Title: Absence of Replication Competent Lentivirus in the Clinic: Analysis of Infused T Cell Products

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ABSTRACT

Exposure to replication competent lentivirus (RCL) is a theoretical safety concern for individuals treated with lentiviral gene therapy. For certain ex vivo gene therapy applications, including cancer immunotherapy trials, RCL detection assays are used to screen the vector product as well as the vector-transduced cells. In this study, we reviewed T cell products screened for RCL using methodology developed in the National Gene Vector Biorepository. All trials utilized third generation lentiviral vectors produced by transient transfection. Samples from 26 clinical trials totaling 460 transduced cell products from 375 subjects were evaluated. All cell products were negative for RCL. A total of 296 of the clinical trial participants were screened for RCL at least 1 month after infusion of the cell product. No research subject has shown evidence of RCL infection. These findings provide further evidence attesting to the safety of third generation lentiviral vectors and that testing T cell products for RCL does not provide added value to screening the lentiviral vector product.

INTRODUCTION

Adoptive therapy with genetically modified T cells using lentiviral vectors is in advanced stages of clinical development for cancer indications by academic investigators and several companies.[1-4] Commercial approval by the US Food and Drug Administration of CTL019, a CD19 CAR T cell for the therapy of relapsed leukemia is expected in 2017. In addition, several centers are testing engineered hematopoietic stem cells and other targets using gene transfer with lentiviral vector technology.[5-9] Thus, detection of replication competent lentivirus (RCL) is emerging as a major issue given the widespread use of lentiviral vector technology.

Detecting RCL in lentiviral vector products is a key release test to ensure patients are not inadvertently exposed to replicating virus. The most likely source of RCL virus would be recombination between vector sequences and the viral genes expressed during vector manufacture.[10-12] Detection of a vector-associated RCL is challenging given this virus is still theoretical and therefore the components of the virus are unknown. Replicating viruses have been described in the manufacture of vectors based on murine leukemia viruses (MLV). Most commonly, these MLV-derived viruses arose through recombination of vector and packaging sequences and decreasing homology between vector and packaging sequences has been shown to decrease virus formation.[13-22] Some recombinant retroviruses have also been shown to contain vector packaging sequences and cellular derived genes.[23, 24] This raises the possibility that a RCL could contain packaging sequences along with endogenous human retroviral[25] or other cellular components. This experience with MLV-based vectors has shaped FDA recommendations for recombinant virus testing, including recommendations for RCL assays.[26]

In the US, a lentiviral vector lot must be screened for RCL prior to clinical use.[27] Research subjects are also continuously monitored after treatment for the presence of RCL. A

third assessment is also required for any cell product cultured ex vivo for more than 4 days, since a putative RCL that was not detected in the vector release assay may be amplified in cell culture and thus become detectable. As the majority of T cell receptor (TCR) and chimeric antigen receptor (CAR) vector trials use cell expansion, RCL screening of the infused T cell product is required for most cancer immunotherapy trials. This requirement presents challenges to the clinical development of T cell applications due to the number of cells that must be tested (1% of the cell product or 10^8 cells, whichever is less),[27, 28] the complexity of assessing RCL in high titer vector,[29] and the associated expense of screening this large number of cells.

RCL detection is also complicated by the similarity between vector and viral particles. Many components of an RCL will be similar to a vector particle (capsid, integrase and reverse transcriptase) so most protein detection methods will not be fruitful. Similarly, assay for reverse transcriptase activity[30, 31] cannot distinguish RCL from vector particles. While vector genomes lack genes used in viral replication, these genes must be expressed in vector producing cells and any carryover of cellular or plasmid DNA into the vector product can lead to false positive molecular assays. Moreover, all non-culture assays to date lack the sensitivity of culture based assays where theoretically one infectious unit can be amplified to large numbers.[11] A number of RCL culture assays have been described including syncytia formation assays capable of detecting a fully competent lentivirus but the sensitivity of this approach in detecting an attenuated virus has not been extensively studied.[32] Marker rescue assays have also been described for HIV-1 but whether a RCL arising from vector production will mobilize the marker is unknown.[33, 34] To date, the most common assays for screening gene therapy products are assays which combine an amplification phase using a cell line capable of expanding attenuated viruses to high titer, with subsequent detection of virus using ELISA or molecular assays. [29, 35-39]

