hypothesis is that a 25-MAB product is better equipped to target the antigenic diversity of RhD. Well over 100 mutations in RhD are known, many of which alter epitope topology. A mixture of MABs could be selected to target a number of these so-called “partial D” variants. However, the large majority of RhD-positive individuals (~98% of whites) are homozygous for wild-type RHD. The inclusion of MABs against rare partial D variants would therefore not be expected to dramatically affect response rates. Another hypothesis is that binding of antibody to multiple epitopes on RhD may enhance the immune response. Past studies suggest a synergistic effect on in vitro phagocytosis of opsonized platelets when anti-RhD MABs with different epitope specificity are combined. The mechanism by which this synergy may occur is uncertain, particularly given that RhD epitopes are small and overlapping and that antibody binding to a single epitope would be expected to block binding of antibody to other epitopes on the same RhD molecule. One wonders whether RBCs exposed to rozrolimupab have more bound anti-RhD than those exposed to a single MAB. A third hypothesis centers on epitope specificity. Previous work has shown epitope specificity to be a determinant of a given MAB’s ability to inhibit opsonized platelet phagocytosis in vitro. How epitope specificity affects this process is unknown, but could relate to the orientation of the MAB on the RBC surface and its interaction with Fc receptors. Perhaps the epitope specificity of 1 or more MABs in rozrolimupab, but not of the MAB tested by Godeau, engenders an inhibitory effect on platelet phagocytosis. To this end it would be of value to understand the individual effects on platelet phagocytosis of each of the component MABs in rozrolimupab. A fourth hypothesis holds that dosing of MAB in the Godeau study (47-95 μg/kg) may have been inadequate. Rozrolimupab showed a 5-fold lower potency than plasma-derived anti-RhD in vitro. A corresponding 4- to 6-fold higher dose (300 μg/kg) was required to achieve a similar clinical response to standard dose (50-75 μg/kg) plasma-derived anti-RhD. It may be that a single anti-RhD MAB with favorable epitope specificity at sufficient doses would yield a response similar to rozrolimupab.

Natural immune responses are polyclonal. It is thus tempting (but unproven) to surmise that a mixture of therapeutic MABs may be better than 1. If this assumption can be established, and its mechanism elucidated, it could have far-reaching implications. Hyperimmune globulin preparations are used for passive immunization against a host of infectious diseases (eg, hepatitis B, cytomegalovirus, rabies). Mixtures of appropriately selected MABs against these organisms could replace plasma-derived therapy. The concept may also be applicable to cancer therapy. The epidermal growth factor receptor (EGFR) is regulated and is a validated therapeutic target in several malignancies. A mixture of 2 anti-EGFR MABs with different epitope specificities was recently shown to be superior to its component MABs alone in inhibiting cancer cell growth in vivo.

Rozrolimupab may someday offer a new therapeutic option for ITP. More importantly, it may represent a new paradigm for use of therapeutic antibodies, but we must first understand whether and why 25 are better than 1.

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

---

**GENE THERAPY**

Comment on Carbonaro et al, page 3677, and on Candotti et al, page 3635

**Gene therapy for ADA-SCID: defining the factors for successful outcome**

H. Bobby Gaspar  
UNIVERSITY COLLEGE LONDON INSTITUTE OF CHILD HEALTH

In this issue of *Blood*, Carbonaro et al1 and Candotti et al2 demonstrate, in the clinical and nonclinical settings, the absolute need for cytoreductive conditioning in successful treatment of adenosine deaminase–deficient severe combined immune deficiency (ADA-SCID) by gene therapy. Intriguingly and in contrast to previously held paradigms, Carbonaro et al suggest that the continued use of enzyme replacement therapy (ERT) after gene therapy may improve immune reconstitution.1

It is now more than 20 years since the first gene therapy trials for genetic diseases were performed on 2 children with ADA-SCID.3 Over the past 2 decades, a succession of clinical trials, each using different vectors and slightly different protocols, have been performed. The early studies were largely lacking in efficacy,1,4 but the most recent 3 studies,5,6 including the new report by Candotti et al,2 convincingly demonstrate that this disease can be corrected long term by gene therapy alone. Until now, the role of cytoreductive conditioning and the cessation of ERT, although thought empirically to be critical for successful gene therapy, have not been evaluated in a comparative manner.

