25.97%	2.41	94.74%	14.58%	4.2	125.60
6	2.57	9.74%	5.66	31.42%	4.73	96.60%	18.26%	4.4	190.58
7	2.74	18.56%	7.47	29.76%	4.42	96.31%	8.39%	4.5	185.79
8	2.03	5.75%	2.74	17.06%	0.42	83.94%	3.02%	5.2	11.34
9	3.38	8.37%	6.87	21.20%	2.91	91.07%	9.36%	4.6	332.26
10	1.74	11.02%	4.14	32.68%	4.54	79.34%	18.72%	5.1	257.09
11	3.39	24.25%	7.99	42.81%	11.05	76.53%	10.30%	3.7	411.69
12	2.02	16.01%	5.15	30.43%	5.68	95.96%	16.85%	4.0	367.74
13	1.81	5.03%	4.21	16.27%	2.77	95.98%	29.28%	4.1	132.24
N	13	13	13	13	13	13	13	13	13
Mean	2.35	12.18%	5.35	29.24%	5.94	89.69%	19.37%	4.3	253.19
SD	0.66	5.90%	1.91	8.59%	4.39	6.73%	10.93%	0.5	140.33
2SD	1.32	11.81%	3.83	17.19%	8.78	13.46%	21.86%	1.0	280.65

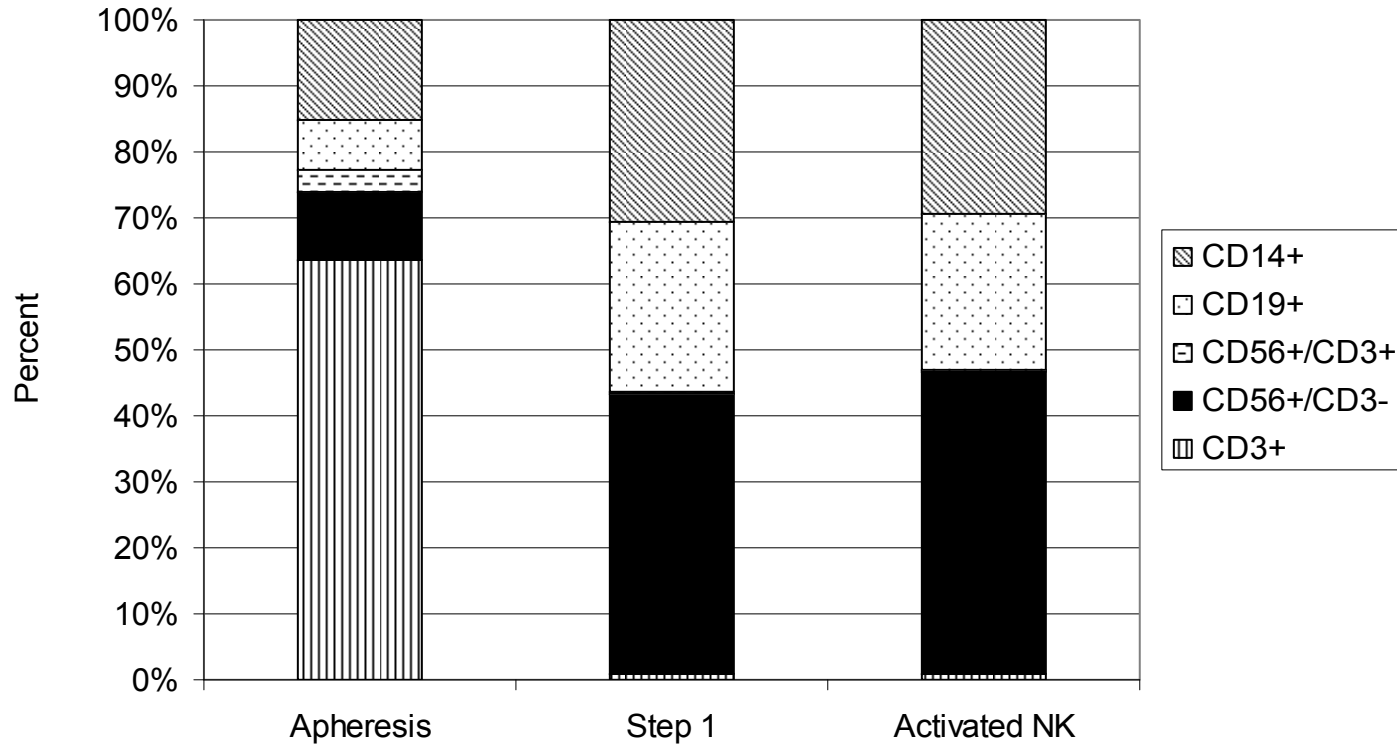
Table 2. Efficiency of *NK Cell Processing II* (CD3 cell-depleted/CD56 cell-enriched product).