Since RCL arising during vector production are still theoretical, their growth rate is unknown, but it is likely to be significantly attenuated compared to wild type lentiviruses due to the absence of accessory genes.[33] Therefore, regulators have required biologic assays to utilize an extended culture period of approximately 3 weeks (a minimum of 5 passages)[27] to amplify any slow growing viruses. Using this stringent screening method, RCL has not been reported in any research or clinical lentiviral vector preparations. The lack of RCL provides support for the overall safety of lentiviral vectors and suggests the multiple safety features incorporated in vector design are effective in limiting RCL development.[40]

Since the advent of lentiviral vectors for clinical application in 2003, a conservative RCL testing approach has been implemented to allow for an optimal risk:benefit for patients, while the field gathered experience with this new vector system. It is unknown whether the testing of infused products adds additional value to the testing of the vector product. In this paper, we review the experience of the National Gene Vector Biorepository (NGVB), a NHLBI funded resource to assist investigators in meeting FDA requirements for gene therapy (www.NGVBCC.org). The NGVB has assisted investigators in testing T cell products for RCL from a variety of clinical trials. To date, none of the products tested were found to contain RCL. The data suggests that RCL testing of infused products does not provide additional assurances of safety and that screening vector products is a sufficient release test for third generation lentiviral vectors.

RESULTS

Lentiviral vectors have much in common with native lentiviruses making it difficult to distinguish vector particles from RCL. Biologic assays which expose permissive cell lines to

vector preparations have been shown to be the preferred method of RCL detection.[11, 35, 36] In

2011, we reported our assay's methodology and performance in testing clinical vector products.[29]

The general components of the RCL assay used in this study are shown in Figure 1. Test articles

and parallel positive and negative controls are added to C8166-45 T cells and the cultures

maintained for three weeks (amplification phase). C8166-45 T cells have been shown to generate

HIV-1 to high titer and also show high transduction with VSV-G (Vesicular Stomatitis Virus –

G) pseudotyped vector.[11, 35] Culture supernatant is then used to inoculate naïve C8166-45 cells

which are cultured for an additional week. Cultures are then screened for RCL using two

methods: (1) p24 ELISA for HIV capsid protein and, (2) a molecular based assay (either psi-gag

PCR[11] or Product Enhanced Reverse Transcriptase,[30] PERT). As the true nature of a RCL

remains theoretical, we chose two methods of virus detection to improve the chance of

identifying an unusual recombinant.

     T cell product analysis. This analysis reviewed RCL testing of cellular products from

June 2011 until August of 2016. All investigators who submitted greater than 10 test articles to

the NGVB were invited to participate, all agreed to submit data. The test articles represent 97.9%

of T cell products tested within this time period. Table 1 summarizes the general characteristics

of the 26 research studies. All trials utilized a third generation lentiviral vector,[41] 4 studies

involved TCR vector while 17 utilized CAR vectors. The trials utilized vectors to transduce T

cell populations or T cell subset populations. All vector utilized in clinical trials were determined

to be RCL negative by the manufacturer.

     Table 2 provides information about RCL testing. A total of 499 assays were performed

during the study period. The T cell manufacturing processes for initial studies included a parallel

non-transduced control, which provided an opportunity to evaluate assay performance for false

positives. After 39 assays of non-transduced control samples were analyzed and found to be RCL negative, the NGVL requested investigators limit subsequent sample submission to transduced cell products only. A total of 460 transduced cell products are included in this analysis. All were RCL negative. The number of T cells within a product varied per protocol. The US FDA requires 1% of the cell product (up to 1×10^8 cells) be screened for RCL and over half of products tested contained or approached the 1×10^8 maximum. Clinical trials with lower numbers were those treating pediatric subjects or transducing T cell subsets. The total number of transduced cells tested from all studies was 2.4×10^{10}.

