



## How do I approach ABO-incompatible hematopoietic progenitor cell transplantation?

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**H**uman leukocyte antigen (HLA) matching is critically important for successful hematopoietic progenitor cell (HPC) transplantation because the rates of engraftment and transplant outcomes are strongly influenced by the degree of match. This is largely because HLA antigens are expressed on immature pluripotent stem cells. In contrast, ABO incompatibility is not a barrier to successful HPC transplantation because ABO blood group antigens are not expressed on pluripotent or early committed HPCs.<sup>1</sup> HLA and ABO antigens are encoded by different genes; thus it is common that potential donors who are fully HLA matched have an ABO incompatibility.<sup>2</sup> Approximately 40 to 50% of HPC transplants are ABO incompatible and are now performed using bone marrow (HPC-M), apheresis-derived peripheral blood progenitor cells (HPC-A), and umbilical cord blood (HPC-C) as HPC sources. Three types of ABO incompatibility exist: major, minor, and bidirectional. A total of 20 to 25% of HPC transplants have a major ABO incompatibility, 20 to 25% a minor ABO incompatibility, and up to 5% a bidirectional incompatibility.<sup>3</sup> Each presents a unique set of potential adverse consequences, including early acute hemolysis that can be ameliorated through HPC product processing to

remove incompatible red blood cells (RBCs) or plasma (Table 1). In these cases, the recipient must be closely monitored both at the time of the infusion and in the posttransplant period because serious events can occur many days later.<sup>4</sup>

Accreditation agencies such as AABB and the Foundation for the Accreditation of Cellular Therapy (FACT) require that all donors be tested for ABO group and D type before the collection of HPC-A or HPC-M<sup>5,6</sup> and that HPC-C be typed after product processing before cryopreservation.<sup>7</sup> The testing and its required documentation in the medical record need to be done in accordance with institutional standard operating procedures. Current FACT standards also require that “for allogeneic cellular therapy products containing RBCs at the time of administration, a test for ABO group and Rh type shall be performed on the first product collected, or on blood obtained from the donor at the time of the first collection.”<sup>5</sup> At our institution, the donor ABO group and D type is tested as part the donor evaluation process. Additional donor ABO group and D testing is performed on each collection day from blood samples obtained at time of collection. If donor blood sample is not available for a particular collection, confirmatory typing is performed on a product sample on the day of product processing. For all fresh HPC products received from outside facilities, confirmatory ABO group and D testing is performed before further product processing. HPC-C units are received in the frozen state and are not subject to repeat testing.

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**ABBREVIATIONS:** FACT = Foundation for the Accreditation of Cellular Therapy; HPC-A = apheresis-derived peripheral blood progenitor cells; HPC-C = umbilical cord blood-derived hematopoietic progenitor cells; HPC-M = marrow-derived hematopoietic progenitor cells; PRCA = pure red blood cell aplasia; TNC = total nucleated cell.

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### MAJOR ABO INCOMPATIBILITY

A major incompatibility exists when the recipient possesses isoagglutinins directed against the corresponding A or B antigens on the donor's RBCs. Major incompatibilities occur between groups A, B, or AB donors and group O recipients and between group AB donors and group A or B recipients. When RBCs are present in the HPC product, the recipient isoagglutinins can bind the corresponding donor RBC antigens and cause immediate hemolysis.<sup>8</sup> Other potential adverse consequences of a major incompatibility include delayed RBC engraftment and pure RBC aplasia (PRCA) secondary to isoagglutinin production by persistent residual recipient B lymphocytes and/or

