Characterizing *in vitro* LAG-3 and PD-1 Exhaustion Marker Kinetics and Therapeutic Blockade System on invariant Natural Killer T (iNKT) cells

Allison Balasko[1]Julie LaJoie[1,2], Keith R. Fowke[1,2,3,4]

[1]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg MB, [2] Department of Medical Microbiology, University of Nairobi, Nairobi Kenya, [3] Department of Community Health Sciences, University of Manitoba, Winnipeg MB, [4] Partners for Health and Development in Africa

Questions? Contact Allison Balasko: umbalask@myumanitoba.ca

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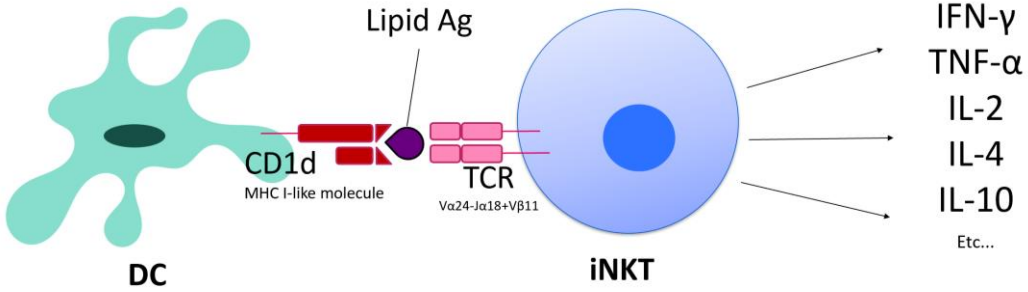
Background

A) One hallmark of chronic HIV infection is immune system exhaustion

- ❑ *Loss of immune system effectiveness*
- ❑ Cellular Immune Exhaustion Markers = **LAG-3, PD-1, etc…**

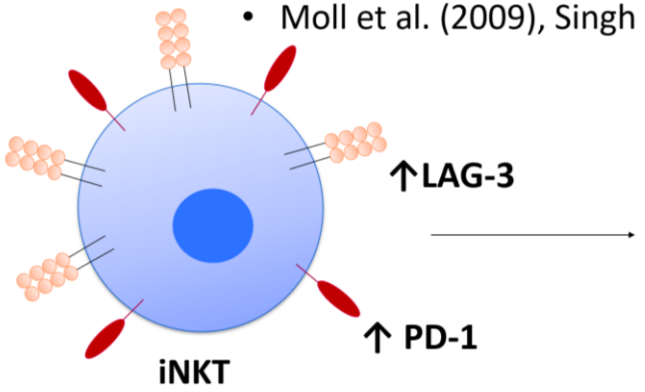
B) Innate Natural Killer T (iNKT) cells are powerful immune cells



- ❑ iNKT cells are quick + critical responders: link innate & adaptive immunity

- ❑ **↑ PD-1 in chronic HIV infection, correlated with ↓ iNKT cell functionality**
  - Moll et al. (2009), Singh et al. (2018)

C) iNKT immune cells are exhausted and dysfunctional in chronic HIV infection



In chronic HIV infection, iNKT cell dysfunction has been correlated with dysfunctional T and NK cell responses

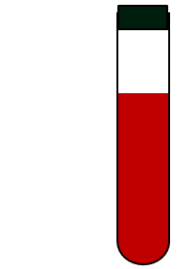
- ❑ **Our lab has shown: ↑LAG-3 correlates with ↓ iNKT cell functionality**

Q1: Can we characterize LAG-3 and PD-1 expression kinetics in relation to iNKT cell activation?

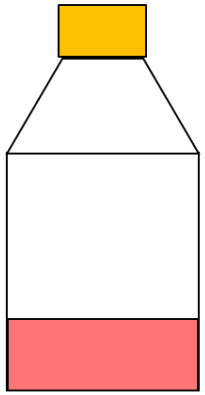
Q2: By blocking PD-1 alone, or in conjunction with LAG-3, can we enhance iNKT immune function?

Hypothesis & Methods

Hypothesis 1: *Expression of exhaustion markers LAG-3 and PD-1 is negatively correlated with iNKT cellular function*



Blood Sample Collection



PBMC Isolation

Exp. 1: Multi-day LAG-3 + PD-1 Kinetics Assay
n = 4

- R10 + IL-2 Media
- 100 ng/ml α-GalCer *(iNKT stimulant)*

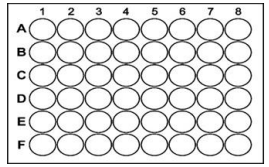
Multi-day Time course

- 24h, 48h
- 4, 7, 10-day
- 10-day + 24h, 48h, 72h rest

iNKT surface LAG-3 and PD-1 expression Assessment via Flow Cytometry

Exp. 2: 10-day Proliferation & Blockade Assay
n = 9

- R10 Media
- 48-well plate
- 100 ng/ml α-GalCer (*iNKT stimulant*)
- 10 ug/ml LAG-3 +/or PD-1 blockade

