CONFLICT OF INTEREST

The authors declare that the consensus agreement was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

G.A.-P. and C.D.P. are supported by NHLBI R01 HL135853 and HL130856. T.M. is supported by NIH NIAID R01 AI116880-01, CIRM RN3-06532, and the UCSF Center for Maternal-Fetal Precision Medicine and has received research support from Ultragenyx Pharmaceuticals. S.N.W. is supported by MRC grants MR/P026494/1 and MR/R015325/1 and SPARKS grant 17UCL01.

REFERENCES

1. U.S. National Institutes of Health. Recombinant DNA Advisory Committee (2000). Prenatal gene tranfer: scientific, medical, and ethical issues: a report of the Recombinant DNA Advisory Committee. Hum. Gene Ther. *11*, 1211–1229.
2. Porada, C.D., Tran, N., Eglitis, M., Moen, R.C., Troutman, L., Flake, A.W., Zhao, Y., Anderson, W.F., and Zanjani, E.D. (1998). In utero gene therapy: transfer and long-term expression of the bacterial neo(r) gene in sheep after direct injection of retroviral vectors into preimmune fetuses. Hum. Gene Ther. *9*, 1571–1585.
3. Frederickson, R.M., and Ylä-Herttuala, S. (2019). Two Decades of Molecular Therapy: The Journey Continues. Mol. Ther. *27*, 1–2.
4. Collins, F.S., and Gottlieb, S. (2018). The Next Phase of Human Gene-Therapy Oversight. N. Engl. J. Med. *379*, 1393–1395.
5. Almeida-Porada, G., Atala, A., and Porada, C.D. (2016). In utero stem cell transplantation and gene therapy: rationale, history, and recent advances toward clinical application. Mol. Ther. Methods Clin. Dev. *5*, 16020.
6. Witt, R., MacKenzie, T.C., and Peranteau, W.H. (2017). Fetal stem cell and gene therapy. Semin. Fetal Neonatal Med. *22*, 410–414.
7. Massaro, G., Mattar, C.N.Z., Wong, A.M.S., Sirka, E., Buckley, S.M.K., Herbert, B.R., Karlsson, S., Perocheau, D.P., Burke, D., Heales, S., et al. (2018). Fetal gene therapy for neurodegenerative disease of infants. Nat. Med. *24*, 1317–1323.
8. Schneider, H., Faschingbauer, F., Schuepbach-Mallepell, S., Körber, I., Wohlfart, S., Dick, A., Wahlbuhl, M., Kowalczyk-Quintas, C., Vigolo, M., Kirby, N., et al. (2018). Prenatal Correction of X-Linked Hypohidrotic Ectodermal Dysplasia. N. Engl. J. Med. *378*, 1604–1610.
9. Chan, J.K.Y., Gil-Farina, I., Johana, N., Rosales, C., Tan, Y.W., Ceiler, J., Mcintosh, J., Ogden, B., Waddington, S.N., Schmidt, M., et al. (2018). Therapeutic expression of human clotting factors IX and X following adeno-associated viral vector-mediated intrauterine gene transfer in early-gestation fetal macaques. FASEB J., Published online December 5, 2018. https://doi.org/10.1096/fj.201801391R.
10. Verma, J., Thomas, D.C., Sharma, S., Jhingan, G., Saxena, R., Kohli, S., Puri, R.D., Bijarnia, S., and Verma, I.C. (2015). Inherited metabolic disorders: prenatal diagnosis of lysosomal storage disorders. Prenat. Diagn. *35*, 1137–1147.