Subject Follow-up. Not all products screened for RCL were infused into the intended research subject due to non-RCL related issues. As shown in Table 3, of the 460 transduced products tested for RCL, 409 were infused (89%). A number of trials utilized multiple products per subject, with the 409 products infused into 375 subjects. To help provide additional validation of the RCL testing method, subjects who underwent RCL testing at time points ≥ 30 days post-infusion were tabulated and listed in Table 2. A total of 296 of the 375 (79%) subjects had a least one RCL test performed after infusion, all analyzes were negative for RCL. In all but one subject, RCL detection of peripheral blood was performed using a PCR for VSV-G envelope DNA.

Assay Performance. The RCL assay used in this paper is composed of two culture phases, an initial amplification phase and a second indicator phase. Culture media is tested at the end of the amplification phase for HIV capsid protein (p24 ELISA). Media from the indicator phase is tested for HIV capsid protein as well as a molecular based assay (psi-gag PCR or PERT). Due to the large number of cells per test articles, over half of the assays contained only 2 test articles per assay. Successful performance of the amplification phase is critical since failure

to complete this portion of the assay requires resubmission of test articles and significantly

delays test completion. Reviewing the 499 RCL test articles analyzed, there were 13 out of

specifications or deviations noted in the amplification phase. Repeat testing was justified by

prospective assay acceptance criteria or following a corrective and preventative action plan and

applied to 11 samples. Defective culture flasks caused loss of cells in 2 test articles; both test

articles contained 1×10^8 cells which were split among 25 flasks. Investigators indicated that the

remaining 9.6×10^7 was sufficient to meet the 1% FDA testing requirement and the assay was

completed without requiring additional test articles. For 2 test articles in one assay, the

amplification phase positive controls where negative requiring resubmission of test articles. Two

additional amplification phases cultures were lost due to technician error. In 3 test articles from

two different investigators, the test articles inhibited the growth of the C8166-45 cells.

Resubmitted test articles also showed inhibition and the assay was completed by increasing the

number of C8166-45 cells at each passage. All resubmitted test articles discussed above

completed the assay successfully and were found to be negative for RCL at the amplification and

indicator phase. During review of all assays for this analysis, 2 assays were found to have a

miscalculation in the number of C8166-45 cells added to the culture; the ratio of test article cell

to C8166-45 was 1:4.35 which is below the minimum of 1:5 specified for this assay.

There were 8 indicator phase assays that failed to meet acceptability criteria. Three of the

assays failed due to technician error. One indicator phase culture grew slowly, and repeat using

reserve material also showed slow growth of C8166-45 cells; sufficient cells were available for

analysis. Four indicator assays were repeated because the positive controls were negative.

Reserve amplification phase media was available for all the indicator phase assays noted above;

resubmission test articles were therefore not needed. All indicator phases cultures using reserve amplification phase media were found to be negative for RCL.

Amplification and Indicator Phase culture media is subjected to p24 ELISA analysis to identify HIV capsid protein, a predicted component of any RCL. Each test article and control is run in duplicate wells. There were no instances when both duplicate wells were positive, which is an acceptance criterion for a positive assay. There were 15 instances when one well was below and one slightly above the limits of detection; 9 occurred in a test article and 6 in a negative control samples. In all cases, repeat analysis with reserve material found the sample to be below the limits of detection (12.5 pg/mL). In addition, the test articles in question were negative by psi-gag PCR or PERT. All test articles tested by ELISA met the criteria for RCL negative samples.

Between June 2011 and March of 2013, 84 test articles were analyzed by the psi-gag PCR assay. One assay did not meet criteria due to a positive signal detected in both the test article and the negative control. The p24 ELISA result for this test article was negative in both the amplification and indicator phase. The assay was repeated and both negative control and test article were negative, test article was considered negative for RCL. After March of 2013, the PERT assay replaced psi-gag PCR. There were 3 PERT assays where the standard curve did not meet acceptability criteria and 1 assay where the positive control did not meet acceptance criteria. The assays were repeated using reserve material and met acceptance criteria. In analyzing 417 test articles by PERT, there were 4 test articles and 2 negative control test articles which were just above baseline. The corresponding p24 analysis was negative and repeat PERT testing using reserve samples was negative for reverse transcriptase. Upon retesting, all test articles met the criteria for RCL negative samples.

