

SLIDE PRESENTATION

379463 - 08 (no electronic copy)

Proposed protocol: Ex-vivo gene transfer of VRX496 into HIV-infected patient-subjects

VRX496 is an HIV vector that contains an anti-HIV antisense sequence

T Cell Isolation

Ex-vivo transduction of T Cells with VRX496

T CELLS

T Cell Expansion

Schematic representation of HIV-based vectors

pNL4-3 (9709bp)

VRX496 (4344bp)

VRX494 (4877bp)

non-coding, dispensable marker sequence from GFP

Safety features of VRX496 for gene transfer into HIV-infected patient-subjects

- Safest approach for gene transfer in HIV-infected patient-subjects - no new sequences are introduced into the patient - the vector is entirely derived from wt-HIV and patient-subjects are laden with wt-HIV
- VRX496 cannot produce a novel pathogenic virus since the vector is a whole derivative of wt-HIV - any recombination event between VRX496 and wt-HIV could not produce a virus that is more pathogenic than wt-HIV
- Targeted expression of anti-HIV antisense payload - antisense payload is Tat and Rev dependent and thus expressed only after wt-HIV infects vector containing cells - no heterologous viral promoters are contained in VRX496
- Antisense payload appears to decrease vector mobilization to cells - expression of anti-envelope antisense results in decreased infection of mobilized vector genomes
- VRX496 contains a triple stop codon in gag - recombination with wt-HIV or helper would likely result in a non-functional gag-pol open reading frame

Possible recombination events between vector and wt-HIV

TRUNCATED ENV, NO TAT

TRUNCATED 3' ENV

TRUNCATED GAG & POL

Result: non-infectious recombinant or wt-HIV

Features of VIRPAC helper construct (VRX170) for VRX496 production

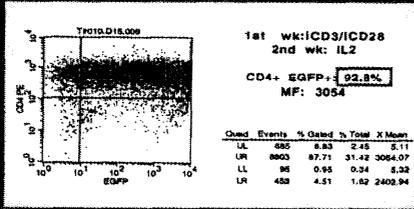
pVRX170 (12010 bps)

- VIRPAC two-plasmid system produces >3-fold higher vector titers (2×10^9 /ml) than commonly used three-plasmid system (7×10^8 /ml) in 293 cells
- Three strong poly A (A_n) and two transcriptional pause sites - transcriptional partition of structural and envelope genes
- Codon degeneration of several regions of the helper protein coding sequence - to decrease packaging of the helper into vector particles - to decrease likelihood of recombination with vector genome

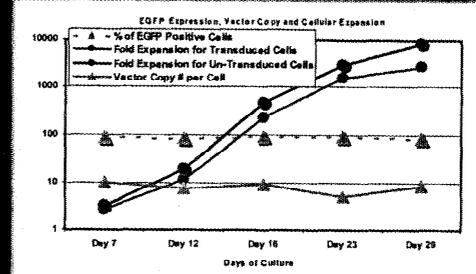
Facilities for raw material production, vector manufacturing and cell processing

- Plasmid raw materials and purified VRX496 vector product are manufactured at VIRxSYS' Clinical Vector Production Facility using cGMP conditions
- Cell processing is performed at the University of Pennsylvania Hospital's Clinical Cell Production Facility using cGMP conditions

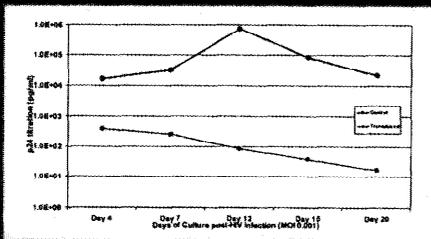
High efficiency transduction of primary human CD4 T cells with VRX494



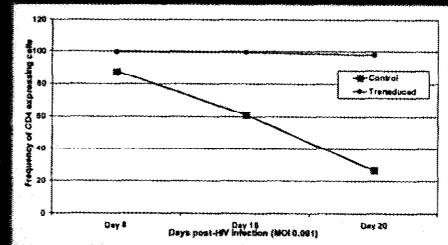
Stable vector transduction of primary CD4 T Cells



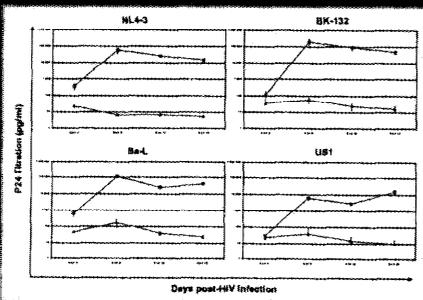
Inhibition of wt-HIV replication by VRX494 containing primary human CD4 T cells



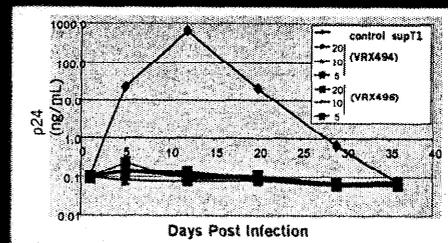
VRX494 containing CD4 T cells are selectively resistant to productive wt-HIV replication

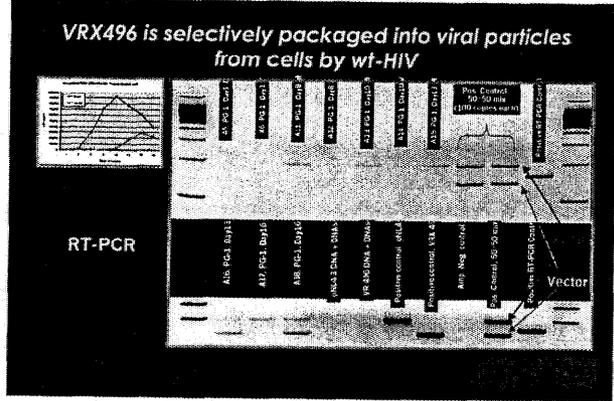
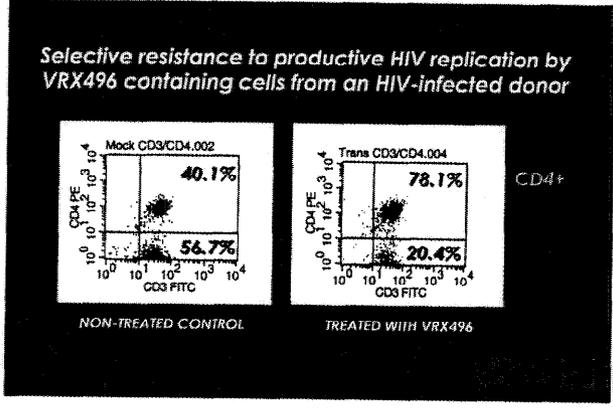
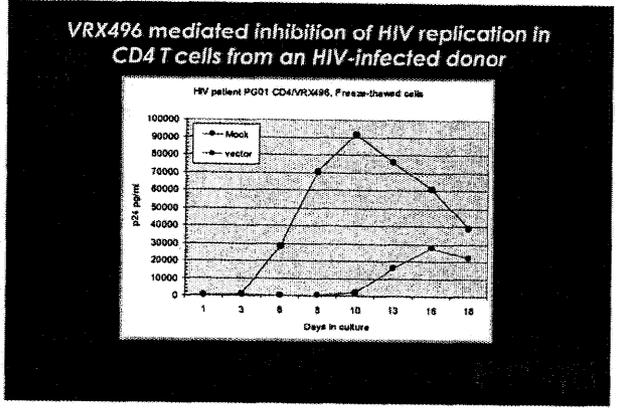
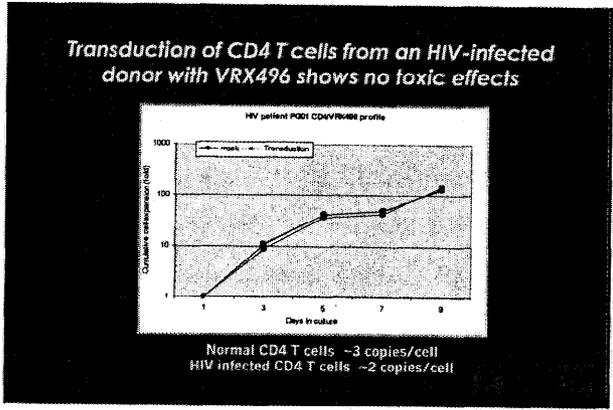
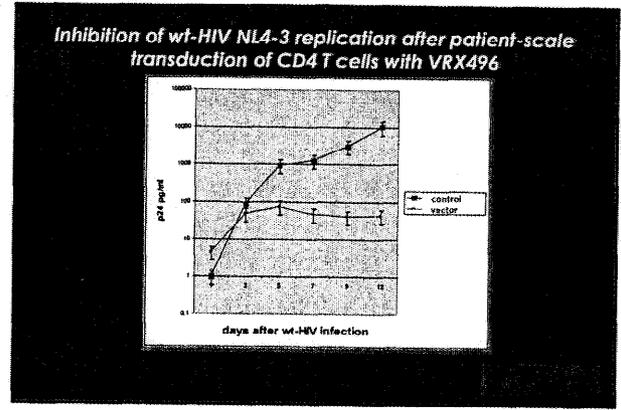
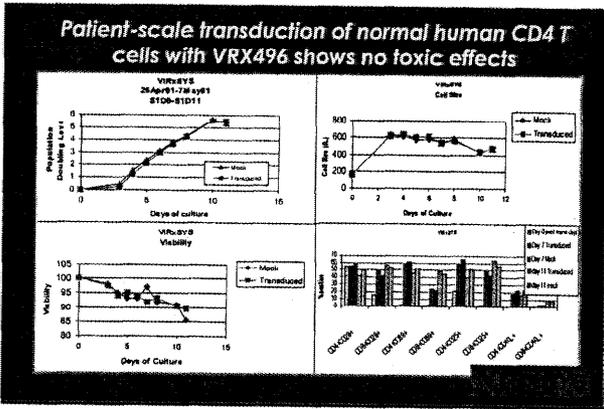