The Future: *In Vivo* CAR T Cell Gene Therapy

Rana Mhaidly[1] and Els Verhoeyen[1,2]

https://doi.org/10.1016/j.ymthe.2019.03.012

Chimeric antigen receptor (CAR) T cells represent a revolutionary therapy for combatting cancers.[1] This novel immunotherapy was only approved in 2017 for treatment of advanced blood cancers in the US and Europe. The most frequently used vehicles for CAR gene delivery in autologous T cells are lentiviral vectors (LVs) pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G-LV). *Ex vivo* gene delivery employing these broad spectrum LVs might, however, lead to off-target transduction of contaminating cancer or antigen-presenting cells with these CARs leading to severe health risks and adverse events.[2] In a new study published in *Molecular Therapy – Methods and Clinical Development*, Jamali et al.[3] limit these risks by using $CD4^+$ or $CD8^+$ T cell targeted LVs for CAR gene transfer. The authors engineered human $CD4^+$ and $CD8^+$ T cell-targeted LVs capable of selective gene delivery into human $CD4^+$ or $CD8^+$ T cells, respectively.[4,5]

The targeting of LVs is based on the principle that fusion-activation of a chimeric envelope should be triggered by the interaction of the ligand displayed on the vector surface with its specific receptor on the target cell. Buchholz and co-workers[3] designed a novel strategy based on the fact that paramyxoviruses separated the two functions, cell binding and virus-cell fusion, into separate glycoproteins. In this case, redirection of the glycoprotein that confers binding to the target cell leaves the fusion glycoprotein (gp) "untouched" and functional. This strategy was initially successful for LVs pseudotyped with measles virus (MV-LVs) retargeted glycoproteins and more recently LVs

[1]Université Côte d'Azur, INSERM, C3M, Nice, France; [2]CIRI - Centre International de Recherche en Infectiologie, Univ Lyon, Université Claude Bernard Lyon 1, INSERM, U1111, CNRS, UMR5308, ENS Lyon, 69007 Lyon, France

Correspondence: Els Verhoeyen, International Center for Infectiology Research, EVIR, Ecole Normale Supérieure de Lyon, 46 Allée d'Italie, 69364 Lyon, France and Université Côte d'Azur, INSERM, C3M, 151 Route St Antoine de Ginestière, Nice, France.
E-mail: els.verhoeyen@ens-lyon.fr

