

**FLT3L and Plerixafor Combination Increases Hematopoietic Stem Cell
Mobilization and Leads to Improved Transplantation Outcome**

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Running title: **FLT3L and Plerixafor Combination in Cell Mobilization**

Abstract

Hematopoietic stem cell (HSC) transplantation has curative potential for patients with hematological malignancies. Clinically, HSCs derived from mobilized peripheral blood are used more frequently than bone marrow. However, current standard mobilizing agents yield grafts that may not contain sufficient HSCs. Here, using murine models, we discovered that FLT3L synergized with **Plerixafor** to mobilize phenotypically defined HSCs, and their combination (**FP**) was superior to G-CSF alone or in combination with **Plerixafor** (**GP**). Additionally, **FP** mobilized more Treg cells, NK cells, and plasmacytoid dendritic cells compared with G-CSF alone or **GP**. Both syngeneic and allogeneic grafts mobilized by **FP** led to long-term survival in transplanted mice. Collectively, **FP** represents a promising novel and potent mobilization regimen with potential clinical application in both the autologous and allogeneic transplantation settings.

Key words: Cell mobilization; Transplantation; FLT3L; Plerixafor

Introduction

Hematological malignancies contribute to almost 10% of the mortalities caused by cancers in the United States [1]. Hematopoietic stem cell transplantation (HSCT) is a well-established treatment with curative potential for a variety of hematological cancers. In addition to bone marrow transplantation (BMT), mobilized peripheral blood provides a rich source of HSCs for transplantation. Successful HSCT depends on a mobilization regimen that provides an adequate number of HSCs for engraftment but is less likely to provoke transplant related complications. However, current mobilization regimens occasionally do not mobilize sufficient numbers of cells into the peripheral blood, or the mobilized immune cells often lead to post transplant complications, such as graft-versus-host disease (GVHD).

The CXCR4 antagonist **Plerixafor** (Mozobil) induces safe and rapid mobilization of clinical grafts capable of promoting robust hematopoietic recovery in HSCT patients [2]. However, the number of hematopoietic stem/progenitor cells (CD34⁺ cells) mobilized by **Plerixafor** alone is far lower than G-CSF, the current gold standard mobilizing regimen, suggesting that combining **Plerixafor** with other agents may be more effective for clinical applications. FLT3 ligand (FLT3L) is a stem-cell specific growth factor which expands and may also mobilize stem cells in mice after administration for ten days, either as a single agent or in combination with other molecules such as IL-8 and G-CSF [3, 4]. Most recently, FLT3L has been introduced into a clinical trial, demonstrating that the

administration of FLT3L on a consecutive 5- or 10-day schedule effectively mobilizes CD34⁺ HSCs in healthy donors [5]. Here we tested the effect of the combination of two clinical grade drugs, FLT3L and Plerixafor, on stem cell mobilization, and evaluated the transplantation outcomes of the mobilized cells in both syngeneic and allogeneic murine models. Our data demonstrate that the combination of FLT3L and Plerixafor is a promising regimen for mobilizing adequate stem cells and other cell subsets that lead to better engraftment and survival following both syngeneic and allogeneic transplantation.

Materials and Methods

Mice

BALB/c and C57BL/6 mice were 8-10 weeks old and purchased from National Cancer Institute-Frederick. All animal work was approved by The Ohio State University Animal Care and Use Committee, and mice were treated in accordance with the institutional guidelines for animal care.

Cell Mobilization

C57BL/6 mice were injected intraperitoneally (i.p.) with recombinant human FLT3L (Celldex Therapeutics or Miltenyi Biotec) (350 µg/kg/day) for 10 consecutive days or G-CSF (Amgen) (150 µg/kg/day) for 5 consecutive days. Twelve hours after the last dose of FLT3L or G-CSF, mice were injected i.p. with PBS or **Plerixafor** (AMD3100, Sigma) (5 mg/kg) and sacrificed 1 hour later. **The peripheral blood was collected retroorbitally while the mice were under deep anesthesia, as described previously [6]. In detail, mice were anesthetized by i.p. injection with 100 uL of a mixture of ketamine (54.3 mg/mL) and xylazine (9.1 mg/mL). Blood was collected from the retro-orbital venous plexus through a microcapillary heparinized tube, followed by euthanization of the mice while they were still deeply anesthetized.** The red blood cells (RBC) were depleted by RBC lysis buffer. The remaining cells were subjected to flow cytometric analysis or transplantation. **The capacity of human FLT3L to bind murine Flt3 receptors and Plerixafor to block murine Cxcr4 receptor has been previously reported [7-10].**

Transplantation

For syngeneic transplantation, C57BL/6 mice were subjected to 9 Gy of irradiation delivered in two fractions separated by 4 hours using RS 2000 X-Ray Irradiator (Rad Source Technologies, Suwanee GP). Four hours later, each mouse was transplanted via tail vein with 2×10^5 mobilized RBC-depleted peripheral blood cells (PBC) from C57BL/6 mice. For allogeneic transplantation, the recipient BALB/c mice were subjected to a lethal irradiation (delivered as aforementioned) and injected with 8×10^5 mobilized RBC-depleted PBC **per mouse** isolated from C57BL/6 mice.

The rationale of using these cell numbers for transplantation is based on previous studies demonstrating that - 90% of mice survived when syngeneically infused with 1×10^6 peripheral nucleated cells mobilized by G-CSF [11, 12], but only around 17% of mice survived if 2.5×10^5 cells mobilized by G-CSF were transplanted [12]. Therefore, for syngeneic transplants, 2×10^5 mobilized cells were used in our experiments to explore whether the combination of FLT3L and Plerixafor has an advantage over G-CSF with a greater mobilizing potential. For allogeneic transplants, a four-fold higher cell number (i.e. 8×10^5 donor cells isolated from C57BL/6 mice) was administered i.v. into each BALB/c recipient, considering the likely graft rejection and GVHD complication in the allogeneic model. The lethally irradiated mice were housed in a sterile environment, and baytril antibiotic was provided in the drinking water to prevent infection-caused mortality.

Following transplantation, mice were monitored daily and the phenotypes of the dying mice were recorded.

Colony formation assay

Colony formation assays were performed using MethoCult[®] GF M3434 (StemCell Technologies, Vancouver, Canada) according to the manufacturer's instructions. Briefly, 4.6×10^4 of mobilized white blood cells were resuspended in 0.3 mL of Iscove's Modified Dulbecco's Media (IMDM, Life Technologies, Grand Island, NY) with 2% FBS and added to 3 mL of MethoCult[®]. The vortexed mixture was then dispensed into three 35 mm dishes at 1.1 mL per plate, which were placed into a 100 mm culture dish and incubated at 37°C, 5% CO₂ for 12 days. Colonies were counted on an inverted microscope (Carl Zeiss Microscopy Thornwood, NY).

Flow cytometric analysis

The mAbs (supplementary data) reactive with murine cell antigens were purchased from BD Biosciences or eBioscience. Cell preparation and analysis were performed as previously described [13]. Mouse HSCs were defined as Lin⁻Sca-1⁺c-Kit⁺ (LSK) cells, and Lin⁻ (lineage negative) cells were gated as shown in Supplemental Figure 1. The immune subsets were gated as CD3⁻NK1.1⁺ for NK cells, CD3⁺CD25⁺Foxp3⁺ for Treg cells, B220⁻CD11c⁺CD11b⁺ for cDCs and CD11b⁻CD11c^{low}PDCA1⁺SiglecH⁺ for pDCs.

Statistics

For continuous endpoints, Student's t test was used to compare two independent groups. Measurements such as frequency and absolute number LSK cells, colony formation, and absolute number of Treg cells, were logarithmically transformed to normalize the data and stabilize the variance. A one-way ANOVA model was applied to multiple comparisons, and a two-way ANOVA model was performed to evaluate the synergistic effect between FLT3L and Plerixafor. Log-rank test was used to compare survival curves. All tests were two sided. *P* values were adjusted for multiple comparisons by Holms' procedure. A *P*-value <0.05 was considered as statistically significant.

Results

FLT3L and Plerixafor combination effectively mobilized Lin⁻Sca-1⁺c-Kit⁺ (LSK) cells into peripheral blood.

