The use of aspirin (ASA) to reduce inflammation does not adversely affect systemic T regulatory cells.

Monika M Kowatsch[1], Julius Oyugi[1,2], Lucy W Mwangi[2], Natasha Hollett[1], Geneviève Boily-Larouche[3], Maureen Akolo[4], John Mungai[4], Joshua Kimani[1,2,4], Julie Lajoie[1,2], Keith R Fowke[1,2,4]

[1]University of Manitoba; [2]University of Nairobi; [3]CIHR, [4]Partners for Health and Development in Africa

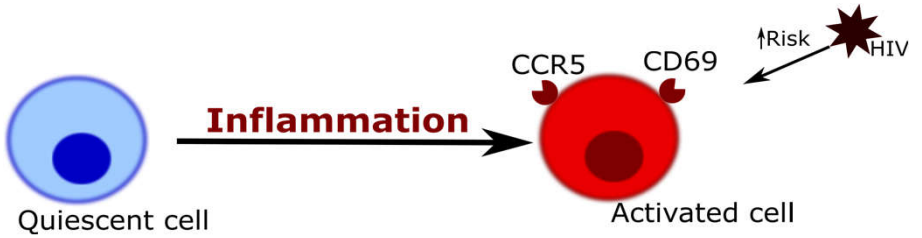
Email contact: Monika M Kowatsch umkowats@myumanitoba.ca

"Conflict of Interest Disclosure: I have no conflicts of interest"

Introduction/Background

Increased inflammation = **increased** risk for HIV acquisition

- STIs: Cause localized genital inflammation
- Microbicides: CAPRISA-004 (1% tenofovir gel)
  - Increased inflammation resulted in increased risk of HIV acquisition



Decreased inflammation = **decreased** risk for HIV acquisition

- HESN (HIV-exposed seronegative) from Majengo cohort
  - Remain HIV uninfected despite intense exposure to HIV
  - Associated with a resting immune state (Immune Quiescence) (Lajoie, Mwangi, & Fowke, 2017)

Aspirin (ASA)

- Non-steroidal anti-inflammatory drug
- Used daily (81mg) for treatment of autoimmune diseases
- Safe, affordable, globally accessible, community accepted

Aspirin and HIV target cells

- Pilot study conducted by our lab found: (Lajoie *et al.*, 2018)
  - Decrease in **expression** of CCR5 on CD4+ T cells in the **blood**
  - 35% decrease in **proportion** CCR5CD4+ T cells at the **genital track**

T regulatory cells (Tregs) and Immune Quiescence

- Tregs are:
  - A key feature of immune quiescence (Lajoie, Mwangi, & Fowke, 2017)
  - Function to keep immune system from being overly activated (Joller et al., 2014)

- Assessing Treg activation and function
  - Treg Activation markers
    - CD69: ability to maintain immune tolerance (Yu et al., 2018)
    - HLA-DR: highly differentiated, induce more vigorous T-cell suppression (Schaier et al., 2013)
  - Treg Function Markers
    - CTLA-4: maintaining T cell homeostasis and tolerance to self (Jain et al., 2010)
    - Helios: Treg stability, restricts effector cytokine expression (Chougnet & Hildeman, 2019)
    - TIGIT: Inhibitory molecule, prevents Tregs from suppressing the immune response (Joller et al., 2014)

- Tregs and Aspirin
  - Based on animal models
    - Mouse: Aspirin increases Treg numbers in the blood (Mondal *et al.*, 2018)
    - Dog: Aspirin has no effect on Treg numbers (Archer *et al.*, 2018)
    - No studies into Treg function with aspirin
    - No human studies on Tregs and aspirin

Gap in Knowledge: what is the effect of daily aspirin on Treg function and number

