Hunting the Cell Loss Data

The Question

Since the placebo treatment consisted of (1) leukapheresis (2) cell processing (3) removal of 2/3 of the cells for freezing (4) refrigeration of the other 1/3 of cells for 36-44 hours and (5) reinfusion of these cells, the obvious question was to quantify the number of cells under discussion.

These data were not presented in any public documents, nor did they become available in the redacted versions of the FDA documents released after approval. This text explains how the numbers were found, and shows the calculations behind the percentage losses quoted in the JNCI paper and in the alternative explanation…..

Starting Point

Table 19: Cumulative Cell Product Parameters Administered in Safety Database

	Sipuleucel –T N=601 Median (range)	Placebo N=303 Median (range)
TNC	9.831×10^9 (0.843×10^9 to 35.974×10^9)	3.384×10^9 (0.093×10^9 to 8.626×10^9)
CD54+	1.877×10^9 (0.108×10^9 to 8.600×10^9)	0.879×10^9 (0.003×10^9 to 6.988×10^9)
CD54+ upregulation Ratio	26.959 (2.900 to 69.648)	2.683 (0.063 to 4.060)

As shown in Table 20, subjects in the sipuleucel-T group received infusions with a higher median cumulative TNC, CD54+ cell count, and CD54 upregulation ratio, compared with subjects in the placebo group. These higher values reflect expected differences between the study product, sipuleucel-T, and the placebo.

http://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM214540.pdf

This table, on p56 of the FDA's Clinical Review, shows data pooled from patients across all the Ph III trials. The highlighted row clearly shows that Provenge patients received 3 times as many cells back in their infusions as placebo patients. This is as expected since 2/3 of placebo cell lots were removed and frozen.[1]

To calculate the net cells lost from placebo patients and account for cells lost during manufacture, the number of cells infused needed to be compared to the number of cells removed in the leukapheresis.

Cell Losses in Manufacturing: Raw Data

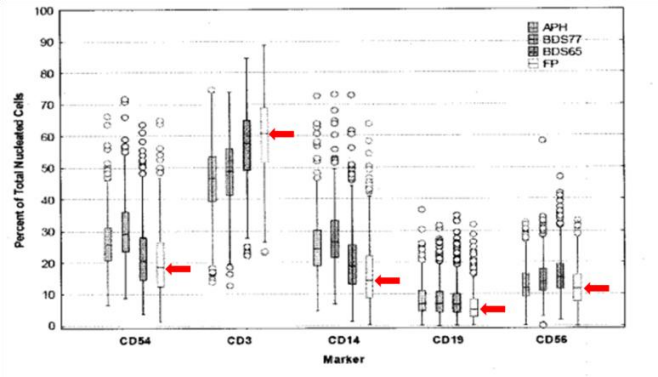

Figure 4. Percentage of leukocyte populations at various manufacturing stages of sipuleucel-T.
Numbers are calculated based on the total nucleated cell counts. Whereas all cell populations decreased when measured by total nucleated cell count, when assessed by percentage of total cell, CD3 positive cells increased. APH = leukapheresis, BDS77 = post first buoyant density centrifugation, BDS65 = post second buoyant density centrifugation just before incubation, FP = final product.