To test the effect of the combination of FLT3L and Plerixafor (FP) on HSC mobilization, both the white blood cell (WBC) counts and HSCs (Lin⁻Sca-1⁺c-Kit⁺, LSK cells, a hematopoietic stem cell-enriched population) in the peripheral blood were analyzed in mice subjected to various mobilization regimens, including the combination of G-CSF and Plerixafor (GP), one of the strongest mobilizing regimens currently used in the clinic. Cell counts showed that mice in the GP group had the highest WBC counts, and the FP group the second highest (Supplemental Figure 2). Compared to PBS-treated mice, Plerixafor-treated mice had modest upregulation of LSK cells, while FLT3L-treated mice mobilized significantly more LSK cells into the blood (Figure 1A). However, the FP combination mobilized significantly higher frequency and greater absolute number of LSK cells, reaching up to 6-fold and 12-fold higher than those of FLT3L alone, respectively (Figure 1A, top and bottom panels). Statistical analysis revealed that FLT3L and Plerixafor showed a synergistic mobilizing effect ($P < 0.05$, Supplemental Figure 3). Although the mice from the GP group had the highest WBC counts, the absolute number of LSK cells was similar to FLT3L alone, since the majority of WBC cells mobilized by GP were neutrophils (Supplemental Figure 4). We also compared the LSK cells mobilized by FP to that by G-CSF alone, which is the regimen most frequently used in the clinic, and data demonstrated that FP mobilized significantly more LSK cells (Figure

1A, $P < 0.05$). Colony formation assays revealed that cells mobilized by **FP** contained significantly more colony-forming units (CFU) than any other regimens, including **GP**, FLT3L alone, and **Plerixafor** alone (Figure 1B), and a synergistic effect was also observed between FLT3L and **Plerixafor** (Supplemental Figure 5, $P < 0.01$).

FLT3L and Plerixafor combination effectively mobilized NK cells, Treg cells and DCs into peripheral blood.

In addition to LSK cells, other immune cell subsets were also assessed. Mobilization with **FP** or FLT3L alone significantly increased natural killer (NK, **CD3⁺NK1.1⁺**) cell percentages to the same extent (Figure 2A, top and left bottom panels), consistent with previous reports of induction of NK cell expansion by FLT3L [14]. Importantly, **FP** administration resulted in much more dramatic increase of NK cells in absolute number than FLT3L alone or any other regimens (Figure 2A, right bottom panel). Although none of the mobilizing agents induced a significant increase of Treg cell frequency (supplemental Figure 6), **FP** led to significantly higher absolute number of Treg cells in mobilized blood than the other regimens (Figure 2B). In agreement with previous findings that FLT3L is able to expand dendritic cells (DCs) both *in vivo* and *in vitro* [15, 16], the two major subsets of DCs, plasmacytoid DC (pDCs) and conventional DC (cDCs), were both significantly increased in proportion for the **FP** group when compared with **GP** (Figure 2C).

FLT3L and Plerixafor combination-mobilized grafts significantly enhanced survival of mice in both syngeneic and allogeneic setting

To test the clinical potential of FP-mobilized grafts, cells mobilized by different regimens were transfused into lethally irradiated syngeneic mice. Mice receiving grafts mobilized by PBS, Plerixafor alone or G-CSF alone died within 21 days, probably due to failure to reconstitute hematopoietic cells, as the frequencies of progenitor or LSK cells were much lower in these groups (Figure 1A). This indicates that fewer progenitor or LSK cells were transplanted for these groups when a consistent number of mobilized cells were infused for each group. In contrast, mice receiving FLT3L or FP-mobilized products survived 100% at day 21, and the survival rate maintained at approximately 70% as far out as 4 months after transplantation (Figure 3A). The engraftment of the cells mobilized by FP was significantly superior to that of the cells mobilized by GP ($P < 0.05$), with the latter having a survival rate of only 35% at 4 months post-transplant. An examination of bone marrow in the surviving mice also showed that mice receiving FP grafts contained a higher frequency of LSK cells than those receiving GP grafts (supplemental Figure 7).

Next we explored the transplantation efficacy of the FP-mobilized cells in an allogeneic transplantation model. Lethally irradiated BALB/c mice were transplanted with peripheral blood cells mobilized by the different regimens from C57BL/6 mice. Based on previous reports [11], more mobilized peripheral blood cells were transplanted in this MHC-mismatched model than in the above syngeneic model (8×10^5 vs. 2×10^5 cells per recipient). All recipients of grafts mobilized by PBS, Plerixafor alone or G-CSF alone died within 3 weeks after transplantation; while 80% and 66% of mice receiving grafts mobilized by FP or FLT3L alone survived to 4 months post-transplantation, respectively

(Figure 3B). The survival rate of the **FP** groups was significantly higher than that observed in the **GP** group (12.5% alive at 4 months post-transplantation, $P < 0.05$). At 4 months post-transplantation, the mice in the **FP** group that remained healthy were sacrificed and found to have both long-term HSCs (LT-HSC, CD135⁻CD34⁻ LSK) and short-term HSCs (ST-HSC, CD135⁻CD34⁺ LSK) [17] originated from donor C57BL/6 mice (H2K^b) dominating in bone marrow (supplemental Figure 8), suggesting that their superior survivals may be due to the better hematopoietic reconstitution resulting from enhanced stem cell mobilization by this regimen.

Discussion

Since mobilized peripheral blood stem cells were demonstrated to have clinical advantages over bone marrow stem cells for HSCT [18], extensive efforts have been invested in the pursuit of optimal mobilizing regimens. Currently G-CSF based regimens (alone or in combination of chemotherapy or other agents) are the most common methods used to mobilize HSCs for autologous transplantation. However, poor mobilization still remains an important problem. In a study of 976 patients who went through mobilization, 29.7% failed to achieve sufficient numbers of stem cells [19]. Here we report the combination of FLT3L and **Plerixafor (FP)** as a novel regimen that is able to induce significantly more robust HSC mobilization, when compared to the current strongest regimen in clinic, the combination of G-CSF and **Plerixafor**. In fact, **FP** leads to 6-fold increase in frequency and 12-fold increase in absolute number of phenotypically defined

stem cells mobilized into peripheral blood (Figure 1). A recent clinical trial has shown the safety and efficiency of FLT3L alone in CD34+ stem cell mobilization in healthy donors [5]. Considering the synergistic effect between FLT3L and Plerixafor reported here, it's tempting to speculate the likelihood of circumventing mobilization failure issue using this regimen in poor mobilizers. And indeed, the lethally irradiated syngeneic mice were rescued with a small amount of mobilized grafts by FP but not the regimens currently used in the clinic, i.e., G-CSF alone or in combination of Plerixafor (Fig 3A), further demonstrating the stem cell mobilizing potential of this regimen.

The allogeneic transplantation setting, on the other hand, requires a sufficient number of HSCs as well as other cell subsets to help overcome allogeneic transplantation-related complications, such as GVHD and engraftment failure due to allograft rejection. Here we showed that FP-mobilized grafts significantly improved the survival of mice after allogeneic transplantation (Fig 3B). The superiority of FP to other regimens may be explained by more HSCs as well as more NK cells, Treg cells and DCs mobilized into peripheral blood to suppress acute GVHD (aGVHD). NK cells have been demonstrated to induce an antitumor response while suppressing the development of GVHD in mice [20]. Treg cells are important immune regulatory cells which have also been shown to generate protective effects against GVHD following allogeneic transplantation [21]. As a result, FP-mobilized grafts might favor a better outcome in MHC-mismatched transplantation compared with grafts mobilized by other regimens. Although cDCs are generally considered to enhance GVHD [22], the simultaneous increase of pDCs may

attenuate the effect of cDCs as pDCs have been shown to inhibit GVHD [23, 24]. Indeed, in our allogeneic model, compared to mice from control groups of PBS, G-CSF alone, and GP, mice transplanted with peripheral blood cells mobilized by FP had lower incidence of aGVHD (characterized by hunched back, inactivity, diarrhea, ruffled fur, and over 10% weight loss). It is also noteworthy that compared to the cells mobilized by other regimens, those mobilized by FP had consistently higher numbers of monocytes ($\text{Gr1}^{\text{med}}\text{CD11b}^+$; Supplemental Figure 4), a population that has been increasingly reported to be involved in both autologous [25] and allogeneic [26] stem cell transplantation as monocytic myeloid-derived suppressor cells (MDSCs) [27]. In allogeneic stem cell transplantation, MDSCs might also mitigate GVHD as NK and Treg cells do. This could be another explanation for the survival advantage observed in mice infused with FP-mobilized cells, although the role of this population in transplantation requires further study. Therefore, we believe that the immune cell subsets and the HSCs mobilized by FP may act together to contribute to the beneficial transplantation outcome in the allogeneic mouse model that we used.