Results

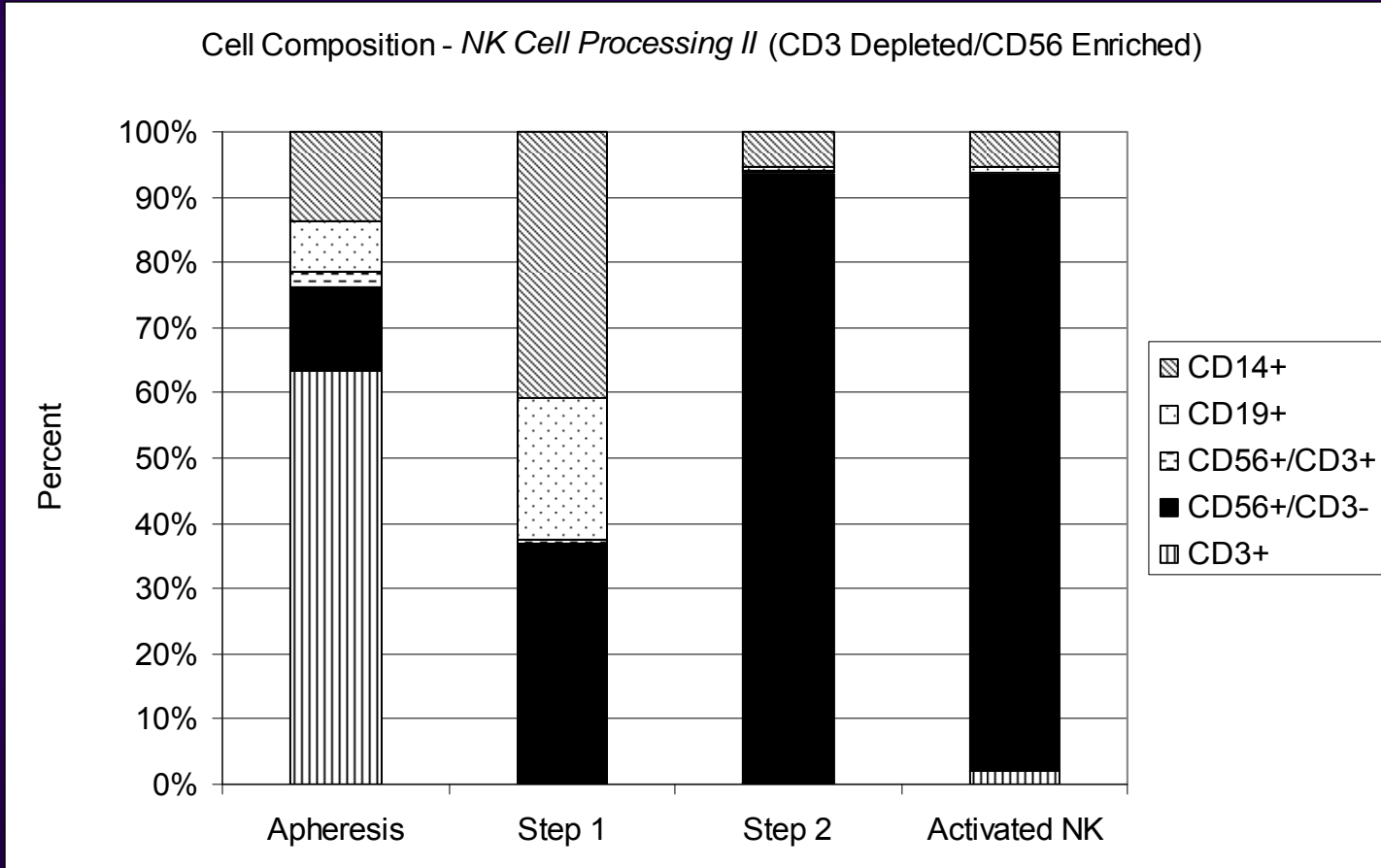
- Viability – 86% (NK 1), 85% (NK 2)
- Gram stain – All ‘No organisms’
- Endotoxin – All ‘<5 EU/kg’
- Micro culture – All neg.