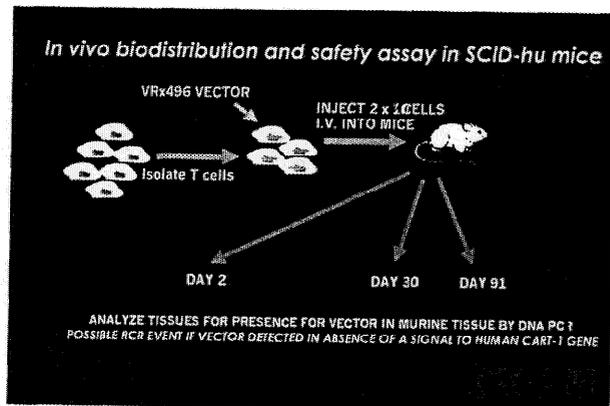
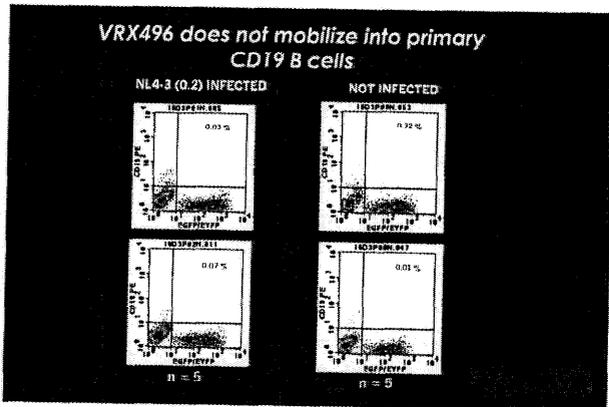
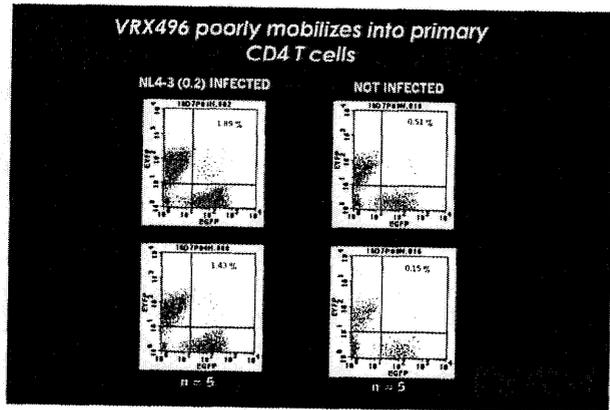
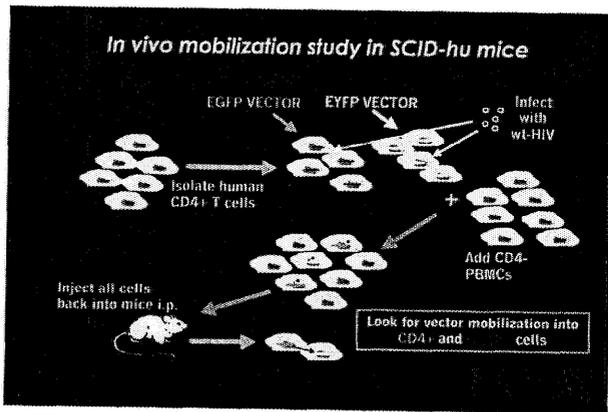
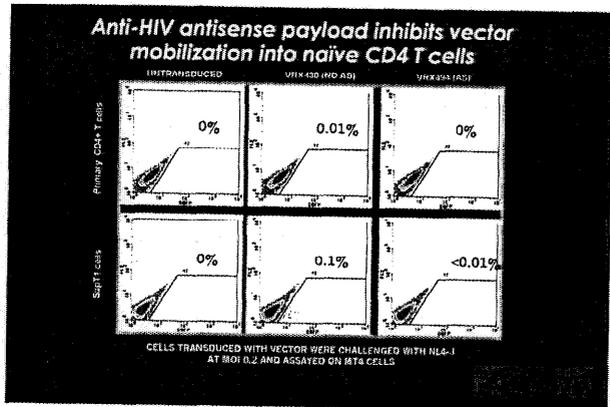
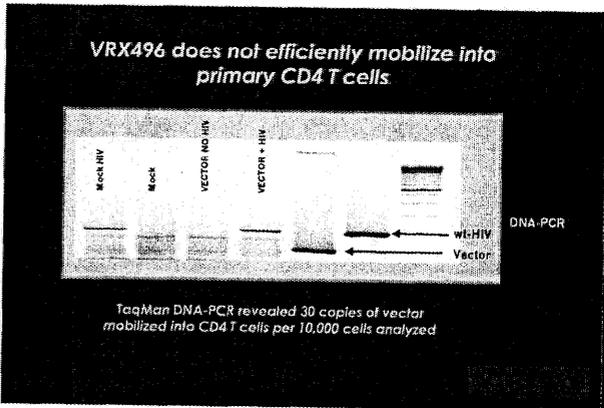
Inhibition of primary HIV strains by VRX494



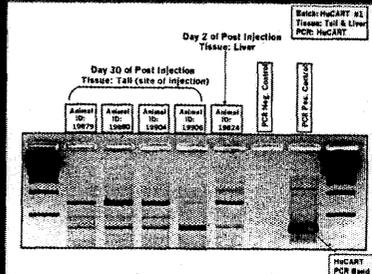
Inhibition of wt-HIV replication by VRX494 and VRX496







HuCARD analysis of G-tag positive tissues from day 30 SCID-hu mice injected with VRX496 CD4 T cells



Sensitivity: 50 copies per ug DNA.

