To the editor:

Outcome of major ABO-incompatible nonmyeloablative hematopoietic stem cell transplantation may be influenced by conditioning regimen

We read with interest the recent paper by Bolan et al documenting delayed donor red cell chimerism following major ABO-incompatible nonmyeloablative compared to myeloablative hematopoietic stem cell transplantation and an associated increased incidence of pure red cell aplasia (PRCA). The authors demonstrated a correlation between a delayed fall in antidonor isohemagglutinin titer and the onset of red cell chimerism, and proposed that the persistence of a greater reservoir of functional host B cells and plasma cells in the setting of nonmyeloablative stem cell transplantation (NST) may be responsible. Of interest, they noted that early severe graft-versus-host disease (GVHD) was associated with a rapid decrease in antidonor isohemagglutinin titer consistent with a graft-versus-plasma cell effect. They also noted that cyclosporine (CsA) withdrawal was necessary before resolution of PRCA and commented that this may relate to its inhibitory action on T- but not B-cell function. Those cases with early evidence of complete donor myeloid chimerism appeared to be at the greatest risk of PRCA.

Our experience with an alternative nonmyeloablative conditioning regimen (alemtuzumab [CAMPATH-1H] 20 mg/d on days −4, fludarabine 25 mg/m²/d on days −7 to −3, and melphalan 140 mg/m² on d −2) differs. We have given transplants from major ABO-incompatible donors to 19 patients using this regimen. All patients received CsA as GVHD prophylaxis from day −1 and continued it for at least 90 days after transplantation before withdrawal. None of the donors or recipients had evidence of alloantibodies prior to transplantation. One patient developed secondary graft failure with subsequent reconstitution of autologous hematopoiesis, confirming the nonmyeloablative nature of the conditioning protocol. Fifteen of the remaining 18 survived for more than 90 days after transplantation (Table 1). The low incidence of GVHD previously reported with this regimen was no different in this subgroup of patients with major ABO-incompatibilities, and there was no apparent association between the occurrence of GVHD and the time to detection of red cell chimerism as assayed by the detection of cells bearing donor red cell ABO antigens (Table 1). Early conversion to full donor myeloid chimerism assessed by means of PCR analysis of informative minisatellite regions is a common feature of this conditioning protocol, perhaps reflecting its greater myelosuppressive activity compared to some of the alternatives reported in the literature. Donor lymphocyte infusions (DLI) for mixed chimerism or residual disease were administered from 6 months after transplantation. In only 1 case (patient 02) was DLI given earlier than this for disease progression (day +144). Despite the relatively prolonged usage of CsA, a low incidence of GVHD, and early full donor myeloid chimerism, none of the patients developed PRCA. Two patients (14 and 15) remained red cell transfusion-dependent for more than 6 months after transplantation. Transfusion with group A red cells may have delayed detection of donor red cells in these cases. Neither had an absolute reticulocytopenia (44 × 10⁹/L and 48 × 10⁹/L, respectively). Restaging bone marrow trephines at 3 and 6 months after transplantation from patient 14 were normocellular with 5% to 10% plasma cells and 21% to 26% nucleated red cell precursors. The patient was subsequently diagnosed with tuberculosis based upon aspiration of an enlarged cervical lymph node and became independent of red cell transfusion following appropriate therapy. Patient 15 had persistent pancytopenia at 6 months after transplantation (neutrophil count 1.6 × 10⁹/L, platelet count 57 × 10⁹/L) and bone marrow trephine showed hypocellularity in all lineages with focal grade III fibrosis.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Patient and diagnosis</th>
