ALLCELLS®

Quality Cells for Quality Science

CD34+ PRODUCTS & MOBILIZED LEUKOPAKS

# A data-driven approach for selecting the right cell products

**CD34 is a cell surface antigen most commonly associated with hematopoietic stem and progenitor cells (HSPCs) with clinical relevance for cell therapies to treat hematopoietic diseases. The CD34+ marker is used therapeutically to enrich donor bone marrow (BM) with HSPCs prior to BM transplantation for treating people with a variety of cancerous (malignant) and noncancerous (benign) diseases like leukemia, Hodgkin's lymphoma, multiple myeloma and primary amyloidosis.**

Often, the key cell population of interest is the true hematopoietic stem cell (HSC), a rare population of precursor cells capable of self-renewal and multilineage differentiation to maintain blood cell homeostasis. HSCs reside within a specialized niche in the bone marrow (BM) microenvironment, which provides critical factors for the survival of quiescent HSCs and the expansion, differentiation and migration of precursors during the process of hematopoietic cell repopulation[1]. CD34 expression correlates with colony forming units (CFUs) in the in vitro colony forming cell (CFC) assay, which demonstrates the proliferative and functional capacity of the hematopoietic progenitor cells[2].

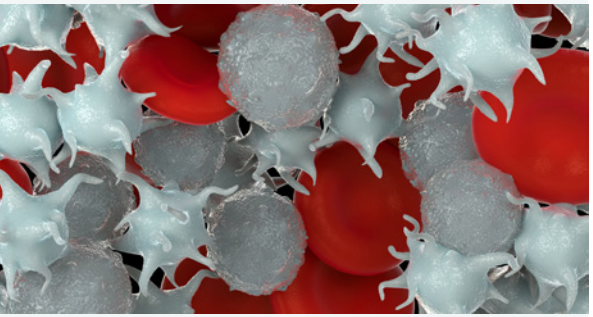
One main obstacle for researchers interested in HSCs is their identification and isolation from a larger, heterogeneous population of cells. Aside from their CD34+ marker expression, HSCs are also characterized by their lack of lineage-specific markers (Lin-). Human HSCs are defined as Lin-CD34+CD38-CD45RA-CD90+CD49f+3.

## Sources of CD34+ Cells

**The three main sources of HSPCs in the human body are: bone marrow, umbilical cord blood and peripheral blood.**

**BONE MARROW** The best-known location for HSPCs is the bone marrow with 1–3% (of total mononuclear cells) CD34+ cell content in normal, healthy donors[4]. Bone marrow is collected from the posterior iliac crest and is an invasive procedure done by needle aspiration. Risks to the donor include bleeding, infection, and localized pain. For these reasons, researchers often prefer to obtain their CD34+ cells from more easily accessible blood sources.

**CORD BLOOD** Umbilical cord blood (UCB) is collected from the umbilical cord and placenta after a baby is delivered and its availability can therefore be variable and potentially limiting. UCB CD34+ cell content is typically <1% of the total mononuclear cells[5]. This decreased CD34+ content and also the smaller volume of cord blood that can be isolated are some disadvantages compared to bone marrow. However, the stem cells from UCB are regarded as being more primitive, having a higher proliferative, engraftment and multipotent potential than those isolated from adult bone marrow, which makes up for their decreased numbers[6].



**PERIPHERAL BLOOD** Peripheral blood is by far the most accessible source of CD34+ cells, and the collection process is much less invasive than bone marrow and more readily available than UCB. However, the normal concentration of CD34+ cells is much lower than in bone marrow or UCB, only 0.01 to 0.05%[7], thus requiring procedures to mobilize stem cells into the circulation prior to collection. Up to 10 to 20 times more CD34+ cells (0.5–1%) can be isolated from a mobilized apheresis collection compared to bone marrow depending upon the mobilization and collection regimes[8]. As a clinical source of HSCs, mobilized peripheral blood (MPB) has emerged as the most widely used stem cell source for hematopoietic transplantation and other novel cellular therapies.

## Mobilized Peripheral Blood

Because HSPCs are not normally circulating in peripheral blood, their collection from peripheral blood requires pre-mobilization of stem cells from the bone marrow niche. Peripheral blood collection is preferable to BM because apheresis does not require hospitalization and is less physically demanding on the donor. Also, there is evidence that mobilized peripheral blood stem cells engraft more rapidly than non-mobilized bone marrow-derived stem cells[9,10]. The preferred mobilization method is dependent on donor characteristics as well as the intended use of the isolated stem cells since it is the most important factor in determining yields and phenotype[11]. Either granulocyte colony stimulating factor (G-CSF), plerixafor, or a combination of the two compounds is used for mobilization, but the specific protocols can vary widely between transplantation programs.