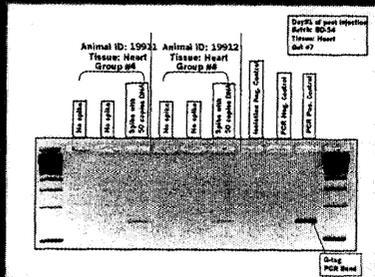
Summary of the Day 30 SCID-hu data

	Heart Vector/Fluoro	Testis Vector/Fluoro	Ovary Vector/Fluoro	Liver Vector/Fluoro	Ing-LN Vector/Fluoro
Group #1	Neg./ND	Neg./ND	Neg./ND	Neg./ND	Neg./ND
Group #2	Neg./ND	Neg./ND	Neg./ND	Neg./ND	Neg./ND
Group #3 (low dose)	5 (+)/ND	4 (+)/ND	4 (+)/ND	7 (+)/ND	8 (+)/ND
Group #4 (high dose)	7 (+)/ND	3 (+)/ND	3 (+)/ND	9 (+)/ND	8 (+)/ND

	Bone Marrow Vector/Fluoro	Lung Vector/Fluoro	Spleen Vector/Fluoro	Tail Vector/Fluoro	Blood Vector/Fluoro
Group #1	Neg./ND	Neg./ND	Neg./ND	Neg./ND	Neg./ND
Group #2	Neg./ND	Neg./ND	Neg./ND	Neg./ND	Neg./ND
Group #3 (low dose)	8 (+)/ND	9 (+)/ND	10 (+)/ND	8 (+)/ND	5 (+)/ND
Group #4 (high dose)	10 (+)/ND	8 (+)/ND	9 (+)/ND	6 (+)/ND	6 (+)/ND

Group 1=medium only; Group 2=no vector cells;
Group 3=low dose VRX496 cells;
Group 4=high dose VRX496 cells

Analysis of SCID-hu mice day 91 post-injection of CD4 T cells transduced with VRX496



Sensitivity: 50 copies per ug DNA

Summary of the Day 91 SCID-hu data

	Heart Vector/Fluoro	Testis Vector/Fluoro	Ovary Vector/Fluoro	Liver Vector/Fluoro	Ing-LN Vector/Fluoro
Group #1	Neg./ND	Neg./ND	Neg./ND	Neg./ND	Neg./ND
Group #2	Neg./ND	Neg./ND	Neg./ND	Neg./ND	Neg./ND
Group #3 (low dose)	10 (+)/ND	5 (+)/ND	5 (+)/ND	10 (+)/ND	10 (+)/ND
Group #4 (high dose)	10 (+)/ND	5 (+)/ND	5 (+)/ND	9 (+)/ND	10 (+)/ND

	Bone Marrow Vector/Fluoro	Lung Vector/Fluoro	Spleen Vector/Fluoro	Tail Vector/Fluoro	Blood Vector/Fluoro
Group #1	Neg./ND	Neg./ND	Neg./ND	Neg./ND	Neg./ND
Group #2	Neg./ND	Neg./ND	Neg./ND	Neg./ND	Neg./ND
Group #3 (low dose)	10 (+)/ND	9 (+)/ND	10 (+)/ND	10 (+)/ND	7 (+)/ND
Group #4 (high dose)	10 (+)/ND	10 (+)/ND	9 (+)/ND	9 (+)/ND	6 (+)/ND

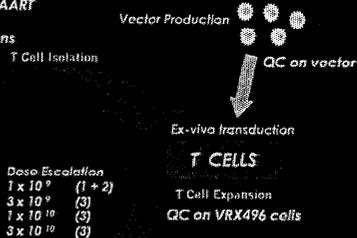
Group 1=medium only; Group 2=no vector cells;
Group 3=low dose VRX496 cells;
Group 4=high dose VRX496 cells

Summary of the proposed clinical trial

Failing or discontinued HAART therapy
No Opportunistic Infections
CD4 200 - 600
VL >500

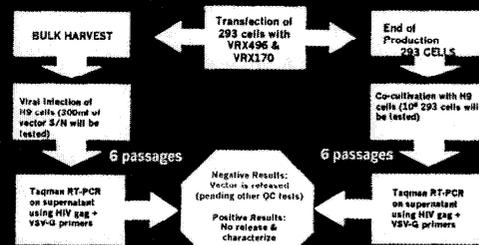


n = 12
(up to 24)



Dose Escalation
1 x 10⁹ (1 + 2)
3 x 10⁹ (3)
1 x 10¹⁰ (3)
3 x 10¹⁰ (3)

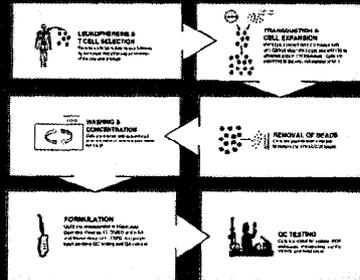
RCR testing of transfected 293 cells & vector product



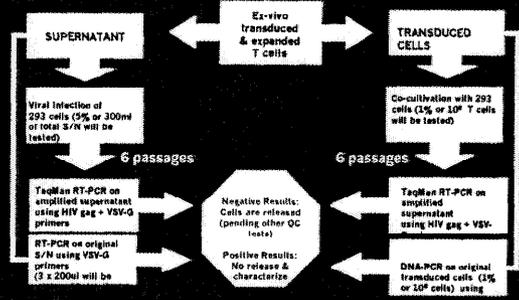
Negative Results: Virus is released (pending other QC tests)
Positive Results: No release & characterize

TaqMan RT-PCR Sensitivity
HIV gag = 1 copy per 10,000 cells
VSV-G = 10 copies per 10,000 cells

Cell processing for ex vivo gene transfer of VRX496 into HIV-infected patient-subjects



RCR testing for VRX496 transduced T cell product



TagMan DNA-PCR Sensitivity for VSV-G: 1 copy per 10,000 cells
 No Functional VSV-G DNA in final cell product
 No Functional VSV-G RNA in supernatant of final cell product

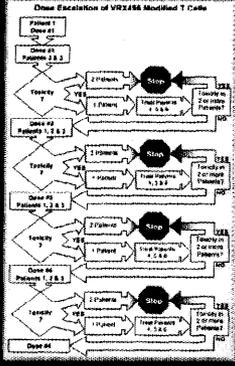
Patient-subject monitoring

- Twenty-eight (28) Days post dosing
- Physical Exam (including vital signs)
 - 12 lead ECG
 - CD4+ T-cell counts
 - Differential Viral Load
 - Anti-HIV immune response - ELISPOT
 - # of VRX496 modified Cells
 - VSV-G RNA in plasma
 - VSV-G antibody response
 - TCR Vβ diversity analysis
 - Chest X-ray
 - Hematology
 - PT/PTT
 - Chemistry
 - Urinalysis
 - Review of Concomitant Medications
 - Adverse Events



Patient-subject monitoring: potential adverse events and toxicities

- If a patient-subject experiences a precipitous increase in viral load of 0.5 log or greater, viral load will be followed for up to 7 days to determine if the increase is a sustained result.
- If a patient-subject experiences a precipitous decrease in CD4+ T-cell count of 50% or greater CD4+ T-cell count will be followed for up to 7 days to determine if the decrease is a sustained result.
- If a patient-subject experiences sustained detection of VSV-G RNA. If VSV-G RNA is sustained then the patient will undergo apheresis for biological RCR testing.
- If a patient-subject demonstrates grade 3 or greater laboratory toxicity the investigator will immediately repeat the test to confirm the result. Laboratory toxicities will be followed until resolution or until no further improvement is anticipated by the Investigator. The DSMB will determine whether any observed toxicities and/or adverse events warrant continuation of the trial.



Summary

- VRX496 can attain very high transduction efficiencies in primary CD4 T cells
- VRX496 can significantly inhibit wt-HIV replication in primary human CD4 T cells
- Safest approach for application of HIV vectors since patients are laden with wt-HIV
- Stringent release testing criteria - no functional VSV-G sequences in cell product
- VRX496 weakly mobilizes to CD4 T cells in vitro and in a SCID-hu mouse model
- No adverse events seen in safety and biodistribution studies in SCID-hu models
- Clinical protocol targeted to HIV patient-subjects that are failing HAART
- Clinical trial is complete when the patients have demonstrated no adverse events:
 - no precipitous and sustained increase in viral load
 - no precipitous and sustained decrease in CD4 T cell count
 - no RCR detection or other significant toxicity associated with VRX496

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Yajin Ni

Kim Lacey

Qiao Yu

Peggy Bennett

Brian Davis

Christine Hardy

Yung-Nien Chang

Title of the Study:

Bio-distribution Study of Vector Transduced Human T
cells in SCID Mice

Purpose of the Study:

To assess potential for dissemination of the vector transduced human T cells to the germ-line and non-target tissues of a single intravenous injection in SCID mice.