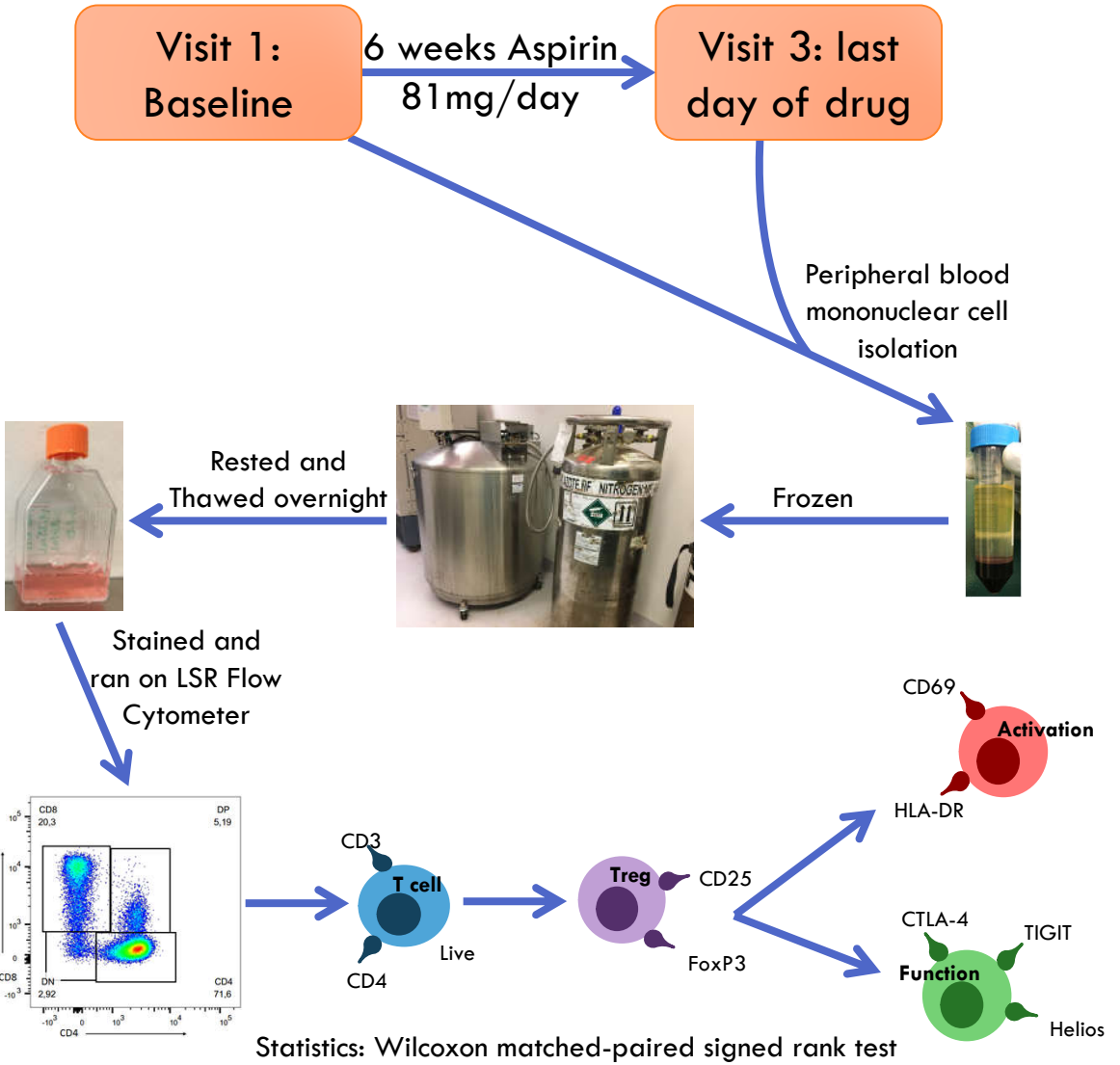
Hypothesis: We expect daily aspirin to decrease HIV target cells without affecting Treg cells in the blood

Participants (N=38)

Participants from Pumwani and Baba Dogo communities

General Characteristics	
Age (mean [SD])	32 [8.0]
Douching (n(%))	21 (55.3)
Contraception	
No Hormonal Contraception (n(%))	12 (31.6)
Depot/DMPA (n(%))	21 (55.3)
Oral HC (n(%))	2 (5.2)
Other (n(%))	2 (5.2)
No information given (n(%))	1 (2.6)
Regular Partner	
Yes (n(%))	30 (78.9)
No (n(%))	4 (10.5)
Not Disclosed (n(%))	4 (10.5)
Times sexual intercourse with regular partner in last 7 days (mean [SD])	1.19 [1.1]
Used condom with regular partner in the last 7 days (n(%))	5 (13.2)

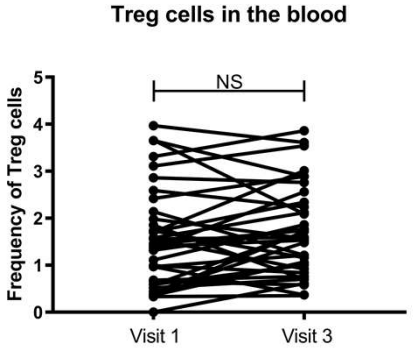
Methods



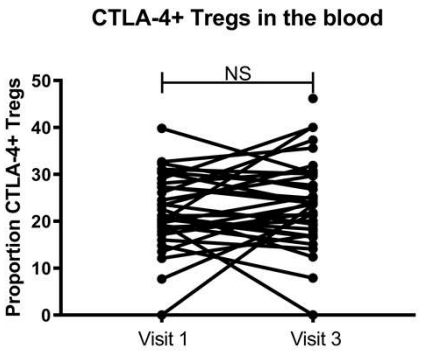
Statistics: Wilcoxon matched-paired signed rank test