**TABLE 1. Types of ABO incompatibility, potential adverse consequences, and recommended interventions**

	Major	Minor	Bidirectional
<b>Definition</b>	<ul style="list-style-type: none"> <li>• Recipient isoagglutinins (anti-A, anti-B, anti-A,B) incompatible with donor RBCs</li> </ul>	<ul style="list-style-type: none"> <li>• Recipient RBCs incompatible with donor isoagglutinins.</li> </ul>	<ul style="list-style-type: none"> <li>• Combination of both incompatibilities</li> </ul>
<b>Donor-recipient ABO pairs</b>	<ul style="list-style-type: none"> <li>• Group A, B, and AB donor and Group O recipient</li> <li>• Group AB donor and group A or B recipient</li> </ul>	<ul style="list-style-type: none"> <li>• Group O donor and group A, B, or AB recipient</li> </ul>	<ul style="list-style-type: none"> <li>• Group A donor and B recipient</li> <li>• Group B donor and A recipient</li> </ul>
<b>Potential adverse consequences</b>	<ul style="list-style-type: none"> <li>• Immediate hemolysis</li> <li>• Delayed RBC engraftment</li> <li>• PRCA</li> </ul>	<ul style="list-style-type: none"> <li>• Immediate hemolysis</li> <li>• Passenger lymphocyte syndrome causing delayed hemolysis</li> </ul>	<ul style="list-style-type: none"> <li>• Combination of potential adverse consequences seen with major and minor incompatibility</li> </ul>
<b>Recommended interventions</b>	<ul style="list-style-type: none"> <li>• RBC reduction if &gt;30 mL RBC and/or if recipient isoagglutinin titers &gt;32</li> <li>• Transfuse ABO appropriate blood products</li> </ul>	<ul style="list-style-type: none"> <li>• Plasma reduction</li> <li>• Close clinical and laboratory observation, between Days +5 and 15 after HPC transplantation for hemolysis (e.g., Hb/Hct, LDH, bilirubin, hemoglobinemia)</li> <li>• Transfuse ABO appropriate blood products</li> </ul>	<ul style="list-style-type: none"> <li>• Combination of interventions used for major and minor incompatibility</li> </ul>
<b>Additional or alternate interventions that may be performed</b>	<ul style="list-style-type: none"> <li>• Recipient's isoagglutinins removal before transplantation via TPE or immunoadsorption</li> </ul>	<ul style="list-style-type: none"> <li>• Replacement of recipient RBCs with donor type via RBC exchange (rarely), rituximab</li> </ul>	<ul style="list-style-type: none"> <li>• Combination of interventions used for major and minor incompatibility</li> </ul>

plasma cells that survived the preparative conditioning regimen that is administered to prevent HPC rejection.<sup>9,10</sup>

In unmanipulated HPC-M, RBCs comprise up to 25 to 35% of the product volume.<sup>11</sup> Because many products can contain 1 to 2 L, the equivalent of 1 or more units of RBCs can be present.<sup>9</sup> In the earlier years of transplantation, approaches to prevent immediate hemolysis from infusion of major mismatched HPC-M included removing recipient isoagglutinins through plasmapheresis using group AB plasma as replacement fluid or by using immunoadsorption columns and/or in vivo adsorption of recipient isoagglutinins by transfusing donor type ABO-incompatible RBCs.<sup>2,4,12,13</sup> These procedures were not without problems and could still be associated with immediate hemolytic transfusion reactions and post-transplant hemolysis due to a rebound in isoagglutinin titers.<sup>4,13</sup> In the 1980s, removal of recipient isoagglutinins by plasma exchange or in vivo adsorption began to fall out of favor after it was shown that removing RBCs from the HPC-M product could effectively prevent acute hemolysis.<sup>14</sup> Most US transplant centers currently do not routinely perform pretransplant plasmapheresis or immunoadsorption. However, several centers, particularly in Europe, still do this either routinely or based on recipient isoagglutinin titers. HPC-A products usually contain very few RBCs (<20 mL). Thus, RBC removal is not usually necessary from these products.

The maximum volume of major mismatched RBCs that can be safely infused is not clearly defined; however, 10 to 30 mL of incompatible RBCs are usually well tolerated by adults.<sup>15</sup> It has been demonstrated that when less than 15 mL of residual RBCs are infused, no clinically significant acute hemolysis occurs.<sup>16</sup>

Accrediting agencies such as FACT and AABB require that institutions establish policies and procedures addressing processing of ABO-incompatible products including the indications for processing and a description of the processing methods to be used for RBC and plasma reduction.<sup>5,6</sup> At many institutions, the indications used for processing HPC products with a major ABO mismatch include the RBC volume or hematocrit (Hct). The maximum allowable RBC volume differs between institutions; it is usually between 10 and 40 mL, and most limit it to 20 to 30 mL or 0.2 to 0.4 mL/kg. The maximum allowable RBC volume at our institution is 30 mL. In addition, institutions should have a policy regarding product infusion if the value is greater than the cutoff. For example, infusion of a product with more than 30 mL of RBCs may be acceptable if it has a very low HPC dose, and further product manipulation is not warranted. We require assessment and approval by the medical director to use these products in such situations. Another option performed at some other centers when RBC volume is slightly above the cutoff value is to split the HPC product and infuse it in few aliquots at 6- to 8-hour intervals.

