Flow Cytometric Analysis of Gut Biopsies

Ian McGowan MD PhD FRCP
University of Pittsburgh
Pittsburgh, PA
USA

Overview

- GALT flow cytometry
- GALT flow data from completed studies
  - MTN studies
  - Non-MTN studies
- Lessons learned and questions for the future

GALT Flow Cytometry

GALT Flow Cytometry

- GALT flow cytometry increasingly used to characterize mucosal cell populations in:
  - HIV pathogenesis studies
  - Evaluation of HIV vaccine responses
  - PreP studies
  - Non-HIV related fields such as inflammatory bowel disease research
- Primary approach is to collect biopsies and to mechanically/enzymatically disassociate into single cells

GALT Flow Data

GALT Flow Data (1)

- HPTN-056
  - No intervention
- RMP-01
  - UC781 gel (Phase 1)
- RMP-02 / MTN-006
  - TFV gel & oral (Phase 1)
- MTN-007
  - TFV gel (Phase 1)
- CHARM-01
  - TFV gel (Phase 1)
- HVTN-MIG study
  - No intervention
- MTN-017
  - TFV gel/oral (Phase 2)
- CHARM-03
  - Maraviroc gel & oral (Phase 1)
- HPTN-069
  - Oral TFV, MVC, FTC (Phase 2)

GALT Flow Data (2)

- ACTG 5330 (Multi-center)
  - The effect of isotretinoin on immune activation among HIV-1-infected subjects with incomplete CD4+ T cell recovery
- Dipyridamole (DP) study (Single center)
  - The effect of DP on HIV-associated immune activation and inflammation
- ACTG 5341s (Multi-center)
  - Size and decay of HIV-1 reservoirs in tissues

