Mass Spectrometry Imaging Reveals Associations Between Antiretrovirals, SHIV, and Cells in Lymph Nodes

475

Elias Rosen[1], Claire Deleage[2], Nicole White[1], Craig Sykes[1], Lourdes Adamson[3], Michelle Mathews[1], Yuri Fedoriw[1], Jacob D. Estes[4], Paul Luciw[3], and Angela DM Kashuba[1]

[1]University of North Carolina at Chapel Hill, [2]Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., [3]University of California at Davis, [4]Oregon Health & Science University

INTRODUCTION

- Recent data suggest that lymph nodes (LN) may harbor HIV infection despite HAART, possibly due to reduced ARV penetration.(1)
- Here, we combine infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry imaging (MSI) and *in situ* hybridization (ISH) and immunohistochemistry (IHC) to evaluate the biodistribution of ARVs relative to viral and target cell expression in LN of rhesus macaques (RM).

METHODS

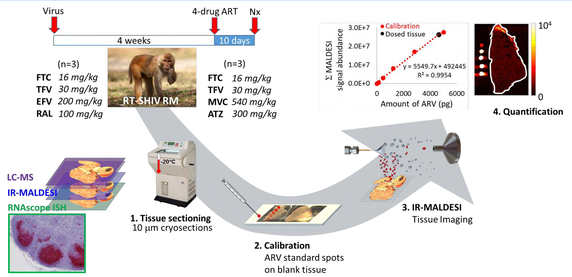


Figure 1 - ***Experimental Workflow of MSI Tissue Analysis by IR-MALDESI***

- RM (N=6) infected with RT-simian-human immunodeficiency virus (RT-SHIV) for 4 weeks. Viral load peaked 14 days post-infection (dpi) at $9.8x10^6$ ($1.5x10^6$-$6.0x10^7$) SIV copies/ml, reduced to $2.3x10^5$ ($5x10^4$-$2.2x10^6$) SIV copies/ml at onset of ART.
- 10 days of ART with emtricitabine (FTC) + tenofovir (TFV) (N=6) in combination with either efavirenz (EFV) + raltegravir (RAL) (N=3; FTER cohort) or maraviroc (MVC) + atazanavir (ATZ) (N=3; FTMA cohort).
- Axillary LNs collected at necropsy (38 dpi), snap frozen, stored at -80°C.
- Tissue accumulation of ARVs and metabolites measured by LC-MS/MS (analyte-specific limits of detection [LOD]: 1-8 ng/ml homogenate) and IR-MALDESI MSI (LOD: 0.05-0.37 ng/mg tissue) from 10 µm thick cryosections (**Figure 1**). Tissue and plasma measurements compared based on assumed tissue density of 1.06 g/ml.
- Subjacent tissue sections analyzed for viral RNA (vRNA) by RNAscope ISH(2) and CD4+ cells by IHC(3).
- Analysis of MSI data performed with MSiReader(4) and MATLAB. MSI and ISH or IHC images combined through color thresholding, spatial resolution matching, feature registration, and image fusion (**Figure 2**).

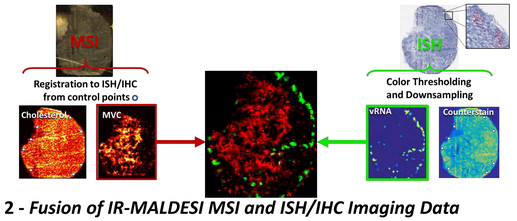


Figure 2 - ***Fusion of IR-MALDESI MSI and ISH/IHC Imaging Data***

RESULTS

- Total penetration of ARVs into LN [LC-MS/MS (µg/g): 0.35-2 TFV, EFV, MVC; 0.05-0.2 FTC, ATZ; 0.02-0.03 RAL] exceeded concentrations in plasma (6 to 10-fold increase) for all ARVs except RAL (8-fold decrease).
- MSI revealed that ARVs accumulated in LN heterogeneously (**Figure 3, Top**), with ARV LN exposure to tissue varying between dosing regimens. FTC and RAL concentrations in LN were below IR-MALDESI LOD.
- Comparison of ARV coverage with spatial distributions of vRNA and CD4 expression is summarized in **Table 1** and shown graphically below (**Figure 3, Bottom**).
- Combination of MSI and ISH showed localization of vRNA in secondary follicles of LN where ARV penetration was absent or poor. Up to half of vRNA expression by area was not co-localized with any ARV.
- ARV co-localization with CD4+ cells was higher than vRNA, and observed predominately in paracortical area of LN.

Table 1- ***Summary of ARV Co-localization with vRNA and CD4.***

Cohort	FTC/TFV/MVC/ATZ	FTC/TFV/EFV/RAL
Estimated vRNA (virions/mm²)	$1.65x10^3$ ($1.58x10^3$ - $1.82x10^4$)	$2.73x10^2$ ($1.92x10^2$ - $2.16x10^4$)
Fractional Coverage of Tissue	74.0% (73.0-75.3%)	99.7% (67.2-99.8%)
Fractional Coverage of vRNA	66.0% (57.5-70.7%)	93.2% (50.3-99.6%)
*Coverage of vRNA > IC_{50}(5,6)	46.5% (41.0-54.5%)	15.7% (2.8-84.5%)
Fractional Coverage of CD4	73.0% (63.5-74.0%)	96.7% (93.5-99.9%)