Some centers decide whether to perform RBC reduction or recipient isoagglutinin removal based on the recipient's isoagglutinin titers and the RBC volume. In one algorithm, RBC reduction is only performed if recipient RBC isoagglutinin titers are 32 or more and more than 20 mL RBCs are present in the HPC product.<sup>11</sup> We do not use RBC isoagglutinin titers as part of our decision-making process, because we feel that isoagglutinin titer testing is subjective and often poorly reproducible as also described by others.<sup>17</sup>

RBC reduction can be performed using several different techniques. These include manual RBC sedimentation using hydroxyethyl starch (HES), manual or automated centrifugation with enrichment of buffy coat cells (i.e., mononuclear cells [MNCs] and granulocytes), manual or automated Ficoll-Hypaque density gradient separation with centrifugation, and semiautomated and automated cell washer and apheresis cell separation.<sup>11,18,19</sup>

Simple centrifugation of the unprocessed product is easiest but least efficient. The HPC product is placed into centrifugation tubes and then centrifuged at rates from 400 to 4000  $\times g$  relative centrifugal force to separate the different cell layers based on relative cell density. The RBCs are at the bottom of the container, followed by granulocytes, MNCs, and platelets (PLTs) on top. The buffy coat can then be removed. This technique is associated with the lowest product purity and is not well favored.<sup>18</sup>

HES sedimentation is performed by adding 6% HES to the HPC-M product in a ratio from 1:4 to 1:8. In one commonly used method, the product bag is attached to sterile tubing connected to a transfer bag using a sterile connecting device. The tubing is clamped, and the product bag was then hung upside down. Sedimentation is allowed to occur. The HES induces RBC rouleaux formation, producing rapid sedimentation of RBCs while the more buoyant nucleated cells fall more slowly. After 30 to 90 minutes the sedimented RBCs are then removed by unclamping the tubing and draining them into the transfer bag. The product bag and tubing are then heat sealed, and the product bag containing the buffy coat layer and suspended plasma is removed and further processed as indicated.<sup>19,20</sup> This has been reported to remove more than 90% of RBCs with 50 to 90% total nucleated cell (TNC) recovery.<sup>21,22</sup> If necessary, a second identical sedimentation procedure can be performed on the drained RBCs fraction and the additional recovered nucleated cells are added back into the original HPC product bag. This method has been reported to increase TNC recovery from a mean of 55.6 to 86.2%.<sup>21</sup>

Density gradient separation enhances the ability to isolate distinct layers of cells by forcing their selective migration, based on relative density, through a viscous solution prepared at a defined specific gravity. In this procedure, a polysaccharide solution with a specific gravity of 1.073 to 1.077, most commonly Ficoll-Hypaque, is

added manually to conical centrifugation tubes. The HPC product diluted in buffer solution is then slowly layered over the top or carefully infused beneath the Ficoll-Hypaque and the tubes are then centrifuged. The MNCs and PLTs have a lower density than the Ficoll-Hypaque and they collect on the top of the Ficoll-Hypaque layer. The RBCs and granulocytes have a greater specific gravity than the Ficoll-Hypaque and they collect below the Ficoll-Hypaque layer.<sup>18</sup> The MNCs layer is then removed and washed two to four times to remove all of the gradient media (which are not FDA approved for human infusion). This procedure removes more than 95% of RBCs and recovers approximately 50 to 70% of the MNCs. This technique can also be semiautomated using instruments such as the Cobe 2991 (CaridianBCT, Lakewood, CO).<sup>23</sup>