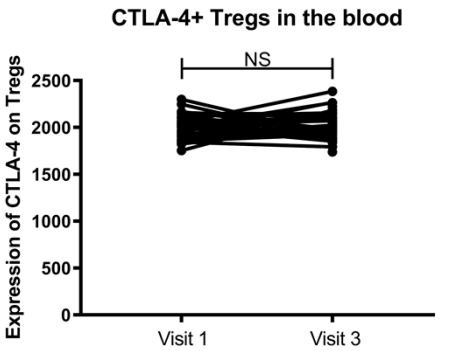
Results: Effect of Aspirin on Treg cells

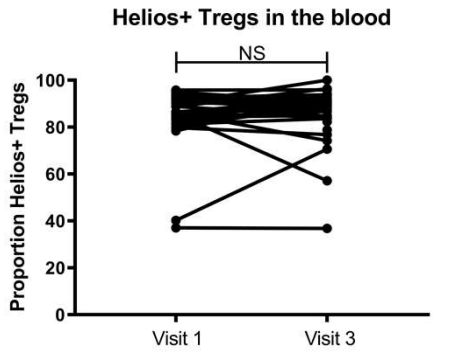
Aspirin did not effect proportion Tregs in the blood



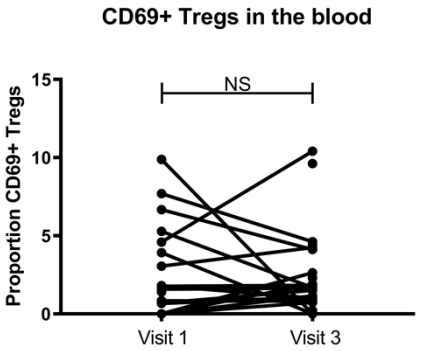
Aspirin had no effect on Treg function markers CTLA-4 and Helios