In summary, in these preclinical studies we describe a novel potent cell mobilization regimen combining FLT3L and Plerixafor, which may have broad clinical applications in both autologous and allogeneic transplantation. Future clinical trials are warranted to further explore the potential of this regimen.

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Disclosure of Potential Conflicts of Interest

HCM is an employee of Celldex Therapeutics. The other authors declare no competing financial interests.

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Figure Legends:

Figure 1. FLT3L and Plerixafor combination effectively mobilized Lin⁻Sca-1⁺c-Kit⁺ (LSK) cells into peripheral blood. (A) Peripheral blood cells in mice treated with indicated mobilizing regimens were harvested, subjected to red blood cell depletion, and stained for HSCs (Lin⁻Sca-1⁺c-Kit⁺, LSK cells). Shown in the top panel are representative data from 1 out of 9 mice with similar results, and in the bottom panel are summary data of 3 mice in one of three experiments with similar data for the frequency and the absolute number of LSK cells. **FP** indicates the combination of FLT3L and **Plerixafor**, and **GP** denotes the combination of G-CSF and **Plerixafor**. (B) Mobilized peripheral blood cells were prepared as in (A) and subjected to colony formation assay. Shown are the summary data of 3 mice for the colony forming units (CFU) larger than 200 cells. For all panels, * indicates $P < 0.05$, ** indicates $P < 0.01$, and error bars represent S.D.

Figure 2. FLT3L and Plerixafor combination effectively mobilized NK cells, Treg cells and DCs into peripheral blood. (A) Peripheral blood cells in mice treated with indicated mobilizing regimens were harvested, subjected to red blood cell depletion, stained for NK cells, and subjected to flow cytometric analysis. Shown are the summary data of 3 mice in one of three experiments with similar data. (B) Mobilized peripheral blood cells were prepared as in (A) and subjected to flow cytometric analysis for Treg cells. Shown are the summary data of 3 mice for the absolute number of Treg cells. (C) **GP**- and **FP**-mobilized peripheral blood cells from 3 mice were pooled together

respectively and stained for cDCs (B220⁻CD11b⁺CD11c⁺) and pDCs (CD11b⁻CD11c^{low}PDCA⁺SiglecH⁺). For all panels, * indicates $P < 0.05$, ** indicates $P < 0.01$, and error bars represent S.D.

Figure 3. FLT3L and Plerixafor combination-mobilized grafts significantly enhanced survival of mice in both syngeneic and allogeneic setting. Survival analysis of lethally irradiated C57BL/6 mice (A) or BALB/c mice (B) receiving peripheral blood (PB) cells from C57BL/6 mice mobilized by the indicated regimens. Irradiated mice without any cell infusion (IR Ctl) served as a control group. A total of 2×10^5 (A) or 8×10^5 PB cells were transplanted for each recipient. Each group contains 5-8 mice.

Figure 1A

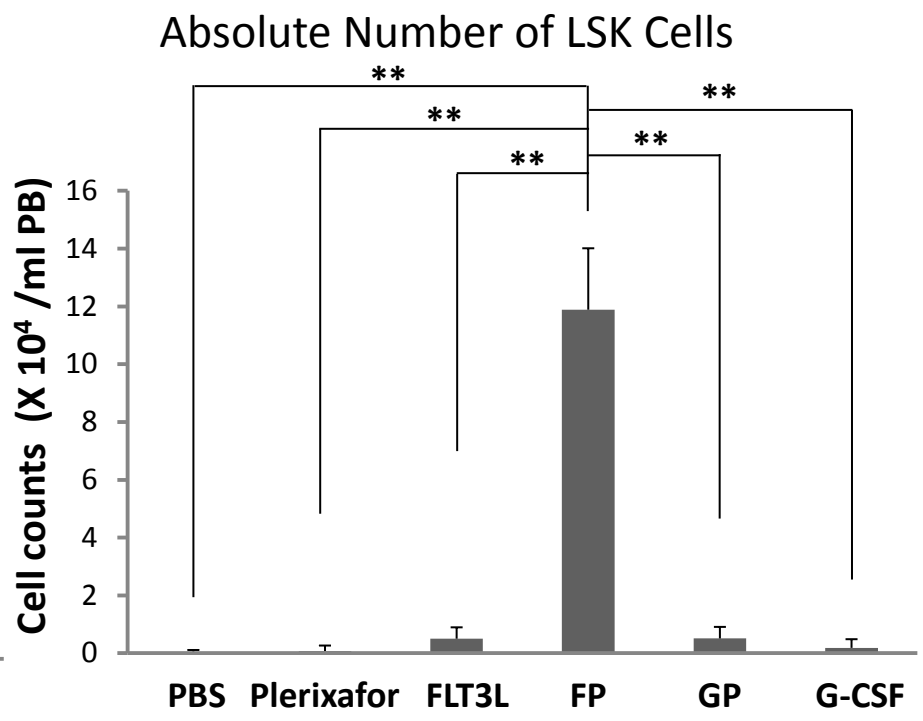
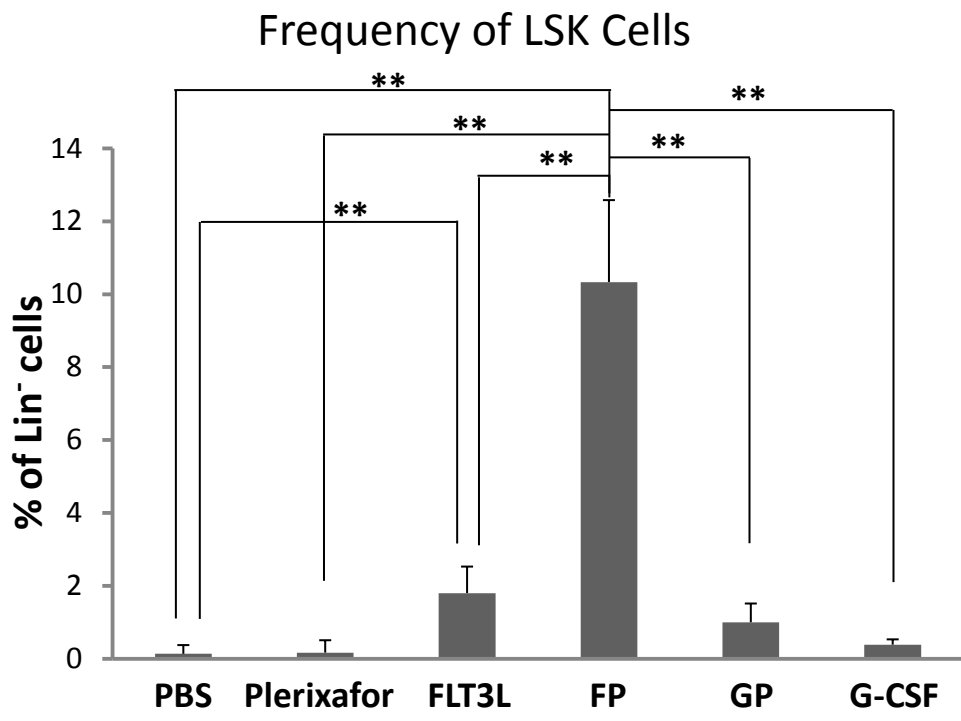
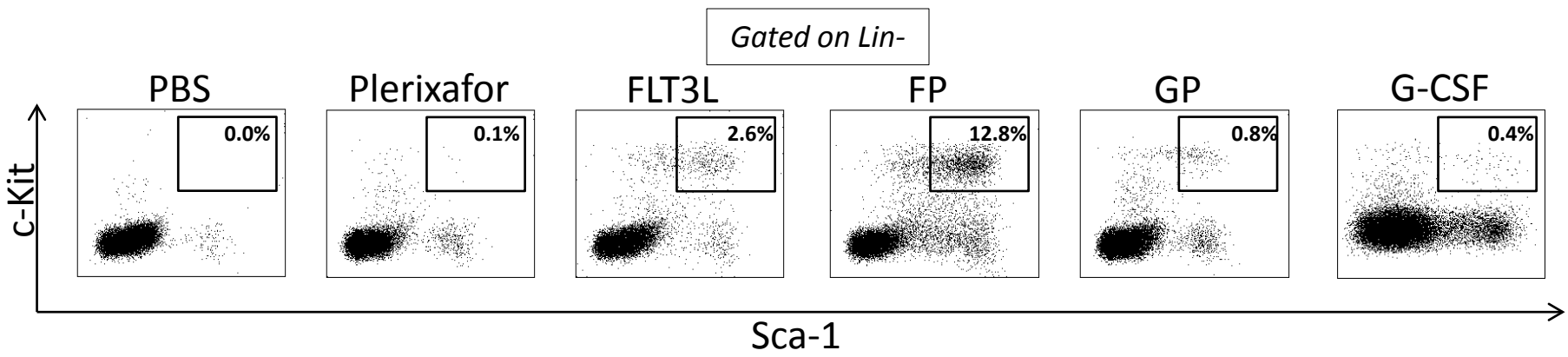