September 6th, 2001

VIRxSYS Corporation
200 Perry Parkway, Suite 1A
Gaithersburg, MD 20877

Animal Model: SCID/SCID mouse

The calculation of dosages is based on the following:

- Average body weight for mice: 20g.
- Average human body weight: 75000g.
- Phase I clinical trial highest dose: 3×10^{10} transduced T cells/patient.
- Phase I clinical trial lowest dose: 10^9 transduced T cells/patient.
- The high dose in the animal studies (20×10^6 cells/mouse) is 2.5x higher than the highest dose of phase I clinical. The low dose in the animal studies (3×10^5 cells/mouse) is equivalent of the starting dose of phase I clinical trial.
- **Infusion media: 1:1=plasma light:5% Dextrose**
- **Mock transduced T cells: cells are transduced by vector storage buffer.**

Study Design:

# Of mice	Animal group#	Day 2	Day 15	Day 30	Day 90	Total# of mice
Infusion Media control	1	5	5	5	5	20
Mock transduced T cells 20×10^6 /mouse	2	5	5	5	5	20
Vector transduced T cells (low dose) 3×10^5 /mouse	3	5	5	5	5	20
Vector transduced T cells (high dose) 20×10^6 /mouse	4	5	5	5	5	20
Total for male mice		20	20	20	20	80
Total for female mice		20	20	20	20	80
Total number of mice		40	40	40	40	160

Tissues have been collected at the time of necropsy:

1. Tail
2. Gonads
3. Inguinal lymph nodes
4. Kidney
5. Spleen
6. Liver
7. Lung
8. Heart
9. Brain
10. Bone marrow
11. Pancreas
12. Quadriceps
13. Adrenal gland
14. Vertebrae
15. Sub maxillary LN

Tissues have been analyzed by DNA-PCR for unique DNA sequence of vector and human specific marker (HuCART gene):

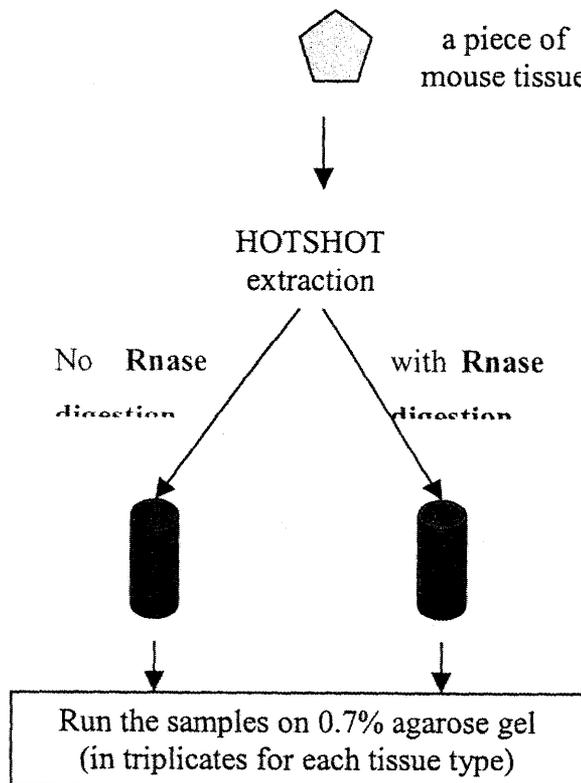
1. Tail
2. Gonads (Testes & Ovary)
3. Inguinal lymph nodes
4. Spleen
5. Liver
6. Lung
7. Heart
8. Bone marrow
9. Blood

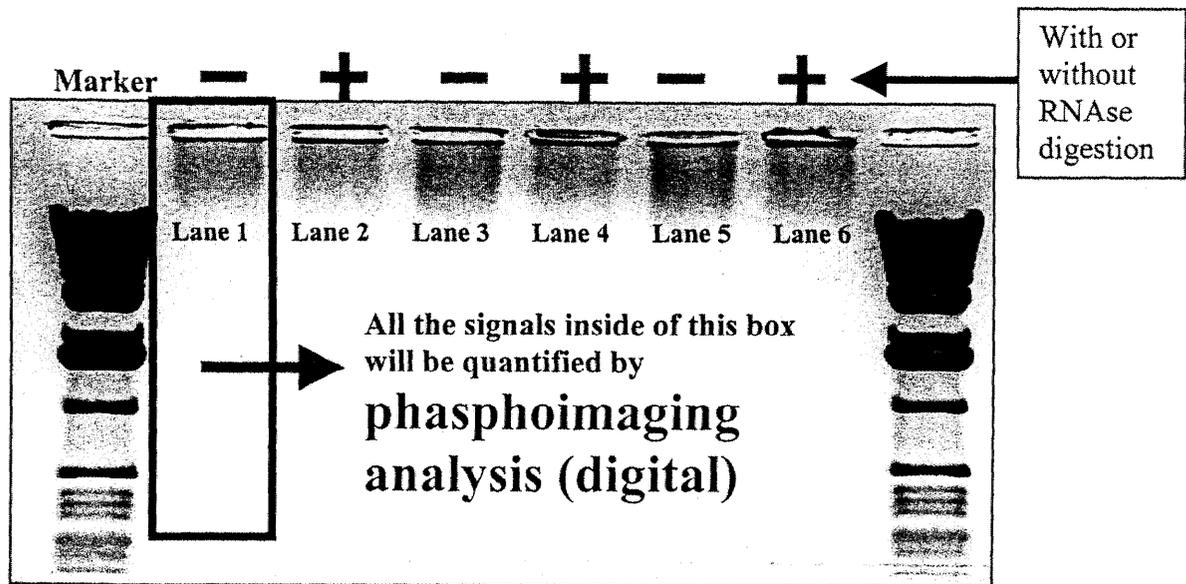
The FDA currently recommends that the sensitivity level of detection assays be less than 100 copies per microgram of DNA per tissue sampled. The methodology should be appropriate to adequately detect the sequence in the tissue samples from preclinical animal studies as well as clinical samples. In order to see if any vector specific sequence has integrated into the host DNA systems, total 3ug of DNA from testing animals need to be tested. One microgram DNA from animal tissue should be spiked with positive control DNA and two micrograms DNA from animal tissue without spiked positive control DNA.

Nucleic acid extraction method: HOTSHOT (G.E. Truett, *et al.* **BioTechniques**, Vol.29, No.1, pp52, 2000). Like any nucleic acid extraction method, both DNA and RNA will be extracted by HOTSHOT method. Because FDA requires total 3 ug DNA (not DNA and RNA) should be analyzed, therefore the following problem needs to be solved.

The problem: what is the % of RNA in our extracted nucleic acid from mouse tissue using HOTSHOT method. We can do RNase digestion and measuring OD260nm after Rnase digestion, but the digested individual RNA nucleotide can contribute to the OD260nm reading. If the input DNA for each PCR reaction is based on OD260nm reading, the DNA input for each PCR reaction will be less than 1ug. In order to meet FDA guideline, we propose the following solution:

Proposed Solution:





The same analysis will be done for all the lanes. Then the following formula will be used to calculate the % of RNA in each tissue type:

$$\begin{aligned}
 X &= \text{Signal Intensity of DNA + RNA} \\
 &= \frac{\text{lane 1} + \text{lane 3} + \text{lane 5}}{3} \\
 Y &= \text{Signal Intensity of DNA only} \\
 &= \frac{\text{Lane 2} + \text{lane 4} + \text{lane 6}}{3}
 \end{aligned}$$

$$\text{Z correction factor} = \frac{X}{Y}$$

For each tissue type, a “Z” correction factor is calculated as above and 1ug DNA input for each PCR reaction will be: “Z” factor X 1ug = Z ug (equivalent of 1ug DNA).

Summary of the "Z" factor calculation for all tissue types for study 1173-100:

1. All tissues have two independent RNase digestions followed by gel electrophoresis.
2. For each independent gel electrophoresis, 3 pairs, i.e. triplicates (with and without RNase digestion) total 6 lanes are analyzed for the "Z" factor.
3. Statistic analysis has been done for each gel electrophoresis.
4. Final "Z" factor is determined upon two independent gel electrophoresis results (total 6 pairs, 12 lanes).
5. After the final statistic analysis, the higher end of the final "Z" factor was taken, as the final "Z" factor for a specific tissue to assure there is enough DNA input for the PCR analysis.