Several different semiautomated and automated techniques are available to prepare buffy coats. The Cobe 2991 instrument can be used to collect buffy coats through either simple centrifugation<sup>18</sup> or density gradient separation.<sup>23</sup> For simple centrifugation the HPC product is placed in a round doughnut-shaped processing bag and centrifuged at around 3000 rpm (2560  $\times g$  centrifugal force) for approximately 10 minutes. After cell packing occurs, the plasma is then expressed into a waste bag until the buffy coat approaches the exit tubing. The waste bag is then clamped off, and additional HPC product is added to the processing bag. The centrifugation and plasma expression steps are then repeated until the processing bag is full. At this point, the buffy coat is diverted into a product collection bag either at a specified rate or for a specified period of time or until the RBCs are about to exit. The collection bag tubing is then clamped, and the buffy coat bag removed. This procedure reportedly recovers more than 80% of MNCs and removes more than 95% of RBCs.<sup>18</sup> With density gradient separation, the Ficoll-Hypaque is fed into the processing bag and centrifugation begun, and the HPC product is then slowly layered over the Ficoll-Hypaque through a peristaltic pump. The cells gradually form layers and the MNC layer can then be collected and washed.<sup>23</sup>

Other apheresis instruments used to separate MNCs include the Cobe Spectra (CaridianBCT) and the CS-3000 (Baxter Fenwal, Deerfield, IL).<sup>24-26</sup> With the Cobe Spectra, one group reported a mean recovery of TNCs of 34% and CD34+ cells of 82%, with a mean RBC reduction of 99% (mean, 4 mL of residual RBCs).<sup>24</sup> Another group reported similar CD34+ cell recovery (88%) and RBC reduction (98%), with a mean MNC recovery of 83%.<sup>25</sup> The CS-3000 device has been reported to have equivalent levels of RBC reduction, with a slightly lower mean MNC recovery of 63%.<sup>26</sup> If major mismatched HPC-M contains less than 125 mL of RBCs, we perform RBCs reduction using HES. If the product contains 125 mL of RBCs or more it is processed by centrifugation at 3000 rpm (2560  $\times g$  centrifugal

force) using the Cobe 2991 cell processor. This cutoff of 125 mL is based on the manufacturer recommendations.<sup>27</sup>

RBC engraftment is usually defined by an absolute reticulocyte count of more than  $30 \times 10^{12}/L$  (>1%) and independence of RBC transfusion.<sup>10</sup> It is evidenced by 100% donor RBC chimerism in the marrow and coincides with the disappearance of recipient isoagglutinins.<sup>28</sup> Delayed RBC engraftment can occur with or without PRCA. It can occur even when there is full neutrophil, T-cell, and PLT engraftment.<sup>28</sup> With major ABO incompatibility, delayed RBC engraftment cannot be predicted by recipient isoagglutinin titers at the time of HPC product infusion.<sup>8,28</sup>

Delayed RBC engraftment has been reported in some series to be more common with nonmyeloablative stem cell transplants. This has been attributed to a higher number of surviving recipient plasma cells that produce isoagglutinins directed against the donor RBCs.<sup>29</sup> In one series, the median time to RBC engraftment was 114 days (range, 28-178 days) with nonmyeloablative versus 40 days (range, 23-73 days) with myeloablative HPC transplants.<sup>28</sup> In another series involving 43 evaluable transplants with major ABO incompatibility, the median time until RBC engraftment was 32 days (range, 12-347 days) versus 20 days (range, 10-152 days) with an ABO-identical graft.<sup>10</sup> The median time to reach undetectable antidonor IgG and IgM titers has been reported to be shorter with matched unrelated donor versus matched related donor transplants and in transplant recipients who develop Grade II to IV graft-versus-host disease (GVHD).<sup>30</sup>

PRCA is defined as reticulocytopenia (<1%) lasting more than 60 days after HPC transplant, with absent erythroid precursors in the marrow in a recipient who has engrafted myeloid precursors, lymphoid precursors and with megakaryocytes present in the marrow. The reported incidence of PRCA after a major ABO mismatch varies between 3 and 29%. This is likely due to differences in preparative regimen, posttransplant immunosuppression, and isoagglutinin specificity.<sup>10,28,31</sup> Several series have shown it is more common with nonmyeloablative transplants and in patients receiving cyclosporine.<sup>8,10,28</sup> Almost all cases of delayed RBC engraftment and PRCA occur in group O recipients of a group A HPC product. Patients with PRCA require significantly increased RBC transfusion support and persistent cases may require treatment with exogenous erythropoietin, donor lymphocyte infusions, or other immunomodulatory interventions.<sup>31-33</sup>

