

unsuccessful previous autologous transplantation ($P < .001$), compared with patients receiving a myeloablative chemotherapy regimen. Even though chemotherapy sensitivity and disease status at transplantation were comparable, my impression is that the patients receiving reduced-intensity conditioning represent a group with a higher likelihood of dying from complications related to the transplantation and to relapse. Thus, I think the “evidence” that reduced-intensity conditioning is associated with a higher relapse rate compared with myeloablative conditioning is flawed and should not be given significant credence.

It is unlikely there will ever be a randomized study evaluating the role of either autologous or allogeneic transplantation for treating relapse or primary progression in pediatric patients with HL. There is a general consensus among pediatric oncologists that high-dose chemotherapy and autologous peripheral stem cell transplantation is effective treatment for

patients with primary refractory HL and for patients with advanced-stage disease at presentation who experience a relapse. Currently there is no indication to use allogeneic transplantation as the first transplantation unless the patient's stem cells cannot be mobilized. The article by Claviez et al provides important information that patients who relapse following an autologous transplantation still have the potential for cure following allogeneic stem cell transplantation. In our center, such patients are generally offered a reduced-intensity conditioning regimen in an effort to reduce the nonrelapse mortality. It is clear from this article that reduced-intensity conditioning is being used more frequently.

Between 1987 and 2001, 36% of patients received reduced-intensity conditioning compared with 75% between 2001 and 2005.

Only a registry study could provide sufficient data to begin to evaluate the role of allogeneic transplantation in the treatment of pediatric patients with HL. However, in

evaluating conclusions drawn from registry data, reported subgroups must be analyzed carefully to ensure that they are comparable. This study clearly shows that allogeneic transplantation following a failed autologous transplantation is feasible and potentially curative for pediatric patients with relapsed HL. However, the comparison between myeloablative and reduced-intensity conditioning is limited in value because the 2 groups have significantly different earlier treatment. It is likely that reduced-intensity conditioning will continue to increase in frequency.

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

REFERENCES

1. Claviez A, Canals C, Dierickx D, et al. Allogeneic hematopoietic stem cell transplantation in children and adolescents with recurrent and refractory Hodgkin lymphoma: an analysis of the European Group for Blood and Marrow Transplantation. *Blood*. 2009;114(10):2060-2067.
2. Bradley M, Cairo M. Stem cell transplantation for pediatric lymphoma: past, present, and future. *Bone Marrow Transplant*. 2008;41(2):149-158.

● ● ● GENE THERAPY

Comment on Mingozzi et al, page 2077

Muscling through AAV immunity

Thierry VandenDriessche UNIVERSITY OF LEUVEN

AAV-1 is one of the most promising vectors for gene delivery to skeletal muscle. In this issue of *Blood*, Mingozzi and colleagues now demonstrate that AAV-1-mediated gene transfer into human skeletal muscle results in activation of capsid-specific T cells.¹

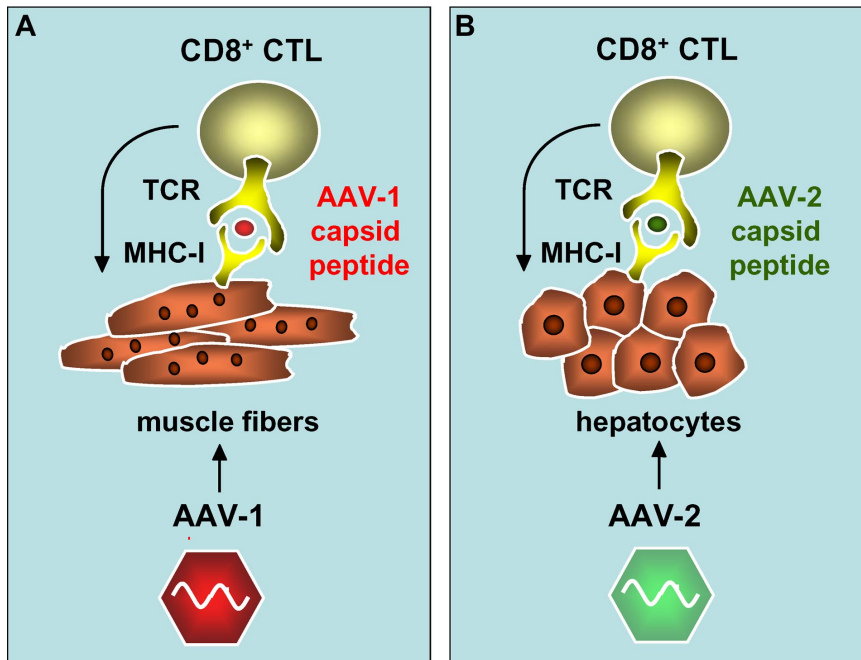
Evidence continues to accumulate demonstrating that gene therapy may be therapeutically effective in patients suffering from different types of hereditary diseases.^{2,3} These recent successes in gene therapy reflect, at least in part, continued improvements in gene delivery technologies. In particular, gene delivery vectors derived from the nonpathogenic adeno-associated virus (AAV) have been used to cure monogenic diseases in preclinical animal models and thus far have yielded some promising results in clinical trials. Despite these advances, the immune response toward the therapeutic protein or the gene-engineered cells themselves represents a potential hurdle that may prevent long-term therapeutic effects. Unfortunately, the assessment of immune responses following gene therapy in preclinical animal models does not always accurately predict the outcome in humans.³

Hence, it is extremely important to fully study the immune consequences of gene transfer in human subjects enrolled in clinical trials.