<th>ABO</th>
<th>Donor</th>
<th>First donor RBC</th>
<th>Full donor myeloid</th>
<th>Full donor T</th>
<th>Full donor B</th>
<th>Acute GVHD</th>
<th>Response and status (d after transplantation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 CLL A→O Sib</td>
<td>151</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>No</td>
<td>CR A (518)</td>
<td></td>
<td></td>
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<tr>
<td>02 MM A→O MUD</td>
<td>44</td>
<td>26*</td>
<td>26*</td>
<td>26*</td>
<td>No</td>
<td>Prog D (427)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>03 LGNHL A→O Sib</td>
<td>64</td>
<td>88*</td>
<td>459</td>
<td>88*</td>
<td>No</td>
<td>CR A (725)</td>
<td></td>
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</tr>
<tr>
<td>04 MM A→O MUD</td>
<td>67</td>
<td>88*</td>
<td>238</td>
<td>88*</td>
<td>No</td>
<td>PR A (725)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>05 HGNHL A→O MUD</td>
<td>49</td>
<td>45*</td>
<td>286*</td>
<td>45*</td>
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<td>32*</td>
<td>335</td>
<td>32*</td>
<td>No</td>
<td>Prog A (438)</td>
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<tr>
<td>07 HD B→O Sib</td>
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<td>43*</td>
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<td>CR A (1496)</td>
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<tr>
<td>08 MM B→O MUD</td>
<td>53</td>
<td>87*</td>
<td>87*</td>
<td>87*</td>
<td>Gd 2</td>
<td>Prog A (731)</td>
<td></td>
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</tr>
<tr>
<td>09 CML A→B Sib</td>
<td>88</td>
<td>195*</td>
<td>728</td>
<td>195*</td>
<td>No</td>
<td>CR A (910)</td>
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<tr>
<td>10 MM A→B MUD</td>
<td>60</td>
<td>25*</td>
<td>469*</td>
<td>25*</td>
<td>Gd 2</td>
<td>Prog A (567)</td>
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<tr>
<td>11 HD B→A Sib</td>
<td>92</td>
<td>32*</td>
<td>32*</td>
<td>32*</td>
<td>No</td>
<td>CRu A (532)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 HD B→A MUD</td>
<td>91</td>
<td>32*</td>
<td>32*</td>
<td>32*</td>
<td>No</td>
<td>CRu A (98)</td>
<td></td>
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<tr>
<td>13 LGNHL AB→A MUD</td>
<td>39</td>
<td>45*</td>
<td>45*</td>
<td>45*</td>
<td>Gd 2</td>
<td>CR A (433)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 MM AB→A Sib</td>
<td>133</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>No</td>
<td>PR A (525)</td>
<td></td>
<td></td>
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<tr>
<td>15 LGNHL AB→A MUD</td>
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<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>No</td>
<td>PR A (238)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CLL indicates chronic lymphocytic leukemia; MM, multiple myeloma; LG/HGNHL, low grade/high grade non-Hodgkin lymphoma; HD, Hodgkin disease; CML, chronic myeloid leukemia; Do, donor; R, recipient; Sib, sibling; MUD, matched unrelated donor; NT, not tested (lack of pretransplant donor material or lack of informative primers); Gd, grade; CR, complete response; Prog, disease progression; PR, partial response; CRu, CR uncertain; A, alive; and D, dead.