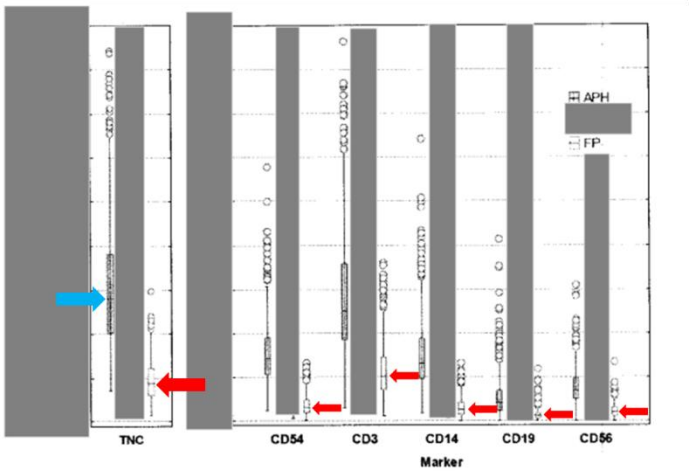

Figure 5. Leukocyte total cell numbers present within the starting leukapheresis material, processed cells, and the final product for autologous vaccination into patients.
These plots show the total cell counts for the several cell types at various manufacturing stages. APH = leukapheresis, [redacted] FP = final product. Data is based on 526 lots.

March 2007 CTGT Advisory Committee Meeting CMC Briefing Document, p9 & 10.
http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4291B1_04a.pdf

Although there is no mention of the cells lost during the manufacturing procedure in the 2010 approval-related documents (unless this has been redacted), I found the charts above in the documents provided to the 2007 advisory committee. They show two charts of the same data: cell counts by percentage in Fig 4, and numeric cell counts in Fig 5. The grey bars on Fig 5 are redactions requested by Dendreon.

- The surface markers represent cell types as follows: CD3 = T Cells, CD14 = monocytes, CD19 = B Cells, CD56 = Natural Killer Cells. CD54 is present on most leukocytes and the composition of this population changes. In Fig 5, left column, TNC = Total Nucleated Cells (also includes granulocytes)
- For each marker, the 4 bars represent cell counts at each of 4 stages of manufacture. The left-hand bar in each set is the cell count in the pouch that arrives from the apheresis center. The right-hand bar is the cell count in the pouch that leaves the manufacturing facility for reinfusion into the patient. The two middle bars (redacted from Fig 5) show counts after the two "buoyant density separations" that precede the incubation. I have added the red arrows to highlight the median values in the finished Provenge product.
- The Total Nucleated Cell plot on the left side of Fig 5 clearly shows that well over half of the cells are lost in manufacture (large blue arrow incoming, large red arrow exiting). Since the scale on the axis and actual cell counts were all redacted, there was no way to know the *numbers* in question from this document alone.
- However, by taking the TNC and CD54+ cumulative cell count numbers from the table (shown above) in the 2010 Clinical Review document, and dividing them by 3 (to get the average number per dose), the median values for the right-hand bars in the TNC and CD54+ plots (red arrows) could be determined. This allowed the redacted scales on Fig5 to be determined (baseline at zero, horizontal lines at $4x10^9$(TNCs) and $2x10^9$(cells by marker) increments). Thus the cell counts in the incoming apheresis product could be read from the chart.
- Addendum 1 to the 2007 AdCom CMC document shows a chart from which the granulocyte numbers (that contribute to the TNC count but are not represented in the leukocyte chart), could also be reverse-engineered. The purpose of the two centrifugation steps is to remove granulocytes from the incoming apheresis cells.
- Combining these, and performing a few, simple calculations, the cell numbers lost during the manufacturing process could be calculated for both the Provenge and placebo patients. Results shown below.

Cell Losses in Manufacturing: Calculations‡

	Apheresis pouch		Provenge dose		% loss during Manufacture Provenge	% loss during Manufacture Placebo
	Cell count ($x10^9$) A	% of TNC	Cell count ($x10^9$) B	% of TNC		
Source	Fig 4	Fig 5	Fig 4	Fig 5	(A-B)/A	(A-B/3)/A
T-Cells (CD3)	5.00	46 %	2.00	62%	60%	87%
Monocytes (CD14)	2.60	24 %	0.48	15%	82%	94%
B-Cells (CD19)	0.80	7 %	0.19	6%	76%	92%
NK cells (CD56)	1.35	12 %	0.38	12%	72%	91%
Other (granulocytes)	1.20	11 %	0.16	5%	87%	96%
TNCs	10.95	100 %	3.21*	100%	71%	90%
Lymphocytes	**7.15**	65%	2.57	80%	**64%**	**88%**
MNCs	9.75	89 %	3.05	95%	**69%**	**90%**

Lymphocytes = T, B and NK Cells, MNCs = Mono-Nuclear Cells (Lymphocytes + monocytes), TNCs = Total Nucleated Cells (MNCs + granulocytes)

* This TNC # checks with the number in Table 19, above ($9.831x10^9$ in 3 doses). Small difference due to different data sets.

