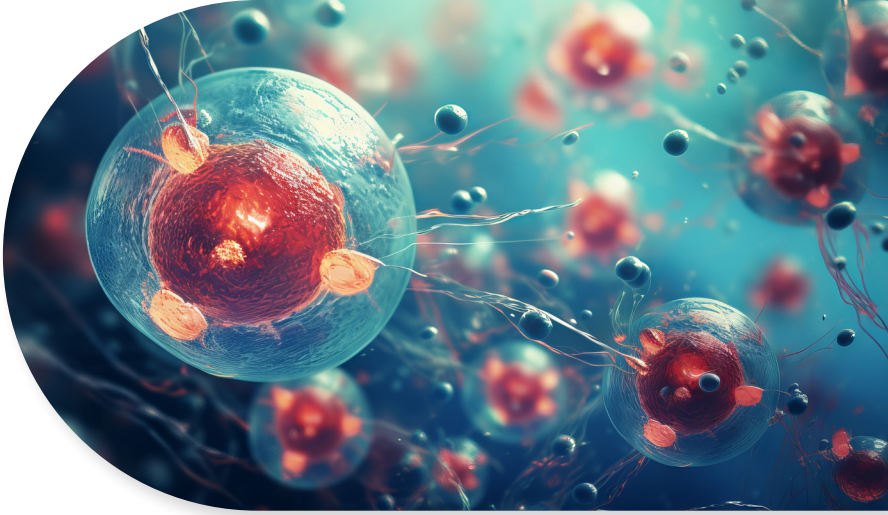
ALLCELLS®



TECHNICAL NOTE

Mobilized Leukopaks with High CD34+ Yield and Low Granulocyte Content

Understand how Discovery Life Sciences' CGT solutions delivers mobilized leukopaks with high CD34+ yield, low granulocyte content, and a reliable 98% collection deliverability rate within just three weeks.

Introduction

Peripheral blood mobilization is an effective method for increasing circulating levels of CD34+ hematopoietic stem and progenitor cells (HSPCs) before leukapheresis collection. During mobilization, donors are treated with one or two FDA-approved agents: granulocyte colony-stimulating factor (G-CSF) and plerixafor. Filgrastim (Neupogen®) is a commonly used form of G-CSF, which stimulate the bone marrow to produce and release HSPCs into the peripheral blood. Plerixafor, marketed as Mozobil®, is a small molecule inhibitor that disrupts stem cells binding to the bone marrow stroma, enabling their release into circulation. Plerixafor is often used in combination with G-CSF in dual mobilization regimens, where they work synergistically to increase HSPC levels compared to treatment with either molecule alone.

There are numerous factors that can affect the CD34+ cell yield including biological donor variability, mobilization regimen and collection parameters. Success hinges on careful consideration of these factors and optimization of key steps of the donor selection, mobilization, and collection process. Discovery Life Sciences comprehensively addresses these variables and consistently delivers high yields of CD34+ HSPCs with the shortest lead time in the market.

Methods

AllCells has been providing mobilized leukopak products to the global scientific community since 1998, offering three mobilization regimens: G-CSF (Neupogen) alone (Figure 1), plerixafor (Mozobil) alone (Figure 2), or a combination of G-CSF with plerixafor (Figure 3), all administered to healthy donors according to IRB-approved guidelines.

Across all regimens, each donor provides two leukapheresis collections on consecutive days following standard protocols using the Spectra Optia® Apheresis System continuous flow centrifugal technology. The yield of CD34+ cells from a single donor is maximized, enabling scalability and reproducibility of experiments.

RegF Regimen

Subcutaneous injection of 10μg/kg/day G-CSF

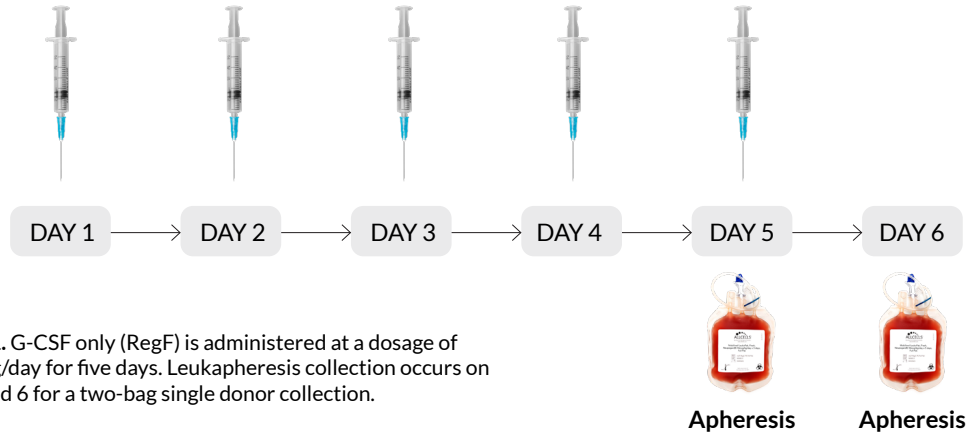


Figure 1. G-CSF only (RegF) is administered at a dosage of 10μg/kg/day for five days. Leukapheresis collection occurs on day 5 and 6 for a two-bag single donor collection.