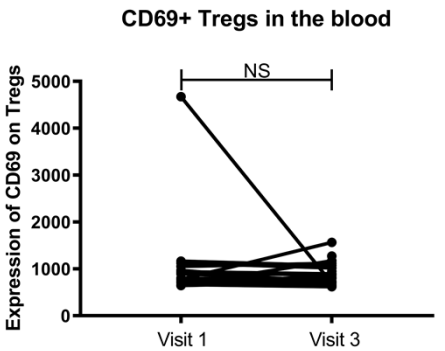


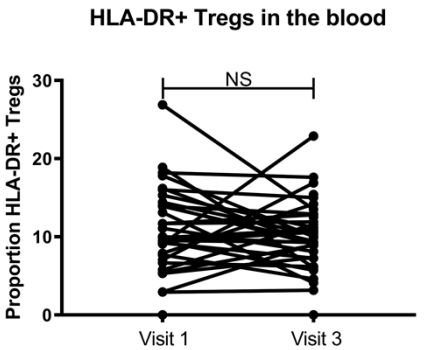


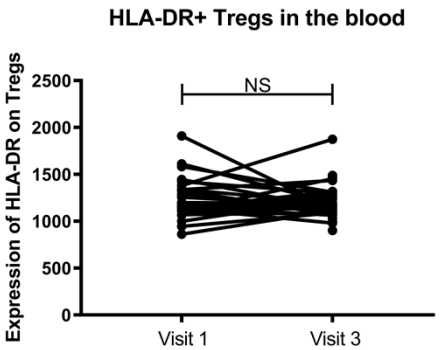


Aspirin did not effect markers of Treg activation CD69 and HLA-DR

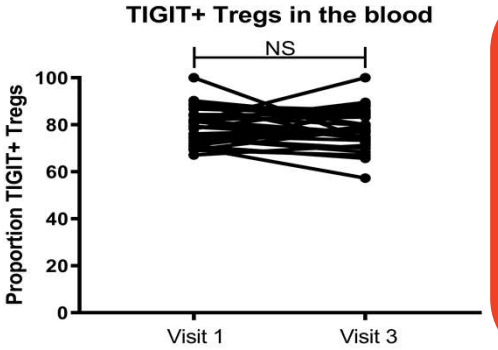


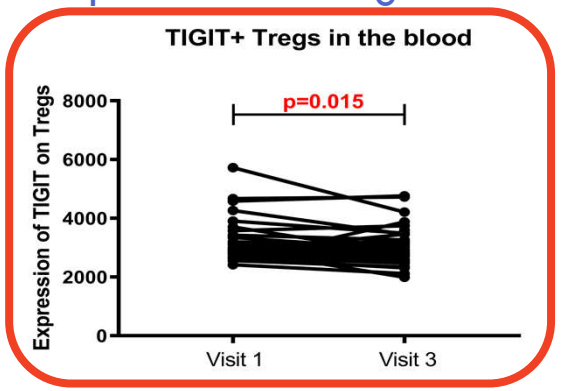






Aspirin decreased TIGIT expression on Treg cells





Effect of 6 weeks low dose ASA on T regulatory cells (Tregs). Visit 1 is before drug, visit 3 is last day of drug. $p<0.05$ were considered significant.

Conclusion and Significance

Conclusion

- In a study of 6 weeks 81mg aspirin
  - Decrease in HIV target cells at the genital tract
  - In respect to tregs in the blood 6 weeks AS treatment **does not**:
    - Alter Treg proportion
    - Alter Treg activation
    - Alter function markers CTLA-4 or Helios
  - 6 weeks aspirin treatment **does**:
    - Reduced the expression of inhibitory molecule TIGIT on a per cell basis

Significance

- As TIGIT is a inhibitory molecule, decreased TIGIT expression results in increased Treg activity, promoting a immune quiescent phenotype
  - Supports ASA's further assessment as a new HIV prevention tool

Thank you!

- Fowke lab members
- Baba Dogo and Pumwani Staff
- Ken Omollo Oduor
- Dr. Yoav Keynan
- UNITID Lab Staff
- Participants
- Funding Sources

References

1. Archer, T. M. *et al.* (2018) 'In vivo effects of aspirin and cyclosporine on regulatory T cells and T-cell cytokine production in healthy dogs', *Veterinary Immunology and Immunopathology*, 197, pp. 63–68. doi: 10.1016/j.vetimm.2018.01.003.
2. Chougnet, C. and Hildeman, D. (2016) 'Helios-controller of Treg stability and function.', *Translational cancer research*. NIH Public Access, 5(Suppl 2), pp. S338–S341. doi: 10.21037/tcr.2016.07.37.
3. Jain, N. *et al.* (2010) 'Dual function of CTLA-4 in regulatory T cells and conventional T cells to prevent multiorgan autoimmunity.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 107(4), pp. 1524–8. doi: 10.1073/pnas.0910341107.
4. Joller, N. *et al.* (2014) 'Treg cells expressing the co-inhibitory molecule TIGIT selectively inhibit pro-inflammatory Th1 and Th17 cell responses', *Immunity*, 40(4), pp. 569–581. doi: 10.1016/j.immuni.2014.02.012.Treg.
5. Lajoie, J. *et al.* (2018) 'Using safe, affordable and accessible non-steroidal anti-inflammatory drugs to reduce the number of HIV target cells in the blood and at the female genital tract.', *Journal of the International AIDS Society*. Wiley-Blackwell, 21(7), p. e25150. doi: 10.1002/jia2.25150.
6. Lajoie, J., Mwangi, L. and Fowke, K. R. (2017) 'Preventing HIV infection without targeting the virus: how reducing HIV target cells at the genital tract is a new approach to HIV prevention.', *AIDS research and therapy*. BioMed Central, 14(1), p. 46. doi: 10.1186/s12981-017-0166-7.
7. Mondal, S. *et al.* (2018) 'Aspirin ameliorates experimental autoimmune encephalomyelitis through interleukin-11-mediated protection of regulatory T cells.', *Science signaling*. American Association for the Advancement of Science, 11(558). doi: 10.1126/scisignal.aar8278.
8. Schaier, M. *et al.* (2013) 'The extent of HLA-DR expression on HLA-DR + Tregs allows the identification of patients with clinically relevant borderline rejection', *Transplant International*. John Wiley & Sons, Ltd (10.1111), 26(3), pp. 290–299. doi: 10.1111/tri.12032.
9. Yu, L. *et al.* (2018) 'CD69 enhances immunosuppressive function of regulatory T-cells and attenuates colitis by prompting IL-10 production', *Cell Death & Disease*. Nature Publishing Group, 9(9), p. 905. doi: 10.1038/s41419-018-0927-9.