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LETTER TO THE EDITOR

Clofarabine and CY do not yield reliable engraftment of hematopoietic stem cells

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Purine analogs, particularly fludarabine, have recognized immunosuppressive properties, making them useful for conditioning patients for engraftment with allogeneic hematopoietic cells. Fludarabine is immunosuppressive enough that its non-transplant use poses significant risks of transfusion-related GVHD after transfusion of unirradiated blood products.[1] Clofarabine has attractive anti-leukemic activity[2,3] and its chemical structure suggests immunosuppressive properties similar to fludarabine.[4] Accordingly, we chose this purine analog to replace fludarabine in combination with CY[5] as non-myeloablative pre-transplant conditioning.

After obtaining institutional review board-approved informed consent, eight patients with poor-prognosis hematological neoplasms were treated with clofarabine 30 mg/m^2 i.v. daily on days −7 through −3 (five doses) and CY 500 mg/m^2 i.v. on days −7 and −6 (Clo-CY). All received sirolimus, tacrolimus and MTX immunoprophylaxis, as described for patients treated with fludarabine and CY.[5] On day 0, all received matched donor (six siblings and two unrelated donors) G-CSF-mobilized progenitors (range $4-5\times10^6$ cells/patient kg body weight, mean 4.86×10^6). Recovery to 500 neutrophils/μL (ANC > 500) was at a median of day 13 (range 7–21 days, but one patient died on day 18 without ANC > 500). Only three of eight developed stable donor engraftment (defined here as 20% peripheral blood donor cells by day 30), though one additional patient engrafted after an additional infusion of donor cells on day 59. Two of three patients engrafting stably (the initial two treated) were the only two of the total eight to have had prior autologous transplants. To verify that this experience was different from our prior data, we analyzed engraftment in two groups of consecutively treated (otherwise unselected except as specified) retrospective controls. All these controls received fludarabine 25 mg/m^2 IV daily days −7 through −3 (five doses) and CY 1000 mg/m^2 IV days −7 and −6 (Flu-CY) and were selected for three criteria deemed relevant to engraftment. All controls received identical immunoprophylaxis to that received by the Clo-CY group, SCT of $<5\times10^6$ cells/patient kg body weight of G-CSF-mobilized CD34$^+$ progenitors, and none had received prior autologous transplantation. In this way, 7 matched sibling SCT recipients (referred to below as MSib-FC) and 43 unrelated donor recipients (matched or mismatched for 1–2/8 HLA Ags–referred to below as URD-FC) were identified and analyzed. All patients in the MSib-FC and 39 of 43 in the URD-FC groups showed stable engraftment. Mean donor engraftment in the three groups are plotted over time in Figure 1. Clo-CY-conditioned patients are shown in solid, and a Clo-CY subgroup without prior autograft (six patients) is also shown. Using mixed-effects analysis of variance models for repeated measures across five time points, Clo-CY patients were found to be significantly slower to engraft than either MSib-FC or URD-FC groups. Specifically, for comparisons (using five days of engraftment testing) of the Clo-CY group vs MSib-FC, $P=0.0260$, and for Clo-CY vs URD-FC, $P=0.0118$. Analysis of the six Clo-CY* patients (no prior autologous transplant–directly comparable to the MSib-FC or URD-FC groups) yielded differences vs MSib-FC $P=0.0009$, and for URD-FC $P=0.0004$. Thus, across all observation dates, both of the two control groups (MSib and URD) were significantly different from the clofarabine-conditioned groups (Clo-CY or Clo-CY*).

In order to establish the relative myelosuppressive properties of the Clo-CY regimen vs Flu-CY, we compared the time taken in achieving ANC > 500 in two groups failing to achieve at least 10% donor engraftment by day 12–18 (our initial assessment of chimerism). Six such patients treated with Flu-CY and similar immunoprophylaxis (one had been excluded from the above analysis because of having received $>5\times10^6$ donor CD34 cells/kg body weight) achieved ANC > 500 at days 8–10 (mean 9 days). Those five patients not engrafting stably after Clo-CY recovered to ANC > 500 at 8–24 days (mean day 17 –one, in fact, expired without recovery of neutrophils on day 18). Although these groups were not statistically different in time taken for neutrophil recovery ($P=0.092$–Mann–Whitney test), it seems safe to conclude that the Clo-CY regimen is at least as myelosuppressive as Flu-CY.

From the above data we conclude that Clo-CY, although significantly myelosuppressive, is insufficiently immunosuppressive to yield reliable engraftment in most patients receiving allo-SCT. Other groups have published effective regimens utilizing clofarabine in allotransplant conditioning,[6–10] but all of these investigators employed co-administered agents that are far more myelosuppressive (high dose melphalan[6,7] or BU[8–10]) or immunosuppressive (alemtuzumab[6]) than that employed in our study. Although the CY dose, which we used was smaller than that in the Flu-CY-treated patients we report above, the regimen is at least as myelosuppressive as Flu-CY. Clo-CY, as described here, employed a lower CY dose than used Flu-CY, thus we cannot formally exclude the possibility that the difference in engraftment seen relates to the CY dose. It seems most reasonable to conclude that clofarabine is significantly less immunosuppressive than fludarabine; however, formal proof of this hypothesis will require other studies. This has, however, other implications for clofarabine

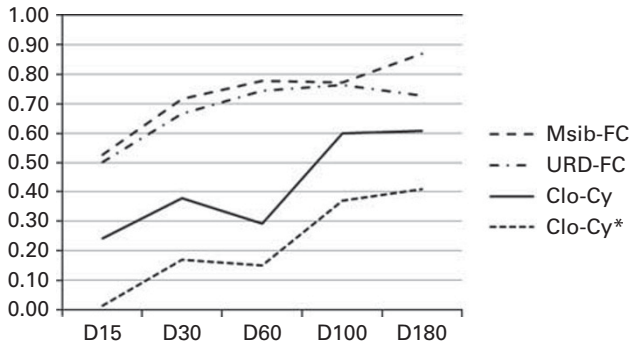

Figure 1. Peripheral blood (PB) engraftment of donor cells post transplant. Whole blood chimerism was determined by RFLP analysis or XY FISH at days 15, 30, 60, 100 and 180 post-transplant. Mean PB donor engraftment is shown for MSib-FC (seven patients), URD-FC (43) and Clo-CY (8) groups. The subgroup Clo-CY* (6), from which two patients who had prior autologous transplants are excluded, is also shown. MSib-FC and URD-FC groups engraft significantly more efficiently and rapidly.

use in non-transplant settings. Unlike the experience with fludarabine,[1] there have been no reports of transfusion-associated GVHD associated with its use, thus irradiation of blood products after its use may be unnecessary (but has not been recommended). For now, it seems most logical not to rely on clofarabine as an immunosuppressive agent for promotion of allogeneic stem cell engraftment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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