December 10, 2018

Division of Dockets Management (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Comments for Docket No. FDA–1999–D–0081: Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up

Dear Sir/Madam:

The American Society of Gene & Cell Therapy (ASGCT) appreciates the opportunity to comment on this guidance document. ASGCT is a professional membership organization for gene and cell therapy with over 3,000 members. Membership consists primarily of scientific researchers, physicians, other professionals, and students in training. Members work in a wide range of settings including universities, hospitals, biotechnology and pharmaceutical companies, and government agencies. The mission of ASGCT is to advance knowledge, awareness, and education leading to the discovery and clinical application of genetic and cellular therapies to alleviate human disease.

FDA's recommendations in this draft guidance are generally welcomed and will provide clarity regarding testing of retroviral vector-based human gene therapy products for replication competent retrovirus. However, ASGCT recommends that RCR/RCL testing post-infusion should only be required in the case of an adverse event.[i-iv] In addition, guidelines for RCR/RCL testing assays (appendices) are problematic in terms of implementation. The following specific comments are provided for FDA consideration:

Section/ Lines	Comment/Issue	Proposed Change
I.	**Introduction**	
92 – 93	Guidance text: "RCR can be generated during the manufacture of a retrovirus vector from any of these genera." Comment: While insertion of lentiviral vectors can lead to growth dysregulation, the risk appears to be significantly lower and through a different mechanism. HIV-1 is not oncogenic. Lentiviral vectors are stripped of accessory regions important in HIV-1 growth and pathogenesis. The lack of accessory genes in HIV-1	Recommended change: Revise this sentence to indicate that RCR has only been observed with early generation gammaretroviral vectors, that RCR has never been observed with modern split-packaging gammaretroviral and

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	suggests any RCL would have a low risk of immunodeficiency. While this remains a theoretical possibility, RCR has never been observed for modern gammaretroviral vectors or lentiviral vectors.[ii–v] Risk of exposure to RCL differs from RCR.[ii,v]	lentiviral packaging systems, and for these reasons the risk of RCR remains only "a theoretical concern" for these and the other genera.
III.	**Recommendations for Product Testing**	
	A. Material for Testing	
	1. Vector Producer Cell Master Bank	
204 - 206	Guidance Text: "Both cells and supernatant from the VPC MCB should be tested for RCR using a cell line permissive for the RCR that could potentially be generated in a given producer cell line." Comment: This guidance presupposes that the nature of RCR that can be generated is known and ignores the potential that RCR can be generated that does not make use of the envelope gene provided as part of the packaging strategy. It remains a theoretical possibility that RCR containing all or part of the vector and/or ancillary packaging genes can be generated using ancestral viral envelope sequences present in the genome of the packaging cells. This approach also presupposes that envelope sequences such as those derived from GALV or VSV-G are capable of generating RCR when combined with all or part of the vector and/or ancillary packaging genes; such RCR have never been observed, and even the potential for such a recombinant to replicate has never been demonstrated. ASGCT recommends that this requirement either be removed altogether, or that the Draft Guidance be revised to make clear that a cell line used for RCR testing should be chosen based on the tropism of the parental virus used to generate the vector, rather than on the "RCR that could potentially be generated in a given producer cell line."	
	3. Ex Vivo Transduced Cells	
244 – 247	Guidance text: "It is possible that RCR may be present in your vector at undetectable levels, which could be amplified during the manufacture of *ex vivo* transduced cells. Therefore, we recommend that each lot of *ex vivo*	Recommended change: "For well-characterized systems, testing of transduced cell products

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	transduced cells and culture supernatant be tested for RCR." Comment: There is little scientific rationale or data indicating testing of ex vivo products protects patients from RCR/RCL exposure. Biologic assays are the gold standard, but are cumbersome and expensive. Alternative methods may not detect RCR/RCL or lead to false positives. Supporting data: Transduced cell products (n=282) screened for RCR from 14 clinical trials were all negative for RCR.[iv] Of the clinical trial participants, 241 were also screened for RCR by analyzing peripheral blood at least one month after infusion, all of which were also negative for RCR.[iv] An additional 95 cell products were negative at the National Gene Vector Biorepository.[iv] Screening clinical cell products for RCR: Unpublished data, courtesy of Dr. Helen Heslop, Baylor College of Medicine: Transduced cell products (n=266) screened for RCR from 17 clinical trials, all negative for RCR; 220 clinical trial participants who received these products (some received more than one product) were also screened for RCR by analyzing peripheral blood at least one month after infusion, all of which were also negative for RCR.	does not add value and is not required."
254 – 258	Guidance Text: "If you have accumulated manufacturing and clinical experience that demonstrates that your transduced cell product is consistently RCR-negative (section III.A.3 of this document), we recommend that you provide this data to support reduction or elimination of testing *ex vivo* genetically modified cells for RCR." Comment: ASGCT supports extending this option both to the vector production setting and to the post-administration setting. We support not testing vectors, with data support, both a sponsor's own data and generally available data. There has been no evidence of RCR/RCL post-infusion of cell products. We recommend RCR/RCL testing post-infusion only in the case of an adverse event. Supporting data: Indiana University experience found over 30 vector products generated in PG13 cell line	

