

## ABSTRACT/INTRODUCTION

In the cell and gene therapy industry, a reliable supply of fresh human hematopoietic stem cells (HSC) is crucial. HSCs are vital for stem cell transplantation, gene therapy, immunotherapies, and regenerative medicine. HSCs reside in the bone marrow, where they can be collected from human donors. Alternatively, they can be mobilized into peripheral blood for easier access. This mobilization is primarily achieved using Filgrastim (Neupogen®), a Granulocyte Colony-Stimulating Factor (G-CSF) that stimulates HSCs proliferation. Plerixafor (Mozobil®), a CXCR4 antagonist, further enhances mobilization by disrupting the interaction between CXCR4 on HSCs and SDF-1 (CXCL12) on the bone marrow microenvironment, promoting their release into circulation.

HSC yields may depend on donor demographics (BMI, weight, age, health) and other biological factors.

## METHODOLOGY

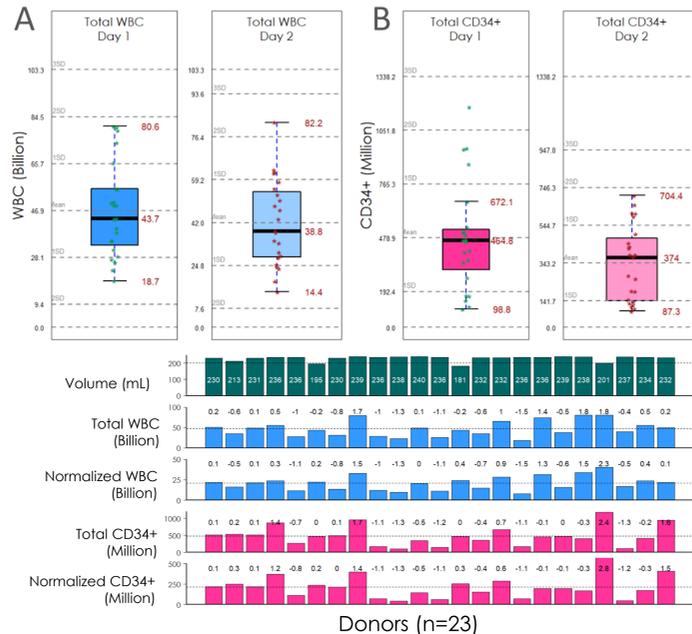
To better understand how donor demographics and mobilization regimens affect white blood cells (WBC) and CD34+ cell yields, we analyzed 23 donors (21 males, 2 females) undergoing single (n=17) or dual (n=6) mobilizations. Donors receive a single (Neupogen, 10 mcg/kg/day for five days) or a dual regimen (Neupogen and additional Mozobil at 240 mcg/kg/day on the last two days). Then, immune cells were collected via apheresis. For six donors, this was their second and final mobilization. Quantification of CD34+ cells was performed using an easyCyte Guava flow cytometer (Cytek Biosciences). We are building our database and the data presented is considered preliminary information.

Regimen Type	Donor Cohorts	First Mobilization (n = 17)	Second Mobilization (n = 6)
Neupogen (n = 16)	Male	11	3
	Female	2	0
Neupogen/Mozobil (n = 7)	Male	4	3
	Female	0	0