Figure 1B

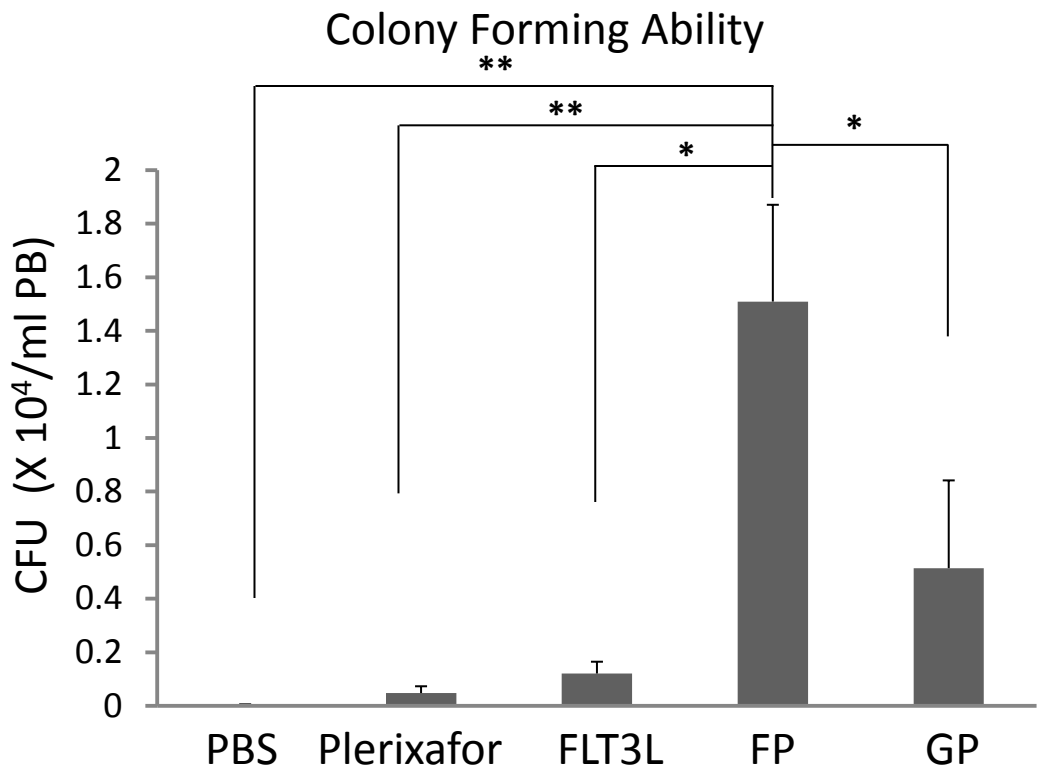


Figure 2A

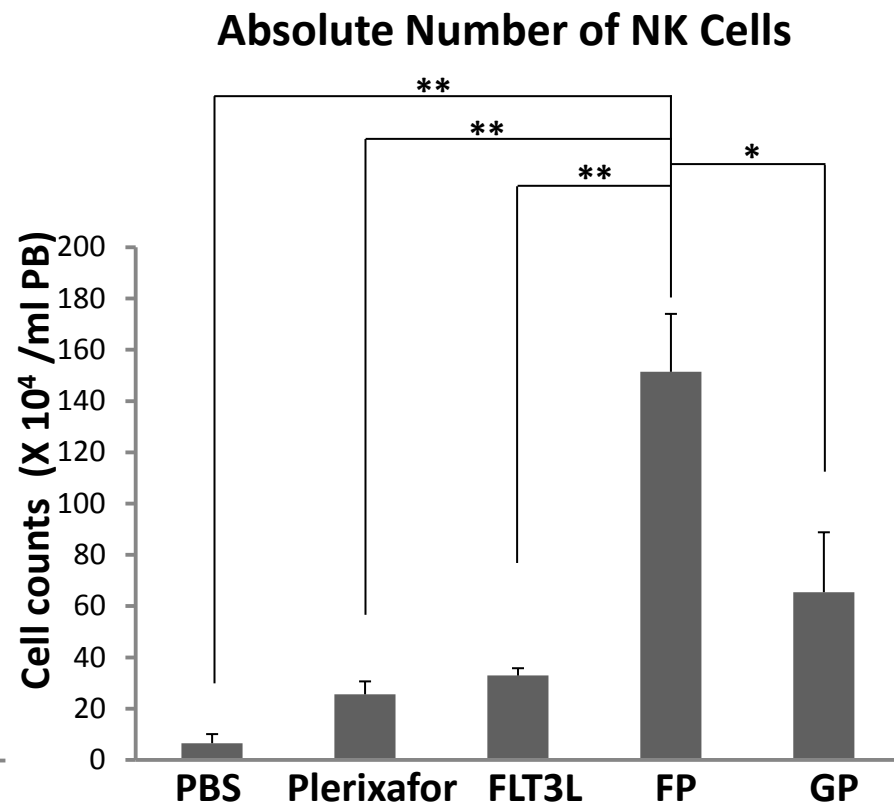
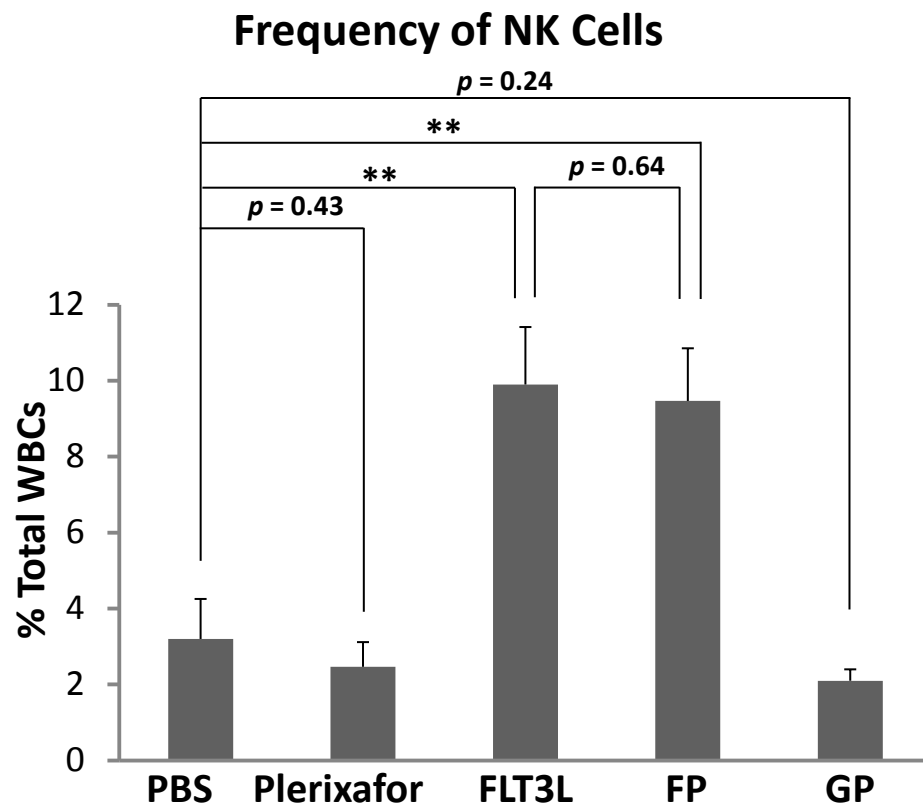
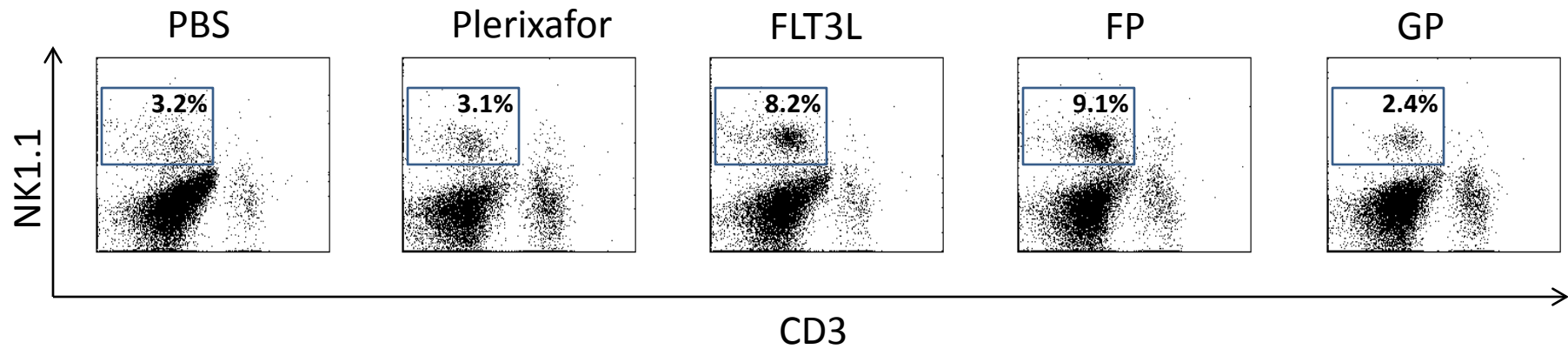


