in early-phase clinical trials. Far less attention in this field has focused on hematologic malignancies, possibly due to the disseminated nature of leukemia in contrast to discrete masses of solid tumor, inferring that leukemia is less suitable as an oncolytic viral target. A notable exception is the investigation of vaccine-strain, live, attenuated measles virus (MV) as oncolytic therapy for B-cell malignancies. However, the high frequency of antiviral immunity due to prior infection, vaccination, or recurrent therapeutic exposure may present an obstacle to any successful systemic virotherapy, but especially with the MV given the widely immunized population.

Castleton et al use bone marrow–derived MSCs as carriers (effectively ALL-targeting producer cells) of the oncolytic MV to circumvent the anti-measles humoral immunity found in most patients which does not seem to be abrogated by their prior chemotherapy. MSCs are an attractive cell carrier as they are easily isolated from a bone marrow aspirate, readily ex vivo–expanded under good manufacturing practice conditions, and may be infused across HLA barriers. The hundreds of clinical trials worldwide over the last 2 decades persuasively support the safety of these cells for clinical applications.

Castleton et al demonstrated that clinically relevant anti-MV immunoglobulin G antibodies persisted in 16 study patients treated in the UK ALL 14 Multicenter Trial, illustrating the impending challenge of using oncolytic measles virotherapy in these patients. In an effort to investigate MSCs as a carrier to overcome the neutralizing effect of the antibodies, they first demonstrated that these cells expressed CD46, the vaccine strain receptor, proving the susceptibility of MSCs to MV infection and the potential to use MSCs as carriers. They generated human MSCs from bone marrow, defining their cells according to the widely accepted International Society for Cellular Therapy (ISCT) criteria, and then optimized the conditions for in vitro MV infection. Importantly, they characterized the time course for maximal viral production within the cells, delineating the conditions needed for production and infusion of the viral-loaded MSCs. Using an established murine model of ALL (Nalm-6 cells [precursor B-lineage leukemia cell line] intravenously infused into SCID mice), they showed by unambiguous bioluminescence that MV-loaded MSCs localize to sites of proven bone marrow ALL after intravenous infusion. Then, using 2-color live cell confocal microscopy of cells in culture, they elegantly demonstrated that viable replicating MV is directly transferred (“handoff”) from the MSCs to Nalm-6 cells by a mechanism that involves, at least in part, an MSC-Nalm-6 cell fusion (see figure). Finally, to demonstrate the clinical applicability of MSC delivery of MV to ALL, they treated SCID mice with established Nalm-6 cell ALL with MV or MV–loaded MSCs, with and without anti-MV antibodies. The original idea that persisting anti-MV antibodies would block any benefit of MV therapy was validated as the survival benefit of MV therapy was abolished by anti-MV antibodies. Additionally, mice receiving MSC-MV showed a benefit, demonstrating that MSCs can deliver the oncolytic virus to ALL cells in vivo. Importantly, the central thesis of this work, the neutralizing effect of anti-MV antibodies could be overcome using cell carriers, was proven correct as mice receiving MSC-MV maintained the observed benefit in the presence of anti-MV antibodies.

These observations have enormous implications for biologic therapy of leukemia. Although the specific message conveyed by this work is that MSCs can be used to deliver oncolytic measles virotherapy to ALL cell targets and shelter the virus from the patient’s humoral immunity, the extension of these results may have far-reaching consequences. First, these data establish proof-of-concept that MSCs can target disseminated leukemia in vivo, opening the door to countless opportunities to target malignant hematopoietic cells or the associated microenvironment. Second, inspection of the survival curves of leukemic mice show that the animals treated with MSC-MV fared better than those treated with MV alone without anti-MV antibodies ($P = 0.02$). Thus, MSCs seem to enhance the targeting of MV to ALL compared with the naked virus, suggesting that MSC virotherapy may be superior to systematic infusions of oncolytic viruses, despite the propensity of these viruses to infect and lyse malignant cells. Given the susceptibility of MSCs to viral infection in vitro and the relative ease of genetically engineering MSCs, the work of Castleton et al will likely spawn a wave of new studies of MSC–based biologic therapy of hematologic malignancies.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Transfusion Medicine

Comment on Cortés-Puch et al, page 1403

Iron-related adverse effects from stored blood

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In this issue of Blood, Cortés-Puch and colleagues investigate a strategy to improve the quality of stored red blood cells (RBCs) by washing the blood, thereby removing “noxious” substances, including cell-free hemoglobin,
transferrin-bound iron, non–transferrin-bound iron (NTBI), and plasma labile iron, that accumulate during storage. Cortés-Puch and colleagues found that in a canine model of *Staphylococcus aureus* pneumonia, transfusion of “old” (42-day) blood was associated with impaired hemodynamics, greater lung injury and shock index, and reduced survival. These outcomes were improved upon when washing RBCs, which was associated with a reduction of NTBI in the circulation. Importantly, washing “fresh” (7-day) RBCs had the opposite effect, reversing the improved survival that was observed after exchange transfusion with younger RBCs; this effect was also related to an increased release of NTBI.