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	were RCR free. There was no RCL detected in 16 vector products, 17 EOP cells, 7 cell lines, in material from six different GMP facilities (20% IU), 1.3 x 10^{14} virions, 1.8 x 10^{9} cells.[ii] Absence of Replication Competent Lentivirus in the Clinic: T-cell products screened from 26 clinical trials, 6 centers, 460 cell products from 375 patients, 275 patients subsequently screened by qPCR for VSV-G env at least one month post T cell infusion were all negative.[iii] Retroviral and lentiviral safety analysis of gene-modified T-cell products and infused HIV and oncology patients: 17 vector lots, 375 manufactured T cell products, and 308 patients post-infusion across both HIV and oncology indications, showing no evidence of RCR/L.[v] Poisson probability model estimates that a single patient, or a group of patients, would need to be followed for at least 52.8 years to observe one positive RCR/L event.[v]	
	C. Assays for Testing	
333-337	Guidance Text: "All assays should include relevant positive and negative controls to assess specificity, sensitivity, and reproducibility of the detection method employed. Each lot of retroviral vector supernatant should be tested for inhibitory effects on detection of RCR by using positive control samples that are added to vector supernatant." Comment: While ASGCT agrees that a positive control is essential for validating any RCR test, an appropriate positive control would, by its nature, be replication-competent. Replication-competent retrovirus represents two very significant risks: risk to the health of laboratory staff; and risk of cross-contaminating vector production. Given the overwhelming evidence that modern split-packaging gammaretroviral and lentiviral packaging systems never generate RCR, and that proper assessment of RCR involves risk due to the need of an RCR-positive control, it is time to substantially reduce the requirements for RCR testing. At the very least, this section should be followed by additional guidance regarding the separation of activities associated with vector production from activities associated with RCR	

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	testing, including the use of physically separated laboratory and storage spaces in order to minimize the risk of cross-contaminating vector production.	
IV.	**Recommendations for Patient Monitoring**	
	A. RCR Testing Schedule	
388 – 393	Guidance text: "Relevant clinical samples should be collected and tested for RCR upon development of an adverse event suggestive of a retrovirus-associated disease. If patients die or develop neoplasms during a gene therapy trial, every effort should be made to assay for RCR in a biopsy sample of the neoplastic tissue or the pertinent autopsy tissue. Sample collection and storage should be compatible with the expected testing strategy." Comment: Given the overwhelming evidence that modern split-packaging gammaretroviral and lentiviral packaging systems never generate RCR, we encourage the FDA to adopt this standard as the default standard for post-dosing RCR testing. We agree that it is still important to collect and archive tissue samples for retrospective RCR testing, but we recommend that such testing should only be conducted for cause.	

Thank you for consideration of these comments. Please do not hesitate to let ASGCT know if you have questions.

Sincerely,

Maritza C. McIntrye, PhD
Chair, ASGCT Clinical Trials and Regulatory Affairs Committee

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[i]Miller A.D., Garcia J.V., von Suhr N., Lynch C.M., Wilson C., Eiden M.V. Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. *J Virol.* 1991; 65(5):2220-4.

[ii]Cornetta K., Yao J., Jasti A., Koop S., Douglas M., Hsu D., Couture L.A., Hawkins T., Duffy L. Replication-competent Lentivirus Analysis of Clinical Grade Vector Products. *Mol. Ther.* 2011; 19(3): 557-566.

[iii]Cornetta K., Duffy L., Turtle C.J., Jensen M., Forman S., Binder-Scholl G., Fry T., A. Chew, Maloney D.G., and June, C.H. Absence of Replication-Competent Lentivirus in the Clinic: Analysis of Infused T Cell Products. *Mol. Ther.* 2017; 26: 280-288.

[iv]Cornetta K., Duffy L., Feldman S., Mackall C.L., Davila M.L., Curran K.J., Junghans R.P., Tang J.Y., Kochenderfer J.N., O'Cearbhaill R., et al. Screening Clinical Cell Products for Replication Competent Retrovirus: The National Gene Vector Biorepository Experience. *Mol. Ther. Methods Clin. Dev.* 2018; 10: 371-378.

[v]Marcucci K. T., Jadlowsky J.K., W-T Hwang W.-T., Suhoski-Davis M., Gonzalez V.E., Kulikovskaya I., Gupta M., Lacey S. F., Plesa G., Chew A., et al. Retroviral and Lentiviral Safety Analysis of Gene-Modified T Cell Products and Infused HIV and Oncology Patients. *Mol. Ther.* 2017; 26: 269-279.