Results – NK Product 1

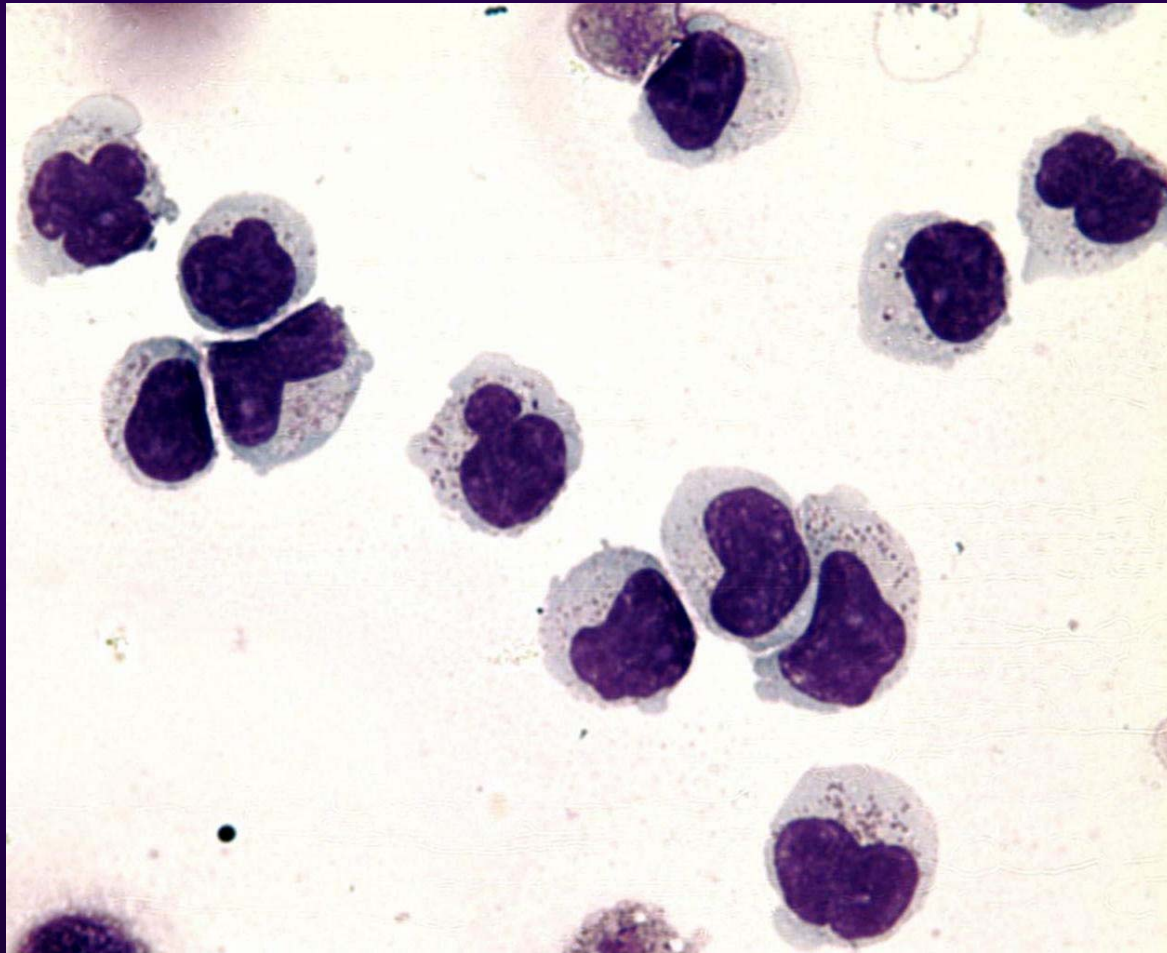
Cell Composition - *NK Cell Processing I* (CD3 Cell Depleted)



