FAST Validation to Study the Baseline and Inducible HIV Reservoir

Marilia R Pinzone[1], Xiaohe Liu[2], Lidia Sambucetti[2], Giuseppe Nunnari[3], and Una O'Doherty[1]

[1]Department of Pathology and Laboratory Medicine, University of Pennsylvania, USA; [2]SRI International, Menlo Park, USA; [3]Division of Infectious Diseases, University of Messina, Italy

Background

"Shock and kill" strategies require highly sensitive methods to detect HIV reactivation upon stimulation with latency reversal agents (LRAs). In this study, we validated that Fiber-optic array scanning technology (FAST) can detect reactivation of latent HIV.

Methods

Resting CD4 T cells from HIV-infected individuals or from PBMCs of uninfected donors were spinoculated with pNL4-3 virus or pLENG1-IRESGFP virus +/- an integrase inhibitor. Cells were cultured with IL-7 for 7 days and stimulated with several compounds, alone or in combination, such as prostratin (300 nM), bryostatin (1 μM), JQ1 (1 μM), or resveratrol (40 μM). After 24 hours, HIV gag or GFP expression was assessed by flow and FAST. Data are presented as mean fold increase of 3 separate experiments.

Results

We validated FAST's ability to detect LRA reversal by comparing it to flow using an *in vitro* model with a high frequency of infected cells. Stimulation with LRAs was associated with an increase in the number of GFP+ or gag+ cells (GPCs). Bryostatin was the most potent compound, inducing a consistent increase in the number of GPCs detected by FAST and flow (3.5- and 4-fold, respectively). Lower fold changes were detected by FAST and flow respectively when using prostratin (1.3-3.3 fold), JQ1 (4.1-1.9 fold), or resveratrol (1.8-3.3 fold). The association of bryostatin and JQ1 was synergistic in increasing the number of GPCs by FAST (5.7-fold increase) and flow (3.8-fold increase).

Side by side comparisons of diluted *in vitro* infected cells as well as patient samples by FAST are shown. All patients had detectable levels of gag+ cells at baseline and after stimulation by FAST, but not by flow. Specifically, flow failed to robustly detect cells that express HIV proteins when the frequency of infected cells is below 1 in 10,000 due to inherent technical limitations.

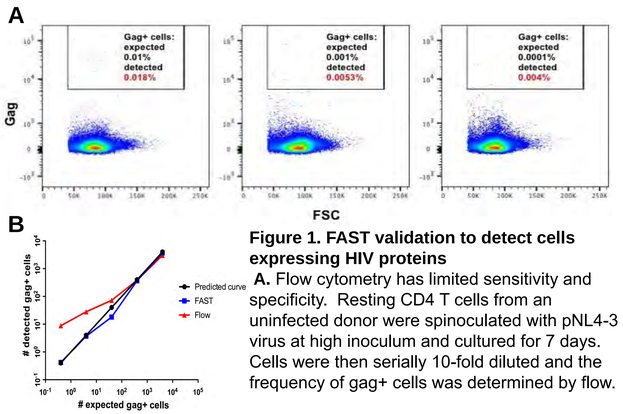
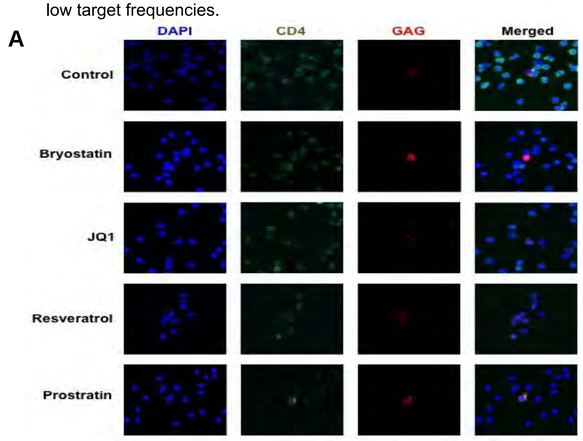


Figure 1. FAST validation to detect cells expressing HIV proteins

A. Flow cytometry has limited sensitivity and specificity. Resting CD4 T cells from an uninfected donor were spinoculated with pNL4-3 virus at high inoculum and cultured for 7 days. Cells were then serially 10-fold diluted and the frequency of gag+ cells was determined by flow.

B. Comparison between FAST and flow cytometry. The same serial dilutions of the previous experiment were plated on slides and the frequency of gag+ cells measured by FAST at SRI. Flow cytometry and FAST correlate well at high inoculum, but FAST can more accurately measure gag+ cells at low target frequencies.