The following is an examples of how "Z" factor derived for a specific tissue:

Results from Gel #1: (Tail, site of injection):

Index	Name	Volume	Adj. Vol.	Std. Deviation			
		CNT*mm2	CNT*mm2				
					Total NA	% of DNA	Z factor
Lane 1	Rep.# 1 D+R	27813	17689	83	100%		1.0
Lane 2	Rep.# 1 DNA only	27642	17518	112		99%	
Lane 3	Rep.# 2 D+R	28946	18822	91	100%		1.1
Lane 4	Rep.# 2 DNA only	27177	17053	91		91%	
Lane 5	Rep.# 3 D+R	30365	20241	176	100%		1.0
Lane 6	Rep.# 3 DNA only	30772	20648	122		102%	
7	Background #1	12009	718	4			
8	Background #2	10574	-718	6			
					Std. Deviation	5%	0.1
					Average	97%	1.0
					95% confidence index = average $\pm 1.96 \times \text{SDV}$		97% $\pm 10\%$ = 87% ~ 107% 1.0 ± 0.2 = 0.8 ~ 1.2

Results from Gel #2: (Tail, site of injection):

Index	Name	Volume CNT*mm2	Adj. Vol. CNT*mm2	Std. Deviation			
Lane 1	Rep.# 1 D+R	339991	110060	1600		Total NA 100%	% of DNA 100%
Lane 2	Rep.# 1 DNA only	312510	82579	1078			75%
Lane 3	Rep.# 2 D+R	303889	73958	1061		100%	
Lane 4	Rep.# 2 DNA only	283371	53441	821			72%
Lane 5	Rep.# 3 D+R	321265	91334	1314		100%	
Lane 6	Rep.# 3 DNA only	287560	57629	832			63%
7	Background #1	226001	-3930	353			
8	Background #2	233860	3930	366			
						Std. Deviation	5%
						Average	70%
						95% confidence Index = average ± 1.96 x SDV	70% ± 10% = 60% ~ 80%
							1.4 ± 0.2 = 1.2 ~1.6

Final statistics analysis for tail (the site of injection):

Std. Deviation for all 6 samples	0.2
Average Z for all 6 samples	1.2
For Tail: 95% confidence	$1.2 \pm 0.4 = 0.8 \sim 1.6$

“Z” factor for tail is: 1.6 (the higher value of the final analysis)

(for a complete calculation of “Z” factors for all the tissues, please see attachment #1)

Z Correction Factor Table:

Mouse Tissue Name	95% confidence	Z factor used for PCR
Blood	0.9 ~ 2.1	2.1
Bone Marrow	0.7 ~ 1.9	1.9
Heart	0.7 ~ 2.3	2.3
Liver	0.9 ~ 2.1	2.1
Lymph Node	0.1 ~ 2.5	2.5
Lung	0.7 ~ 2.3	2.3
Ovary	1.3 ~ 2.1	2.1
Testes	0.7 ~ 1.9	1.9
Spleen	0.4 ~ 2.8	2.8
Tail	0.8 ~ 1.6	1.6

- The FDA currently recommends that the sensitivity level of detection assays be less than 100 copies per microgram of DNA per tissue sampled.
- VIRxSYS sensitivity for the detection of vector specific sequence after input DNA corrected by above "Z" factor:
50 copies per ug DNA from mouse tissue
- Vector specific sequence detected by DNA-PCR: a fragment of GFP coding sequence, G-tag. Size of PCR product: **142 bp**
- Human specific sequence detected by DNA-PCR: 3' untranslated region of human CART-1 gene. Size of PCR product: **156 bp**.

Preparation of the positive spike plasmid DNA:

Stock: VRX496, 10^9 copies/10ul Lot#: VRX496pEXP06
Prepared on 4-25-2001 by K. Schonely & Y. Li
Diluents: ss-DNA: salmon sperm DNA, 1ng/ml

↓
Take 10ul from stock + 990ul ss-DNA (1ng/ml) → tube#1: 10^7 copies/10ul

↓
Take 10ul from tube#1 + 990ul ss-DNA (1ng/ml) → tube#2: 10^5 copies/10ul

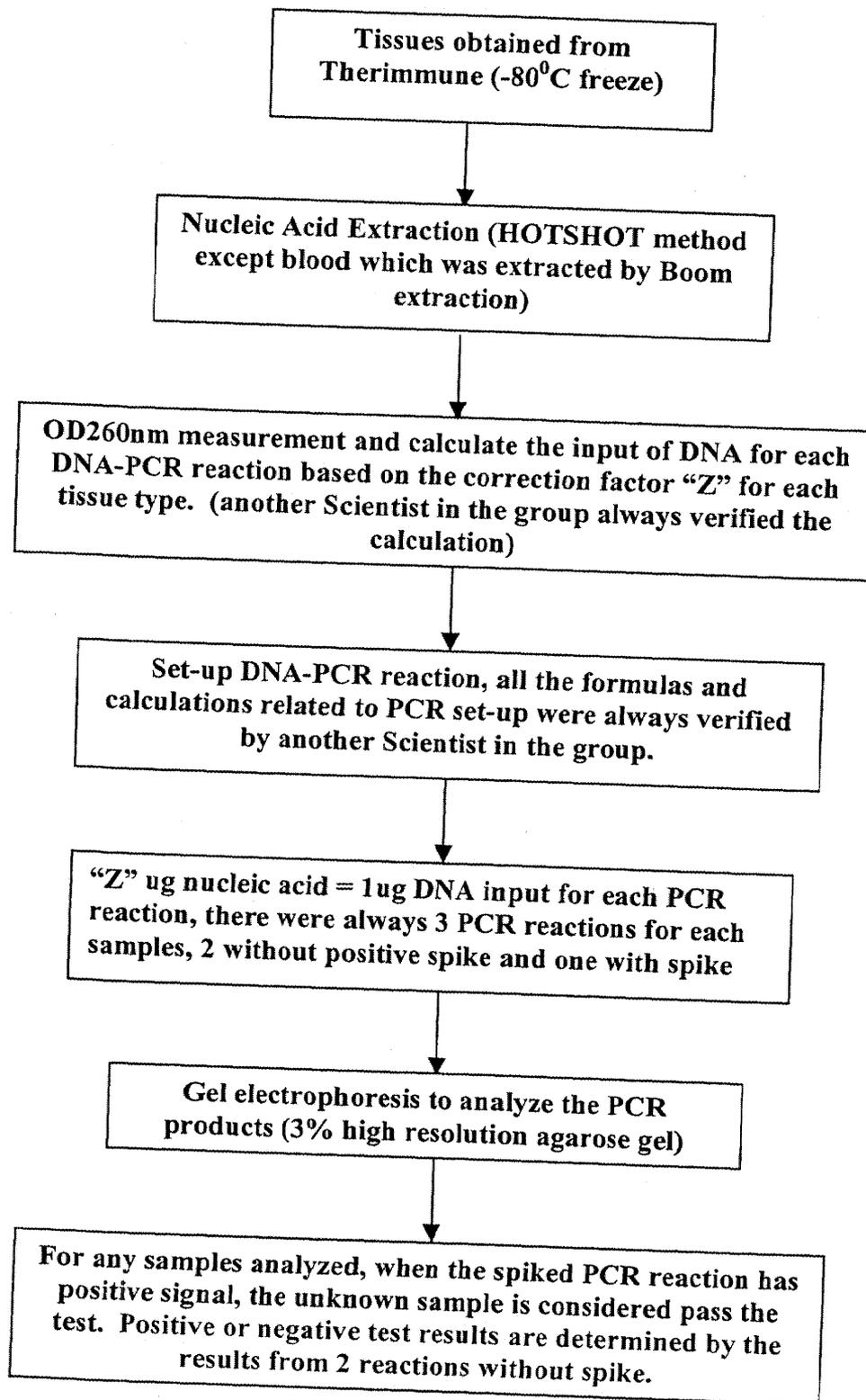
↓
Take 10ul from tube#2 + 990ul ss-DNA (1ng/ml) → tube#3: 10^3 copies/10ul

↓
Take 100ul from tube#3 + 900ul ss-DNA (1ng/ml) → tube#4: 10^2 copies/10ul

↓
Take 5ul (50 copies) from tube#4 add to each PCR reaction

Positive spike DNA is prepared each day from original stock and disposed daily into bleach container to avoid any potential contamination.