In this issue, Mingozzi et al describe the immune consequences of AAV-mediated skeletal muscle gene delivery in humans. Their findings were based on an open-label dose escalation study in which an AAV-1 vector encoding a truncated lipoprotein lipase (LPL^{S447X}) was administered into the limb muscles of subjects suffering from LPL-deficiency.⁴ These patients typically have abnormal lipid breakdown consisting of elevated triglyceride (TG) levels in the blood. A transient decrease in plasma TG levels was apparent in some of the subjects, suggesting a possible therapeutic effect. However, because dietary fluctuations may potentially interfere with TG levels and the number of patients was limited, conclusions regarding efficacy will

need to be confirmed in subsequent larger trials.

The authors noted that one of the subjects who received the highest vector dose experienced a transient increase in muscle enzyme creatine phosphokinase (CPK). This elevation in CPK appeared to be associated with a therapeutic effect whereby TG levels transiently returned to baseline. Activation of AAV-1 capsid-specific CD4⁺ and CD8⁺ T-cell responses may have contributed to the clearance of the AAV-1-transduced muscle fibers. Interestingly, 50% of the subjects enrolled in this trial developed an AAV-1 capsid-specific T-cell response, whereas LPL-specific T-cell responses could not be detected. Increasing vector doses resulted in a more rapid onset of capsid-specific T-cell responses and an apparent shift toward mixed CD8⁺ and CD4⁺ AAV-1 capsid-specific T-cell responses. In



Activation of AAV-1 and AAV-2 capsid-specific CD8⁺ cytotoxic T cells (CTL) following cross-presentation of capsid-derived antigenic peptides onto major histocompatibility complex class I antigens (MHC I) in transduced muscle fibers (A)¹ and hepatocytes (B).^{3,5,6}

contrast, the T-cell responses in the low-dose cohort was delayed and driven primarily by CD4⁺ instead of CD8⁺ T-cell capsid epitopes. Hence, AAV-1–transduced muscle fibers likely presented the capsid-derived antigenic peptides in association with major histocompatibility complex class I (MHC I) to CD8⁺ cytotoxic T cells, with subsequent elimination of transduced muscle cells, in a vector dose-dependent manner. Previous natural exposure to wild-type AAV in humans could possibly increase the likelihood of inducing these capsid-specific T-cell responses. Induction of AAV-1 capsid-specific MHC I–restricted CD8⁺ T-cell responses would de facto require “cross-presentation” of the exogenous AAV particles via the endogenous antigen presentation pathways, particularly because AAV-1 capsid antigens are not expressed de novo in transduced cells.

There are striking similarities between the results obtained in this trial and previous observations in a gene therapy trial for hemophilia B, which further strengthens the under-

lying hypothesis. In this trial, administration of AAV-2 vectors encoding clotting factor IX (FIX) resulted in a dose-dependent T-cell response that cleared the transduced hepatocytes expressing FIX. This is consistent with the observation of a decline in FIX expression levels coincident with transient elevation of liver enzymes in the plasma.^{3,5} This T-cell response was again directed at the AAV capsid-derived antigenic peptides presented in association with MHC I on the surface of the transduced hepatocytes, possibly via “cross-presentation.”⁶ Similarly, there was no evidence for T-cell responses against FIX, consistent with the lack of T-cell responses against LPL.

It has been proposed that the use of vectors derived from alternative AAV serotypes may reduce the likelihood of inducing capsid-specific T-cell responses, particularly if uptake of the vector particles by antigen-presenting cells is impaired. Because this uptake is likely mediated by heparan sulfate proteoglycan receptors (HSPGRs), AAV serotypes that ex-

hibit reduced binding to HSPGRs may reduce activation of capsid-specific T cells.⁷ However, the present results challenge this notion by demonstrating that the use of AAV-1, which binds poorly to HSPGRs, does not prevent induction of a capsid-specific T-cell response.

This study has important implications for the design of future trials using AAV vectors, and suggests that switching AAV serotypes may not suffice to render the transduced cells impervious to immune attack, particularly because T-cell epitopes are relatively conserved among distinct AAV serotypes. Whether the T-cell responses to capsid curtail long-term transgene expression in some or all cases requires further investigation. The use of transient immune suppression and/or lower vector doses in conjunction with more robust transgene expression cassettes may potentially overcome some of these T-cell immune responses while maintaining stable transgene expression. The present study provides a conceptual framework to further test these hypotheses in future trials.

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

REFERENCES

- Mingozzi F, Meulenbergh JJ, Hui DJ, et al. AAV-1–mediated gene transfer to skeletal muscle in humans results in dose-dependent activation of capsid-specific T cells. *Blood*. 2009;114(10):2077–2086.
- Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber’s congenital amaurosis. *N Engl J Med*. 2008;358(21):2240–2248.
- Manno CS, Pierce GF, Arruda VR, et al. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med*. 2006;12(3):342–347.
- Stroes ES, Nierman MC, Meulenbergh JJ, et al. Intramuscular administration of AAV1-lipoprotein lipase S447X lowers triglycerides in lipoprotein lipase-deficient patients. *Arterioscler Thromb Vasc Biol*. 2008;28(12):2303–2304.
- Mingozzi F, Maus MV, Hui DJ, et al. CD8(+) T-cell responses to adeno-associated virus capsid in humans. *Nat Med*. 2007;13(4):419–422.
- Pien GC, Basner-Tschakarjan E, Hui DJ, et al. Capsid antigen presentation flags human hepatocytes for destruction after transduction by adeno-associated viral vectors. *J Clin Invest*. 2009;119(6):1688–1695.
- Vandenbergh LH, Wang L, Somanathan S, et al. Heparin binding directs activation of T cells against adeno-associated virus serotype 2 capsid. *Nat Med*. 2006;12(8):967–971.