MTN
microbicide trials network

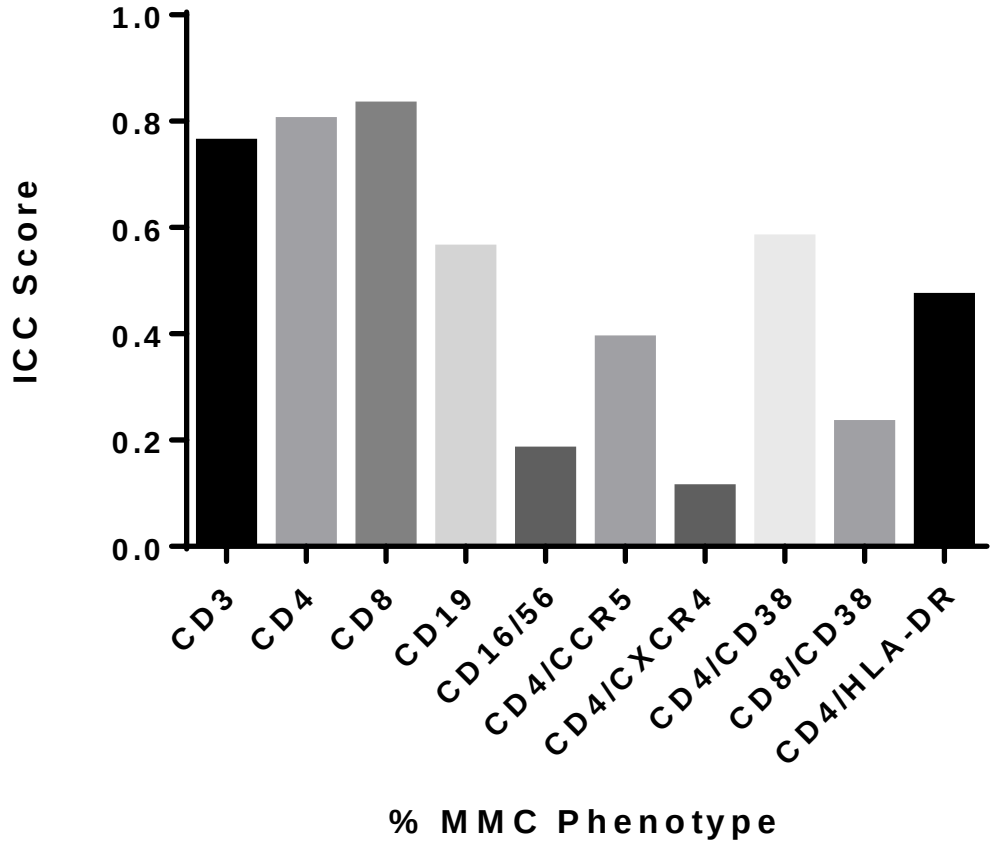
HPTN-056

- Population
  - HIV-negative (N=8)
  - HIV-positive (N=8)
- Center(s)
  - Single
- Sampling
  - Colon
  - 10 cm and 30 cm
  - BL, +2/52 +4/52
- Panel
  - CD3, CD4, CD8, CD45,
  - HLA-DR, CD38,
  - CXCR4, CCR5, DC-SIGN
  - CD19
  - CD16, CD56

McGowan et al. JAIDS 2007

MTN microbicide trials network

HPTN-056 ICC Scores



McGowan et al. JAIDS 2007

RMP-01

- Population
  - HIV-negative (N=36)
- Center(s)
  - Single
- Sampling
  - Colon
  - 10 cm and 30 cm
  - BL, post single dose, and post seven doses
- Products (1:1:1)
  - UC781 gel (0.1%)
  - UC781 gel (0.25%)
  - HEC placebo
- Panel
  - CD4, CD8, CD45,
  - HLA-DR, CD38
  - CXCR4, CCR5

Anton PA et al. PLoS One 2011

MTN microbicide trials network

RMP-01

- Single dose
  - UC781(0.1%) vs HEC (p >0.05)
  - UC781 (0.25%) vs. HEC (p >0.05)
- 7 day exposure
  - UC781(0.1%) vs HEC (p >0.05)
  - UC781 (0.25%) vs. HEC
    - CCR5 RFI on CD4 (p = 0.025)
    - CCR5/CXCR4 on CD4 (p = 0.020)

Anton PA et al. PLoS One 2011

MTN microbicide trials network

RMP-02 / MTN-006

- Population
  - HIV-negative (N=18)
- Center(s)
  - 2 sites
  - Samples shipped to UCLA for analysis
- Sampling
  - Colon (15 cm)
  - BL, post single dose, and post seven doses
- Products (2:1)
  - TFV gel (1%)
  - HEC placebo
- Panel
  - CD4, CD8, CD45,
  - HLA-DR, CD38
  - CXCR4, CCR5

Anton PA et al. AIDS Res Hum Retroviruses 2012

MTN microbicide trials network

RMP-02 / MTN-006

	Oral	Single	7 day
	P values		
CD3+ on CD45+	0.20	0.90	0.32
CD4+ on CD45+	0.46	0.75	0.20
CD8+ on CD45+	0.08	0.44	0.71
CD38+ on CD4+	0.72	0.37	0.86
CD38 RFI on CD4+	0.76	0.42	0.81
HLA- DR+ on CD4+	0.53	0.92	0.84
HLA- DR+ CD38+ on CD4+	0.26	0.93	0.97
CCR5+ on CD4+	0.15	0.29	0.94
CCR5 RFI on CD4+	0.13	0.45	0.83