Two clinical studies have shown the long-term success of hematopoietic autologous stem...
cell (HSC) gene therapy in correction of ADA-SCID. In both cases, the use of chemotherapy (either busulfan 4 mg/kg or melphalan 140 mg/kg) was thought to remove autologous ADA-deficient HSCs and thereby promote the engraftment of gene-modified cells. However, in both studies, no comparative group was available to determine the absolute need for conditioning. In their report, Candotti et al describe 10 patients treated in 2 different phases of the same trial. In the first phase, for safety reasons, patients were treated without cytoreductive conditioning and with ongoing ERT. In these 4 individuals, minimal or no engraftment of gene-modified cells was seen. After reports emerging from successful studies in Europe, the trial protocol was amended to include a low dose of intravenous busulphan (65-90 mg/m²) and cessation of ERT. In 6 patients treated in the second phase, 3 were able to stop ERT long term and had significant engraftment of gene-corrected cells in the periphery with sustained immune recovery. Importantly, because the same vectors and transduction protocols were used in the 2 phases of the study, a direct comparison could be made and demonstrates that conditioning is required for gene-modified cell engraftment.

One caveat to this conclusion is that cessation of ERT was another variable in the second phase of this study. However, the murine studies of Carbonaro et al well and truly put this argument to bed. In a series of well-constructed comparative experiments in ADA-/- mice (which accurately recapitulate the human immunodeficiency) these studies show the need for cytoreduction in promoting gene-modified cell engraftment. Unconditioned mice did not engraft at all with or without ERT and increased conditioning (in this case increased levels of total body irradiation) also enhanced the levels of gene-modified cell engraftment although in the clinical setting this needs to be balanced against the toxicity associated with increased conditioning.

The other major issue in the implementation of clinical gene therapy trials has been whether the use of ERT promotes or negates the engraftment of gene-modified cells. Contrasting anecdotal clinical data are available, with one report suggesting that ERT cessation allowed gene-modified cell development whereas others, including the present Candotti et al report, show that ERT usage may actually promote gene-modified cell proliferation. To address this formally, Carbonaro et al performed a series of experiments in which ERT was continued for either 1 or 4 months after gene transfer. The first finding was that continued use of ERT did not blunt gene-modified cell engraftment. However, the most striking results were seen in experiments that most accurately represent the clinical scenario, which is the use of gene-modified lin-ve progenitor cells in nonmyeloablated (200 cGy) hosts. In this setting, they found that in contrast to mice that had stopped ERT, mice receiving ERT for 1 or 4 months after gene transfer had significantly increased levels of gene-modified cells in the thymus. The reasons for this are not clear but may relate to the role of exogenous ADA in detoxifying the marrow or thymic stromal microenvironment, thereby permitting the engraftment and survival of gene-modified cells. These are important data especially because, as the authors state, the use of ERT for a short time period after gene therapy may not only protect patients in the period of lymphopenia after chemotherapy, but may also promote engraftment of gene-corrected cells. Clearly there is a need to validate these findings in the clinical setting.

One important feature is the safety profile associated with these studies. For ADA-SCID, more than 40 patients have been treated worldwide with 100% survival and an overall efficacy, as determined by cessation of ERT, of more than 70%. For any high-risk interventional modality, these are impressive data and compare very favorably with matched allogeneic HSC transplants for ADA-SCID. Nevertheless, all of these studies have been conducted with gammaretroviral vectors with intact viral promoter sequences that are known to have caused insertional mutagenesis in other gene therapy trials. Although no transformation events have been seen in treated ADA-SCID patients, integration events into known proto-oncogenes have been reported for all 3 of the trials conducted. The use of self-inactivating lentiviral vectors with internal mammary promoters, which have been shown in preclinical models to have safer insertional profiles, may alleviate these concerns, but equal or improved clinical efficacy also needs to be demonstrated. The studies of Candotti et al and Carbonaro et al are important in providing us with information on how best to achieve efficacy in future clinical studies.

Conflict-of-interest disclosure: The author has been an occasional consultant for Enzon Inc, former manufacturers of PEG-ADA.

REFERENCES