DISCUSSION

Inadvertent exposure of subjects to pathogens that contaminate gene therapy products is a top safety concern. Lentiviral vectors have been designed to minimize the chance of recombination and, to date, RCL has not been detected in research materials generated with third generation vector systems. In 2011, we also showed that 16 lentiviral vector products manufactured for clinical trial use had no evidence of RCL.[29] Continued testing at the NGVB has failed to detect RCL using material submitted from a variety of manufacturing facilities. In this manuscript, we provide further evidence for the biosafety of lentiviral vector production systems. Using a sensitive RCL assay, there was no evidence of replicating virus in 460 T cell products. The products tested span 26 clinical trials at 6 different institutions. Follow-up analysis of treated research subjects also found no evidence of RCL. Given the lack of documented RCL in a large number of T cell products, the design features of third generation lentiviral vectors, and the lack of documented RCL in clinical vector lots, RCL testing of T cell products does not appear to provide additional assurances of safety and testing requirements should be re-evaluated. Our findings also have implications for research laboratories and suggest re-evaluation of biosafety requirements for third generation lentiviral vectors.

In developing a detection assay for RCL, we developed an assay that was exhaustive by intent. The assay uses both p24 ELISA and a molecular detection assay (psi-gag PCR or PERT) to provide redundancy in order to minimize false positive or false negative results due to technical error. Since we do not know the components of a RCL, two assays aimed at different components of the RCL may also increase the chance of RCL detection. For example, a RCL containing endogenous human retroviral or other unpredicted sequences may not be recognized

in assays aimed at detecting HIV-1. The addition of the indicator phase also ensures that the assay detects a true RCL by requiring passage from amplification phase cells to naïve C8166-45 cells. The assay has been validated to detect 1 IU of RCL per 10^6 test article cells, and in this study, there was a total of 2.4 x 10^{10} transduced T cells tested.

The design of third generation lentiviral vectors suggest the risk of RCL is significantly lower than that of MLV-based vectors.[40] The ability to segregate the vector components onto four plasmids, the use of self-inactivating (SIN) LTR's, and retention of Rev dependence all contributes to the safety profile of lentiviral vectors.[41, 42] HIV-1 also depends on a number of accessory genes and regulatory sequences that are deleted in lentiviral vector systems, further limiting the growth potential of any RCL. Furthermore, lentiviral vectors are generally produced by transient transfection which limits the time for recombination events. Whether the experience in vector products generated by transient transfection will extend to lentiviral packaging cell lines awaits further studies. The most current lentiviral lines do suppress gene expression until shortly before vector harvest and also incorporate many of the safety features described above.[43, 44] This predicts a significantly greater safety profile than the MLV-based packaging cell lines but additional experience is required to establish whether these will consistently generate RCL-free vector products. Similarly, the propensity for RCL development in HIV-1 based vectors with significant differences in vector design, method of manufacture, or envelope will require bridging or full validation studies. Also, our findings do not extend to non-HIV-1 based lentiviruses or other retroviral vector systems.

In general, the RCL assay described here performed well. Of the 499 test articles analyzed there were 11 test articles that required resubmission due to technical issues. Three of the 11 required resubmission due to growth inhibition of the C8166-45 cells. Growth inhibition

appeared to be related to the cellular product; there were no apparent issues of microbial contamination, samples were run on different assays, and were obtained from different investigators, and there was no evidence of p24 in the amplification phase media. The assays were repeated and increasing the number of cells at each split allowed the assays to be completed. Interestingly, the three products in questions (representing 3 of 499 products test or 0.6%) were CD4 T cell subset samples.

There were occasions when a single replicate well in p24 or PERT was above background will all other analyses for p24 and PERT were negative. This occurred in test articles and negative controls. Given the greater number of test articles run per assays compared to the negative control, there is no evidence of a higher frequency occurring in test articles. Both p24 ELISA and PERT assays are performed in plates, the most likely cause of the sporadic positive wells is aerosolization of positive control materials during set up and handling of the plates.

In addition to testing the T cell products, we collected data from the 26 clinical trials on post-infusion RCL testing. The US FDA requires subjects infused with lentiviral transduced products be monitored for RCL but currently there is no guidance requiring a specific assay. Interestingly, each investigator independently chose qPCR for the VSV-G envelope as the preferred method for RCL monitoring. To date, no evidence of VSV-G envelope DNA has been reported in the subjects enrolled in the 26 clinical trials surveyed.

