SINGLE-CELL QUANTIFICATION OF HIV-1 AND LENTIVIRAL VECTOR IN GENE THERAPY STUDIES (424)

Amanda M. Buck[1], Joanna Donnatelli[1], Corinna Gartner[1], Tyler-Marie Deveau[1], Isaac Thomas[1], Julie Beegle[2], Kim Athony-Gonda[3], Merhdad Abedi[2], Joseph Anderson[2], Timothy J. Henrich[1]

[1]Division of Experimental Medicine, University of California San Francisco, San Francisco, CA, [2]Department of Medicine, University of California Davis, Davis, CA, [3]Caring Cross, Gaithersburg, MD

Amanda M. Buck, BS
University of California San Francisco
Amanda.Buck@ucsf.edu

BACKGROUND

Lentiviral-based vector therapies, which include many CAR-T and gene-modified cell therapies, are at the forefront of HIV-1 cure research. However, sequence homology between lentiviral vectors and HIV-1 creates challenges with quantifying cellular HIV-1 burden and lentiviral vector DNA. As a result, we developed a novel single-cell in-droplet (scd)PCR method to co-quantify genomic HIV-1 DNA and lentiviral vector DNA (from CAR-T and gene-modified autologous stem cell transplant(SCT)) with single-cell resolution. This approach provides a high-throughput platform to determine if gene modified cells can become infected *in vivo*, a critical unknown question.

PURPOSE

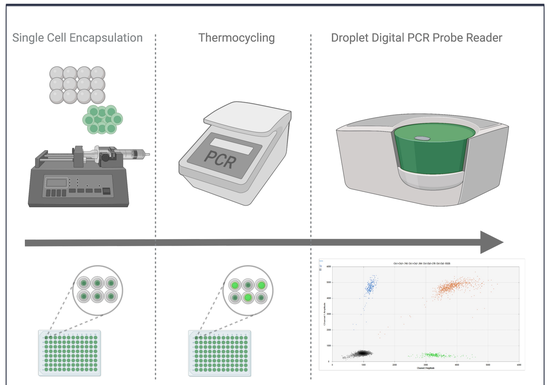
Gene-modification has become the next step in HIV-1 cure therapies, and a common way to achieve this cellular change is through the use of lentiviral vectors containing the modified genes. In other medical conditions, quantification is not an issue. However, the shared homology of these viruses creates a dual quantification problem that needs to be addressed.

METHODS

We tested HIV-infected and uninfected cell lines transduced (8E5, A301) or transfected (293T) with the HTVL-based 1-TAX gene modification vector that codes for a CCR5 siRNA, Rhesus macaque–human chimeric Trim5-alpha gene, HIV-1 TAR decoy, and a truncated CD25 molecule (for modified stem cell modification selection) and tested cross-reactivity with multiple HIV-1 DNA qPCR and ddPCR assays [1]. The 1-TAX gene modification vector is currently being used in the AMC 097 trial (PWH on ART receiving gene modified autologous stem cell transplant for hematological malignancies).

Next, we created a primer and probe set that quantified the MNDU3 promoter region of the 1-TAX vector, a common component of lentiviral gene delivery vectors, that did not cross-react with the human genome and that of HIV-1. We then screened 22 published HIV-1 DNA quantitative assays to determine the optimal method to quantify HIV DNA in cells from human studies that do not cross-react with the 1-TAX vector or similar lentiviral vectors for bulk quantitation or subsequent single-cell quantitation.

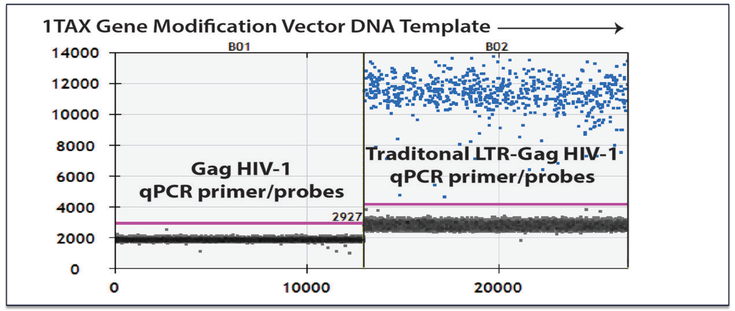
Finally, we developed a multiplexed ddPCR method to co-quantify genomic HIV-1 DNA and MNDU3 promoter on both bulk lysates and within single cells using a multiplexed scdPCR assay [2] to enable a high throughput single-cell resolution.



Using scdPCR allows for high throughput analysis at a single-cell level. The first step involves encapsulation of individual cells with ddPCR amplification reagents in oil droplets. After single-cell encapsulation, the plate is thermocycled allowing for primer probe amplification of MNDU3 and HIV-Gag DNA. Each droplet was then evaluated in the ddPCR reader to quantify single cells that harbor genomic 1-TAX vector, HIV, or both.