+Censored data point.

*First time point tested.

†Isohemagglutinin titers available in 6 cases (median, 128; range, 16-512).
Bolan et al noted that PRCA appeared to be more common in cases with incompatible donor-A antigens, in which host antidonor isohemagglutinins declined less rapidly. Seven of the cases we report involved group A donors with either group O (n = 5) or group B (n = 2) recipients. Pretransplant isohemagglutinin titers were not significantly lower in these NST recipients than those reported by Bolan et al. The differences in outcome between the studies are therefore unlikely to reflect differences in pretransplant serological characteristics, although it remains possible that differences in A antigen expression on the red cell surface according to group A subtype may be important. The lack of available data on isohemagglutinin titers in all cases in both our patients and those of Bolan et al make definitive conclusions difficult. In addition, we do not have data available on the rate of decline of titers following transplantation for direct comparison between the 2 conditioning regimens.

The addition of mycophenolate mofetil to CsA as GVHD prophylaxis resulted in no differences in serologic endpoints in the study of Bolan et al despite its activity against B cells. Although the small sample number makes interpretation difficult, it may, however, be significant that neither of the 2 patients with delayed red cell recovery receiving mycophenolate mofetil subsequent developed PRCA. Since B cells express the target antigen for alemtuzumab (CD52), its inclusion in the conditioning regimen would be expected to result in greater activity against the B-cell compartment. Elimination of recipient B cells and earlier complete B-cell donor chimerism might then result in earlier detection of donor red cell chimerism. Complete donor B-cell chimerism (assessed by means of PCR analysis of informative minisatellite regions of cells enriched by magnetic sorting of CD19-Dynabead [Dynal, Oslo, Norway] labeled cells) occurred early following transplantation (at the first time point analysed in all patients in whom data was available [n = 12]). This may not accurately reflect the situation in all tissues but could provide part of the explanation for the differences reported. Only 3 of the 15 patients in our series had first donor red cell chimeraism detected after day 100, and in 2 of these (patient 14 and 15) alternative causes of prolonged red cell transfusion requirements may have delayed detection of donor red cell antigens, particularly as they both involved group A recipients of group AB grafts (low density B antigen) receiving group A red cells.

Thus it is likely that the incidence of delayed donor red cell chimerism and PRCA may differ not only in myeloablative compared to nonmyeloablative transplants, but also according to the type of nonmyeloablative conditioning regimen and perhaps also the degree of B-cell suppression achieved.

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References


Response:

Parameters of erythropoietic function after major ABO-incompatible hematopoietic stem cell transplantation: implications following nonmyeloablative conditioning

We appreciate the interest in our work by Peggs et al and thank the editor for the opportunity to respond to their observations.

In our report,1 we specifically highlighted the critical impact that the transplant approach and conditioning regimen have on host-donor immune interactions and hematopoiesis following major ABO-incompatible hematopoietic stem cell transplantation. We compared prospectively a cohort of patients receiving nonmyeloablative conditioning with a concurrent series receiving myeloablative conditioning, and we identified differences between our regimen and previous cases described following reduced intensity conditioning.2 It is important to note that in contrast to conventional myeloablative transplants, where a relatively small number of “standardized” regimens are utilized, there exists a wide diversity of reduced intensity regimens currently under clinical evaluation. Whereas the fludarabine- and cyclophosphamide-based regimen used in our study was clearly nonmyeloablative,3 documented in all patients by a substantial and often prolonged degree of autologous hematopoietic recovery, the data in Table 1 from Peggs et al indicate that all patients had 100% donor myeloid engraftment (no autologous myeloid chimeraism) at the first time point tested, raising the possibility that the relatively high-dose melphalan (140 mg/m²), fludarabine, and alemtuzumab (CAMPATH-1H) regimen actually “myeloablated” many patients. Although data relevant to the effect are not provided, one could speculate that this potent conditioning regimen resulted in substantial eradication of host isohemagglutinin-producing cells analogous to that observed in our patients receiving myeloablative conditioning with cytoxan and total body irradiation. The interesting possibility that alemtuzumab, an agent with profound antilymphocyte activity employed as a therapy of acquired immune cytopenias including pure red cell aplasia (PRCA) outside the transplant setting,4 provided clinically significant additional activity against host isohemagglutinin-producing cells distinct from the potent effects of other agents used in this conditioning regimen cannot be determined from the limited data provided. Further interpretation of Peggs et al’s observations is impaired by the fact that chimeraism assays were often first obtained at distant posttransplantation intervals, and relevant data such as reticulocyte counts, isohemagglutinin titers, and forward and reverse ABO type were either not obtained or were evaluated retrospectively in a nonsystematic fashion.

We would like to emphasize that the serologic assessments in our study were determined using standard blood banking tests that are routinely performed during pretransfusion testing and should be readily accessible to clinicians who may wish to monitor these events in their own patients. We found that the initial posttransplantation appearance of circulating donor red blood cells (RBCs) (onset of donor RBC chimeraism) following nonmyeloablative conditioning was strongly correlated with the decline of host-type antidonor isohemagglutinins to levels causing minimal agglutination (1+ +) of donor-type RBCs with undiluted patient plasma. This
relationship, similar to that previously described following myelo-
ablative conditioning, is consistent with antibody-mediated inhibi-
tion of donor erythropoiesis by persistent circulating isohemagglu-
tinins. However, the pace of decline of isohemagglutinins was
markedly delayed following our nonmyeloablative regimen, and,
consequently, the appearance of donor-type RBCs in peripher-
al blood was also markedly delayed in the patients receiving
nonmyeloablative conditioning. Similar data have recently been
described in patients receiving an alternative nonmyeloablative
conditioning regimen.7