B

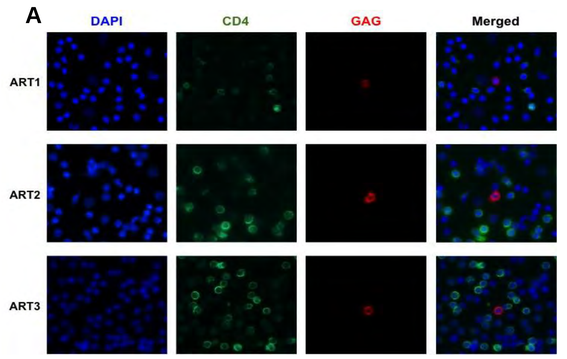
Fold increase in gag+ cells	FAST	Flow
JQ1	4.1	1.9
Bryostatin	3.5	4
Resveratrol	1.8	3.3
Prostratin	1.3	3.3
JQ1+bryostatin	5.7	3.8

Figure 2. FAST can be used to detect the effect of latency reversal agents (LRAs)

Resting CD4 T cells from uninfected donors were spinoculated with pNL4-3 virus or pLENG1-IRESGFP virus +/- an integrase inhibitor. Cells were cultured with IL-7 for 7 days and stimulated with several compounds, alone or in combination, such as prostratin (300 nM), bryostatin (1 μM), JQ1 (1 μM), or resveratrol (40 μM). After 24 hours, HIV gag or GFP expression was assessed by flow and FAST.

A. Some representative images of gag+ cells by FAST

B. Fold increase in the number of gag+ cells as detected by FAST and flow. The association of JQ1 and bryostatin synergistically and consistently increased the number of cells expressing HIV proteins.



B

	ART1	ART2	ART3	ART4	ART5
Gag+ CD4- cells	13	2	5	79	67
PBMCs (millions)	48.6	27.2	34.5	45	33
Gag+ cells per million	0.27	0.07	0.14	1.76	2.03
Integrated HIV DNA per million	354	54	573	23	1360
Gag+ cells per provirus (%)	0.075	0.13	0.025	8	0.15

Figure 3. Patients on ART have detectable levels of gag+ cells at baseline by FAST

A. Peripheral blood mononuclear cells (PBMCs) from five ART patients were adhered to slides, fixed, permeabilized and stained for intracellular gag (red), CD4 (green) and DAPI (blue). Slides were scanned by FAST. Some representative images from three patients are shown.

B. Patients on ART have variable levels of gag+ cells, ranging from 0.07 to 2 per million PBMCs.

The frequency of gag+ CD4- cells was determined by FAST. The levels of integrated HIV DNA were measured by *Alu-gag* PCR.

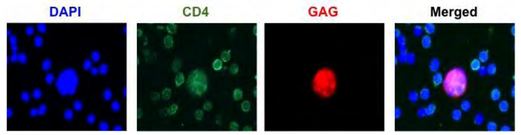


Figure 4. FAST identifies syncytia of cells expressing HIV proteins.

Here we show a representative image of a syncytium of gag+ CD4 T cells identified by FAST after LRA exposure. These aggregates are missed during flow cytometry analysis because they are too large to pass through flow cell and would presumably be lysed during the process.

Conclusions

FAST detects HIV proteins expressed in resting CD4+ T cells at baseline and after stimulation providing a tool to assess HIV reactivation upon stimulation with LRAs. We find a correlation between FAST and flow at high inoculum, but flow loses sensitivity and specificity at low infection frequencies.

FAST has benefits over flow cytometry, including the ability to capture events that can be missed by flow, such as syncytia of cells expressing HIV proteins. In conclusion, FAST represents a novel sensitive assay, which can help characterizing the baseline and inducible reservoir of HIV-infected patients.

	1	2	3	4	5	6	7	8	9	10
A	**4905**	3118	7597	2244	7337	9140	12616	7560	11004	**18491**
B	16045	**21823**	25466	27818	31516	34319	37490	38070	**40828**	43292
C	**11847**	16023	**17640**	20457	24040	27542	30682	**33704**	36060	**36826**
D	8303	11800	14033	15550	17261	19564	22824	26832	29891	31752
E	**7578**	10696	**7906**	7561	9724	13134	15865	**17567**	20377	**25671**
F	7111	**8274**	6429	5977	8158	9645	10992	12682	**16472**	20664
G	**4399**	4073	3956	4855	4377	5702	6240	8386	11139	**13759**

	1 slide
The absolute cell count on a single slide	1,162,679
The estimated count using results from the darker shaded regions (bold numbers)	1,259,459
Average results	**6 slides**
Average absolute count	731,700 (±38,782)
Average estimated count using results from the darker shaded regions	760,237 (±48,557)

Figure 5. Algorithm to estimate the total number of cells on slides reducing the scanning time

Here we show the number of cells on one representative slide with 1,163,000 cells adhered divided into 70 regions. The number of cells identified per region is shown. Currently, the whole slide is scanned in ~20 hours and nuclei counted by DAPI staining. We show that we can obtain consistent adherence with a similar number of cells per slide (731,700 ±5%). We also validated that we can estimate the number of cells by counting representative regions and multiply by an area correction factor. Using the depicted algorithm, we estimated 760,000 ± 49,000 per slide, which is close to the real count of 732,000 ± 39,000 in a series of 6 slides. Evaluation of alternate algorithms is underway.

References: Demaster L, et al. J Virol 2015; Baxter A, et al. Cell Host & Microbe 2016

Acknowledgements: Nicholas Radio, Thermo Fisher

Poster #283

Funding:

NIAID award UM1AI126617, co-funded by NIDA, NIMH and NINDS

R01-AI120011