The American Medical Association has identified overuse of 5 medical treatments, including blood transfusions along with cardiac stents, ear tubes, antibiotics, and the induction of birth in pregnant women, and has highlighted the danger of unnecessary transfusion. Although blood transfusion has long been considered to be a safe and effective therapy for patients with anemia, increasing evidence has identified adverse patient outcomes that are associated with RBC transfusion. Rather than conferring a benefit, blood transfusions may in fact be injurious.

Retrospective, observational studies have suggested that the etiology of a number of the adverse events associated with RBC therapy may be due to storage lesions in banked blood. Residual plasma and white blood cells present in stored blood contain inflammatory mediators, free radicals, macromolecule oxidation products, dead and broken cells and vesicles, deranged electrolytes, and other components contributing to “storage lesions.”

Measures such as leukoreduction and improved storage solutions have generally been able to help with this situation, albeit to limited extents. The possibility that an iron effect might be a risk of blood transfusion has been proposed and investigated previously. Hod and colleagues found that in a mouse model, transfusions with stored RBCs increased NTBI and initiated inflammation compared with mice transfused with fresh RBCs. They subsequently reported that in human volunteers reinfused with either fresh (3- to 7-day) or old (40- to 42-day) autologous blood, significant differences between the fresh and older transfusions were found only in iron parameters and markers of extravascular hemolysis. The mechanism by which these observed effects were achieved remain elusive; nevertheless, circulating NTBI derived from rapid clearance of transfused, older RBCs may promote transfusion-related complications, including risk of infection (see figure).

It has long been known that a significant storage lesion is present in stored RBCs that impairs the intended benefit of a blood transfusion. The depletion of intracellular diphosphoglycerate (2,3 DPG) in stored RBCs results in a left shift of the oxygen dissociation for hemoglobin, so that although transfusion of RBCs increases oxygen carrying capacity acutely, the transfused RBCs give up oxygen reluctantly to the tissues in the peripheral circulation until after a time-dependent repletion of intracellular 2,3 DPG occurs in vivo in the transfused RBC. It is therefore likely that any perceived patient benefit observed acutely (within 6 hours or so) from a blood transfusion is due to an increase in volume rather than any increase in oxygen consumption as a consequence of an increase in oxygen delivery. On the risk side, definitive evidence for whether transfusion of stored RBCs is causal for increased morbidity and mortality, rather than being simply associated with adverse patient outcomes, awaits completion of an important ongoing, prospective, randomized trial in adult patients undergoing open heart surgery. The relative benefits/risks of blood are therefore important elements in any decision to transfuse, especially when alternative therapies are available for the management of anemia. In the meantime, changes in strategies of blood inventory management, such as preferential issuing of fresher RBCs or washing RBCs, must wait until such evidence is in hand.

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![Proposed mechanistic pathway (the “iron hypothesis”) explaining how transfusion of older stored RBCs may induce adverse effects in patients.](https://example.com/pathway.png)


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**Comment on Bachireddy et al, page 1412**

**Reversing CD8⁺ T-cell exhaustion with DLI**

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In this issue of *Blood*, Bachireddy et al explain why infusion of donor CD4 T cells induces remission in some patients with persistent chronic myeloid leukemia (CML) after allogeneic hematopoietic cell transplantation (HCT), but not in others.¹

In a landmark report, Kolb et al² described 3 patients with recurrent CML after bone marrow transplantation who were treated with interferon-α and buffy coat cells from the marrow donor. All 3 patients had a durable complete hematologic and cytogenetic remission. A subsequent study showed that donor lymphocyte infusion (DLI) induced durable complete remission in ~75% of patients with persistent or recurrent chronic-phase CML after allogeneic HCT.³ In this study, however, ~40% of the patients developed graft-versus-host disease (GVHD), and in another study, 76% of the patients developed acute or chronic GVHD.⁴

In 1995, Giralt et al⁵ reported preliminary results suggesting that treatment with lower numbers of donor cells and depletion of CD8-positive T cells from DLI might decrease the risk of GVHD, without loss of anti-leukemic efficacy. Subsequent studies at the Dana-Farber Cancer Institute supported this hypothesis. In a study by Alyea et al,⁶ 15 of the 19 patients with cytogenetic or hematologic persistence of CML after allogeneic HCT had a complete cytogenetic response after CD8-depleted DLI, and only 32% of patients in the trial developed acute or chronic GVHD. These results demonstrated that donor CD4 T cells can induce an anti-leukemic response in patients with CML, but they raised 2 related questions. First, if hematopoietic stem cells and their malignant CML counterparts do not express major histocompatibility complex class II molecules that present antigens to CD4 T cells, how do donor CD4 T cells eliminate these cells in the recipient? Second, why is CD8-depleted DLI effective in some patients with CML, but not others?

Bachireddy et al⁵ compared the characteristics of cells from 29 patients categorized according to their response to DLI and report 3 main findings. (1) Before DLI, the numbers of CD8 T cells in the marrow were higher in responders than in nonresponders (B), and a gene expression profile indicating exhaustion is observed only in responders. See Figure 6 in the article by Bachireddy et al that begins on page 1412.

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