A Quantitative Approach to Evaluate ARV Proximity to Virus and Cells in Lymph Nodes

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INTRODUCTION

- Recent data suggest that lymph nodes (LN) may harbor HIV infection despite HAART, possibly due to reduced ARV penetration.[1]
- Understanding potential consequences of diverse ARV accumulation requires quantitative methods to characterize ARV proximity to virus and target cells.
- We have developed a novel analytical based on a combination of mass spectrometry imaging (MSI), *in situ* hybridization (ISH) and immunohistochemistry (IHC) to better understand the consequences of heterogeneous ARV distribution in LN.

METHODS

Non-human Primate (NHP) Study

- Rhesus macaques (N=6) infected with RT-simian-human immunodeficiency virus (RT-SHIV) for 4 weeks. Viral load peaked 14 days post-infection (dpi) at median (range) $9.8x10^6$ ($1.5x10^6$-$6.0x10^7$) 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.

Multimodal Imaging of NHP Tissues

- Tissue accumulation of ARVs and metabolites measured simultaneously by IR-MALDESI MSI (LOD: 0.05-0.37 ng/mg tissue) from 10 μm thick cryosections at 0.1 mm spatial resolution.
- Subjacent tissue sections analyzed for viral RNA (vRNA) by RNAscope ISH[2] and CD4+ cells by IHC[3] to create a multimodal imaging approach (**Figure 1**).

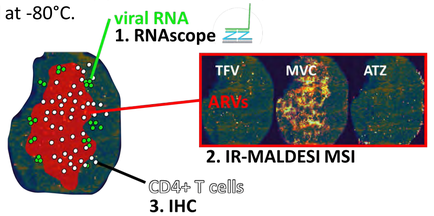


Figure 1 – *Combined imaging approach for LN tissue*

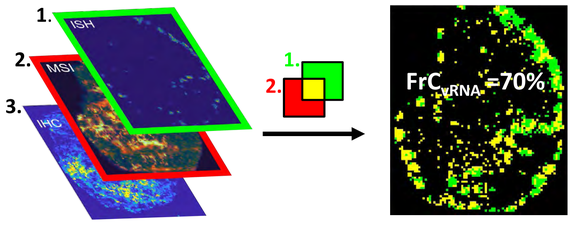


Figure 2 - *Fusion of IR-MALDESI MSI and ISH/IHC Imaging Data*

Combination of Imaging Data

- MSI and ISH or IHC images combined through color thresholding, spatial resolution matching, and feature registration with MSiReader[4] and MATLAB. (**Figure 2**).
- Co-localization and fractional coverage (FrC) evaluated from fused images of co-registered data. In binary fused images, regions of overlap between ARVs (red) and vRNA or CD4 (green) are yellow.

Proximity Assessment

- Spatial relationships evaluated by nearest neighbor proximity search (NNS) on co-registered images using MATLAB.
- NNS algorithm determines minimum distance ($Dist_{NN}$) between each individual pixel response associated with any defined target (green) and the closest pixel associated with measurable ARVs (red).
- Nearest neighbor distances (**Figure 3**) evaluated for each ARV separately as well as for entire regimen.

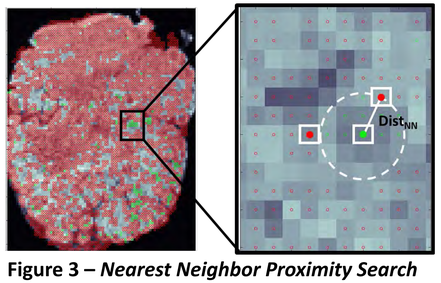


Figure 3 – *Nearest Neighbor Proximity Search*

RESULTS

- MSI simultaneously measured all detectable ARVs (FTC, RAL < limits of detection: 0.05-0.37 ng/mg tissue) and the blood biomarker, HEME.
- Representative fused images of cumulative ARV LN response and either HEME, vRNA, or CD4 are shown in **Figure 4** along with accompanying binary images to highlight areas of direct co-localization.