MozB Regimen

Subcutaneous injection of 240μg/kg Plerixafor

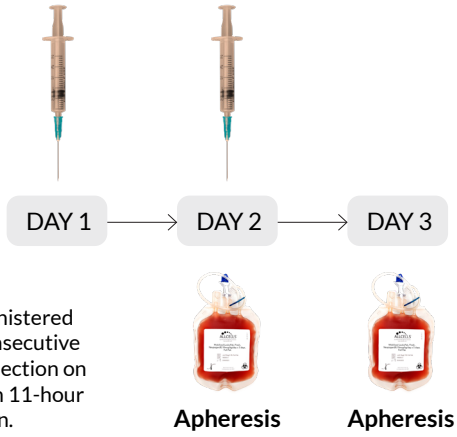


Figure 2. Plerixafor only (MozB) is administered at a dosage of 240μg/kg/day for two consecutive evenings, followed by leukapheresis collection on days 2 and 3 in the mornings. There is an 11-hour interval between injection and collection.

RegH Regimen

Subcutaneous injection of 10μg/kg/day G-CSF Subcutaneous injection of 240μg/kg Plerixafor

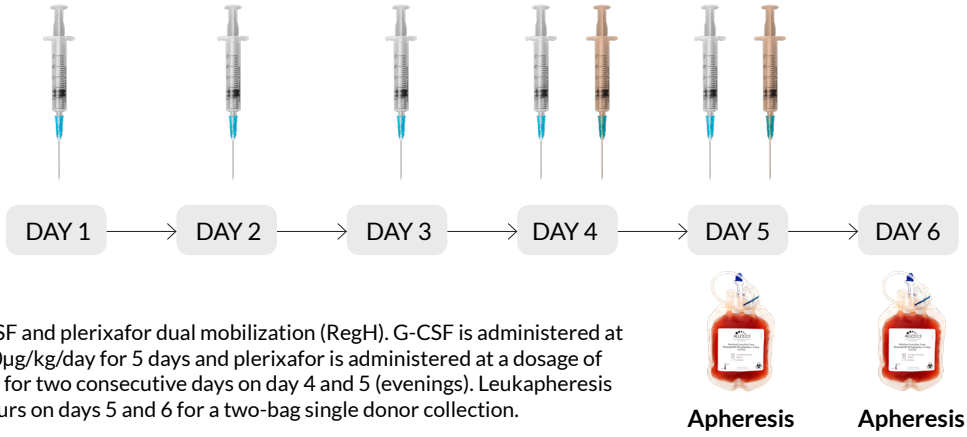


Figure 3. G-CSF and plerixafor dual mobilization (RegH). G-CSF is administered at a dosage of 10μg/kg/day for 5 days and plerixafor is administered at a dosage of 240μg/kg/day for two consecutive days on day 4 and 5 (evenings). Leukapheresis collection occurs on days 5 and 6 for a two-bag single donor collection.

CD34+ Enumeration Across Mobilization Regimens

AllCells uses the International Society of Hematotherapy and Graft Engineering (ISHAGE) methodology for CD34+ cell enumeration using flow cytometry. This international gold standard method ensures consistency and accuracy in CD34+ cell enumeration across different laboratories and research settings, allowing for reliable comparison of stem cell products, and is used extensively for clinical decision-making in stem cell transplantation procedures.

In Figure 4, the average percentage of CD34+ cells quantified using the ISHAGE method across mobilized leukopaks collected from the three regimens is shown. Each data point represents the average of the two leukapheresis collections from each donor. As expected, the highest percentage of CD34+ cells is observed in the RegH dual mobilization regimen, with an average of 1.29%, followed by RegF at 1.07%, and finally, MozB at 0.43%. In comparison, the % CD34 cells in non-mobilized leukapheresis collections is ~0.01-0.05%[1].