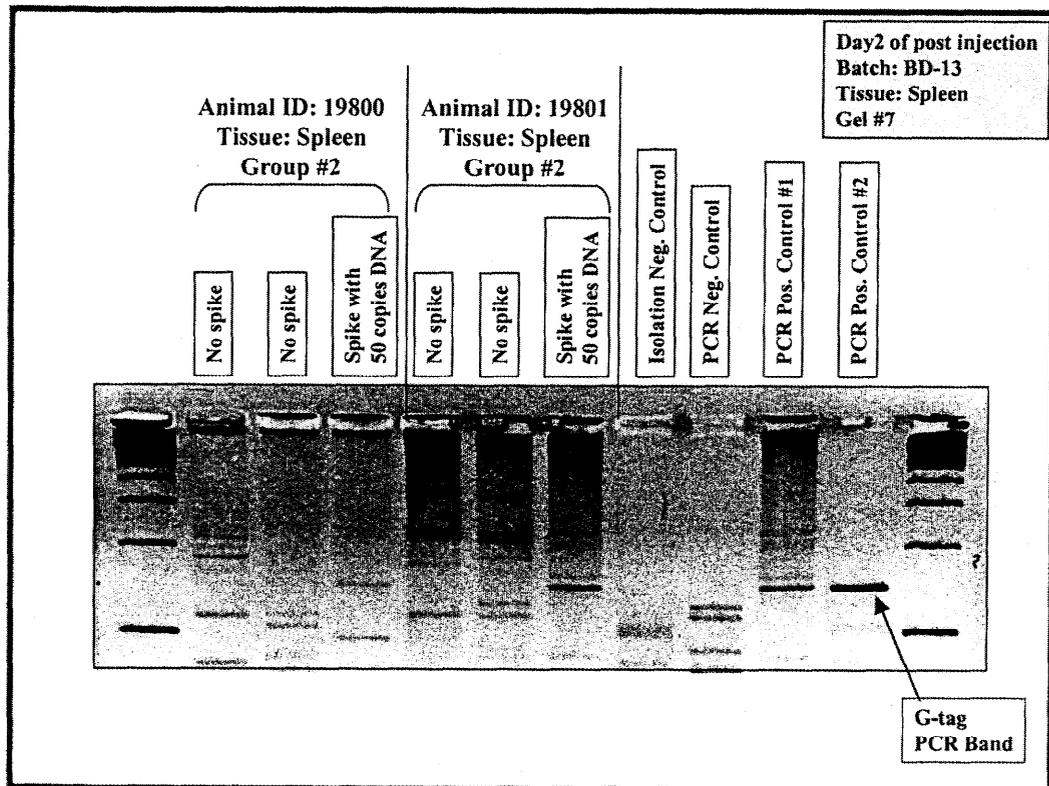
Sample processing and DNA-PCR analysis work flow:



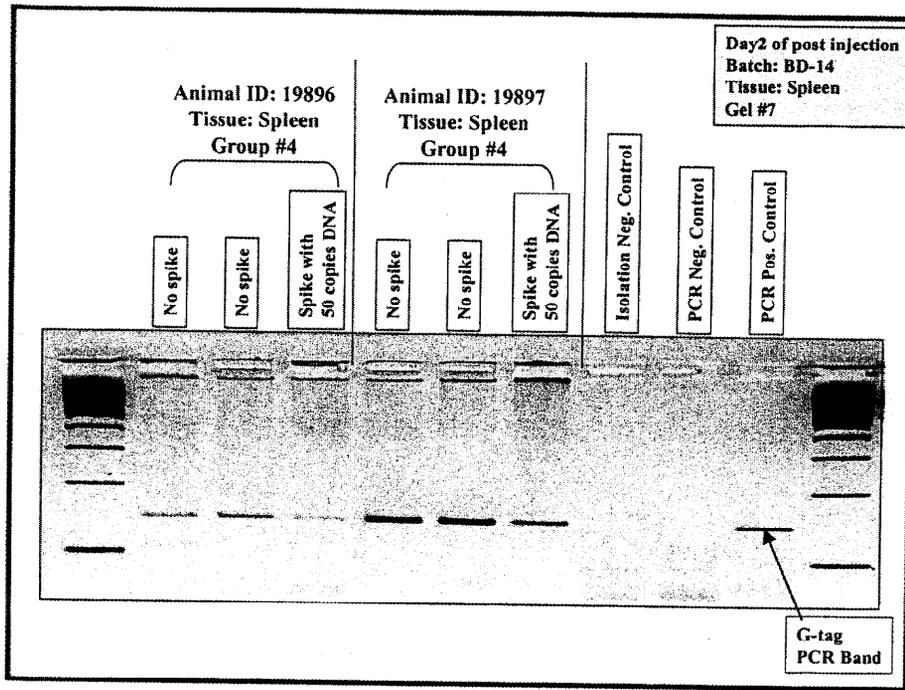
Representative data of DNA-PCR analysis:

Day 2 of Post-Injection:

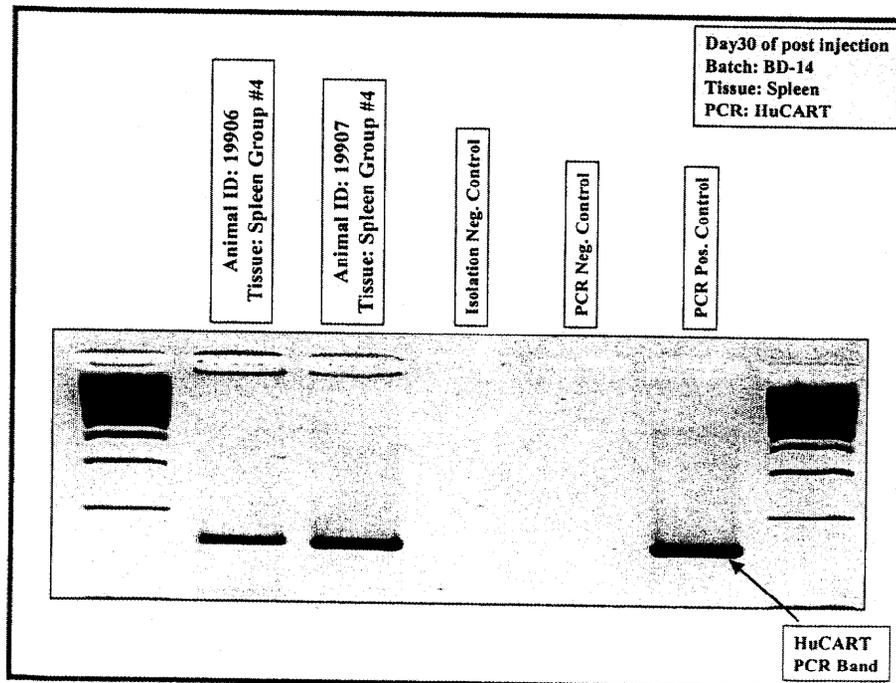
- Samples from control group, PCR detection: Vector sequence (GFP)



Samples from high dose group, PCR detection: Vector sequence (GFP)