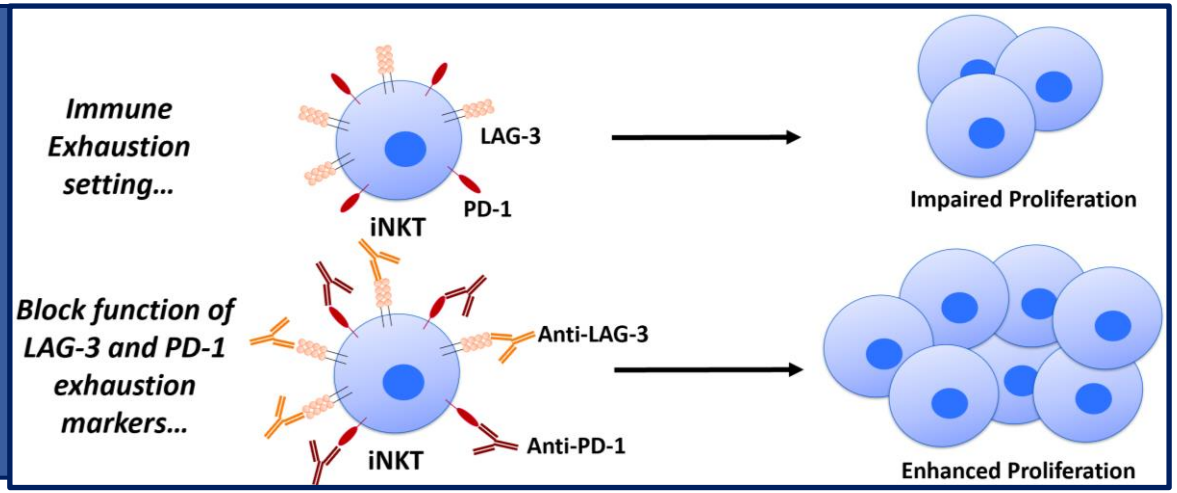


Replenished media, antibody blockades + stimulant

Day 4 Day 7

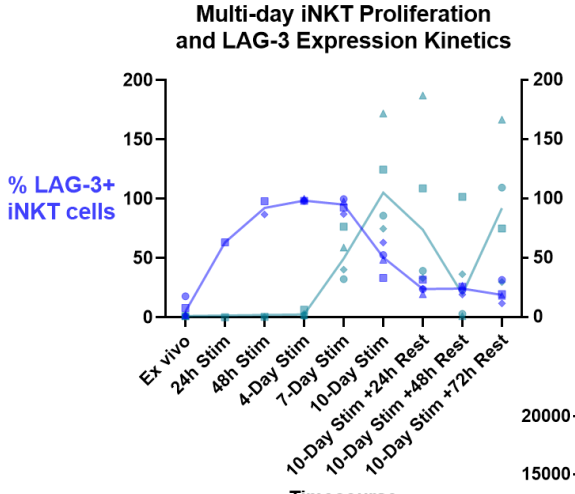
iNKT Proliferation Assessment via Flow Cytometry

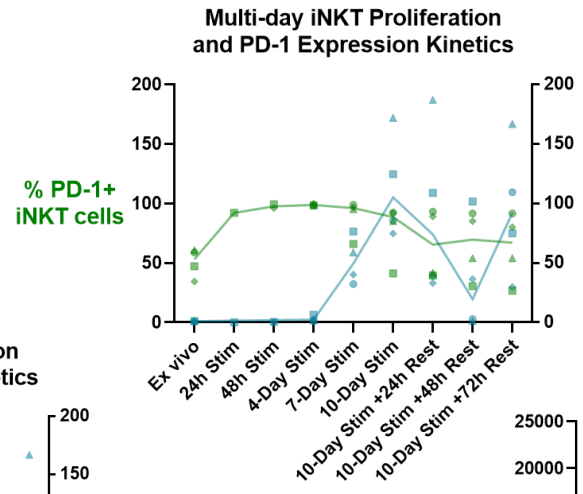
Hypothesis 2: *Blocking PD-1 and/or LAG-3 via an antibody blockade system will enhance cell function* in vitro *by measure of proliferative ability*