AllCells offers three Mobilized Leukopak products, which are apheresis collections from healthy donors treated with different mobilization regimens, including G-CSF (Neupogen®), plerixafor (Mozobil®), or a combination G-CSF with plerixafor through IRB approved protocols. AllCells has the shortest lead times in the industry, capable of delivering Mobilized Leukopak products to researchers exactly when they need them.

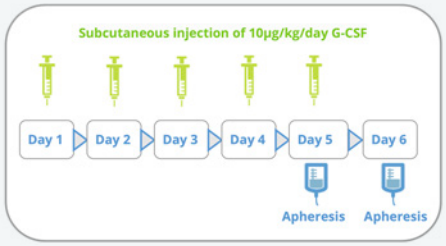



**Figure 1:**
*RegF regimen with a subcutaneous injection of 10µg/kg/day G-CSF for 5 days. Mobilized peripheral blood is collected by apheresis on days 5 and 6.*

**G-CSF REGIMEN (REGF)** Granulocyte colony stimulating factor (G-CSF, Neupogen®) is a cytokine responsible for the proliferation of neutrophils and can directly modulate expression of proteases that degrade proteins anchoring stem cells to the marrow stroma. Protease-independent mechanisms include indirect regulation of CXCR4 expression on HSPCs, which also contributes to the release of stem cells into the circulation. Although the actual mechanism of G-CSF action is most likely a combination of several pathways, research implicates the CXCR4/SDF-1α pathway as a critical determinant of HSPC mobilization[9].

Mobilization of cells expressing the CD34 marker peaks in the peripheral blood around Day 4 of subcutaneous G-CSF injections of 10µg/kg/day. Thus, leukapheresis is initiated on Day 5 and 6 for a two-bag single donor collection. A target number of CD34+ cells to be collected is usually ≥4 × $10^6$/kg[9].

**MOZOBIL REGIMEN (MOZA)** The CXCR4 chemokine receptor expressed on CD34+ cells facilitates adhesion of CD34+ stem cells to the bone marrow stroma through interactions with the chemokine stromal-derived factor-1α (SDF-1α). Plerixafor, also known as Mozobil®, is a small molecule that reversibly inhibits binding of SDF-1α to CXCR4 receptor resulting in mobilization of CD34+ cells from the marrow into the circulation.

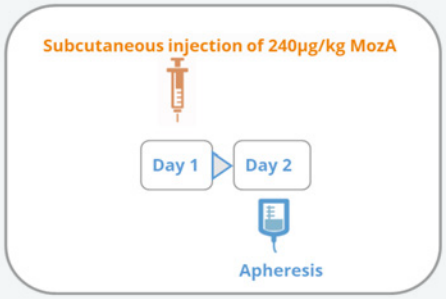



**Figure 2:**
*MozA regimen with a subcutaneous injection of 240µg/kg MozA for a single day followed by mobilized peripheral blood collection by apheresis the next day.*

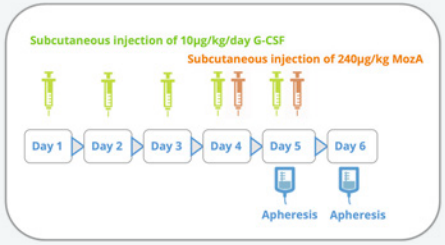



**Figure 3:**
*RegH regimen with subcutaneous injection of 10µg/kg/day of G-CSF for 5 days and 240µg/kg/day of MozA on days 4 and 5. Mobilized peripheral blood is collected by apheresis on days 5 and 6.*

In comparison to G-CSF mobilization, the Mozobil® regimen requires only 1 injection of 240 µg/kg prior and apheresis is performed on the following day.

**NEUPOGEN® + MOZOBIL® REGIMEN (REGH)** Mozobil® has been found to act as an adjuvant, boosting mobilization compared to G-CSF alone. The combination of Mozobil™ and G-CSF is well tolerated by donors resulting in rapid and synergistic mobilization of CD34+ cells as well as CD3+, CD4+ and CD8+ and CD56+ cells compared to G-CSF alone. This regimen not only mobilized more CD34+ cells per leukapheresis than Neupogen™ alone, but research indicates there is a significant increase in the percentage of primitive progenitor cells mobilized with a high repopulation capacity[12].