## MINOR INCOMPATIBILITY

A minor incompatibility exists when the donor possesses isoagglutinins directed against the corresponding A or B antigens on the recipient's RBCs. Minor incompatibilities occur between group O donors and group A, B, or AB recipients and between group A or B donors and group AB

recipients. When present, the passive transfer of isoagglutinins present in an unmanipulated HPC product can result in acute hemolysis.<sup>11</sup> This is especially likely to occur if the donor has high-titer isoagglutinins and/or the recipient has a small plasma volume relative to the volume of plasma infused. When a minor ABO mismatch exists, plasma reduction is performed on the HPC product to remove donor antibody and thus prevent acute hemolysis.<sup>9,11</sup> The policy at our center is to perform plasma reduction on all ABO minor mismatched HPC products. There are no standards or guidelines to the use of any "trigger points" such as isoagglutinin titers or plasma volume.

Plasma reduction can be performed using simple centrifugation followed by plasma expression or using semiautomated or automated buffy coat enrichment techniques where plasma reduction is an inherent part of the procedure.<sup>18</sup> Simple centrifugation involves centrifugation at a rate between 400 and 4000  $\times g$  centrifugal force either at 4°C in a refrigerated centrifuge or at room temperature. This can be performed on the product bag or using conical tubes. The latter, however, involves an open system.<sup>19</sup> If the product bag is centrifuged, it is placed in a plasma volume extractor after the centrifugation step, and the plasma is then manually expressed. In our center we centrifuge the product bag at 1800 rpm (900  $\times g$  centrifugal force) for 10 minutes at 20°C. We then express off the supernatant plasma until approximately 1 inch of residual plasma is below the top of the extractor, being careful to minimize buffy coat (which contains the HPC) loss.

Transplantation of minor ABO-incompatible HPC product can also result in delayed hemolysis due to the "passenger lymphocyte syndrome." This complication is caused by viable B lymphocytes present in the HPC product producing isoagglutinins directed against residual recipient RBCs.<sup>3,11</sup> Although it can occur with both myeloablative and nonmyeloablative transplants,<sup>34,35</sup> it appears to be more frequent with nonmyeloablative transplants.<sup>34-36</sup> Hemolysis typically occurs between Days 5 and 15 posttransplant.<sup>34,35,37</sup> Recipient serologic testing usually demonstrates a positive direct antiglobulin test (DAT), the presence of donor derived isoagglutinins 1 to 3 weeks posttransplant, and an eluate demonstrating antibodies with the same specificity as those in the serum.<sup>35</sup> These serologic findings, however, have also been seen in some patients who do not experience clinically significant hemolysis.<sup>34,35</sup> Most recipients of a minor mismatched HPC transplant develop a positive DAT, but only 10 to 15% DAT-positive recipients develop hemolysis.<sup>38</sup> Many cases of delayed hemolysis are mild, but brisk hemolysis can occur within a matter of hours. Death has also been reported.<sup>39</sup> The hemolysis subsides as the residual recipient RBCs are destroyed or replaced by newly produced donor type RBCs and by transfused RBCs.<sup>40</sup> Risk factors for developing passenger lymphocyte syndrome include an HPC-A product (rather than HPC-M) and the use of

cyclosporine without methotrexate for posttransplant GVHD prophylaxis.<sup>37,41,42</sup> This is presumably because methotrexate not only inhibits proliferation of T cells but also of isoagglutinin-producing B cells.<sup>41</sup> Passenger lymphocyte-mediated hemolysis has also been reported to be more common in recipients of matched related donor versus matched unrelated donor transplants.<sup>42</sup> The management is usually supportive care and RBC transfusion. In rare cases, RBC exchange has been performed.<sup>35</sup> Isolated case reports also describe the successful use of rituximab and/or methotrexate, although in one case hemolysis rapidly recurred 3 days after a single dose of 375 mg/kg rituximab.<sup>37</sup>

The management approach at our institution to minimize the adverse effects of hemolysis due to passenger lymphocyte syndrome is to work closely with the transplant team to monitor the recipient for increasing total and indirect bilirubin, increasing lactate dehydrogenase (LDH), hemoglobinemia, or a sudden unexplained decrease in Hct. We provide additional RBC transfusion support as indicated by the degree of hemolysis and advocate additional aggressive hydration with severe hemolysis.