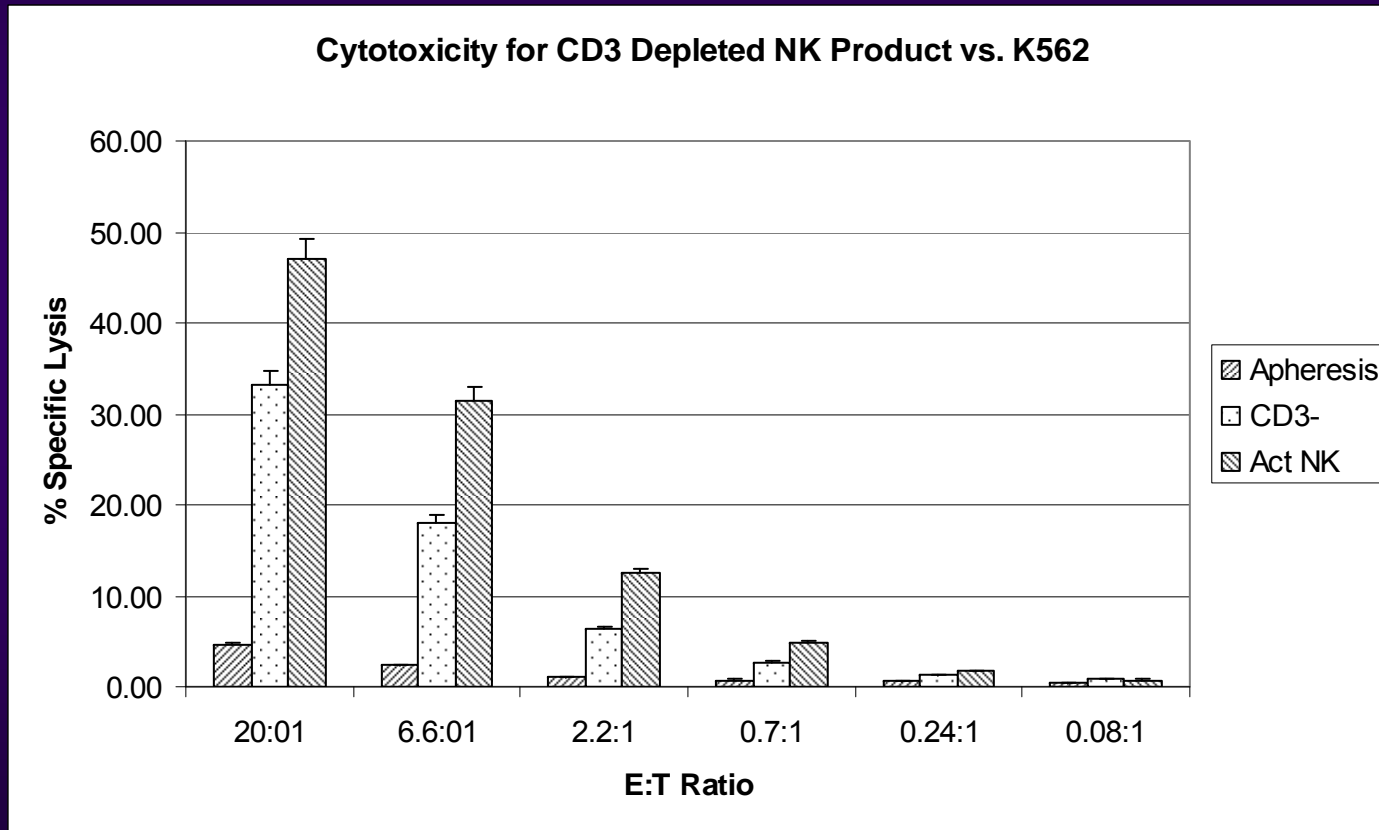
Results – NK Product 2



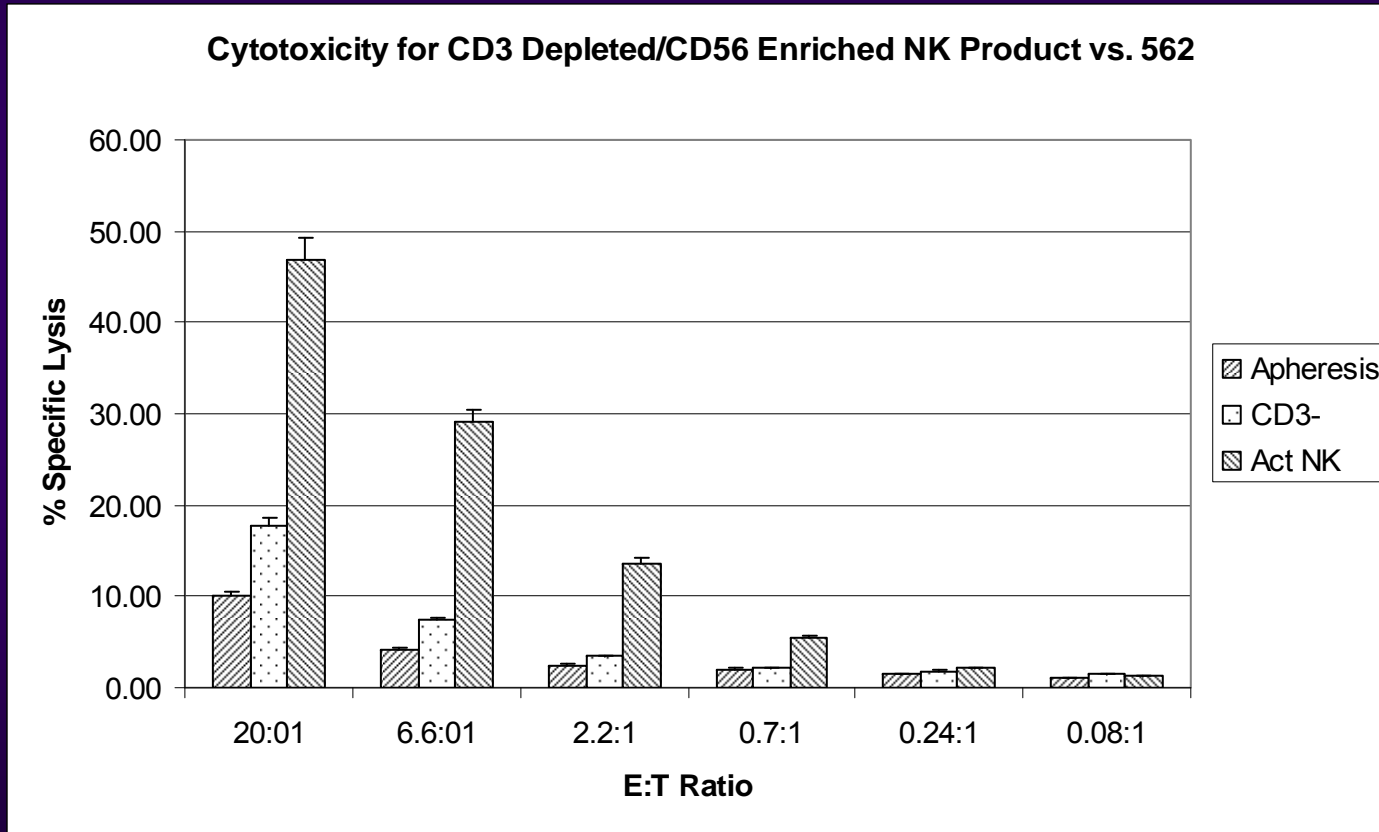
Results – NK Product 2



Results – NK Product 1



Results – NK Product 2



Conclusions (Part I)

- Clinical (GMP) production is feasible
- Efficacy apparent*
- Best source (PB, UCB)?
- Best type of product (CD3-depleted v. CD3-depleted/CD56-enriched v. other)?

*Miller JS, Soignier Y, Panoskaltsis-Mortari A, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in cancer patients. *Blood* 2005; 105 (8): 3051-3057.

Stability/Shipping of Fresh Cells

- Many novel cell therapies must be administered as fresh products
- Problematic when manufacturing site is distant from clinical site
- Validation of shipping and in-transit activation of NK cells – assessment of short-term stability “with a twist”

Stability Program Considerations

- Type of stability program – SHORT-TERM
- Type of products – FRESH ALLO-NK CELLS
- Phase of the study – EARLY PHASE (I/II)
- Length of storage to be assessed – < 24 HRS.
- Storage conditions – WARM (BT/CX)
- Type of testing – TO BE SHOWN
- Frequency of sampling – PRE/POST-SHIPPING