Donors are dosed with G-CSF (10µg/kg/day) alone for 3 days and then with both G-CSF (10µg/kg/day) and Mozobil® (240 µg/kg) for an additional 2 days for a total of 5 days of injections in this regimen. As with the G-CSF alone protocol, apheresis is collected on Day 5 and Day 6 to obtain a total of two apheresis bags per donor.

This table summarizes the average TNC, CD34+ counts and average frequency of CD34+ cells based on ISHAGE protocols for the three mobilization regimes obtained from the AllCells Mobilized Leukopaks.

| Mobilization Regimen | Average TNC Count ($x10^{10}$ cells) | Average $CD34^+$ Count ($x10^8$ cells) | $CD34^+$ Frequency (%) |
|---|---|---|---|
| RegF | 5.77 | 4.62 | 0.8 |
| RegH | 7.83 | 11.04 | 1.4 |
| MozA | 3.82 | 1.26 | 0.3 |

## Why Should I Purchase a 2nd Mobilized Leukopak?

As described above, for the RegF and RegH regimens, two apheresis collections are performed per donor based on standard protocols. There are several advantages to purchasing both apheresis collections from a single donor including decreased donor-to-donor variation in the starting cell population. You can also maximize the yield of CD34+ HSPCs for experiments from a single-source donor allowing for scalability and reproducibility of experiments. Utilizing the same cell source for multiple experiments enables direct comparisons to be made in the data generated. Figure 4 shows the TNC and CD34+ yield comparisons between Day 1 and Day 2 of apheresis.

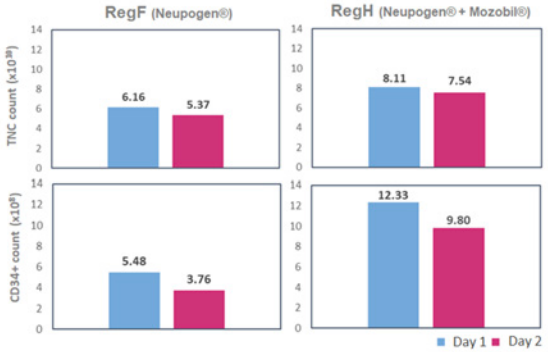



**Figure 4:** *The average TNC ($10^{10}$ cells) and the CD34+ count ($10^8$ cells) for Day 1 and 2 apheresis collections for RegF and RegH mobilization regimens are very comparable.*

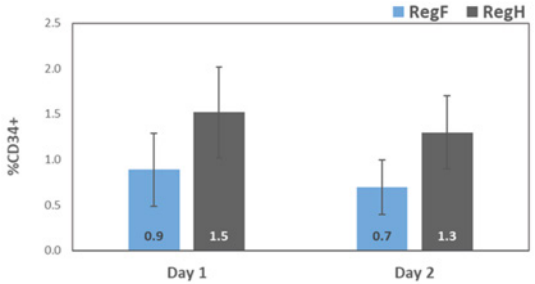



**Figure 5:** Comparison of Day 1 and 2 paired collections of CD34+ frequency (%) for RegF and RegH mobilization regimens.

Figure 5 shows AllCells data demonstrating no significant difference between Day 1 and Day 2 of apheresis in the % CD34+ cell content for RegF (Neupogen®) and RegH (Neupogen® and Mozobil®) regimens.

Additionally, there is a significant cost savings to purchasing two Mobilized Leukopaks from a single donor compared to one. On average, a savings of ~40% on the purchase of the second collection bag can be expected for both RegF and RegH Leukopaks.

## The AllCells Advantage: Focus on Quality

AllCells' quality-controlled and continuously optimized processes from donor recruitment to delivery ensure that you receive reproducible and consistently high-quality cells, minimizing your experimental variability.

- State-of-the-art apheresis equipment, protocols and extensive clinical expertise deliver optimal cell quality.
- Isolation and cryopreservation processes performed immediately onsite after collection ensures the highest possible cell viability.
- Depending on requirements, Mobilized Leukopaks can be available in as little as 3 weeks. This is the shortest lead time in the industry ensuring your cells are there when you need them.
- Validated AeroSafe Global Packaging for cryopreserved shipments to minimize transient warming events impacting cell quality and performance.

**Contact us today at info@allcells.com or 510.726.2700 to learn more and/or get a quote.**

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