We noted that in the nonmyeloablative setting, the clinical conse-
quences of these events are determined by the competency of pretrans-
plantation recipient erythropoietic function and the relative timing
between conversion to full donor hematopoiesis (absent recipient
hematopoiesis) and the decline of antidonor isohemagglutinins to
clinically insignificant levels. Patients converting to full donor hem-
atopoiesis before isohemagglutinins decline to low levels will lack compe-
tent erythropoiesis from either recipient or donor, and develop PRCA.
In our report, this event occurred in association with an early conversion
to full donor myeloid chimerism; however, PRCA can also develop in
patients with a delayed conversion when circulating isohemagglutinins
remain persistently elevated. Indeed, we have observed long-lasting
PRCA develop more than 3 months after the transplant in this setting.
Unfortunately, the data provided by Peggs et al lack any assessments of
posttransplantation isohemagglutinin levels, and these relationships
cannot be evaluated in their patients. Interpretation of their observations
is further hampered because myeloid chimerism assessments were not
determined in any of their 3 patients with delayed donor RBC
chimerism (more than 100 days after transplant), and reticulocyte counts
were provided at only a single time point in the 2 RBC transfusion-
dependent patients. We question the assertion that donor-type group A
RBC might not have been detected in 2 patients in Table 1 due to
repetitive transfusion with recipient-type group A RBC. Based on the
sensitivity of serologic assays8,9 and the lack of molecular data in these
patients, we believe it is equally likely that the delayed detection of
donor-type RBCs was due to impaired donor erythropoietic function.

We agree that the information regarding pretransplantation
isohemagglutinin titers presented in Table 1 is limited. Further,
these data are presented in a manner that preclude comparisons
with patient outcomes, either in their study or our own. While we
described a significant correlation between pretransplantation recipi-
ent isohemagglutinin titers and the time until undiluted patient
plasma caused only minimal agglutination of donor-type RBC's
following transplantation, we noted that a variety of other factors,
including ABO blood type and the occurrence of graft-versus-host
disease (GVHD) also impacted posttransplantation isohemagglu-
tinin levels. Isohemagglutinin levels may also have decreased more
rapidly in their cohort due to the frequent use of matched-unrelated
donors,10 or because their patients had hematologic malignancies
and may have received significant prior chemotherapy.11

Finally, we have recently observed resolution of PRCA during
slow tapering of cyclosporine (CsA), in contrast to our study where
PRCA resolved only after CsA discontinuation. While the use of
conditioning agents that decrease the activity of host isohemagglutinin-
producing cells may decrease the incidence of delayed donor red
chimerism and PRCA following major ABO-incompatible nonmy-
eoablative transplantation, adjustment of GVHD prophylaxis and
other methods to enhance donor immune effects against these cell
populations may be required to effectively treat PRCA that
develops in this setting.

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The opinions and assertions are the private views of the authors and do not
represent the official views of the United States government, the Department
of Health and Human Services, or the Department of Defense.

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To the editor:

Effects of adenosine 5'-diphosphate (ADP) receptor blockade on platelet
aggregation under flow

We read with interest the article presented by Dr Turner and
colleagues demonstrating the effects of P2Y1 and P2Y12 adenosine
5'-diphosphate (ADP) receptor blockade on platelet aggregation
and thrombus formation under flow conditions.1 However, we have
serious concerns about their statement, “blockade of P2Y1 alone or
blockade of P2Y1 alone did not reduce thrombus formation on
VWF-collagen surface,” not only because these results are not in
agreement with previously published findings using prodrugs of
thienopyridine antiplatelet agents2 that inhibit the P2Y12 receptor,
but also because they are not in agreement with our own

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