- Evaluation of data – TO BE SHOWN

Cell Processing

- Non-mobilized PB MNC
- CD3-depletion
- Culture:
 - X-VIVO 15
 - 10% AB serum
 - IL-2 (1000 IU/mL)
- Post-shipping
 - Wash and re-suspend in 5% HSA

Shipping & Receipt

- Packaged for transport
 - Insulated cardboard shipping container
 - Temperature stabilizing packs (brought to $\sim 35^{\circ}\text{C}$)
 - Continuous temperature monitoring device (DataLogger)
- Shipped overnight
 - Mpls, MN to Columbus, OH to Mpls, MN
- Received
 - Product examined
 - Temperature data downloaded

QC Testing

- Immunophenotype (flow cytometry)
- Nucleated cell count
- Viability (flow cytometry)
- Gram stain
- Endotoxin
- Bacterial/fungal culture
- Cytotoxicity

Methods

Determination of Specifications

Good manufacturing practices production of natural killer cells for immunotherapy: a six-year single-institution experience

David H. McKenna, Jr., Darin Sumstad, Nancy Bostrom, Diane M. Kadidlo, Susan Fautsch, Sarah McNearney, Rose DeWaard, Philip B. McGlave, Daniel J. Weisdorf, John E. Wagner, Jeffrey McCullough, and Jeffrey S. Miller

