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To the editor:

Toxic effects of sorafenib when given early after allogeneic hematopoietic stem cell transplantation

We read with interest the article by Metzelder et al showing sorafenib had antileukemic activity and could be given safely to patients with FLT-3 mutated AML relapsing after allogeneic stem cell transplantation (ASCT).[1] Because sorafenib delays progression of renal cell carcinoma, we administered this drug to patients who had progression of metastatic kidney cancer after an ASCT. Besides the classic sorafenib hand-foot syndrome, we also observed new-onset, biopsy-confirmed chronic graft-versus-host disease (cGVHD) of the skin (including one case of sclerodermoid cGVHD) and exacerbations of preexisting chronic skin GHVD in 4 of 7 patients treated with 400 mg of sorafenib given orally twice daily. Metzelder et al also noted cGVHD occurred in 2 of 4 patients receiving sorafenib after ASCT, although the temporal association of this drug with cGVHD is unclear from their study. Although the authors speculate sorafenib might be effective when given prophylactically after an ASCT to reduce FLT-3 mutated AML leukemia burden, it is important to note that the initiation of sorafenib in the 4 patients in their study was delayed months after the transplantation (87-322 days). In vitro and murine findings from our laboratory raise the concern that sorafenib may result in substantial toxicity and increase the risk of GVHD when this drug is administered early after a T cell–replete ASCT.

Using a major histocompatibility complex (MHC)–matched murine model of ASCT, we explored whether sorafenib would slow tumor progression potentially facilitating GVT effects in mice with established RENCA tumors. Balb/C mice conditioned with 950cGy total body irradiation received either a T cell–depleted (bone marrow alone) or T cell–replete (bone marrow plus splenocytes) ASCT from MHC-matched, minor antigen–mismatched B10.d2 donors. Nontransplanted tumor-bearing Balb/C control mice that received sorafenib by oral gavage (60 mg/kg/day) had no evidence of drug toxicity and had slower tumor growth which improved survival compared with mice not receiving sorafenib (median survival 49 vs 34 days, respectively; $P = .04$). In recipients of a T cell–depleted ASCT, sorafenib by oral gavage was not associated with overt toxicities and also delayed tumor progression and

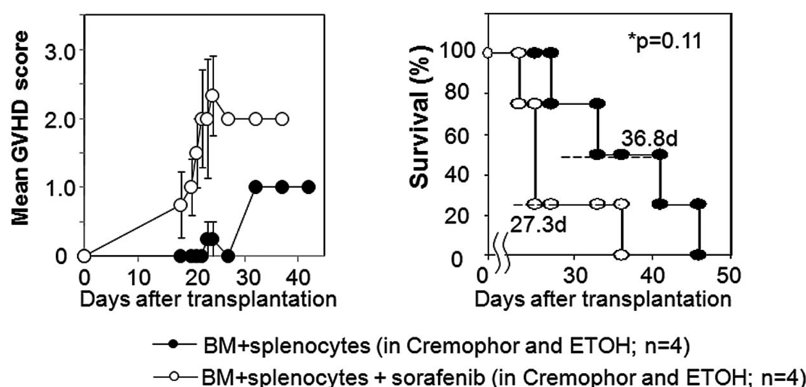

Figure 1. Sorafenib worsens GVHD and shortens survival when given after a T cell–replete allogeneic SCT in mice with RENCA tumors. GVHD score and survival in tumor bearing mice undergoing allogeneic SCT using BM + splenocytes with or with sorafenib given by oral gavage after transplantation. GVHD score was assessed by the following symptoms: alopecia (0-4 points), hunched posture (0-2 points), ear or eye irritation (0-1 points). Error bars (left panel) show standard error of the mean.

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improved survival compared with nonsorafenib controls (median survival 42 vs 31 days; $P < .01$). Remarkably, T cell–depleted transplant recipients that received sorafenib had no evidence of organ toxicity or GVHD at autopsy. In contrast, there was a surprising and significant increase in clinical GVHD (Figure 1) and histologically confirmed severe skin and liver GVHD ($P = .0023$) with a trend toward shortened survival when sorafenib was administered to recipients of a T cell–replete SCT (Figure 1). Blood samples showed a nonsignificant increase in the percentage of $CD3^+$ T cells in mice that received a T cell–replete ASCT with sorafenib versus without sorafenib (62% ± 29% vs 28% ± 10%; $P = .26$).