Anton PA et al. AIDS Res Hum Retroviruses 2012

MTN microbicide trials network

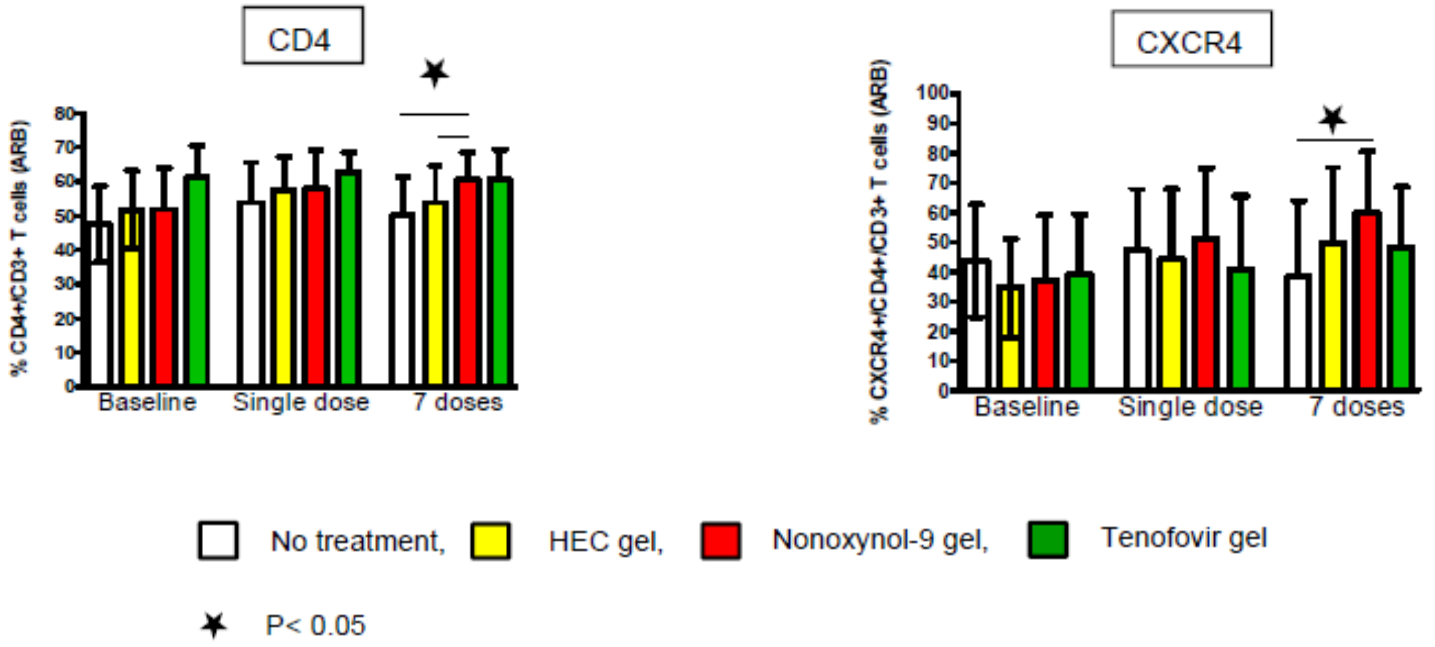
MTN-007

- Population
  - HIV-negative (N=60)
- Center(s)
  - 3 centers
  - Samples shipped to Pittsburgh
- Sampling
  - Anoscopic
  - Flex sig (15 cm)
  - BL, post SD, and post 7D
- Products (1:1:1:1)
  - TFV gel (1.0%)
  - N9 gel (2.0%)
  - HEC placebo
  - No Rx
- Panel
  - CD3, CD4, CD8, CD45,
  - CD69
  - CXCR4, CCR5

McGowan I et al. PLoS One 2013

MTN microbicide trials network

MTN-007



7doses of TFV gel versus HEC gel associated with a significant increase in CD45+/CD3+ cells isolated from Flex sig biopsies (57% versus 42.8%; p = 0.04)

McGowan I et al. PLoS One 2013

CHARM-01

- Population
  - HIV-negative (N=14)
- Center(s)
  - 2 centers
  - Samples shipped to Pittsburgh
- Sampling
  - Flex sig (15 cm)
  - BL, and post 7D of each formulation
- Products (crossover)
  - TFV gel (1.0%)
  - RG TFV gel (1.0%)
  - RS TFV gel (1.0%)
- Panel
  - CD3, CD4, CD8, CD45,
  - CD69
  - CXCR4, CCR5