TRANSFUSION Volume 47, March 2007

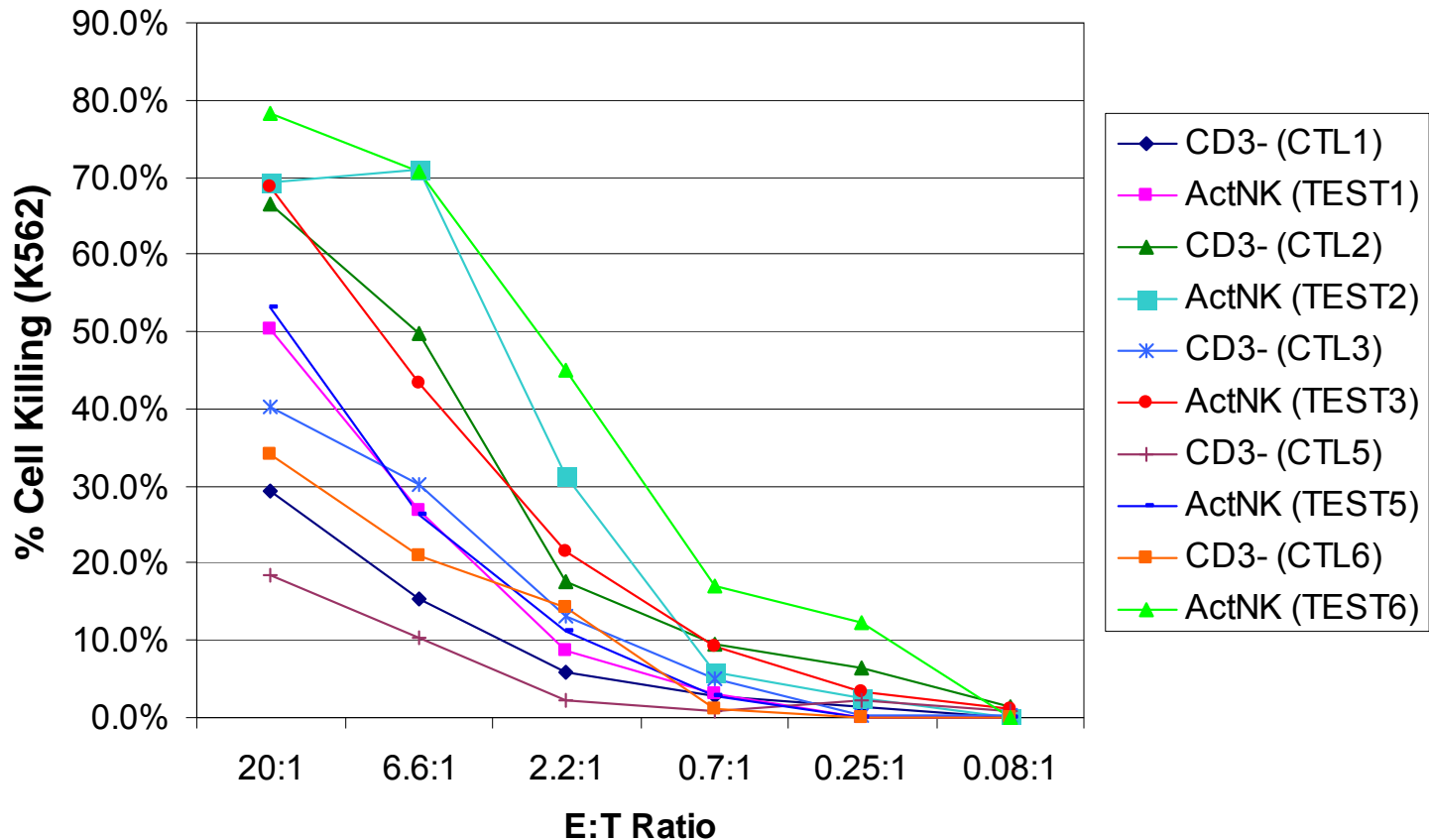
RESULTS: A total of 70 NK cell therapy products have been manufactured for patient infusion since 2000. For the CD3 cell-depleted NK cell products, the mean purity, recovery, and viability were 38, 79, and 86 percent, respectively. For the CD3 cell-depleted/CD56 cell-enriched NK cell products, the mean purity, recovery, and viability were 90, 19, and 85 percent, respectively. Gram stain, sterility, and endotoxin testing were all within acceptable limits for established lot release. Compared to the resting processed cells, IL-2 activation significantly increased the function of cells in cytotoxicity assays.

