Molecular Therapy
Editorial

Seek and You Will Not Find: Ending the Hunt for Replication-Competent Retroviruses during Human Gene Therapy

In this issue of *Molecular Therapy*, two articles[1,2] and the related Letter to the Editor[3] provide extensive data on replication-competent retrovirus (RCR) and replication-competent lentivirus (RCL) monitoring in T cells genetically modified with retroviral or lentiviral vectors and in patient follow-up samples; no evidence for RCR or RCL has yet been found. These and previous studies[4,5] argue that T cell products from an RCR- or RCL-negative vector lot need no longer be routinely subjected to this expensive and burdensome testing and that patient follow-up samples can be archived rather than tested. This change would significantly reduce the cost of these potent therapies without adversely affecting their safety.

When integrating viruses such as retroviruses and lentiviruses were first used to transduce cellular products in clinical trials, investigators and regulatory agencies feared that a recombination event could lead to generation of a novel RCR or RCL during cell product manufacturing or in the patients receiving them. Such an event would increase genotoxicity and the risk of malignant transformation. A primate gene transfer study in the early 1990s, in which 3 of 10 animals who received hematopoietic stem cells transduced with a retroviral vector contaminated with replication-competent virus developed an aggressive T cell lymphoproliferative disease,[6] confirmed this risk. The US Food and Drug Administration (FDA) therefore published guidance for monitoring clinical vector lots, manufactured cell products, and patients post-infusion using biologic or PCR-based testing to detect RCR or RCL.

In the 25 years since this guidance was published, retroviral packaging cell line and vector designs have minimized the homology between vector and packaging cell sequences and have segregated packaging genes so that the generation of RCR is extremely unlikely. The segregation of vector components into four plasmids for lentiviral production has similarly ensured that, to date, RCL generation remains only a theoretical possibility.[5] In addition, there are now long-term safety data from clinical trials, all of which used vector supernatants that were released for use only after negative tests for RCR/RCL. In 2012, Bear et al.[4] published a multicenter review reporting that RCR screening using amplification in HEK293 cells and analysis with the S^+L^- focus assay were consistently negative from 30 master cell banks (MCBs) and 42 viral supernatant lots used in clinical trials at 4 centers. These vectors were used to generate 297 genetically-modified T cell products, all of which were themselves RCR negative. RCRs were also absent in 629 patient follow-up samples.[4] In 2011, the National Gene Vector Biorepository (NGVB) similarly reported that 16 lentiviral vector products manufactured for clinical trial use had no evidence of RCL.

The two articles and the Letter to the Editor in this issue of *Molecular Therapy* provide additional safety data to assuage concerns. A report from the NGVB discusses the screening of samples from 26 trials that used third-generation lentiviral vectors produced by transient transfection to genetically modify T cells.[1] Over 450 transduced T cell products manufactured for 375 subjects were screened for RCL, and all tests were negative. In addition, 296 of the subjects that received the T cell product were screened for RCL (using PCR for *env*) at least 1 month after infusion of the cell product. All tests were negative.[1] A second report from the University of Pennsylvania presents data between 2001 and 2017 for eight different investigational products used in clinical trial subjects with hematological malignancies, solid tumors, or HIV.[2] They analyzed 17 vector lots (15 lentiviral and 2 retroviral), 375 manufactured T cell products, and 308 patient samples after infusion. These analyses were also consistently negative.[2] Finally, a letter from Lyon et al.[3] updates the Baylor College of Medicine experience with 9 different retroviral vector lots tested for RCR. Again, all were negative. These vectors were used in 17 clinical trials to manufacture 266 T cell lines, all of which tested negative for RCR using a co-culture assay. A total of 549 patient samples from 220 patients infused with these products have been assayed, and all have been negative.[3]

Table 1. Published reports of RCR/RCL Testing

	Vector Products		Gene-Modified T Cell Lines		Patient Follow-Up Studies	
Study	RCL	RCR	RCL	RCR	RCL	RCR
Bear et al., 2012[4]	–	42 vector lots negative	–	297 negative	–	629 negative
Cornetta et al., 2017[5]	26 vector lots negative	–	460 negative	–	296 negative	–
Marcucci et al., 2017[2]	15 vector lots negative	2 vector lots negative	351 negative	24 negative	288 negative	20 negative
Lyon et al., 2017[3]	–	9 vector lots negative	–	266 negative	–	549 negative
Total	41 negative	53 negative	811 negative	587 negative	584 negative	1495 negative

The results from these three studies as well as the previous 2012 report from Bear et al.[2] are summarized in Table 1. They provide compelling safety data attesting to the low risk of generating RCR or RCL with current retroviral PG13 packaging cells and vectors and third-generation lentiviral vectors using VSV-G and transient transfection, although we do not yet know whether newly emerging lentiviral packaging cell lines and viral envelopes will have similar characteristics. Nonetheless, these published studies show no measurable risk that any RCR-/RCL-negative supernatants will lead to unanticipated replicative recombinants following gene transfer. While testing genetically modified T cell products for RCL or RCR provides no discernible added value over testing of the retroviral or lentiviral vector, it imposes a substantial resource and financial burden—usually over $10,000 for each patient.

The recent FDA approval of two genetically modified T cell products targeting CD19 with chimeric antigen receptors[7–9] has led to intense debate about cost and accessibility,[7] a debate that will only become more intense as the number and reach of such therapies becomes broader. Eliminating the requirement for RCR/RCL testing of the genetically modified T cell product and routine testing of patient follow-up samples would significantly reduce the cost of developing and implementing these potent therapies without adversely affecting their safety. It is, therefore, now time to review the RCR and RCL guidelines that have been in place for 25 years. We should base our new practices on current evidence rather than past fears.

AUTHOR CONTRIBUTIONS

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