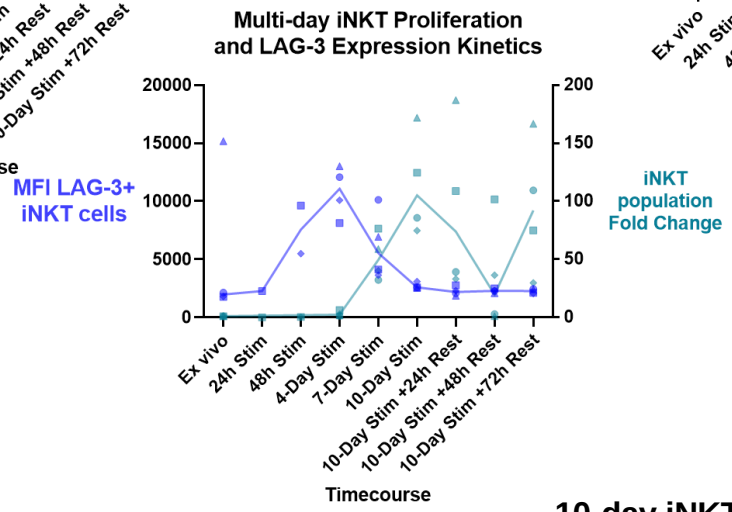


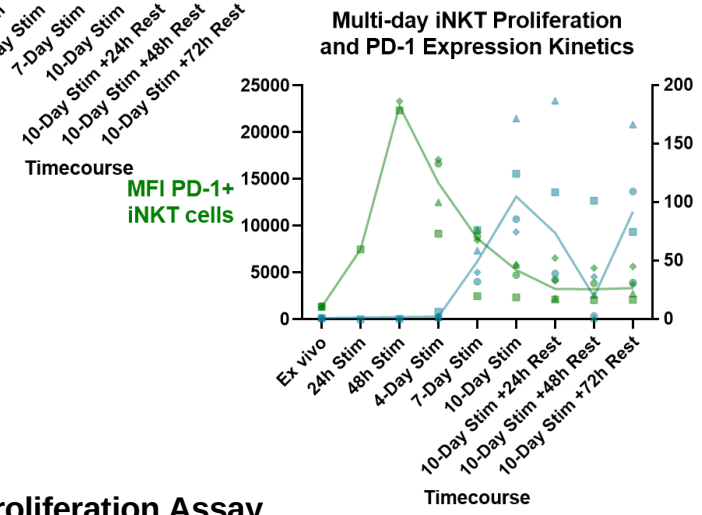
Results

LAG-3 and PD-1 Kinetics Assay (n=4): *LAG-3 percent and median fluorescence intensity (MFI) peaked at Day 7 and 4, respectively, with a steep decrease by peak iNKT proliferation (Day 10). PD-1 percent expression remained relatively elevated, while MFI peaked at Day 4 with a steep decrease at peak iNKT proliferation.*

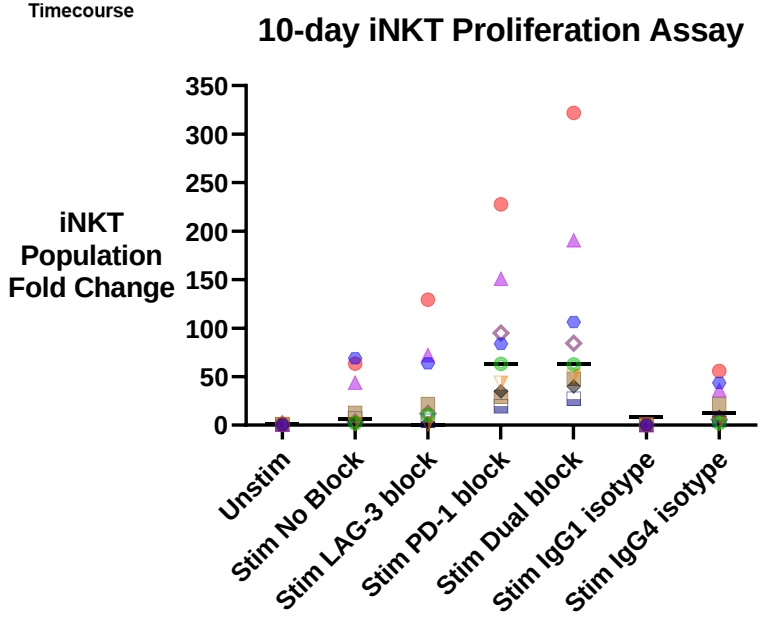








iNKT Proliferation +/- PD-1 and/or LAG-3 Blockade Assay (n=9): *The presence of the anti-PD-1 blockade causes substantial iNKT cell proliferation when compared to the no blockade condition. The anti-PD-1 + anti-LAG-3 dual blockade system shows enhanced proliferative capacity in most donors, when compared to the single PD-1 blockade condition.*



Implications of Study

- ❑ **First study to characterize multi-day LAG-3 and PD-1 expression kinetics on iNKT cells upon activation**
- ❑ **In chronic HIV infection, iNKT cells are exhausted with upregulated LAG-3 and/or PD-1**
- ❑ **We believe that by blocking PD-1 and/or LAG-3 via antibody blockade immunotherapy, will can reverse iNKT cell exhaustion and enhance cellular function**
- ❑ **This novel proliferation model works as proof that the iNKT cell population in HIV-positive individuals has potential to be restored**

Overall Goal: Reverse HIV-mediated dysregulation of iNKT function in an effort to boost viral control in a functional HIV cure approach, as well as ameliorate the immune response against opportunistic infections