Figure 2B

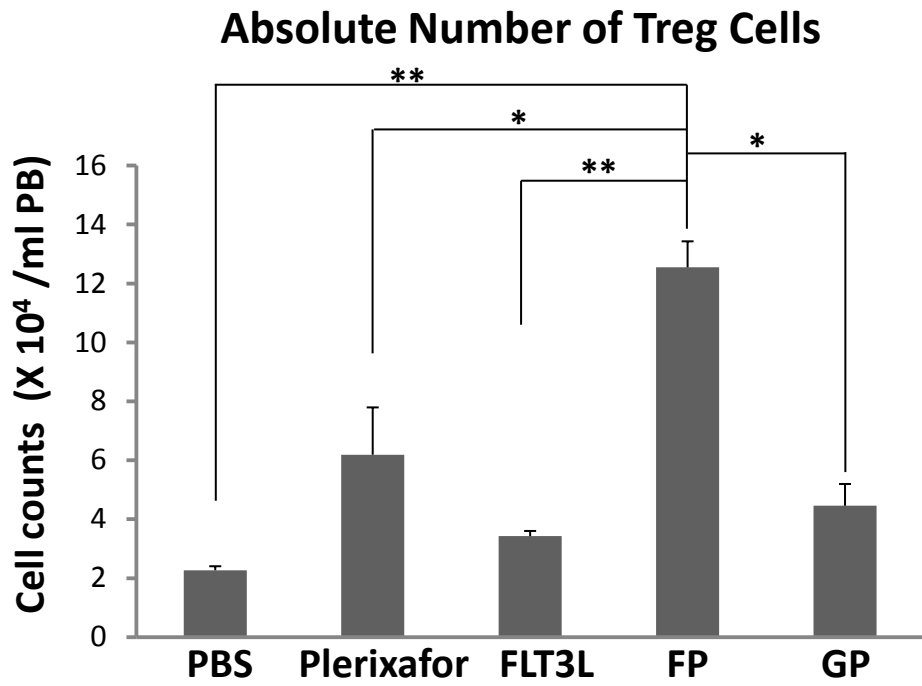
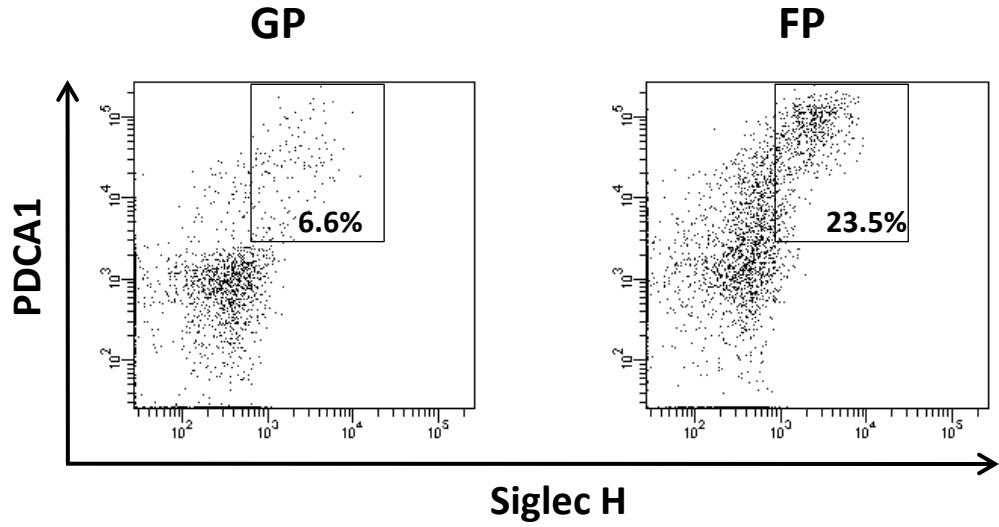


Figure 2C

pDCs
(Gated on CD11b⁻CD11c^{low})



cDCs
(Gated on B220⁻)

