

## Analysis of Dendritic Cell Content Within Allografts Mobilized Following G-CSF or GM-CSF Does Not Reveal Significant Differences in DC1 or DC2 Subsets

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**Origin of Study** USA

**Type of Study** FLOW-CYTOMETRIC ANALYSIS OF DENDRITIC CELL CONTENT

**Objectives** Analyze dendritic cell (DC) content within leukapheresis products obtained from peripheral blood progenitor cell (PBPC) donors following mobilization with granulocyte colony-stimulating factor (G-CSF, filgrastim) alone, granulocyte-macrophage colony-stimulating factor (GM-CSF, sargramostim) alone, or a combination of filgrastim and sargramostim.

Relate any differences found in DC content to the incidence of moderate to severe (grades 2–4) acute graft-versus-host disease (GVHD) in recipients of PBPC allografts mobilized with different cytokine regimens

**Study Design** Aliquots of cells taken from the original leukapheresis product were thawed for this analysis.

DC1 was identified among viable cells as lineage negative (lin<sup>-</sup>) CD1c<sup>+</sup> cells and DC2 as Lin<sup>-</sup>,BDCA-2<sup>+</sup> cells using flow cytometry.

The expression of the costimulatory molecule CD86 was analyzed on both DC subsets contained within the allografts.

**Patients** All donors had to have an HLA-identical sibling recipient, be medically eligible, and be willing and able to provide informed consent. Sixty-five donors received filgrastim 10 µg/kg daily, 70 received filgrastim 10 µg/kg plus sargramostim 5 µg/kg daily, 10 received sargramostim 10 µg/kg daily, and 21 received sargramostim 15 µg/kg daily. Leukapheresis was started on day 5 except for the last group (day 6).

**Observations** Ten leukapheresis products were analyzed in each group. The results of the analysis are given in the table below:

**Median Dendritic Cell Content of Allografts**

CYTOKINE	DC1 × 10 <sup>6</sup> /kg	DC2 × 10 <sup>6</sup> /kg	DC1/DC2
Filgrastim + sargramostim	1.2	1.6	0.71
Filgrastim alone	0.91	0.78	1.43
Sargramostim alone	0.88	0.68	1.29
<i>P</i> value	NS	0.007 <sup>a</sup>	NS

<sup>a</sup> Probability value for filgrastim + sargramostim vs filgrastim or sargramostim alone; all other comparisons are nonsignificant (NS).

The leukapheresis products obtained from the group treated with a combination of filgrastim and sargramostim contained the highest quantity of DC2. There were no significant differences, however, in total DC1 or DC2 content in either the filgrastim-alone or sargramostim-alone groups, nor were there significant differences in the DC1/DC2 ratio.

The proportion of DC2 expressing CD86 in the filgrastim-mobilized group was significantly higher than in the sargramostim-mobilized group (mean, 15% vs 0.1%). No differences were observed in CD86 expression on DC1 subsets within any group.

## Dendritic Cell Content Within Allografts Mobilized Following G-CSF or GM-CSF Mobilization

### Conclusions

This analysis did not reveal significant differences in DC1 or DC2 content between filgrastim- or sargramostim-mobilized leukapheresis products. Further analysis of qualitative differences in DC, T cells, or other effector cells mobilized by various cytokines or chemokines appears warranted in order to understand why apparent differences in the risk of GVHD exist.

### Discussion

Patients receiving HLA-identical sibling PBPC allografts mobilized by using sargramostim (Leukine, Prokine) have been reported to have a reduced risk of moderate to severe (grades 2–4) GVHD compared with recipients of allografts mobilized by filgrastim (Neupogen) alone or combined with sargramostim. To test the hypothesis that one of the reasons for the difference in risk may be related to differences in DC content, Devine and colleagues analyzed DC content in leukapheresis products obtained from PBPC donors following mobilization with the three different cytokine regimens.

Donors had to have an HLA-identical sibling recipient, be medically eligible, and be willing and able to provide informed consent. In addition to having an HLA-identical sibling donor available, recipients had to be between 18 and 70 years old and have an advanced hematological malignancy, ECOG performance status of 0–1, and adequate organ function.

The conditioning regimen consisted of cyclophosphamide (60 mg/kg per day) on days –3 and –2; single-dose total body irradiation (550 cGy) at 30 cGy/min on day –1; and PBPC transplantation on day 0. GVHD prophylaxis was cyclosporine at 3 mg/kg actual body weight, beginning day 1, with a target level of 200–400 ng/mL. No methotrexate was given. Growth factor post transplantation was filgrastim at 5 µg/kg subcutaneously beginning on day +1 and continuing until the absolute neutrophil count (ANC) was > 1,500/µL for 3 consecutive days.

As in previous reports, the incidence of grades 2–4 GVHD was lower in the two cohorts receiving sargramostim alone. Two of 10 patients (20%) in the 10-mg sargramostim cohort and 2 of 21 patients (10%) in the 15-mg sargramostim cohort had grade 2 GVHD; none of the patients in either cohort had grade 3 or 4 GVHD. These results compare to combined totals for grades 2–4 GVHD of 32 (49%) patients in the filgrastim and 48 (69%) in the filgrastim + sargramostim group.

No significant difference was found in total DC1 or DC2 content or in the DC1/DC2 ratio in allografts mobilized using either filgrastim or sargramostim, but allografts mobilized following filgrastim + sargramostim had the highest quantity of DC2. The researchers advised that since frozen cells were analyzed and post-thaw viability was low, the results should be interpreted with caution. They called for further analysis of both quantitative and qualitative differences in DC, T cell, and other effector cells mobilized by various cytokines and chemokine inhibitors.

### Key Points

- The incidence of grades 2–4 GVHD was lowest in patients receiving sargramostim alone.
- No significant difference was found in the total DC1 or DC2 content or in the DC1/DC2 ratio in allografts mobilized using either filgrastim or sargramostim.
- Further analysis of quantitative and qualitative differences in DC, T cell, and other effector cells mobilized by cytokines and chemokine inhibitors is warranted.

### References

- Arpinati M, Green CL, Heimfeld S, Heuser JE, Anasetti C. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood* 2000;95:2484–2490.
- Devine SM, Adkins DR, Khoury H, et al. Impact of GM-CSF mobilization on the composition of donor allografts and the risk of acute GVHD following HLA-matched hematopoietic cell transplantation. *Blood* 2002;100:175a. Abstract 655.
- Devine SM, Ritchey J, DiPersio JF. Analysis of dendritic cell content within allografts mobilized following G-CSF or GM-CSF does not reveal significant differences in DC1 or DC2 subsets. Poster presented at the 45th Annual Meeting of the American Society of Hematology; December 6–9, 2003; San Diego, Calif. Abstract 1679.