McGowan I et al. PLoS One 2015

MTN microbicide trials network

CHARM-01

Flow parameter	Enrollment(n = 14) Mean (SD), Median (25%, 75%)	Mean at 7th Dose N, Mean (SD), Median (25%, 75%)	Change at 7th Dose(n = 13) Mean (SE)	*P* value**
% $CD3^+$ from $CD45^+$	44.4 (17.4), 47.2 (34.6, 58.9)			
Enrollment vs. RF D7		11, 53.8 (16.4), 54.8 (46.9, 66.2)	12.23 (5.89)	0.0380
Enrollment vs. HEC/VF D7		12, 54.6 (18.9), 58.3 (49.8, 63.4)	11.98 (3.46)	0.0005
% $CXCR4^+$ from $CD4^+$	71.5 (16.1), 70.3 (57.9, 84.5)			
Enrollment vs. HEC/VF D7		12, 61.1 (23.2), 61.4 (55.0, 79.5)	-10.68 (5.40)	0.0480
Change at 7th Dose (RGVF v HEC/VF)			16.4 (6.70)	0.0142
% $CD69^+$ from $CD4^+$	83.4 (6.0), 83.9 (80.4, 87.4)			
Enrollment vs. HEC/VF D7		12, 80.5 (4.9), 82.3 (78.7, 83.5)	-2.27 (0.97)	0.0188
% $CXCR4^+$ and $CCR5^+$ from $CD4^+$	55.6 (11.9), 55.1 (43.8, 66.2)			
Enrollment vs. HEC/VF D7		12, 45.4 (15.9), 48.7 (41.2, 53.9)	-10.16 (4.20)	0.0157
Change at 7th Dose (RGVF v HEC/VF)			15.70 (5.58)	0.0049
% $CXCR4^+$ from $CD8^+$	51.2 (17.1), 47.8 (40.2, 68.1)			
Enrollment vs. HEC/VF D7		12, 39.3 (18.8), 38.3 (33.9, 54.2)	-13.38 (4.58)	0.0035
% $CD69^+$ from $CD8^+$	85.7 (6.1), 84.7 (82.1, 90.5)			
Enrollment vs. RGVF D7		13, 71.4 (26.6), 80.2 (78.1, 84.3)	-13.54 (6.17)	0.0283
Change at 7th Dose (RF v RGVF)			12.80 (6.02)	0.0336
% $CXCR4^+$ and $CCR5^+$ from $CD8^+$	43.2 (13.1), 42.5 (35.6, 48.0)			
Enrollment vs. HEC/VF D7		12, 31.5 (13.8), 34.0 (29.1, 37.7)	-12.51 (3.66)	0.0006