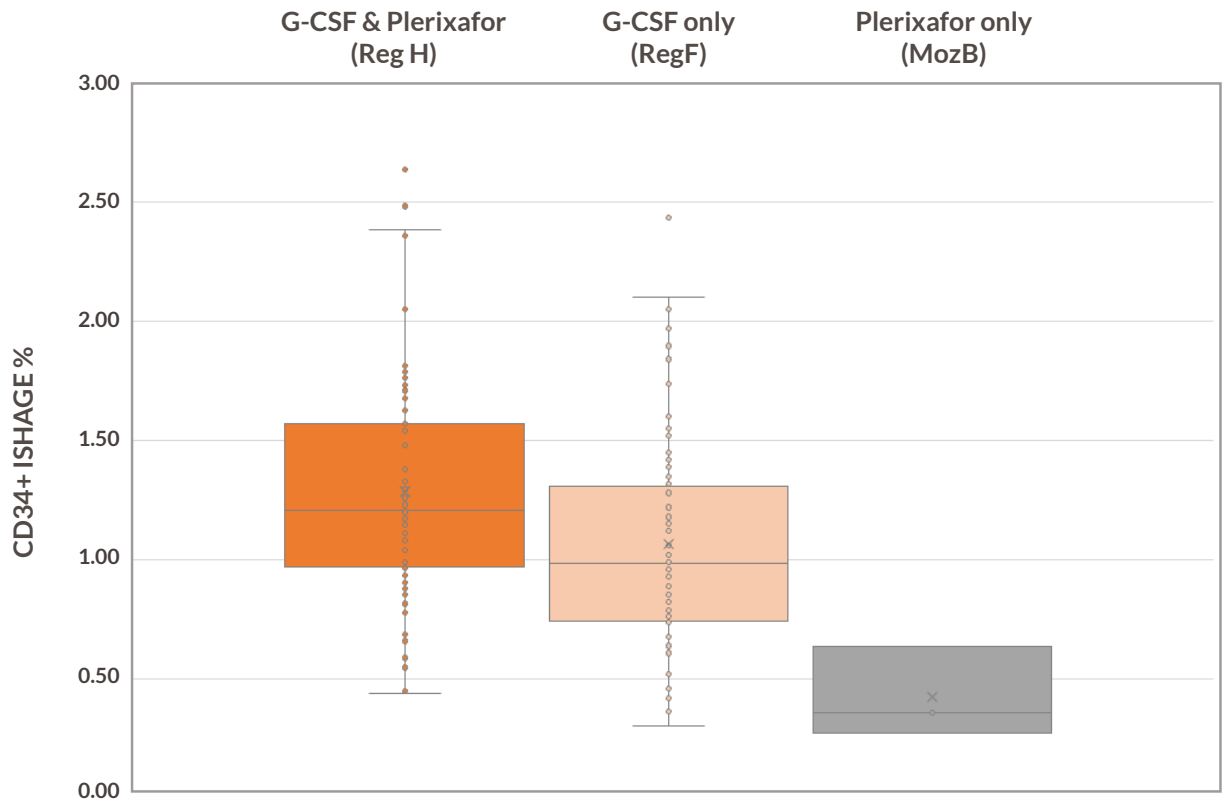


Figure 4. Average percentage of CD34+ cells for each mobilization regimen is depicted with RegH (dual mobilization) representing n=78, RegF (G-CSF only) n=88, and MozB (Plerixafor only) n=3.

It is evident that the % of CD34+ cells isolated during collection is influenced by both donor variability and the chosen mobilization regimen.

Additional factors affecting CD34+ yield include the expertise of the clinical collection team and the donor selection process. Some individuals inherently exhibit higher levels of CD34+ cells and certain donor attributes like age and BMI can also impact CD34+ levels. Therefore, the capacity to identify and collect from specific donors, as well as potentially recalling them for repeat donation at additional timepoints, represents a significant advantage for researchers. Since the molecular profile, cellular composition, and expected cellular yield of the donor is already known from previous donations, this contributes to reduced variability and raw material consistency for downstream applications.

Granulocyte Contamination

The same mobilization agents used to increase the circulating levels of CD34+ HSPCs also increase the percentage of granulocytes (neutrophils, eosinophils, and basophils) present in a mobilized leukopak. G-CSF, for example, is also known to stimulate the production and release of granulocytes from the bone marrow into the bloodstream. Plerixafor, has a more targeted effect on HSPCs compared to G-CSF, but may still have some influence on granulocyte levels.

As proinflammatory cells, granulocytes have the potential to become activated, which may negatively impact other cell types in the mobilized leukopak. These cells also cryopreserve poorly and can release DNA and lysosomal enzymes upon thawing, causing damage to other cells. Studies show granulocyte contamination adversely affects T cell functionality[2] and reduces the ability of NK cells to degranulate and secrete cytokines[3]. Therefore, it is necessary to control their levels during collection to minimize their impact on downstream applications.

Figure 5 shows the average % of granulocytes present in non-mobilized leukopaks is 3.3%, while in mobilized leukopak collections that percentage increases to 8.86% and 9.29% for RegH and RegF, respectively.