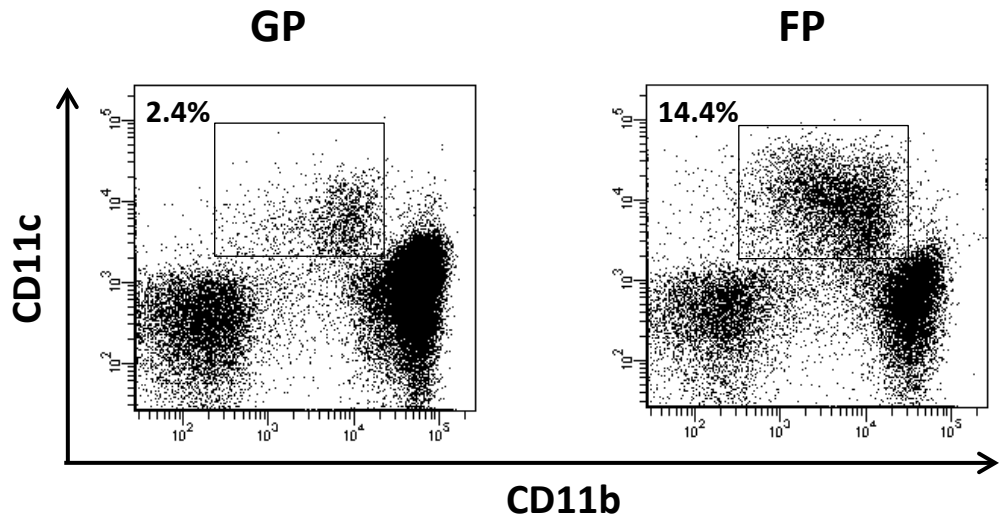
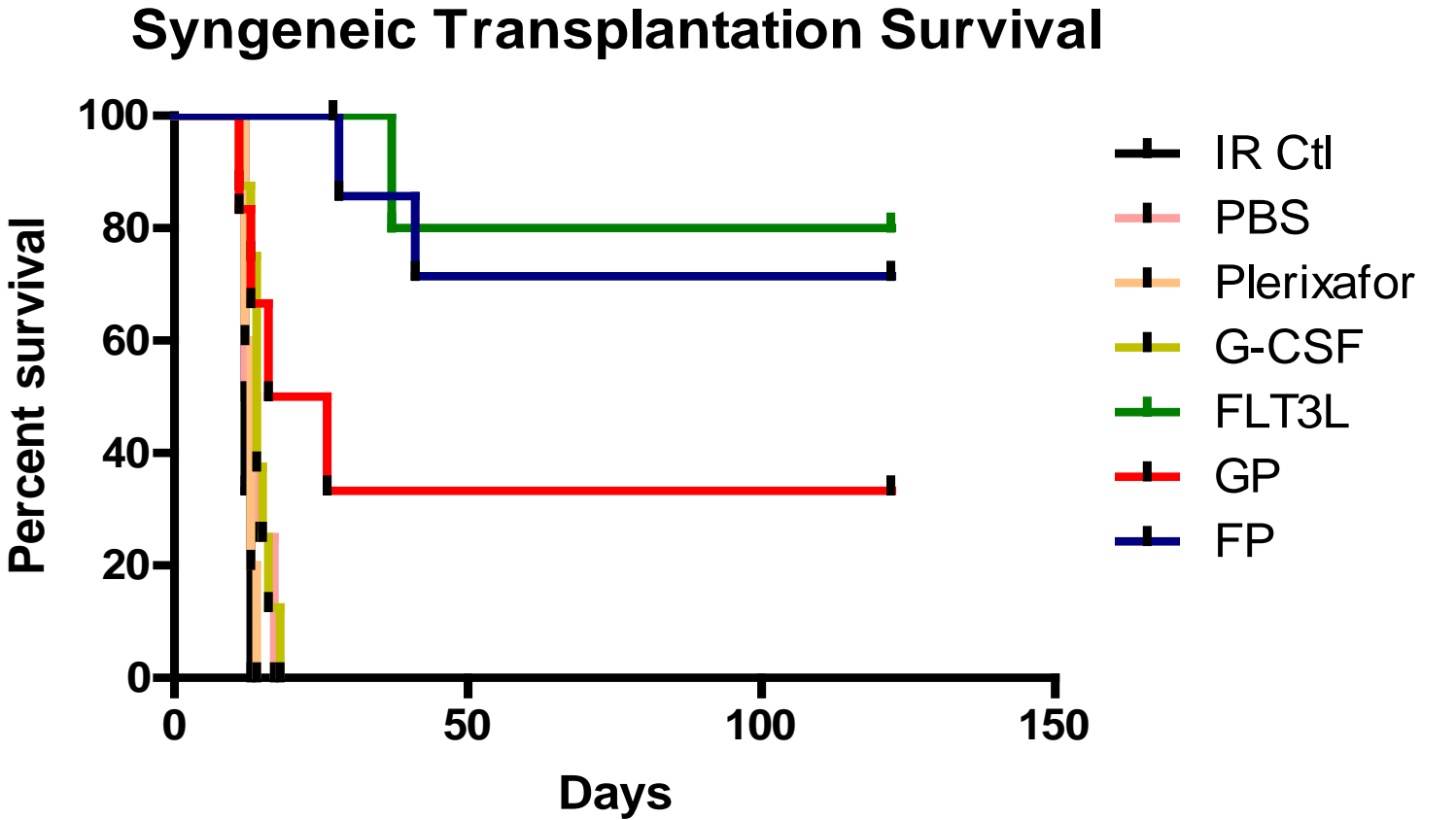
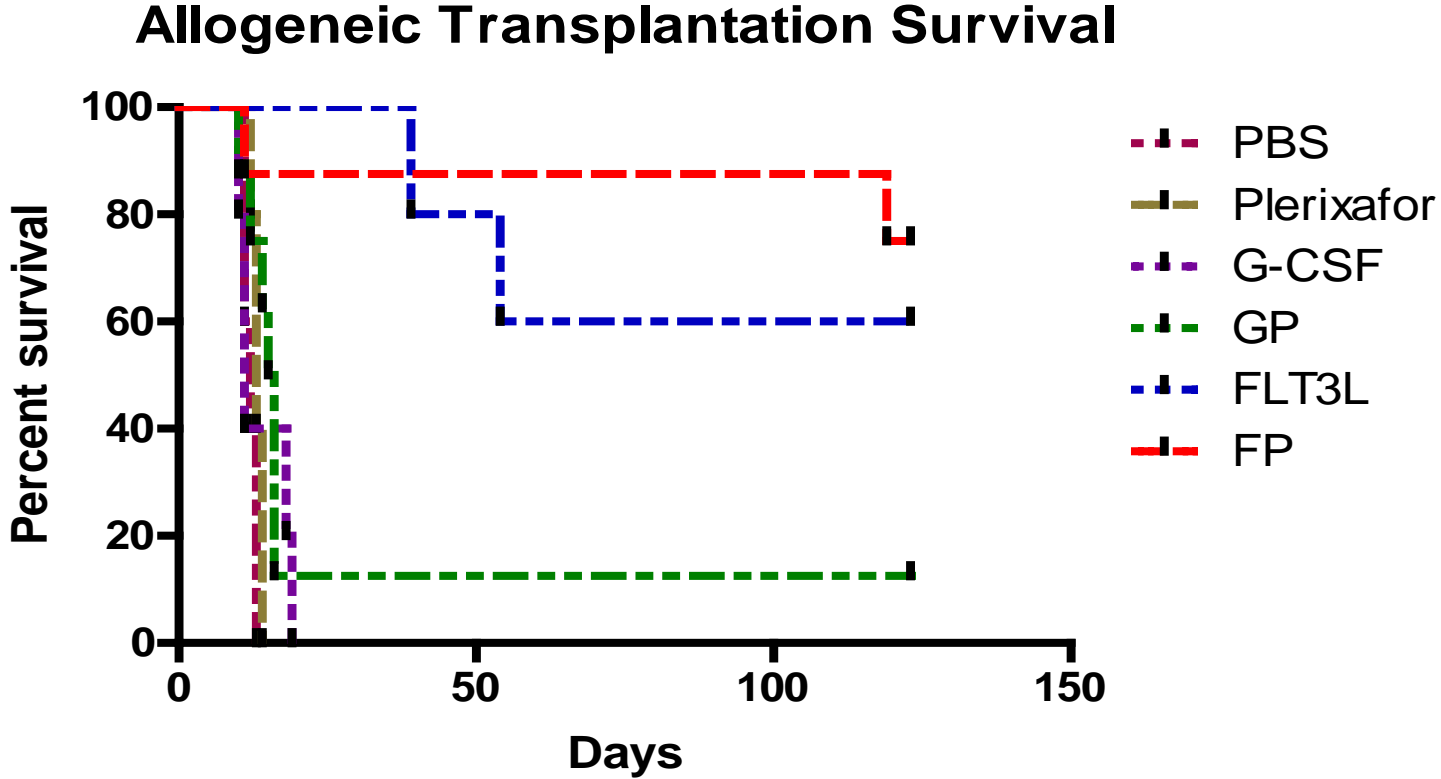


Figure 3A



FP vs GP: $p < 0.05$
FP vs FLT3L: $p = 0.53$
FP vs G-CSF: $p < 0.01$
FP vs Plerixafor: $p < 0.001$
FP vs PBS: $p < 0.001$

Figure 3B



FP vs GP: $p < 0.05$
FP vs FLT3L: $p = 0.41$
FP vs G-CSF: $p < 0.01$
FP vs Plerixafor: $p < 0.01$
FP vs PBS: $p < 0.001$

FLT3L and Plerixafor Combination Increases Hematopoietic Stem Cell Mobilization and Leads to Improved Transplantation Outcome

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Supplemental Information

Supplemental materials and methods:

Antibodies

Antibodies to CD3, CD11b, CD19, Gr1, NK1.1, FOXP3, CD25, CD34, CD135, CD117, Sca-1, Lin, PDCA, Siglec H, B220, and CD11c were purchased from BD Biosciences (San Jose, CA) or eBioscience (San Diego, CA).

Legends for supplemental figures:

Supplemental Figure 1. Flow cytometric analysis of Lin⁻ (lineage negative) cells in peripheral blood mobilized by PBS, Plerixafor, FLT3L, the combination of FLT3L and Plerixafor (FP), and the combination of G-CSF and Plerixafor (GP).

Supplemental Figure 2. White blood cell counts in peripheral blood mobilized by different regimens. Mice were treated with different mobilizing regimens as described in the Materials and Methods section, and total white blood cells were enumerated by microscopic examination of blood after red blood cell lysis. The combination of G-CSF and Plerixafor (GP) regimen led to highest number of white blood cells in peripheral blood after mobilization. Shown are the summarized data from 3 mice in one of two experiments with similar data. Error bars represent S.D. ** indicates $p < 0.01$. FP indicates the combination of FLT3L and Plerixafor.

Supplemental Figure 3. Synergistic effect of FLT3L and Plerixafor on LSK cell mobilization. The absolute amount of LSK cells mobilized into peripheral blood was determined as described in Figure 1A. The increase of LSK cells by each mobilization regimen is presented as fold ratio to PBS control. The paired bars compare the additive effect of FLT3L and Plerixafor each alone (left, composite bar) versus the effect of the combination (right, black bar).

A two-way ANOVA model was applied to evaluate the synergistic effect of FLT3L and Plerixafor. $P < 0.05$ was obtained for the synergistic effect test.

Supplemental Figure 4. Neutrophils comprise the majority of cells in the peripheral blood mobilized by the combination of G-CSF and Plerixafor (GP). The constitution of immune cell subsets in mobilized peripheral blood was determined by flow cytometric analysis with corresponding antibodies (B cell: CD3⁻CD19⁺, T cell: CD3⁺NK1.1⁻; NK cell: CD3⁻NK1.1⁺; Neutrophil: Gr1^{high}CD11b⁺; Monocyte: Gr1^{med}CD11b⁺; DC: CD11c⁺). Shown are the summarized data from 3 mice in one of two experiments with similar data. Error bars represent S.D. FP indicates the combination of FLT3L and Plerixafor.