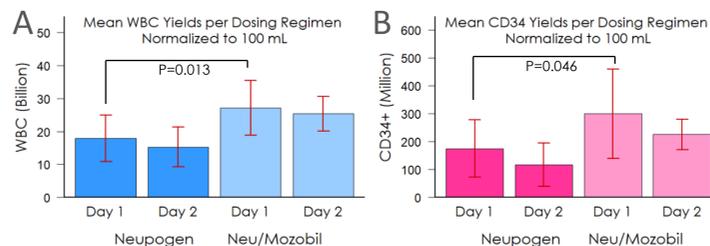


## RESULTS

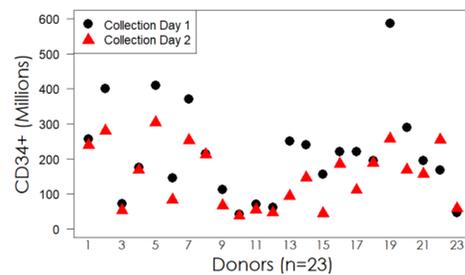
WBC yields ranged from 18 - 82 billion, and CD34+ cells yields from 0.98 - 1.2 billion per donor (Fig 1, top). Because some of the factors influencing yields are collection time and leukopak volume, we normalized yields to 100 mL for better comparisons of yields per donor (Fig 1, bottom). Dual dosing regimen yields were higher (1.5-fold for WBC, 1.7-fold for CD34+) than a single regimen (Fig 2). Yields per donor decreased significantly between Collection Days 1 and 2 (p=0.0013) (Fig 3). Male donors had higher yields than females (1.8-fold for WBC, 4-fold for CD34+) (Fig 4). Mean yields declined based on age, except for the 65-year-old donor, which is an outlier. The decrease in yields was not significant for WBC. (Fig 5). Most donors had higher lymphocyte percentages on collection day 1 (16 out of 23), with 6 showing a difference of 15% or more between the two days. In contrast, monocytes exhibited an opposite trend (Fig 6). First-time donors had higher WBC yields than second-time donors (20 vs. 16 billion/100 mL, p=0.018 in day 1). Changes were also observed for CD34+ total yields (206 versus 138 million/100 mL for Neupogen, and a reverse trend of 185 versus 377 million/100 mL for Neupogen/Mozobil), but the differences are not significant (Fig 7).



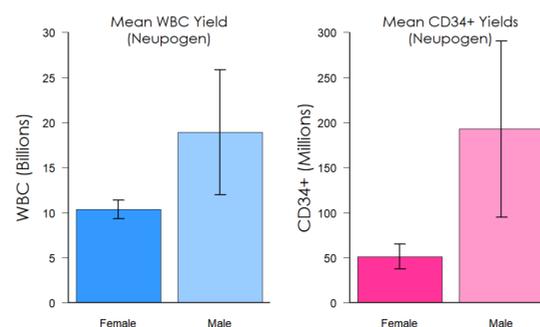
**Fig 1. Top.** Distributions of (A) total WBC and (B) total CD34+ yields observed on collection days 1 and 2. N = 23 in all cases. **Bottom.** Data normalization. Since not every leukopak has the same volume, the WBC and CD34+ yields were normalized to cell productions per each 100 mL for each donor. For Total and normalized WBC and CD34+ bar plots, values on each bar represent Z Scores, and indication of how far each donor is from the means. Normalizing to 100 mL adjust the distributions. Horizontal dotted lines indicate means.



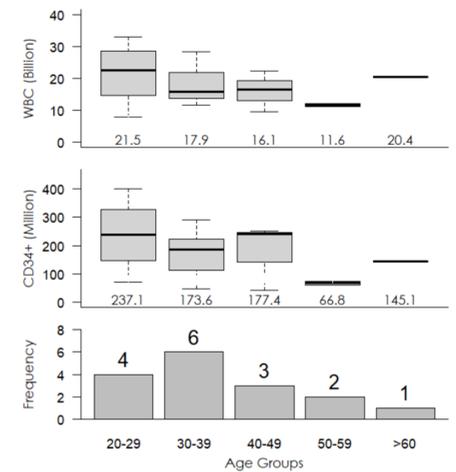
**Fig 2. (A)** WBC and **(B)** CD34+ mean yields per dosing regimen and per collection day. Error bars represent one standard deviation above and below mean. On collection days 1 and 2. Neu/Moz regimen produces higher WBC yields than Neu alone (p=0.013), it is not significant for CD34+ cells.



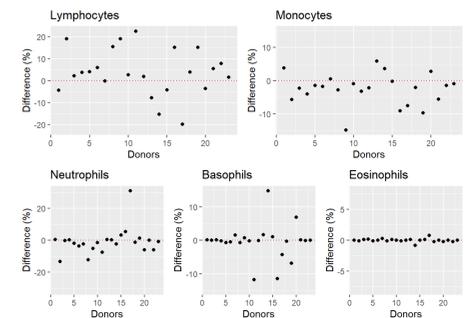
**Fig 3.** CD34+ cells yields comparisons between collection day 1 and collection day 2. The differences in CD34+ yields are strongly significant (p = 0.0013).