In summary, RCL has not been detected in third generation lentiviral vector products manufactured for clinical use.[29] We now add to that experience by analyzing T cell products used in cancer immunotherapy. In 460 products tested, using a vigorous biologic assay for RCL, there was no evidence of RCL. Participants evaluated post-infusion of T cell products were also without evidence of RCL exposure. These findings suggest that current vector design and vector

product screening provide a high level of assurance regarding the absence of replicating virus.

Therefore, screening T cell products for RCL does not add additional assurance of safety and

should no longer be required when the lentiviral vector product has been successfully screened

for RCL.

MATERIALS AND METHODS

Collection of Study Data. The NGVB is a National Heart, Lung and Blood Institute

sponsored resource that assist gene therapy investigators in meeting FDA required testing

(www.NGVBCC.org) and all T cell product testing was performed centrally at the NGVB. For

this study, RCL assays performed between June 2011 and August 2016 were reviewed and all

investigators with at least one study of greater than 10 subjects were invited to participate. Only

samples intended for in vivo administration were included in the analysis. Investigators agreeing

to participate were sent a list of their studies along with the test articles name and the dates of

assay initiation and completion. Participants were asked to supply the following information (1)

lentiviral vector type (second or third generation); (2) target cell type; (3) transgene type (TCR,

CAR, other); (4) if the product was administered to the subject; (5) the clinical vector product

was shown to be RCL free prior to use in the clinical trial; and (6) if the subject was screened for

RCL at $\geq$ 30 days after product administration. For subjects screened for RCL after product

administration, the testing was not done centrally and the method of screening was at the

investigators discretion. All were screened by VSV-G except for one patient tested by serology

in a clinical pathology laboratory for HIV-1 p24 antigen.

Cell Line and Positive Control Preparation. The C8166-45 (derived from human

umbilical cord lymphocytes) cell line was obtained from the AIDS Research and Reference

Reagent Program (Rockville, MD) and HEK293T cells were obtained from the American Type Culture Collection (Manassas, VA). The former was maintained in RPMI 1640 media while the latter were maintained in Dulbecco's modified Eagle medium (DMEM). Both media were obtained from Invitrogen, Carlsbad, CA and supplemented with 10 % fetal calf serum (FCS; Hyclone, Logan, UT), 2 mM L-glutamine (Invitrogen), and 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen)), (RPMI10 and D10 respectively). D10 also contains sodium pyruvate 100 mM and L-glutamine 200 mM. C8166-45 cells are maintained in cell banks and only those cells at $\leq$ 15 passages at the start of an assay are utilized. The attenuated HIV-1 positive control is generated by transfecting HEK293T cells with the pR8.71 plasmid (provided from Cell Genesys, 112 µg plasmid per 5x 10^6 cells/) followed by a media change at 24 hours. Cell free supernatant (0.45 µm filter) is collected 48 hours after transfection. Material is stored at < -70° C then thawed for potency assessment by preparing thirty nine 50-mL flat bottom culture tube with 1 x 10^6 C8166-45 cells per tube and incubating cells overnight. On day 0, 6 tubes are centrifuged and cells are resuspended in 1 mL of virus with Polybrene at 8 µg/mL. The six dilutions tested range from 10^{-3} to 10^{-8} of the frozen viral stock. Three negative control tubes are also prepared. After a 4-hour incubation, cells are pelleted, resuspended in RPMI, and transferred to 6-well plates. Cells are maintained in log-phase growth for at 12 days after which time cells are pelleted, and the media is filtered (0.45 µm) and assessed for p24 by ELISA. Cultures are read as positive or negative based on the lower limits of detection of the ELISA assay (12.5 pg/mL).