Results

Assay	Result/ Mean	Specification	# Runs Passed
NK Cell Phenotype (% CD3-/CD56+)	29.91%	$\geq 20\%$	5/6
T (CD3+) Cell Dose (E+05/Kg)	9.81E+04/kg	<5.00E+05/kg	6/6
Viability (7-AAD)	92.91%	>70%	6/6
Gram Stain	No organisms	No organisms	6/6
Endotoxin (LAL Method)	<3.15EU/kg	<5EU/kg	5/5
Bacterial/Fungal Culture (BacTec)	No Growth	No Growth	6/6

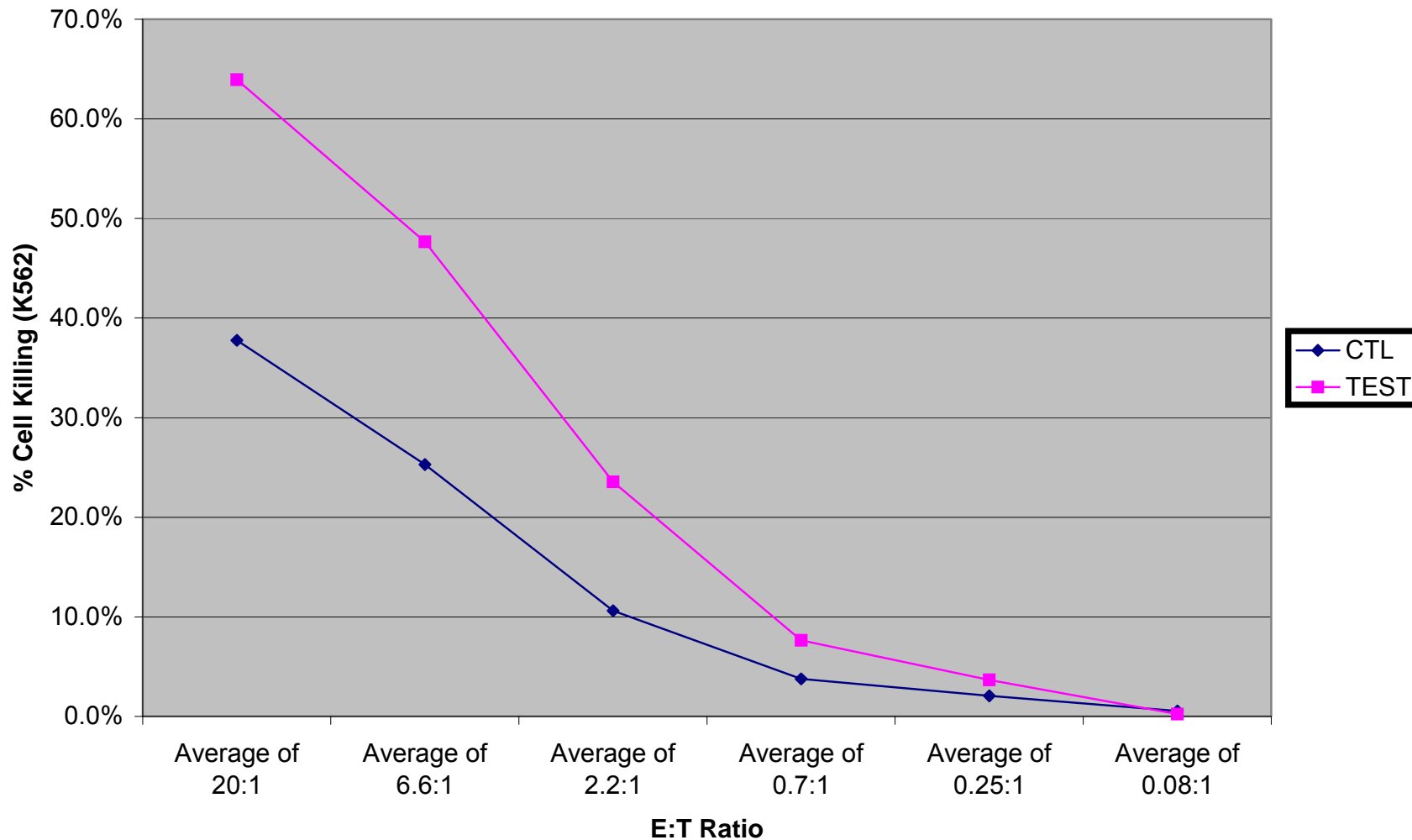
Results

Cytotoxicity



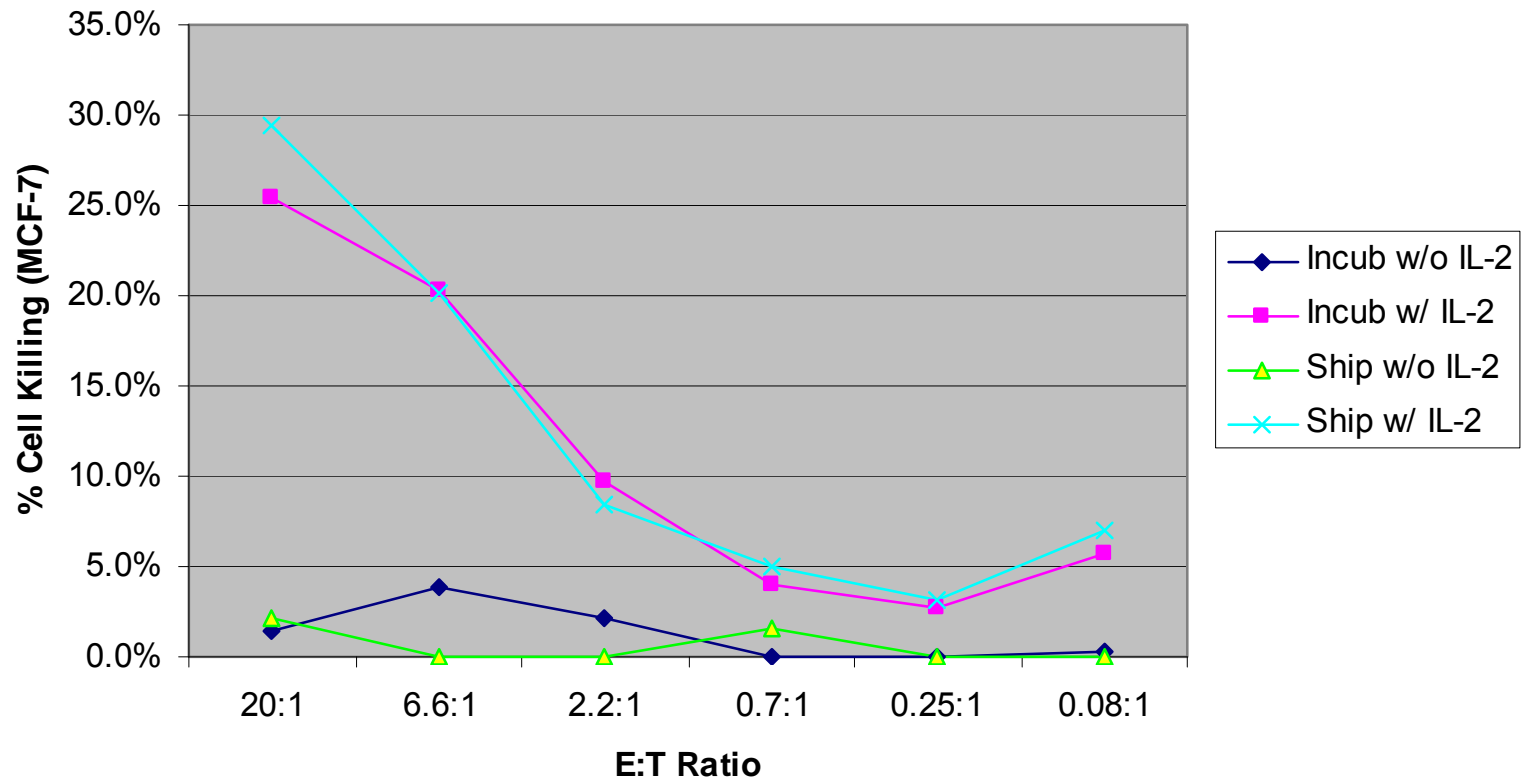
Results

Cytotoxicity (Mean)



Results

NK Cells Shipping and Incubation w/ & w/o IL-2



Results

Shipping Temperature

- Length of shipping ranged from 14-19 hours
- Final temp: 26.9 °C – 32.5 °C
- Sample data (run #2)

Shipping temperature data:	
Hours 1 → 3	33.1°C → 33.5°C
Hours 3 → 6	33.5°C → 33.1°C
Hours 6 → 9	33.1°C → 32.4°C
Hours 9 → 12	32.4°C → 31.6°C
Hours 12 → 15	31.6°C → 30.9°C
Final temp.- 17hrs	30.5°C

Conclusions (Part II)

- Requirement for fresh CT product at clinical site distant from that of manufacturing need not be a limitation
- NK cells can be shipped successfully
 - Shipping containers can be warmed to approach BT
 - Incubator not essential (i.e., control of CO₂ not critical)
 - As expected, IL-2 necessary to activate NK cells

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