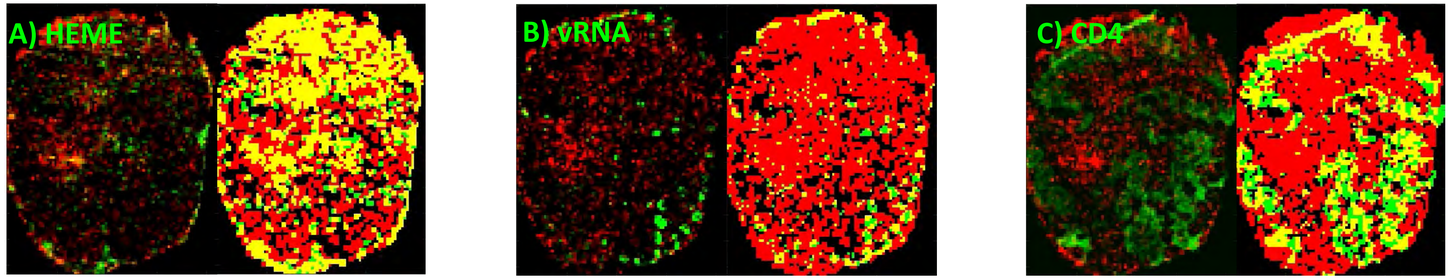


Figure 4 - *Spatial distribution of ARVs, vRNA, and CD4 in representative NHP LN sample*. Co-localization of IR-MALDESI MSI total ARV penetration (red) with A) HEME, B) vRNA, or C) CD4 distributions (green). Adjacent binary images show regions of overlap in yellow.

- Distributions of nearest-neighbor distances across all study tissues are summarized in **Figure 5**.
- Based on NNS analysis between ARVs and HEME (reflecting ARV in the vasculature), 57% of all ARVs in LN were ≤0.1 mm from HEME (**Figure 5A**). The greatest tissue penetration was found for TFV and EFV (up to 1.7 mm from HEME).
- The degree of colocalization between ARVs and vRNA varied (TFV: 1-9%; ATZ: 4-16%; MVC: 54-68%; EFV: 89-99%). Yet NNS analysis indicated that >95% of all vRNA was ≤0.1 mm from a detectable ARV response in each cohort (**Figure 5B**).
- However, proximity of vRNA to ARV concentrations > in vitro IC_{50} values[5,6] was farther (vRNA≤0.3 mm: FTER=0-60%; FTMA=88-97%). Similar results were observed for NNS analysis of CD4+ T cells (**Figure 5C**. CD4≤0.3 mm: FTER=0-94%; FTMA=96-99%).

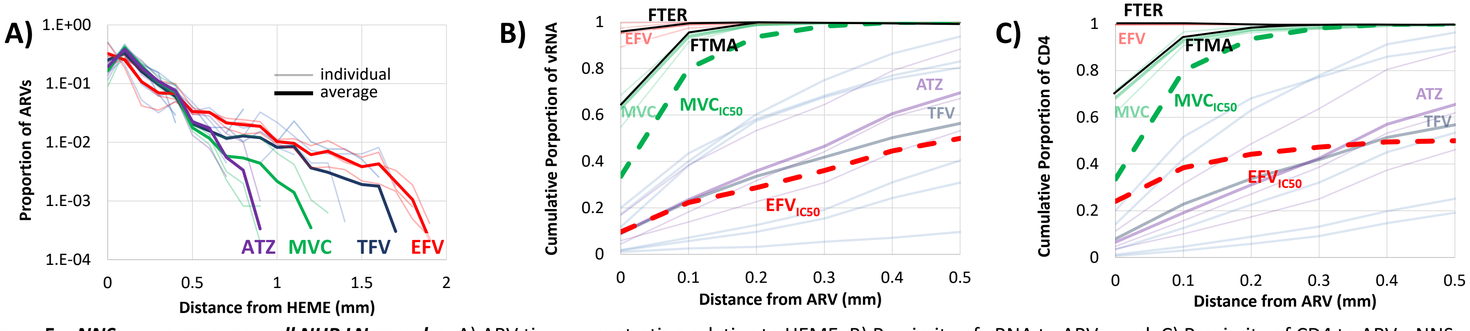


Figure 5 – *NNS summary across all NHP LN samples.* A) ARV tissue penetration relative to HEME; B) Proximity of vRNA to ARVs; and, C) Proximity of CD4 to ARVs. NNS distance to detectable ARVs shown for individual samples (thin lines) and mean distributions (bold solid line), along with distance to ARV > IC_{50} values (dashed line).

CONCLUSIONS

- Multimodal approach quantifying spatial relationships between drug and targets (blood, virus, and T cells).
- ARV coverage extends to >73% of the LN, better for CD4 (>64%) than vRNA (>58%), but may not be adequate everywhere relative to known inhibitory concentrations.
- Flexible framework allows ARVs to be evaluated individually or in aggregate, offers a tool help optimize pharmacokinetics and pharmacodynamics to ARV treatment.

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