**Reflects ARV with largest proportion of coverage exceeding in vitro IC_{50} values (FTMA: MVC; FTER: EFV)*

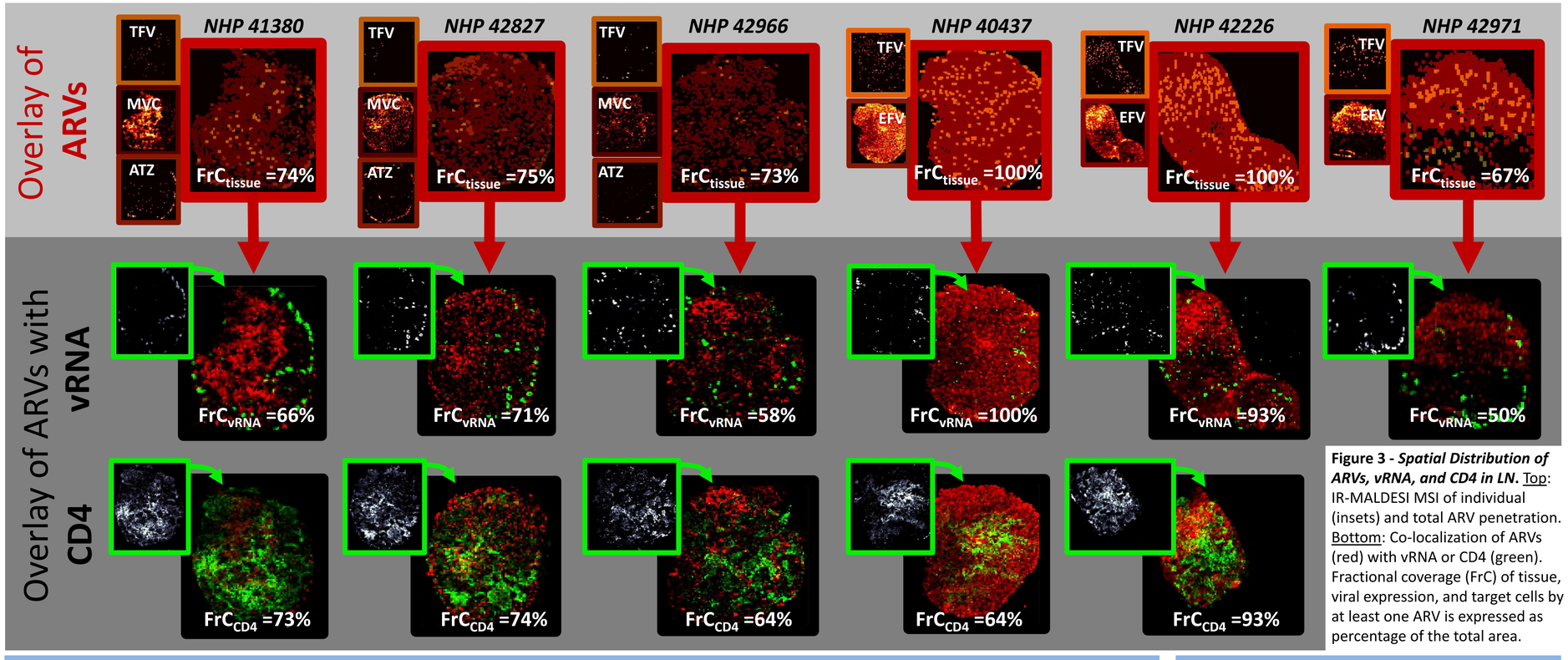


Figure 3 - ***Spatial Distribution of ARVs, vRNA, and CD4 in LN.*** Top: IR-MALDESI MSI of individual (insets) and total ARV penetration. Bottom: Co-localization of ARVs (red) with vRNA or CD4 (green). Fractional coverage (FrC) of tissue, viral expression, and target cells by at least one ARV is expressed as percentage of the total area.

CONCLUSIONS

- ISH vRNA response was sensitive to ARV regimen, with lowest expression found in LNs where EFV penetrated throughout the parenchyma and highest expression in LNs where MVC and ATZ distributions showed discrete regions of accumulation.
- Fusing MSI and ISH imaging modalities offers a means of evaluating drug distribution in the context of viral replication, and provides a path to spatially resolve pharmacokinetic-pharmacodynamic relationships.
- The influence of drug concentration and effect within a tissue compartment, otherwise lost by tissue homogenization, can be elucidated with this approach.
- Future work will extend this approach to evaluate the role that collagen deposition plays in the disposition of ARVs in LN tissue and also compare these results in RM with human samples from the National NeuroAIDS Tissue Consortium.

REFERENCES

1. Lorenzo-Redondo, R., et al. (2016). *Nature* 530(7588), 51-56N.
2. Deleage, C., et al. (2016). *Pathogens and Immunity* 1(1): 68-106.
3. Estes, J.D. et al. (2017). *Nature Medicine* 23, 1271.
4. Robichaud, G., et al. (2013). *Journal of the American Society for Mass Spectrometry* 24(5): 718-721.
5. Dorr, P., et al. (2005). *Antimicrob. Agents Chemother.* 49, 4721-4732.
6. Ramanathan, S., *et al.* (2016). *JAIDS Journal of Acquired Immune Deficiency Syndromes* 72, 281-288.

ACKNOWLEDGEMENTS

Funding Sources: R01AI111891, P30AI050410