‡Cell numbers and calculations based on median values. Some patients lost far more, and others far fewer cells.

Cells Removed in Leukapheresis

On p28 of the FDA's clinical review , the baseline lymphocyte counts of the patients in IMPACT were given as $1.44x10^3$ and $1.41x10^3$ per µL for the Provenge and placebo patients, respectively. An average adult male has 5.25L of blood. Combining these, the values in the table below could be calculated

	Baseline White Blood Cells		Cells removed from patient by apheresis	
	% (approx)	# in 5.25 L man ($x10^9$)	Cell number ($x10^9$) A	% of Baseline
Lymphocytes	23.5%*	7.48	**7.15**	**95.6%**
Monocytes (CD14)	~9%‡	2.84	2.60	~92%
MNCs	~33%‡	~10.32	9.75	~94.5%

* Lymphocytes are 23.5% of baseline WBCs from p28 of the FDA's clinical review (1.425/6.065=23.5%)

‡Monocytes typically represent 2-10% of white blood cells. Since no baseline counts are provided for the trial subjects, 9% has been used as an approximation, although this is on the high end of the range. Due to the large number of monocytes known to be in the apheresis product, using a lower approximation would imply that >100% were removed during leukapheresis.

Summary

Provenge Intervention: Repeated 3 times: days 0, 14 and 28.

- ❑ Day 1: over 90% of circulating mononuclear cells removed by apheresis
- ❑ Days 2-3: ~68% of cells lost in processing. Cells incubated at 37˚C with antigen for 36-40hrs
- ❑ Day 4: Remaining cells (~32% of those harvested) reinfused

Cumulative cells "lost": $20.1x10^9$ mononuclear cells (of which $13.7x10^9$ lymphocytes)

"Placebo" Intervention: Repeated 3 times: days 0, 14 and 28.

- ❑ Day 1: over 90% of circulating mononuclear cells removed by apheresis
- ❑ Days 2-3: ~68% of lost in processing, a further 2/3 removed and frozen.
- ❑ Days 2-3: Remaining cells stored at 2-8˚C for 36-40hrs (+transit time)
- ❑ Day 4: Cells (~11% of those harvested) of unknown viability (many or all likely to have died) reinfused

Cumulative cells "lost": $26.2\text{-}29.3x10^9$ mononuclear cells ($18.9\text{-}21.5x10^9$ lymphocytes)

Key Findings

LEUKAPHERESIS: Trial subjects had over 95% of their circulating lymphocytes harvested in each of 3 leukaphereses over 1 month. (JNCI paper uses "over 90%")

- **PROVENGE**: 64% of lymphocytes lost in manufacture. This leaves just 36% of the harvested lymphocytes for reinfusion into patients.
  - ★ **Cumulative cells "lost" in 1 month: 20 billion MNCs** (of which 14 billion lymphocytes)
- **PLACEBO**: 64% of lymphocytes lost in manufacture, minus a *further* 2/3 of cells for freezing. 12% of harvested lymphocytes returned to patients. It is not known whether these cells were viable, as our experience suggests many may be dead after 40-58 hours at 2-8˚C.
  - ★ **Cumulative cells "lost" in 1 month: 26-29 billion MNCs** (of which 19-22 billion lymphocytes)

*MNCs = mononuclear cells

Footnotes:

1. Whereas the difference between arms in mean and max counts is 3-4 fold, the difference between minimal values is nearly tenfold – comparing the spread of cell counts around the medians could yield valuable insights.