McGowan I et al. PLoS One 2015

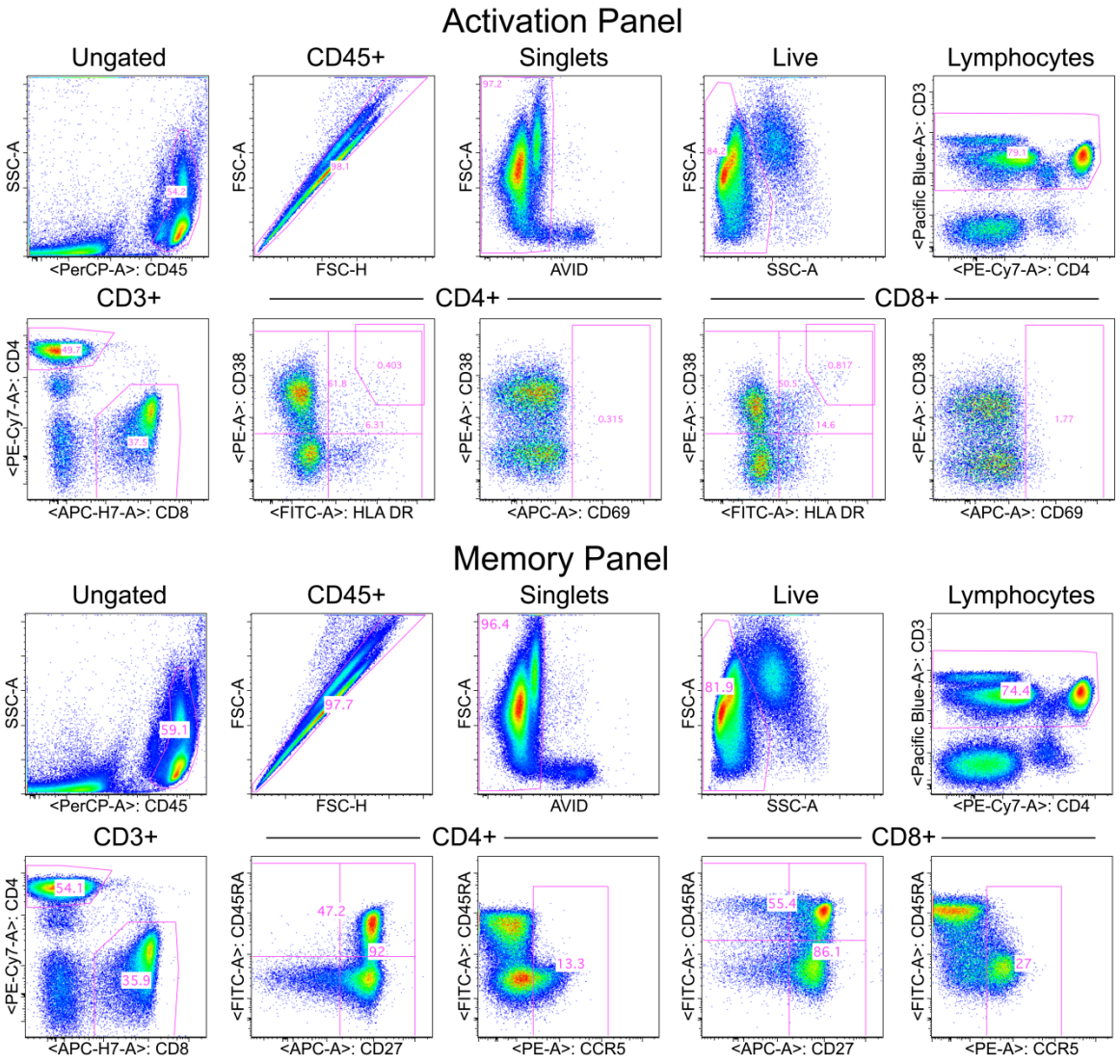
HVTN-MIG

- Feasibility of multi-site flow cytometric processing of GALT samples
- Clinical sites
  - UCLA (N = 18)
  - Pittsburgh (N = 17)
- Samples
  - PBMC, qPBMC, MMC
- Flow analysis
  - Fred Hutchinson CRC

McGowan I et al. PLoS One 2015

MTN microbicide trials network

MIG Panel

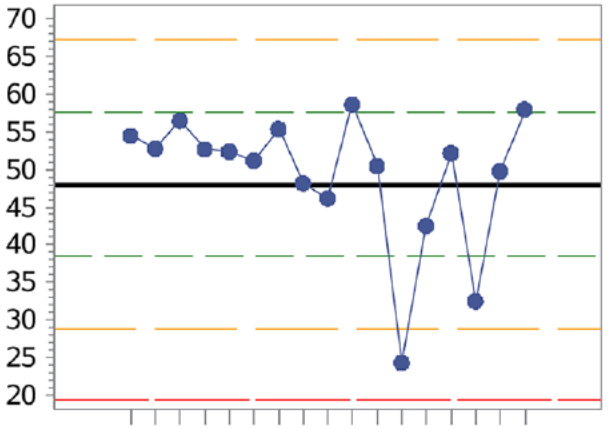

Activation Panel
Ungated
CD45+
Singlets
Live
Lymphocytes
SSC-A
<PerCP-A>: CD45
FSC-A
FSC-H
AVID
SSC-A
<Pacific Blue-A>: CD3
<PE-Cy7-A>: CD4
CD3+
CD4+
CD8+
<PE-Cy7-A>: CD4
<APC-H7-A>: CD8
<PE-A>: CD38
<FITC-A>: HLA DR
<APC-A>: CD69
Memory Panel
Ungated
CD45+
Singlets
Live
Lymphocytes
59.1
97.7
96.4
81.9
74.4
CD3+
CD4+
CD8+
54.1
35.9
47.2
92
13.3
55.4
86.1
27
<FITC-A>: CD45RA
<APC-A>: CD27
<PE-A>: CCR5