RCL Assays. *Amplification Phase.* The RCL assay used here has previously been reported for screening lentiviral vector products[29] and was used with the modifications described in the text below. Control triplicate negative and positive control cultures are prepared on day – 1

by adding 12 mL of RPMI10 and 5 X 10^6 C8166-45 cells into six 50 mL culture tubes (flat-bottom 10-cm^2). Test article flasks are prepared on day -1 based on the number of cells to be tested. A minimum of 1 test cell to 5 C8166-45 is required for the assay. Most investigators submit 10^8 cells which require twenty five 300 cm^2 flasks. On Day 0, the negative control culture media is replaced with 1 mL of RPMI10 and Polybrene (8 µg/mL) and the three positive controls are inoculated with 1 mL of RPMI10, 5 IU of R8.71 virus, and 8 µg/mL Polybrene. On day 0 the test article cells are introduced into the flasks containing C8166-45 cells. After four hours, cultures are centrifuged and media replace with RPMI10. Cells are passed for 3 weeks with a minimum of 5 splits. Cultures in larger flasks are split into decreasing size flasks with cells cultured in 75 cm^2 flasks by the end of week 3. The media is not conducive for primary T cell growth and C8166-45 cell predominate at the end of the amplification phase.

Indicator Phase. At the end of the 3 week culture, test article and negative and positive control culture cells are resuspended in fresh RPMI10 and media is collected after 24 hours then filtered (0.45 µm). Naïve C8166-45 cells (1 x 10^6 C8166-45 cells in 4 mL) are incubated overnight then cultured with the filtered media for 4 hours in the presence of Polybrene (8 µg/mL). For a test article, the material from all flasks are pooled and used to inoculate 2 test article indicator phase flasks. After 6 days in cultures, cells are resuspended in fresh media and collected 24 hours later, filtered (0.45 µm) and analyzed for evidence of RCL. All samples in this paper were assessed for RCL using the p24 ELISA assay, a commercially available kit (Alliance HIV-1 p24 ELISA kit, Perkin Elmer, Waltham, MA) as previously described.[29] All measurements were done in duplicate. For test articles, any value above the limits of detection (12.5 pg/mL) is considered positive. For the positive controls, values above the upper limits of detection (100 pg/mL) are expected.

The psi-gag PCR assays detects recombinants between the psi and gag viral genes and is described in Sastry et al.[11] with a modification to include a second PCR for human beta globin to validate that the test article DNA is of sufficient quantity and quality. The psi-gag PCR assay required PCR amplification followed by Southern blot analysis with a P^{32}-labeled probes and added significant time to the assay turnaround. A validation study was performed demonstrating equivalency of the PERT assay[30] to the psi-gag PCR assay, samples after March 2013 were evaluated by PERT. The limits of detection in the PERT assay is 100 RT molecules per 25 uL; positive controls at the end of the indicator phase are expect to be in excess of 10^4 RT molecules per 25 uL.

Acceptance criteria is defined as follows. At the end of the indicator phase, the assay is acceptable if (1) all 3 negative controls are negative for p24 antigen; (2) all 3 negative controls are negative for psi-gag sequences or PERT; (3) at least one of the positive controls flasks are positive for p24 antigen and psi-gag at the indicator phases. If the controls are acceptable and the 2 test article flasks are negative for p24 antigen and psi-gag PCR/PERT then the test article is reported as negative for RCL. If the test article is positive for both p24 and psi-gag, the sample is interpreted as RCL positive. If both test article flasks are positive in one assay but negative in the other (ex. positive for p24 and negative for psi-gag PCR/PERT), the indicator phase is repeated and extended for > 14 days before samples are harvested and tested. If one of two test article flasks are positive in one assay and negative in the other assay then both assays are repeated with reserve samples (ex. one flask above and one below background in the ELISA assay and both flasks negative in psi-gag PCR/PERT). The only time the indicator phase is not repeated is when one of the 10 test article analyses is above background (each test article has 2 flasks from the indicator phase, each flask is tested in duplicate for 24 and in triplicate by PERT for a total of 10

analyses per test article). In this case, the discrepant sample is retested from reserve material from the initial indicator phase. If the repeat testing is negative, the sample is considered negative for RCL.

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FIGURE LEGENDS

Figure 1. <u>Schematic representation of the RCL Assay</u>. T cell products are incubated with C8166-45 cells at a ratio of 1:5. Over half of test articles submitted contained approximately 1×10^8 T cells and were divided into twenty five 300 cm^2 flasks. Cultures are passed a minimum of 5 passaged using increasingly smaller vessels. After 3 weeks, cells are placed in fresh media and conditioned media is harvested after 24 hours. Media from all amplification cultures are pooled and two aliquots are then incubated with naïve C8166-45 cells. After 7 days, culture media is analyzed for p24 antigen by ELISA and either the psi-gag PCR assay or PERT. The figure depicts amplification of a low titer RCL present in the test article.