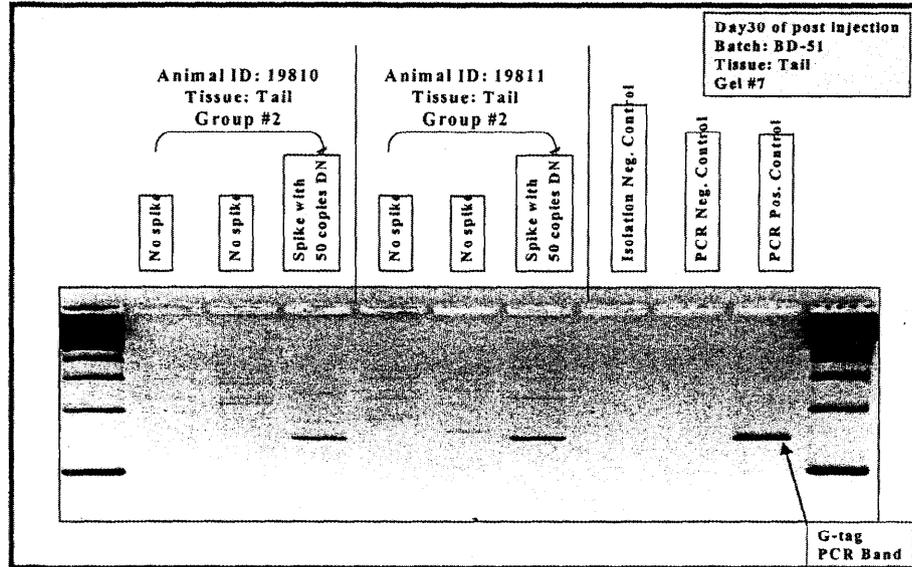


PCR detection: Human cell marker (HuCART) for above GFP positive samples

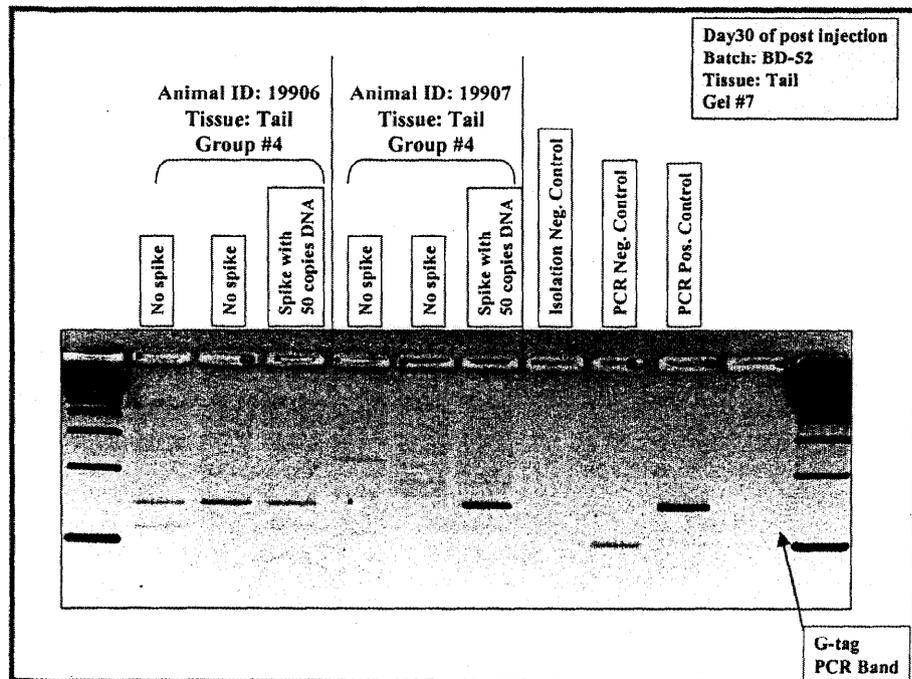


Day 30 of Post-Injection:

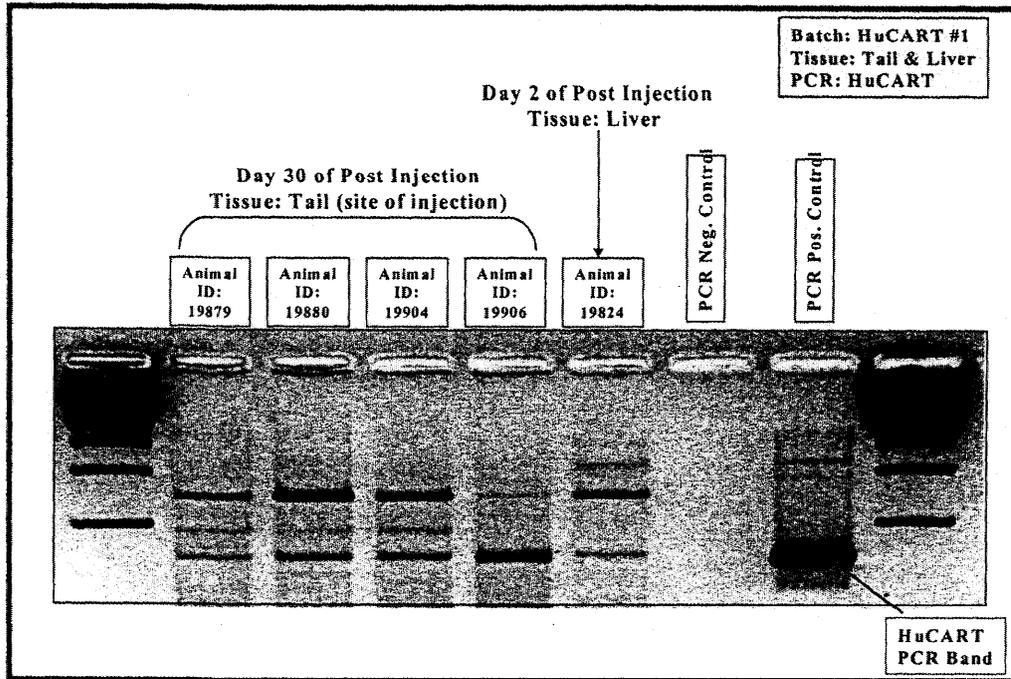
- Samples from control group, PCR detection: Vector sequence



- Samples from high dose group, PCR detection: Vector sequence



- **PCR detection: Human cell marker (HuCART) for GFP positive samples**



Summary of the PCR analysis for study 1173-100:

Day 2 of Post Injection

Note:

1. Group #1: Infusion Media control. Number of mice: 10.
2. Group #2: Mock transduced T cells 20×10^6 /mouse. Number of mice: 10.
3. Group #3: Vector transduced T cells (low dose) 3×10^5 /mouse. Number of mice: 10.
4. Vector transduced T cells (high dose) 20×10^6 /mouse. Number of mice: 10.
5. 10 mice per group, 5 mice for each gender.

	Heart Vector/Human	Testes Vector/Human	Ovary Vector/Human	Liver Vector/Human	Ing-LN Vector/Human
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #3 (low dose)	1 (-) / ND 9 +/9 +	4 (-) / ND 1 +/1 +	5 (-) / ND	10 (+) / 10 (+)	6 (-) / ND 4 (+) / 4 (+)
Group #4 (high dose)	10 (+) / 10 (+)	5 (+) / 5 (+)	2 (-) / ND 3 (+) / 3 (+)	10 (+) / 10 (+)	1 (-) / ND 9 (+) / 9 (+)

	Bone Marrow Vector/Human	Lung Vector/Human	Spleen Vector/Human	Tail Vector/Human	Blood Vector/Human
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	7 (-) / ND 3 False Pos.*
Group #3 (low dose)	10 (+) / 10 (+)	10 (+) / 10 (+)	10 (+) / 10 (+)	4 (-) / ND 6 (+) / 6 (+)	2 (-) / ND 8 (+) / 8 (+)
Group #4 (high dose)	10 (+) / 10 (+)	10 (+) / 10 (+)	10 (+) / 10 (+)	10 (+) / 10 (+)	1, Fail** 9 (+) / 9 (+)

*3 blood samples from group #2 have shown false positive signals for G-tag sequence. However, due to the limitation of sample availability, those three samples could not be repeated for PCR analysis.

**1 blood sample from group #4 has shown inhibitory effect for PCR reaction and data obtained.

Day 30 of Post Injection

	Heart Vector/Human	Testes Vector/Human	Ovary Vector/Human	Liver Vector/Human	Ing-LN Vector/Human
Group #1	Neg. / ND				
Group #2	Neg. / ND				
Group #3 (low dose)	5 (-) / ND 5 (+) / 5 (+)	4 (-) / ND 1 (+) / 1 (+)	4 (-) / ND 1 (+) / 1 (+)	7 (-) / ND 3 (+) / 3 (+)	6 (-) / ND 4 (+) / 4 (+)
Group #4 (high dose)	7 (-) / ND 3 (+) / 3 (+)	3 (-) / ND 2 (+) / 2 (+)	2 (-) / ND 3 (+) / 3 (+)	9 (-) / ND 1 (+) / 1 (+)	8 (-) / ND 2 (+) / 2 (+)

	Bone Marrow Vector/Human	Lung Vector/Human	Spleen Vector/Human	Tail Vector/Human	Blood Vector/Human
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND * 5 Failed
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND ** 1 Failed
Group #3 (low dose)	8 (-) / ND 2 (+) / 2 (+)	9 (-) / ND 1 (+) / 1 (+)	10 (-) / ND	8 (-) / ND 2 (+) / 2 (+)	5(-) / ND 3 (+) / 3 (+) ***2 Failed
Group #4 (high dose)	10 (-) / ND	6 (-) / ND 4 (+) / 4 (+)	9 (-) / ND 1 (+) / 1 (+)	6 (-) / ND 4 (+) / 4 (+)	6 (-) / ND 4 (+) / 4 (+)

Notes:

***5 samples in group #1 have failed PCR and could not be repeated due to the availability of the samples.**

**** 1 sample in group #2 has failed PCR and could not be repeated due to the availability of the sample.**

*****2 blood samples from group 3 failed PCR amplification and no sample available for repeat.**

Conclusions:

1. At post-injection day2 and day 30, all tissues analyzed from all the animals in control groups (group #1 and #2) are negative for VIRxSYS vector specific sequence by DNA-PCR analysis.
2. At post-injection day2, all the tissues analyzed are positive for VIRxSYS vector specific sequence by DNA-PCR analysis.
3. All the samples positive for vector specific sequence are also positive for human specific sequence as detected by DNA-PCR.