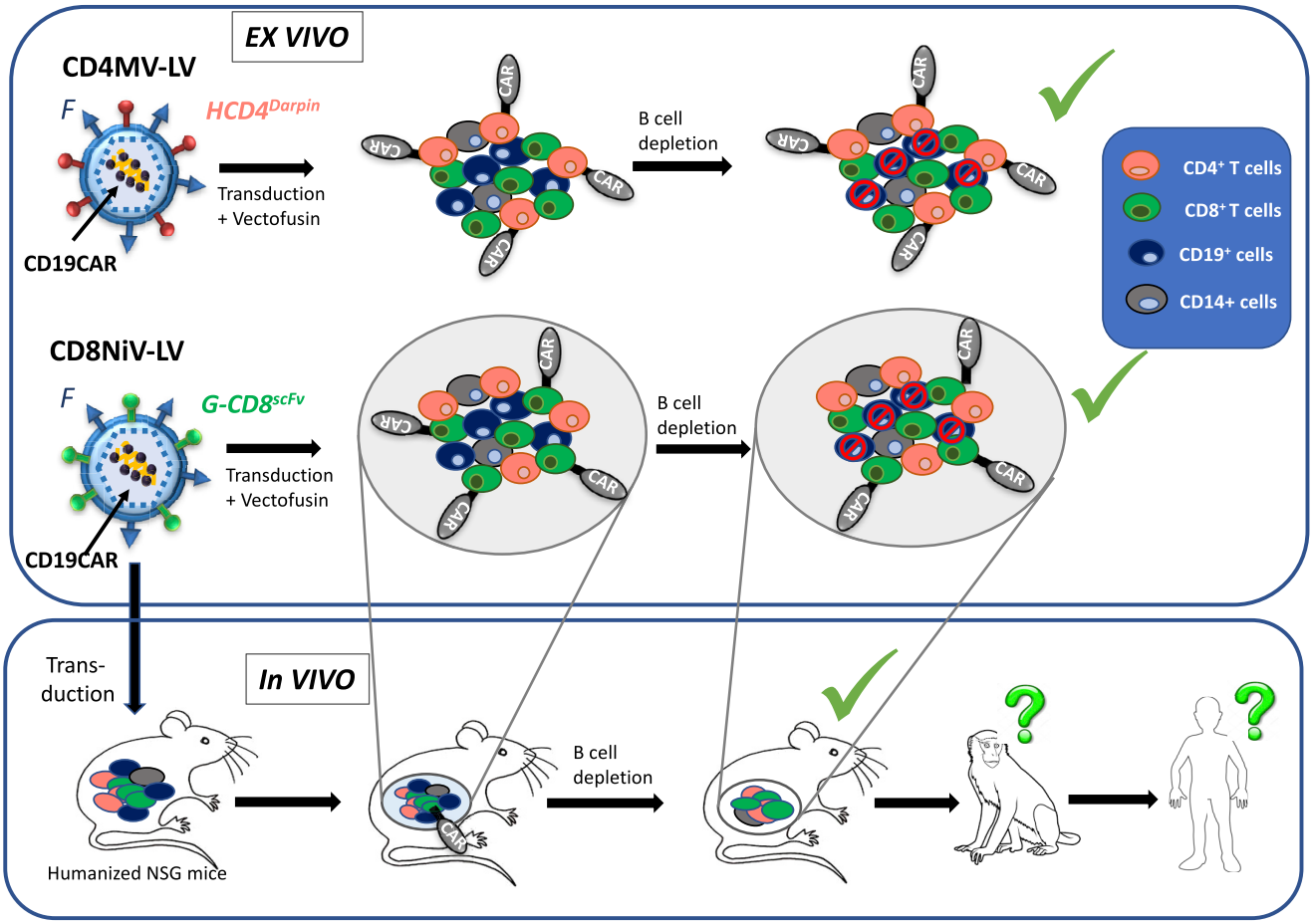

Figure 1. Toward *In Vivo* CAR T Cell Therapy

Exclusive transduction of $CD4^+$ or $CD8^+$ T cells in total PBMCs with an anti-CD19 CAR encoding $CD4^{darpin}$MV- and $CD8^{scFv}$NiV-retargeted lentiviral vectors, respectively. CAR T cells were able to specifically deplete healthy and cancer $CD19^+$ cells *ex vivo*. *In vivo* injection of $CD8^{scFv}$NiV-retargeted lentiviral vectors into non-obese diabetic/severe combined immunodeficient (NOD/SCID) $\gamma C^{-/-}$ mice that were engrafted with $hCD34^+$ stem and progenitor cells resulted in expression of the CD19 directed CAR solely on $CD8^+$ cells *in vivo* which, upon encounter with $CD19^+$ B cells, were strongly expanded and resulted in B cell depletion *in vivo*. This represents a first step toward *in vivo* CAR therapy but evaluation in non-human primates will be required before these tools can enter the clinic for patient treatment.

pseudotyped with receptor-targeted Nipah virus glycoproteins (NiV-LVs). These effectively enter into cells when using cell surface proteins as receptors that bring them sufficiently close to the cell membrane.[6]

Jamali et al.[3] demonstrated exclusive transduction of $CD4^+$ or $CD8^+$ T cells in total peripheral blood mononuclear cells (PBMCs) with an anti-CD19 CAR encoding CD4MV- or CD8NiV-retargeted LVs, respectively. Moreover, in the presence of an LV facilitating agent, Vectofusin, transduction with CD19-CAR-targeted LVs was comparable to T cell transduction levels achieved with VSVG-LVs currently used in the clinic. This was achieved without compromising the specificity for the target T cells (Figure 1). Although such a targeted approach will make *ex vivo* CAR T cell therapy safer, it remains a highly personalized treatment that likely requires a costly clinical manufacturing grade and labor-intensive *ex vivo* T cell manufacturing process. These considerations highlight the obstacles associated with manufacturing of this kind of patient-specific therapy. Therefore, implementation of CAR T cell therapy for routine use in the clinic remains a real challenge and this new therapeutic product may be out of reach for many cancer patients in need of this novel therapy. A future approach avoiding the above concerns may be *in vivo* CAR T cell generation.