Table 1. Clinical Protocols Submitting Transduced T Cell Products for Replication Competent Lentivirus Testing.

Study Number	Clinical Trial #	Principle Investigator	Initial Assay Initiated	Final Assay Completed	Vector Generation	Transgene class	Transduced Cell Type
11-2	NCT01350401	June	8/8/11	10/28/13	Third	TCR	T cell
11-3	NCT01352286	June	6/27/11	4/15/13	Third	TCR	T cell
11-4	NCT01343043	June	12/6/12	2/13/13	Third	TCR	T cell
11-11	NCT01626495	June	7/11/12	2/20/14	Third	CAR-T	T cell
11-12	NCT01029366	June	12/15/11	9/9/13	Third	CAR-T	T cell
11-13	NCT01551043	June	12/15/11	12/15/11	Third	CAR-T	T cell
12-4	NCT01626495	June	7/2/14	11/9/16	Third	CAR-T	T cell
12-16	NCT01683279	Jensen	12/8/14	3/16/15	Third	CAR-T	CD4, CD8
13-4	NCT01747486	June	3/21/13	4/18/16	Third	CAR-T	T cell
13-12	NCT01567891, NCT01352286, NCT01350401, NCT01343043	Binder-Scholl	9/5/15	3/9/16	Third	TCR	T cell
13-15	NCT01318317, NCT01815749	Forman	2/13/14	2/13/14	Third	CAR-T	memory T
14-9	NCT02030847	June	4/2/14	8/4/16	Third	CAR-T	T cell
14-10	NCT02030834	June	5/5/14	5/2/16	Third	CAR-T	T cell
14-12	NCT01865617	Turtle	5/28/14	8/24/15	Third	CAR-T	CD4, CD8
14-18	NCT02315612	Fry	1/26/15	6/13/16	Third	CAR-T	T cell
14-27	NCT01815749	Forman	2/12/15	2/12/15	Third	CAR-T	memory T
15-9	NCT02146924[a]	Forman	3/30/14	10/26/15	Third	CAR-T	memory T
15-10	NCT02153580	Forman	3/30/15	10/26/15	Third	CAR-T	memory T
15-11	NCT02051257	Forman	3/30/15	10/26/15	Third	CAR-T	memory T
15-26	NCT02028455	Jensen	12/8/14	3/18/16	Third	CAR-T	CD4, CD8
15-36	NCT02208362	Forman	10/26/15	10/26/15	Third	CAR-T	memory T
16-1	NCT02311621	Jensen	4/4/16	6/17/16	Third	CAR-T	CD4, CD8

[a] 1 patient treated on a compassionate use basis linked to this study; TCR = T cell receptor; CAR-T = chimeric antigen receptor T cell; T = T cell.

Table 2. Number of RCL Assays Performed by Study and the Number of Cell Analyzed.