4. As showing below, there are 4 tissues at day 30 of post-injection have higher percentage positive signals for vector specific sequence in the low dose group than in the high dose group, however the signals are very low as detected by DNA-PCR analysis and the signals were at the border line of the detection limit. This may be caused by animal variation and the detection limit variation for each individual PCR reaction.

5. Data analysis: (% of positive for vector specific sequence)

Day2 of post-injection:

	Heart	Testes	Ovary	Liver	Ing-LN
Group #3 (low dose)	90%	20%	0%	100%	40%
Group #4 (high dose)	100%	100%	60%	100%	90%
	Bone Marrow	Lung	Spleen	Tail	Blood
Group #3 (low dose)	100%	100%	100%	60%	80%
Group #4 (high dose)	100%	100%	100%	100%	100%

Day30 of post-injection:

	Heart	Testes	Ovary	Liver	Ing-LN
Group #3 (low dose)	50%	20%	20%	30%	40%
Group #4 (high dose)	30%	40%	60%	10%	20%
	Bone Marrow	Lung	Spleen	Tail	Blood
Group #3 (low dose)	20%	10%	0%	20%	30%
Group #4 (high dose)	0%	40%	10%	40%	40%

Summary of the PCR analysis for Day 91 of Post-Injection:

	Heart Vector/Human	Testes Vector/Human	Ovary Vector/Human	Liver Vector/Human	Ing-LN Vector/Human
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #3 (low dose)	10 (-) / ND	5 (-) / ND	5 (-) / ND	10 (-) / ND	10 (-) / ND
Group #4 (high dose)	10 (-) / ND	5 (-) / ND	5 (-) / ND	9 (-) / ND 1 ^a (+) / 1 (+)	10 (-) / ND

	Bone Marrow Vector/Human	Lung Vector/Human	Spleen Vector/Human	Tail Vector/Human	Blood Vector/Human
Group #1	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #2	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND	Neg. / ND
Group #3 (low dose)	10 (-) / ND	9 (-) / ND 1 ^b (+) / 1 (+)	10 (-) / ND	10 (-) / ND	7 (-) / ND *3 failed
Group #4 (high dose)	10 (-) / ND	10 (-) / ND	9 (-) / ND 1 ^c (+) / 1 (+)	9 (-) / ND 1 ^d (+) / 1 (+)	6 (-) / ND *4 failed

* Failed PCR reactions and no samples available for repeat PCR assay.

Note:

1^a: 3 out of 6 independent sample isolations showed positive PCR signals for VIRxSYS vector specific sequence.

1^b: 3 out of 7 independent sample isolations showed positive PCR signals for VIRxSYS vector specific sequence.

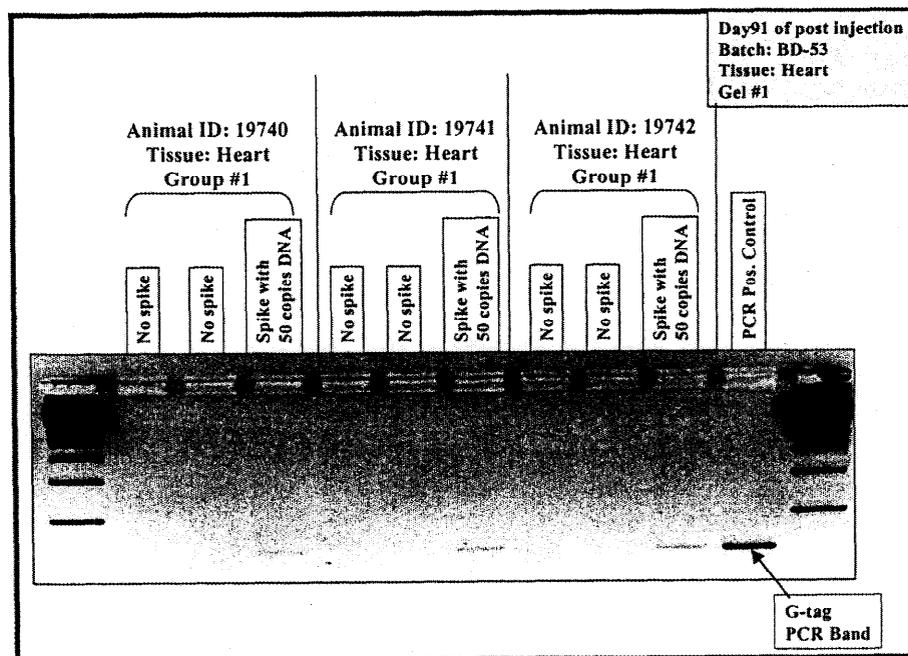
1^c: 2 out of 4 independent sample isolations showed positive PCR signals for VIRxSYS vector specific sequence.

1^d: 3 out of 6 independent sample isolations showed positive PCR signals for VIRxSYS vector specific sequence

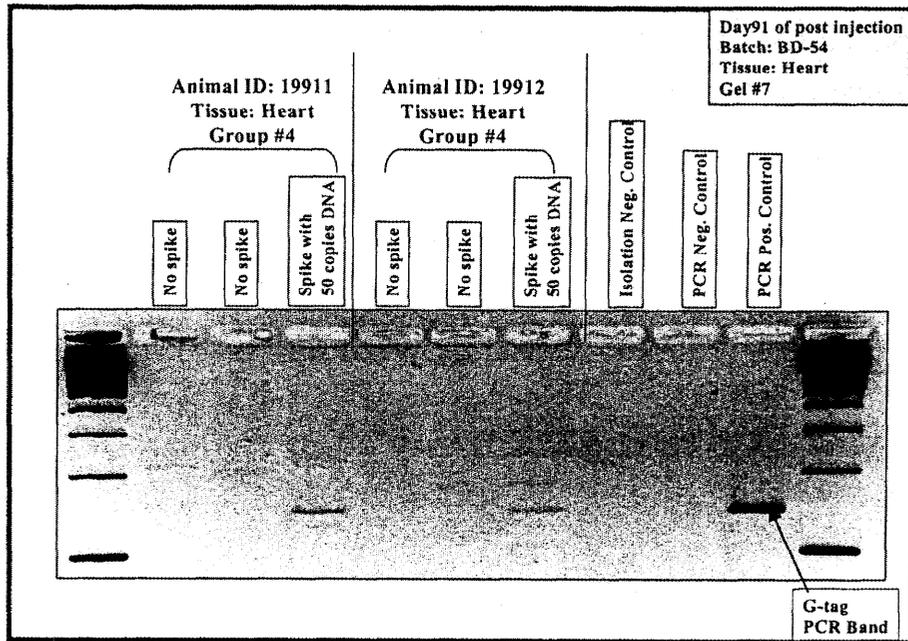
Overall: In groups 3 and 4, there were only 4 tissues out of 173 tissue samples that showed borderline positive signals for vector specific sequence.

Data representative of DNA-PCR analysis from Day 91 post-injection:

- **Samples from control group, PCR detection: Vector sequence**



● **Samples from high dose group, PCR detection:
Vector sequence**



Conclusions:

1. At post-injection day 91, all tissues analyzed from all the animals in control groups (group #1 and #2) are negative for VIRxSYS vector specific sequence by DNA-PCR analysis.
2. At post-injection day 91, there were only 4 tissue samples out of 173 injected samples that showed weak positive signals (at the border line of detection limit) for VIRxSYS vector specific sequence by DNA-PCR analysis.
3. All the samples positive for vector specific sequence are also positive for human specific sequence as detected by two rounds of DNA-PCR analysis.
4. When two rounds of DNA-PCR analysis was performed on the control animal tissues from group 1, the human specific marker is still negative after two rounds of PCR amplification.
5. Three of the four vector sequence positive samples were from highly perfused organs (Liver, Lung, and Spleen). One was from the injection site (tail). This could be due to the test article being accidentally injected into the tail muscle tissue instead of the tail-vein.