qPBMC Data

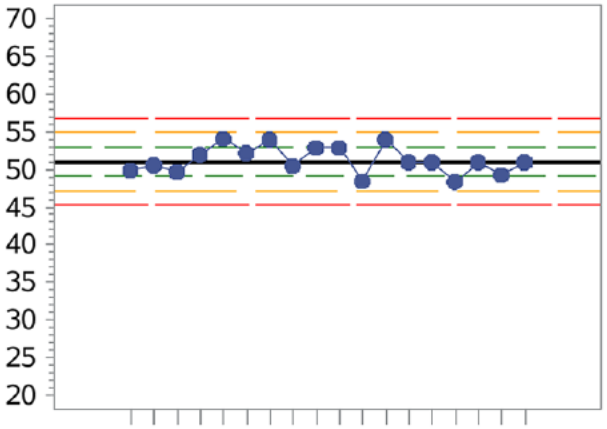
Activation Panel

CD4+ Percentage of CD3+ Cells

MWRI

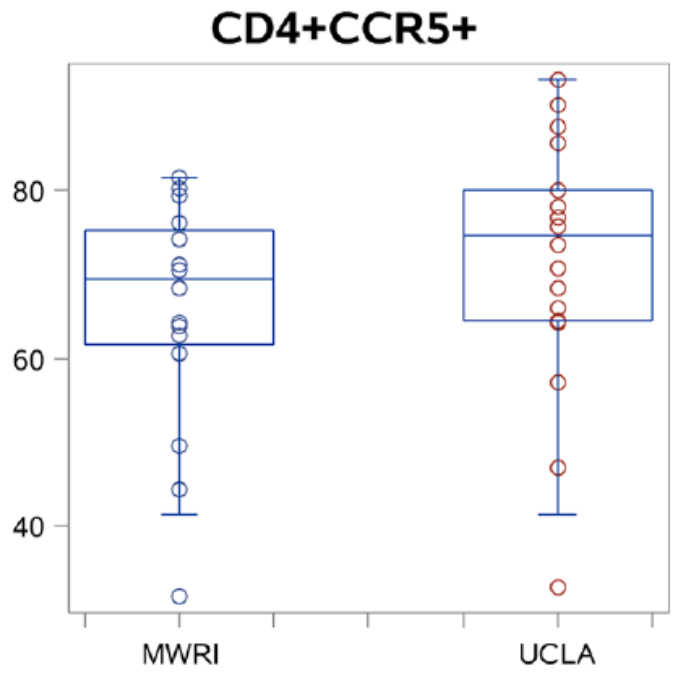


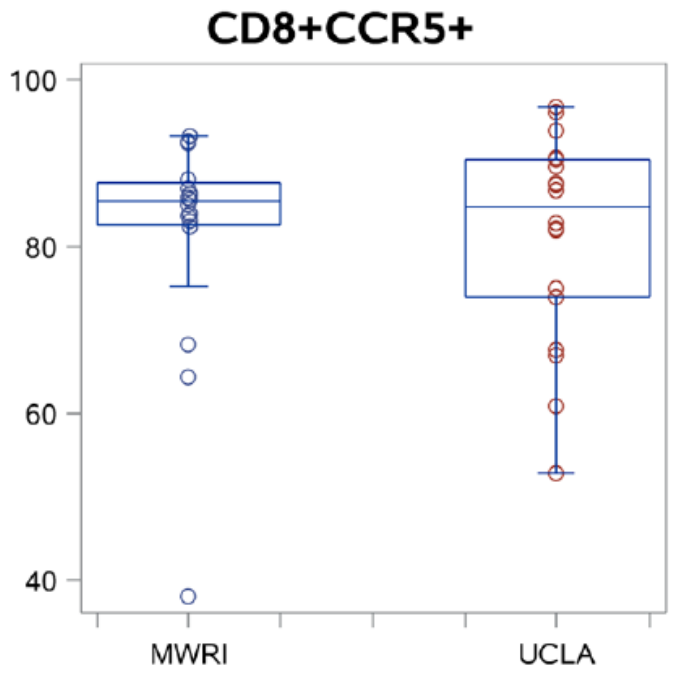
UCLA



McGowan I et al. PLoS One 2015

MMC Data





McGowan I et al. PLoS One 2015

MIG Summary

- Ranges for qPBMC CD4/CD8 equivalent at both sites
- Significant differences for the majority of other parameters
- Standardized protocols can reduce but not eliminate variability between sites
- HSV seropositivity influences T cell phenotype
- FMO not routinely required

Lessons Learned

Lessons Learned

- GALT flow cytometry is challenging and variable
- Cryopreservation and centralized analysis may provide more stable data
- Unclear whether flow cytometry routinely required in microbicide studies
- Further data may clarify the situation
  - MTN-017, HPTN-069, CHARM-03

MTN
microbicide trials network

Future Questions

Future Questions

- What studies require flow cytometry?
- What panels should be included?
- Multi-center studies
  - Should sample processing/staining and/or analysis be centralized?
  - Could cryopreservation of tissue samples or isolated MMC lead to more robust data?

Acknowledgements

The Microbicide Trials Network is funded by the National Institute of Allergy and Infectious Diseases (UM1AI068633, UM1AI068615, UM1AI106707), with co-funding from the National Institute of Child Health and Human Development and the National Institute of Mental Health, all components of the U.S. National Institutes of Health.