Study Number	Principle Investigator	# Samples Assayed	# negative control samples	# transduced samples	Total number of control cells tested	Total number of transduced cells tests	Average number of transduced cells per assay	Median number of cells per assay
11-2	June	5	2	3	2.00E+08	3.00E+08	1.00E+08	1.00E+08
11-3	June	43	20	23	1.88E+09	2.05E+09	8.90E+07	1.00E+08
11-4	June	4	2	2	2.00E+08	2.00E+08	1.00E+08	1.00E+08
11-11	June	33	5	28	4.07E+08	2.24E+09	7.98E+07	1.00E+08
11-12	June	27	9	18	4.11E+08	1.27E+09	7.04E+07	9.80E+07
11-13	June	2	1	1	5.00E+07	5.00E+07	5.00E+07	5.00E+07
12-4	June	41	0	41	0	3.20E+09	7.80E+07	1.00E+08
12-16	Jensen	3	0	3	0	8.75E+07	2.92E+07	3.07E+07
13-4	June	35	0	35	0	2.64E+09	7.53E+07	8.73E+07
13-12	Binder-Scholl	36	0	36	0	3.60E+09	1.00E+08	1.00E+08
13-15	Forman	6	0	6	0	9.60E+07	1.60E+07	1.65E+07
14-9	June	30	0	30	0	2.39E+09	8.11E+07	1.00E+08
14-10	June	39	0	39	0	3.25E+09	8.33E+07	1.00E+08
14-12	Turtle	90	0	90	0	1.73E+09	1.92E+07	2.00E+07
14-18	Fry	14	0	14	0	1.20E+08	8.57E+06	1.00E+07
14-27	Forman	7	0	7	0	5.09E+07	7.26E+06	5.80E+06
15-9	Forman	6	0	6	0	3.00E+07	5.00E+06	4.38E+06
15-10	Forman	6	0	6	0	3.63E+07	6.04E+06	3.13E+06
15-11	Forman	11	0	11	0	1.14E+08	1.03E+07	1.00E+07
15-26	Jensen	45	0	45	0	3.65E+08	8.30E+06	7.50E+06
15-36	Forman	2	0	2	0	5.75E+06	2.88E+06	2.88E+06
16-1	Jensen	14	0	14	0	1.49E+08	1.06E+06	1.00E+07
TOTAL		**499**	**39**	**460**	**3.15E+09**	**2.40E+10**		
MEAN							**4.64E+07**	**5.26E+07**

Table 3. Follow-up Testing for Replication Competent Lentivirus (RCL) in Subjects Infused with Gene Modified T Cell Products.

IU VPF Study Number	Principle Investigator	# Products Infused	# Subjects Infused	# Subjects with RCL Follow-up*	Method of RCL Detection	Level of Sensitivity per DNA
11-2	June	2	2	1	VSV-G DNA PCR	25 copies per 1 ug
11-3	June	19	17	16	VSV-G DNA PCR	25 copies per 1 ug
11-4	June	2	2	2	VSV-G DNA PCR	25 copies per 1 ug
11-11	June	24	24	21	VSV-G DNA PCR	25 copies per 1 ug
11-12	June	14	13	13	VSV-G DNA PCR	25 copies per 1 ug
11-13	June	1	1	0	VSV-G DNA PCR	25 copies per 1 ug
12-4	June	36	36	34	VSV-G DNA PCR	25 copies per 1 ug
12-16	Jensen	3	3	2	VSV-G DNA PCR	10 copies per 50ng
13-4	June	32	32	23	VSV-G DNA PCR	25 copies per 1 ug
13-12	Binder-Scholl	31	31	24	VSV-G DNA PCR	5 copies per 100 ng
13-15	Forman	5	5	5	VSV-G DNA PCR	2.5 copes per 50 ng
14-9	June	25	25	14	VSV-G DNA PCR	25 copies per 1 ug
14-10	June	34	34	30	VSV-G DNA PCR	25 copies per 1 ug
14-12	Turtle	76	76	49	VSV-G DNA PCR	10 copies per 1 ug
14-18	Fry	14	14	11	VSV-G DNA PCR	10 copies per 200 ng
14-27	Forman	7	7	6	VSV-G DNA PCR	2.5 copes per 50 ng
15-9	Forman	6	4	3**	VSV-G DNA PCR	2.5 copes per 50 ng
15-10	Forman	6	6	5	VSV-G DNA PCR	2.5 copes per 50 ng
15-11	Forman	11	11	11	VSV-G DNA PCR	2.5 copes per 50 ng
15-26	Jensen	45	23	19	VSV-G DNA PCR	10 copies per 50ng
15-36	Forman	2	2	2	VSV-G DNA PCR	2.5 copes per 50 ng
16-1	Jensen	14	7	5	VSV-G DNA PCR	10 copies per 50ng
TOTAL		**409**	**375**	**293**		

* ≥ 30 days post infusion; ** 2 subjects tested by PCR, 1 subject tested by serology

The safety of lentiviral vectors is a factor in their acceptance as clinical therapies. In this issue of *Molecular Therapy*, Cornetta et al. (2017) screened 460 cell products for replication competent lentivirus (RCL); none were positive. The low risk of RCL suggests revisions to US FDA testing guidelines are warranted.