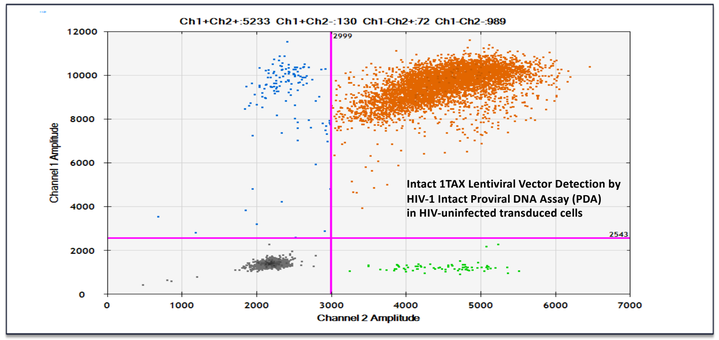
RESULTS

Traditional HIV-1 DNA PCR Methods Cross React with Lentiviral Vector



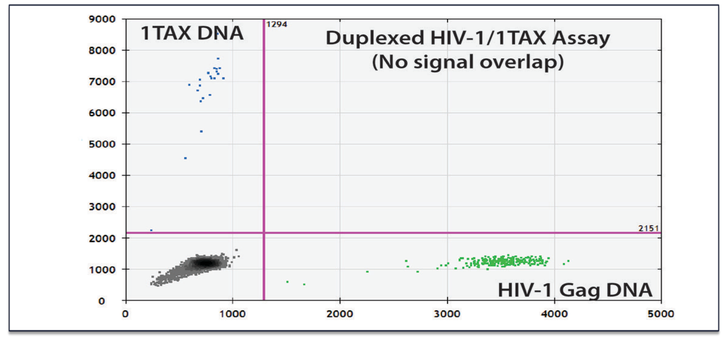
- **HIV-uninfected, vector-transduced lab cell lines have almost 100% cross-reactivity with traditional PCR (HIV-1 LTR, LTR-Gag junction/Psi, and Env regions) assays compared to the left.**
- **Only 1 out of 22 published HIV-1 qPCR assays, that targets downstream Gag region with broad group coverage[3], did not cross-react with gene modification vector DNA as shown in left panel and was amenable to duplexing in scdPCR.**

IPDA Quantifies Lentivirus Gene Modification Vector DNA



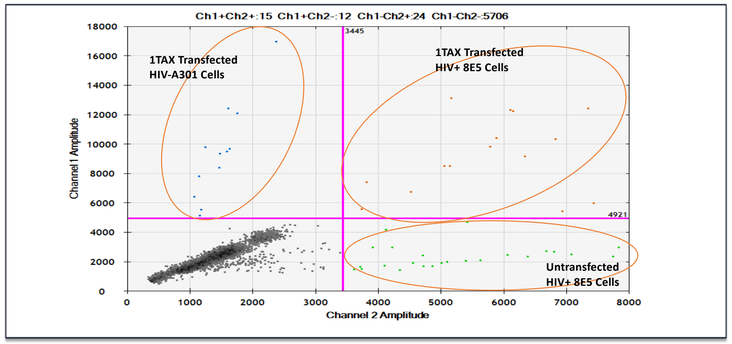
- **IPDA, which co-targets Psi/Env, quantified nearly 100% of lentiviral vector DNA (to right quadrant).**
- **While this assay could potentially be used to see the amount of intact lentiviral vector in sample due to the 100% cross-reactivity, it cannot differentiate between HIV-1 and HTLV in the setting of these therapies.**

Developed Duplex Assay to Co-Detect HIV-1 and Lentiviral Vector DNA



- **Duplex DNA assay of HIV-1 Gag region and MNDU3 promoter region of the lentiviral vector for droplet-digital (dd)PCR with minimal cross-reactivity.**
- **Used bulk cell lysates from combinations of 293T cells transfected with: a) HIV-1 plasmid, b) 1TAX (lentiviral vector), c) HIV-1 and 1TAX, d) non-transfected.**

Applied Duplexed ddPCR Assay to single-cell-in-droplet PCR (scdPCR)



- **Applied the duplexed ddPCR assay in our scdPCR platform for use with individually-encapsulated, lentiviral transduced HIV+ 8E5 cells.**

Acknowledgments

This research was funded by CHRP Grant H22BD4484 and R01AI141003 awarded to TJH, and the initial study was supported through the the AIDS Malignancy Consortium (AMC). Merhdad Abedi, MD and Kim Gonda, PhD are also acknowledged for the part they played in this research, along with the laboratory of Timothy Henrich at UCSF.

References

[1] Anderson, et. Al Molecular Therapy 2009.
[2] Yucha, et. Al eBioMedicine 2017.
[3] Ometto, et. Al AIDS 2002.

CONCLUSIONS & FUTURE DIRECTIONS

- We are able to reliably and simultaneously quantify HIV-1 DNA and lentiviral vector DNA in cell lysates and in individually-encapsulated cells using this duplex dd/scdPCR assay.
- This method also allows in-depth characterization of residual HIV-1 burden and the potential for transduced cells to become infected *in vivo* across a range of gene-modification studies.
- We will apply this novel method to detect HIV-1 and lentiviral vector burden in individual cells (including AMC-097 gene therapy and duoCAR-T Cell therapy samples).