Supplemental Figure 5. Synergistic effect of FLT3L and Plerixafor on the colony forming units (CFU) in mobilized cells. The numbers of colony forming units in mobilized blood were determined as described in Figure 1B. The increase of CFU by each mobilization regimen is presented as fold ratio to PBS control. The paired bars compare the additive effect of FLT3L and Plerixafor each alone (left, composite bar) versus the effect of the combination (right, black bar). A two-way ANOVA model was applied to evaluate the synergistic effect of FLT3L and Plerixafor. $P < 0.001$ was obtained for the synergistic effect test.

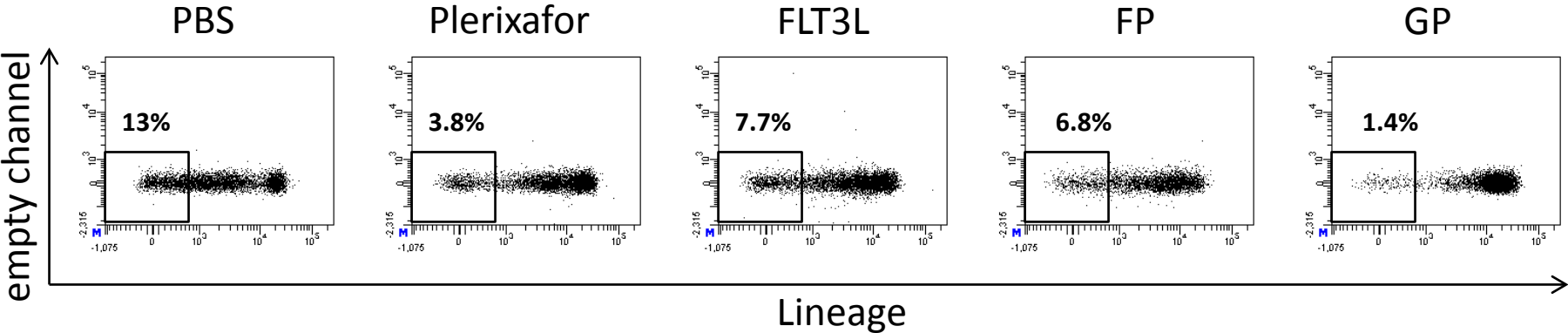
Supplemental Figure 6. Frequencies of Treg cells in the mobilized peripheral blood. Mice were treated with different mobilizing regimens as described in the Materials and Methods section, and total white blood cells were stained and subjected to flow cytometric analysis of

Treg cells. None of the regimens increased Treg cell percentages in mobilized peripheral blood (right). Shown are the summarized data from 3 mice. Error bars represent S.D.

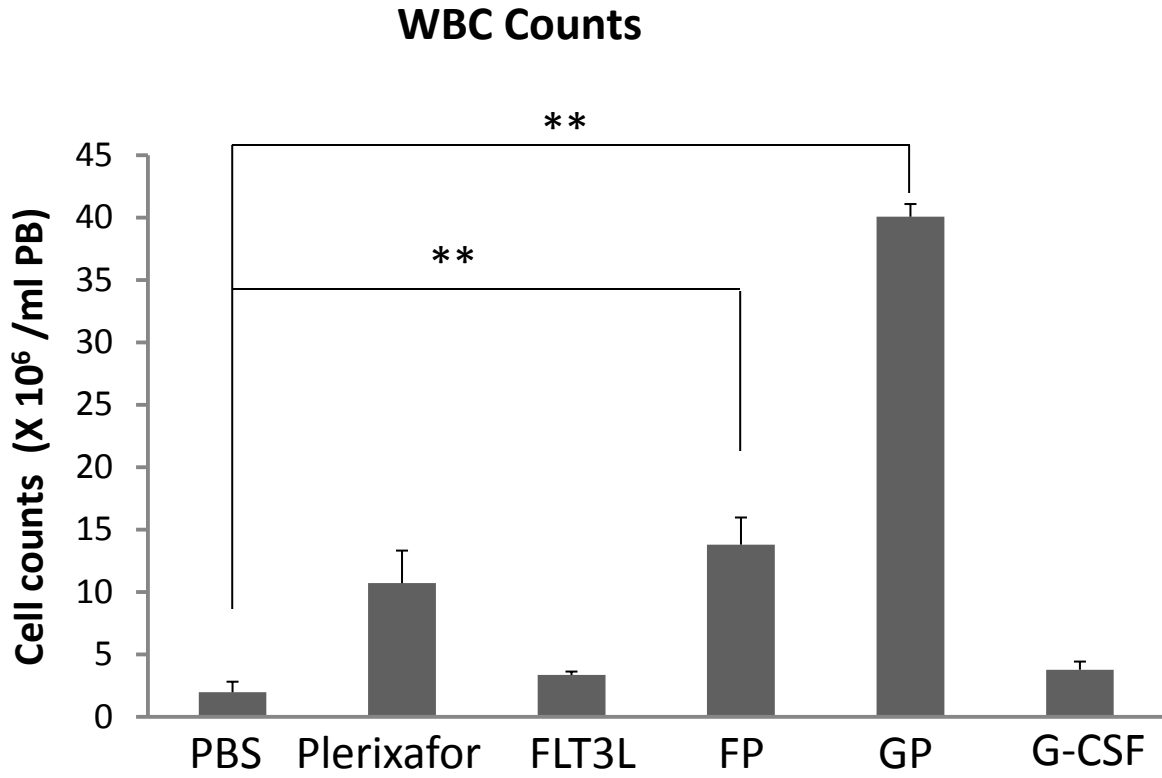
Supplemental Figure 7. LSK flow cytometric analysis of the mice with syngeneic transplantation of mobilized peripheral blood cells depleted of red blood cells. Lethally irradiated C57BL/6 mice were transplanted with grafts mobilized by different regimens. At four months post-transplantation, the surviving mice that were recipients of grafts mobilized by the combination of FLT3L and **Plerixafor (FP)** or the combination of G-CSF and **Plerixafor (GP)** were sacrificed, and the HSCs in the bone marrow were determined using flow cytometry. Shown are representative data from 1 out of 3 mice with similar results. Syngeneic mice receiving grafts mobilized by FP generated more HSCs in bone marrow than the mice receiving grafts from mobilized by **GP**.

Supplemental Figure 8. Mice receiving allogeneic grafts mobilized by the combination of FLT3L and **Plerixafor (FP) established both long-term HSCs and short-term HSCs in bone marrow.** Lethally irradiated BALB/c mice were transplanted with grafts from C57BL/6 mice that were mobilized by indicated regimens. At four months post-transplantation, the surviving mice were sacrificed. The long-term HSCs (LT-HSCs, defined as $\text{Lin}^- \text{Sca-1}^+ \text{c-Kit}^+ \text{CD34}^- \text{CD135}^-$) as well as the short-term HSCs (ST-HSCs, defined as $\text{Lin}^- \text{Sca-1}^+ \text{c-Kit}^+ \text{CD34}^+ \text{CD135}^-$) in the bone marrow were determined by flow cytometric analysis. All cells were gated on H2K^b (C57BL/6 source). Shown are representative data from 1 out of 5 mice with similar results.