Although the exact mechanism through which this agent enhances GVHD in vivo remains under investigation, correlative in vitro studies using human peripheral blood mononuclear cells obtained from healthy volunteers revealed OKT3-induced T-cell proliferation increased significantly in the presence of sorafenib (median stimulation index 4.6; range, 1.8-10.8; $P = .035$). These preclinical murine studies and early observations in humans raise the concern that sorafenib may exacerbate GVHD, and imply the early or prophylactic use of this agent after a T-cell replete ASCT for FLT-3-ITD–positive AML should be pursued with caution and should be given only in the context of a clinical trial.

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To the editor:

Connecting the dots: monocyte/DC and NK subsets in human peripheral blood

A recent paper in *Blood* reported that lineage (CD3/14/19)–negative $CD56^+$ mononuclear cells in human peripheral blood contain 2 populations: bona fide $CD7^+$ natural killer (NK) cells and $CD7^-$ monocyte/dendritic cells (DCs).[1] Whereas this study delivers a valuable improvement in the definition of peripheral blood NK cells, it leaves unresolved the question of which monocyte/dendritic cells were found to express CD56.

Approaching this problem from the other direction, we characterized the CD56 expression of monocytes and blood DC subsets from healthy volunteers. Figure 1 revisits the analysis of Milush et al with additional intermediate plots to show the location of monocytes and DCs.

Figure 1A shows the distribution of CD56 among lineage (CD3/14/19/20)–negative cells, as previously illustrated by Milush and colleagues, in comparison with isotype control. Anti-CD56 clone MY31 (IgG1) from Becton Dickinson was used.

Figure 1B then maps on top of these dot plots (now shown in gray as background) the major populations of $CD14^+$ monocytes, $CD16^+$ monocytes/DC, $CD123^+$ plasmacytoid DCs (PDCs), and CD11c myeloid DCs,[2] to reveal where CD56 expression appears above isotype.

Figure 1C shows the key results as histograms. A defined population, 12.8% to 15.4% (range; n = 3), of CD11c myeloid DCs express CD56. A small population of $CD14^+$ monocytes also express CD56 as previously described.[3,4] $CD16^+$ monocytes and $CD123^+$ PDCs do not express significant CD56. Because Milush and colleagues used $CD14^+$ in their lineage cocktail, $CD14^+$ monocytes are excluded. We therefore identify the $CD56^+$ cells described in their article as a subset of $CD11c^+$ myeloid DCs.

A further contribution of these authors was to show that, although certain DCs fall into lymphoid side scatter gates and express CD56, these cells do not express CD7. CD7 antibodies are therefore a potentially useful addition to the lineage cocktail for DC biologists eager to exclude NK cells from their analysis. The utility of this strategy critically requires that no other DCs express CD7. We confirmed that $CD11c^+$ myeloid DCs are negative for CD7 but found a population, 15.2% to 22.8% (range; n = 3), of $CD123^+$ PDCs expressing CD7 (Figure 1C). Within the lineage-negative DR^+ gate, approximately 10% of $CD34^+$ peripheral blood stem cells also expressed CD7, although these are not typically included as a subset of DCs.

Finally, we compared the addition of CD7 and CD56 singly or in combination to a lineage cocktail of CD3/14/19/20 (Figure 1D) in a plot of HLA-DR versus lineage. It is evident that the addition of both antibodies improves the separation of NK cells (depicted in black) from the DR^+ $lineage^-$ region containing monocytes and DCs (background, gray). This is particularly useful for excluding activated NK cells that express HLA-DR. However, as we have now shown, the caveat is that CD7 and CD56 each removes a population of $CD123^+$ PDCs or $CD11c^+$ myeloid DCs, respectively. Whether these subpopulations of DCs have distinct functional properties remains to be determined.

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