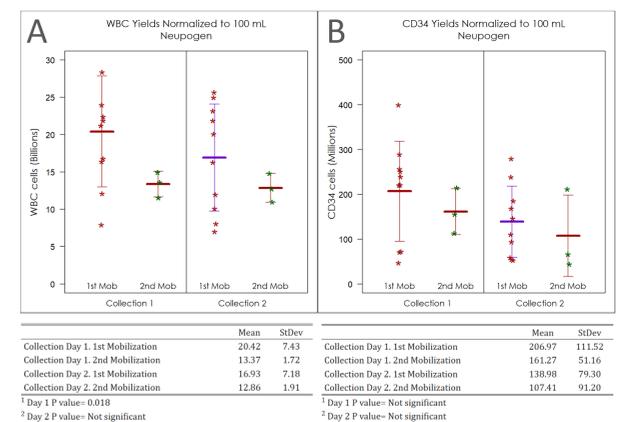
**Fig 4.** Mean WBC and CD34+ yields per gender, Neupogen dosing regimen. Note: No female donors received the double Neu/Mozobil dose. Error bars represent ±1 standard deviation.



**Fig 5.** Day 1 collection data in Neupogen regimen, presented by donor age group: Top, distribution of WBC yields (per 100 mL), with mean values indicated below each box plot. Middle, distribution of CD34+ cell yields (per 100 mL), with mean values indicated below each box plot. Bottom, frequency of donors within each age group, highlighting sample size variations. Based on the mean values, we observe an age-dependent decrease in cell production, but the differences are not statistically significant.



**Fig 6.** Percent change of different immune cell populations between collection days 1 and 2. Values above 0 indicate a higher percentage on day 1, while values below 0 indicate a higher percentage on day 2.



**Fig 7.** Mean (A) WBC and (B) CD34+ yields in Neupogen regimen normalized to 100 mL. Graph shows the mean yields of first-time donors (n=10) versus second-time donors (n=3). The graph also compares collection day 1 versus collection day 2.

## CONCLUSIONS

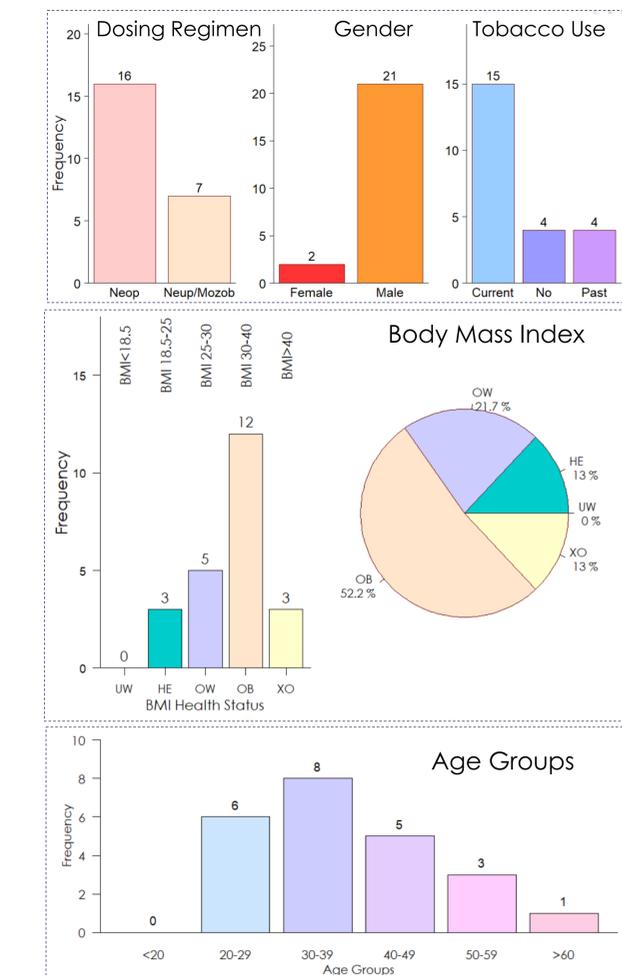
Mobilization involves intensive treatment with expensive medications, making it essential to have reliable donors and consistent results to ensure the effectiveness and success of the process. Our findings reveal significant variations in WBC and CD34+ cell yields depending on donor demographics and mobilization strategies. These insights underscore the importance of product quality and consistency, while also promoting more effective and cost-efficient advancements in the gene and cell therapy industry. The data presented shows some tendencies based on age, dosing regimen and mobilization on immune cells percentages, among other parameters. However, our n values (n=23) are too small for proper analysis for comparisons among categories. We need to analyze additional mobilization collection data to test if the differences are significant.

## REFERENCES

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Frequencies of donor demographics and dosing regimens. UW, underweight, HE, healthy, OW, overweight, OB, obese, XO, extreme obese.

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