Supplemental Figure 1

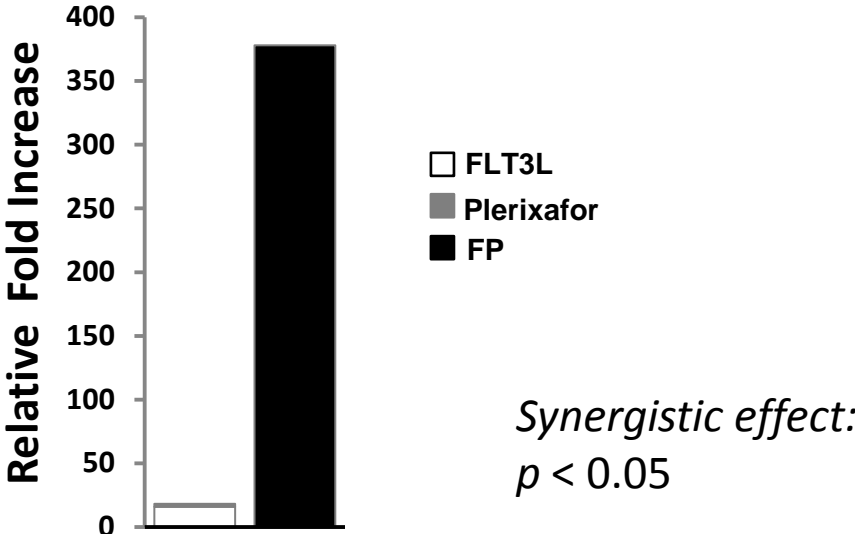


Supplemental Figure 2

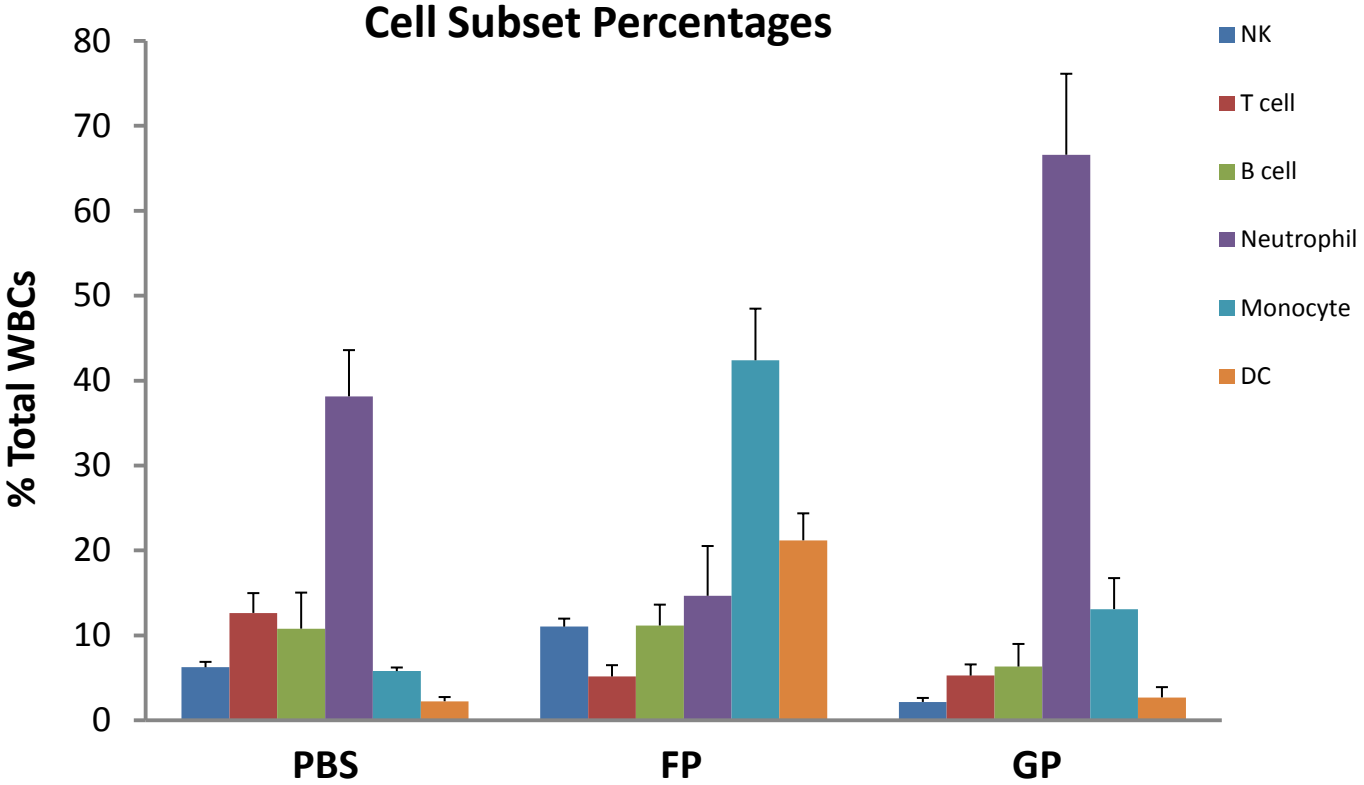


Supplemental Figure 3

Synergistic Effect on LSK Cell Mobilization

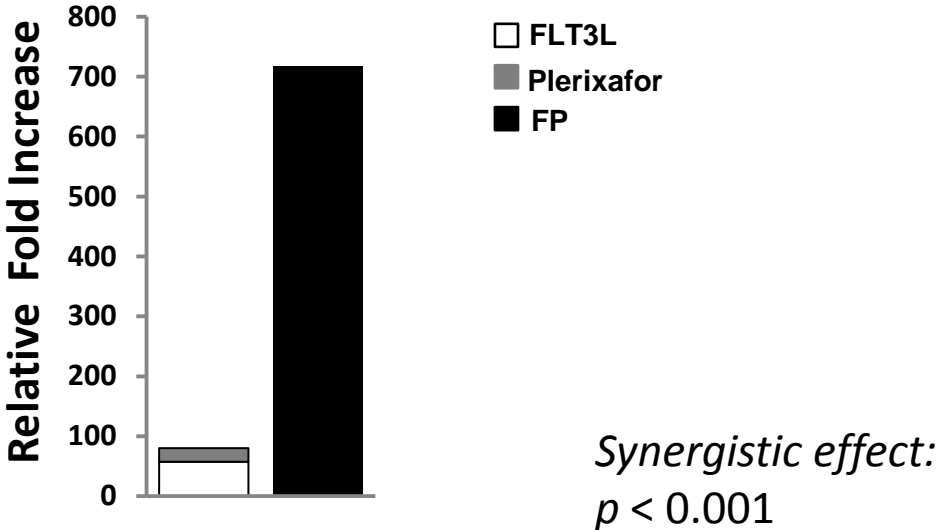


Supplemental Figure 4

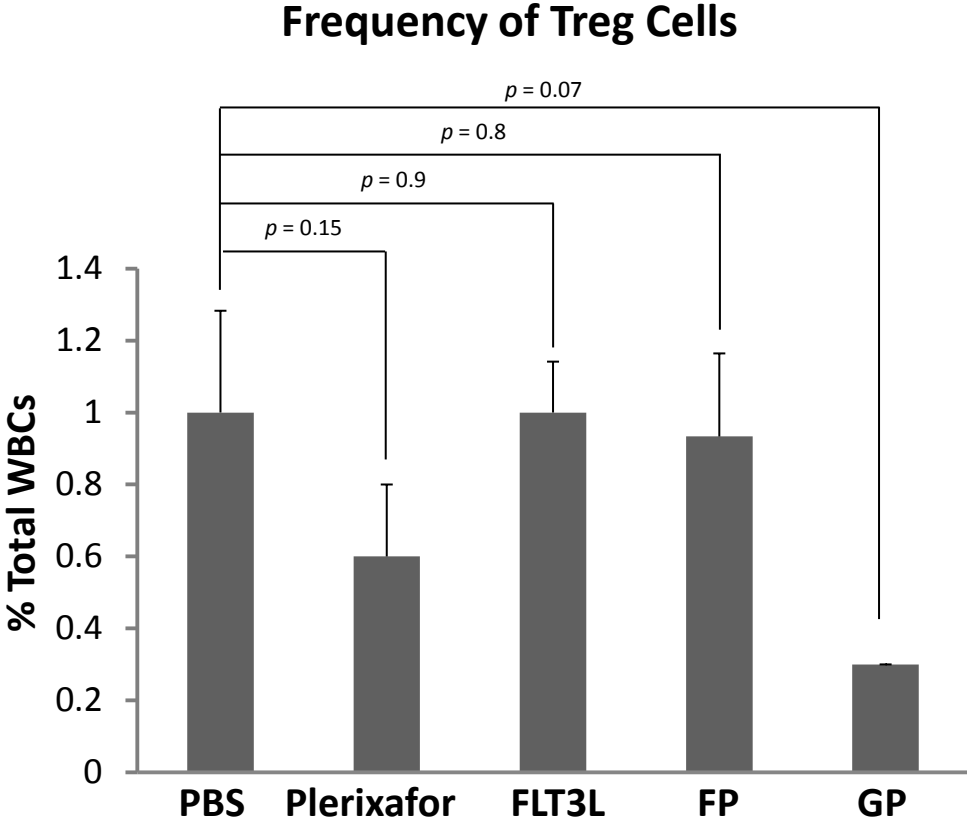


Supplemental Figure 5

Synergistic Effect on CFU Generation



Supplemental Figure 6



Supplemental Figure 8

FP