### BIDIRECTIONAL INCOMPATIBILITY

Bidirectional incompatibility involves both a major and a minor ABO incompatibility. This occurs in the combination of group A donor and B recipient, as well as the combination of group B donor and A recipient. The potential consequences of these are similar to both those of major and minor incompatibilities (Table 1). Therefore, these products usually require both RBCs and plasma reduction. Most RBC reduction techniques also remove plasma.<sup>11</sup> Thus, at our institution, we perform RBC reduction for bidirectional HPC-M products according to previously outlined guidelines, but not an additional plasma reduction, because this is an inherent part of the procedure. For bidirectional mismatch HPC-A products, which

usually contain less than 20 mL of RBCs, we perform plasma reduction as previously outlined.

### TRANSFUSION SUPPORT FOR ABO-INCOMPATIBLE TRANSPLANTS

When choosing the ABO type of RBCs, PLTs, and plasma for transfusion, both the donor and the recipient ABO type must be considered to minimize the risk of hemolysis of the transfused RBCs, the residual recipient RBCs, and the donor-derived RBCs. Donor-compatible plasma is used to avoid isoagglutinins directed against donor RBCs to minimize the risk of acute hemolysis and delayed RBC engraftment. Each institution must develop its own standard operating procedures for choosing the ABO type of products transfused to recipients of ABO-incompatible transplants.

Guidelines at our center are adapted from those in the 16th edition of the AABB technical manual<sup>43</sup> and are presented in Table 2. The technical manual divides the transplant period into three phases. Phase I commences at the time the recipient is prepared for HPC transplant. Phase II commences at the initiation of induction chemotherapy and ends 1) for RBCs, when the DAT is negative and anti-donor isoagglutinins are no longer detectable (i.e., the serum or back type is the donor ABO type) and 2) for plasma, when the recipient's RBCs are no longer detectable (i.e., the cell or front type is the donor ABO type). Phase III occurs when both the cell and the serum types are consistent with the donor ABO type. We follow the technical manual guidelines for transfusion of RBCs, plasma, and PLTs in Phases I, II, and III with the exception that Phase II commences at the time of HPC product infusion rather than at the time of initiation of induction chemotherapy. Similarly, seven of 10 European institutions surveyed in a recent article transfuse only recipient-type products until the day of HPC transplant irrespective of the kind of ABO mismatch and conditioning regimen.<sup>44</sup>

**TABLE 2. Transfusion support for patients undergoing ABO-incompatible allogeneic HPC transplantation\***

Recipient	Donor	Phase I, all components	Phase II				Phase III, all components
			RBCs	First-choice PLTs	Next-choice PLTs†	FFP	
O	A	Recipient	O	A	AB; B; O	A, AB	Donor
O	B	Recipient	O	B	AB; A; O	B, AB	Donor
O	AB	Recipient	O	AB	A; B; O	AB	Donor
A	O	Recipient	O	A	AB; B; O	A, AB	Donor
A	B	Recipient	O	AB	A; B; O	AB	Donor
A	AB	Recipient	A	AB	A; B; O	AB	Donor
B	O	Recipient	O	B	AB; A; O	B, AB	Donor
B	A	Recipient	O	AB	B; A; O	AB	Donor
B	AB	Recipient	B	AB	B; A; O	AB	Donor
AB	O	Recipient	O	AB	A; B; O	AB	Donor
AB	A	Recipient	A	AB	A; B; O	AB	Donor
AB	B	Recipient	B	AB	B; A; O	AB	Donor

\* From Szczepiorkowski et al.<sup>43</sup>

† PLTs should be selected in the order selected.

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## CONFLICT OF INTEREST

Both authors declare that there are no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

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