The Buchholz team[7] has very recently performed a first step toward *in vivo* reprogramming of CAR T cells using CD8NiV-targeted LVs. A single systemic injection of CD19-CAR-encoding CD8-LVs into immune-deficient mice engrafted with a human blood system generated *in vivo* CAR T cells, which effectively wiped out the human B cells (Figure 1).[7] This outcome supports the feasibility of *in vivo* CAR T cell therapy. Remarkably, NiV-LVs could be produced at up to two orders of magnitude higher titers compared to their MV-based counterparts and were at

least 10,000-fold less effectively neutralized than MV glycoprotein pseudotyped LVs by human serum, underlining their potential as a medicine that is applicable for *in vivo* delivery.[4]

However, some of the CD8NiV-LV injected humanized mice developed a cytokine storm equivalent to some CAR T cell treated patients, which warrants further preclinical testing of the *in vivo* approach. A next logical step will be to test the performance of *in vivo* CAR T cells therapy in mice carrying patient derived tumor xenografts (PDX mice models). However, humanized mice do not recapitulate a fully functional immune system and evaluation in large animal models such as non-human primates may be needed before a first clinical trial on *in vivo* CAR T cell gene therapy can commence (Figure 1). Nonetheless, once these steps have been successfully completed future CAR T cell therapy might consist of a single injection of a vectorized medicine into the blood stream, circumventing the current cost-intensive *ex vivo* production of CAR T cells, and thus making this therapy more broadly available to patients.

REFERENCES

1. Guedan, S., Calderon, H., Posey, A.D., Jr., and Maus, M.V. (2018). Engineering and Design of Chimeric Antigen Receptors. Mol. Ther. Methods Clin. Dev. *12*, 145–156.

2. Ruella, M., Xu, J., Barrett, D.M., Fraietta, J.A., Reich, T.J., Ambrose, D.E., Klichinsky, M., Shestova, O., Patel, P.R., Kulikovskaya, I., et al. (2018). Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. Nat. Med. *24*, 1499–1503.

3. Jamali, A., Kapitza, L., Schaser, T., Johnston, I.C.D., Buchholz, C.J., and Hartmann, J. (2019). Highly efficient and selective CAR-gene transfer using CD4- and CD8-targeted lentiviral vectors. Mol. Ther. Methods Clin. Dev., Published online March 16, 2019. https://doi.org/10.1016/j.omtm.2019.03.003.

4. Bender, R.R., Muth, A., Schneider, I.C., Friedel, T., Hartmann, J., Plückthun, A., Maisner, A., and Buchholz, C.J. (2016). Receptor-Targeted Nipah Virus Glycoproteins Improve Cell-Type Selective Gene Delivery and Reveal a Preference for Membrane-Proximal Cell Attachment. PLoS Pathog. *12*, e1005641.

5. Zhou, Q., Uhlig, K.M., Muth, A., Kimpel, J., Lévy, C., Münch, R.C., Seifried, J., Pfeiffer, A., Trkola, A., Coulibaly, C., et al. (2015). Exclusive Transduction of Human CD4+ T Cells upon Systemic Delivery of CD4-Targeted Lentiviral Vectors. J. Immunol. *195*, 2493–2501.

6. Frank, A.M., and Buchholz, C.J. (2018). Surface-Engineered Lentiviral Vectors for Selective Gene Transfer into Subtypes of Lymphocytes. Mol. Ther. Methods Clin. Dev. *12*, 19–31.

7. Pfeiffer, A., Thalheimer, F.B., Hartmann, S., Frank, A.M., Bender, R.R., Danisch, S., Costa, C., Wels, W.S., Modlich, U., Stripecke, R., et al. (2018). *In vivo* generation of human CD19-CAR T cells results in B-cell depletion and signs of cytokine release syndrome. EMBO Mol. Med. *10*, 10.