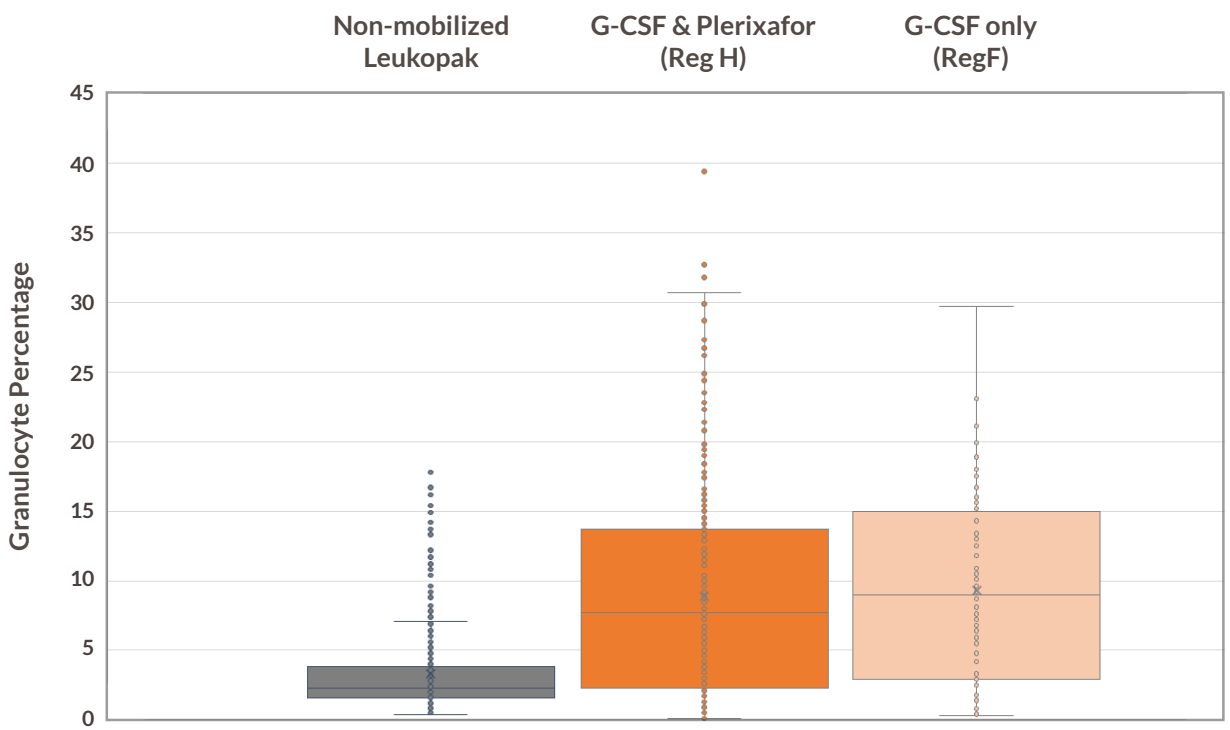


Figure 5. Average % of granulocytes present in non-mobilized leukopaks (n= 551) compared to mobilized leukopaks collected using either the RegH (n=378) or RegF (n=90) mobilization regimen.

Our decades of expertise have allowed us to refine our collection protocols and implement stringent control measures to limit granulocyte levels during collection, which allows us to successfully maintain one of the lowest percentages of granulocyte contamination in the industry. Across both RegH and RegF, 99% of all collections demonstrated granulocyte levels below 30%, with 61% below 10%.

The AllCells Advantage

As a proven market leader in mobilized apheresis collections, AllCells delivers mobilized leukopaks with high CD34+ yield and low granulocyte content with the shortest lead time on the market of just three weeks. Our expertise in mobilization, collection, and processing, coupled with a large, highly recallable, and scalable donor ecosystem supports our mission to advance research and clinical efforts through the provision of high-quality products and services.

Our global donor network provides access to thousands of recallable and highly characterized donors with high-value attributes for different program requirements. We have a dedicated donor management team to assist in selecting the ideal donors to meet the specific requirements of any program.

98%
Collection Deliverability Rate

AllCells collects mobilized leukopaks from multiple wholly owned and operated, FDA-registered, IRB-approved facilities across the US, which are staffed by experienced clinicians dedicated to providing donors with the best possible experience throughout the collection process. Our adjacently located processing labs have capabilities to execute cell isolation, processing, and cryopreservation of numerous cell types immediately post-apheresis to maximize product quality. With reliability and recallability embedded into our processes, we have achieved a proven 98% collection deliverability rate, ensuring on-time and in-full delivery of products.

Learn more about our mobilized leukopak products and services to advance your programs and accelerate scientific discoveries.

Contact us at orders@allcells.com or visit our website at allcells.com

References

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