HIV Vaccine Development in 2009

Where are we and where are we going?

The Importance of an HIV Vaccine

- HIV one of the greatest challenges to health and development that we face;
- Nowhere in the world is the pandemic under control despite effective prevention and treatment;
- The toll of the HIV pandemic in 2007
  - 33.2 million people living with HIV
  - 2.5 million new infections
  - 2.1 million deaths

25 Years of HIV Vaccine Research

	Scientific Highlights
1st Wave 1983-1994	•Discovery of HIV •Demonstration of neutralizing antibody (Nab) to Env and CMI to HIV •CD4 identified as primary HIV receptor •1st clinical trial of an HIV vaccine •1st viral vector for and HIV vaccine developed •Hypervariability of HIV described •Env immunization elicits Nab for lab strains but not primary isolates •No go decision for gp120 vaccines chills HIV vaccine investments
2nd Wave 1995-2007	•Broadly neutralizing monoclonal antibodies (BnMabs) to HIV discovered •CCR5 identified as HIV co-receptor •CMI to HIV correlated with viral control – CMI vaccine strategies developed •BnMabs protect macaques against challenge with chimeric HIV-SIV •1st efficacy trial of gp120 vaccines (Vaxgen) show no protection •Structures of BnMabs bound to gp120 understood •Gobal HIV Vaccine Enterprise established •Efficacy trial of leading CMI vaccine shows no evidence of effectiveness • Chill around HIV vaccines
3rd Wave 2008-	•Refocus on understanding correlates of protection in humans •Refocus on understanding mucosal immunity •Application of powerful new systems biology tools on HIV vaccines • Attraction of new talent and new ideas to the HIV vaccine field

STEP Trial Results

- Utilized Adenovirus 5 vector with HIV gag, pol and nef genes
- Stopped early because of “futility”
- Induced high level of T cell responses
- No effect on HIV incidence or viral load

Study Group	Person Years Of Follow up	HIV Incidence
Vaccine	619	3.07
Placebo	622	1.77

STEP Trial Analysis Stratified By Pre-existing Ad5 Antibody

Ad5 Titer	Vaccine	Placebo	Relative Incidence	95% CI
<18	4.0	4.0	1	0.5-2.0
19-200	4.4	2.2	2.1	0.6-9.3
201-1000	6.1	3.0	2.0	0.8-5.9
>1000	4.4	1.2	3.5	0.7-35

Current HIV Vaccine Trials

- Env targeting Vaxgen trial in Thailand is the only Phase III trial currently
- Two Phase IIB trials of the most promising T cell vaccine have been stopped
- Two Phase II trials target T cell responses
- Twenty-one Phase I/II trials in progress all are directed at T cell responses
- No neutralizing antibody trials
- No mucosal vaccine trials

Why is an HIV Vaccine So Challenging?

- HIV is a retrovirus
- HIV infection does not induce completely protective immunity
- HIV is hypervariable
- HIV has immune evasion mechanisms
- HIV only infects humans
- HIV is sexually transmitted
- Window of opportunity for prevention after exposure extremely brief

Why Does and HIV Vaccine Seem Achievable?

- HIV is inefficiently transmitted (per sexual exposure risk is <1:1000)
- Usually only one viral clone transmitted
- Host immune response correlates with viral control in HIV infected individuals
- Highly HIV exposed uninfected individuals appear to be resistant to infection
- Broadly neutralizing HIV monoclonal antibodies have been produced
- Animal models show some protection

Time to Vaccine Development

	Agent Linked to Disease	Vaccine Licensed in US	Intervening Years
B. pertussis	1906	1948	42
Polio	1908	1955	47
Measles	1953	1963	10
Hepatitis B	1965	1981	16
H. influenza	1889	1981	92
S. typhi	1884	1989	105
VZV	1953	1995	42
Rotavirus	1973	2006	33
HPV	1981	2006	25
HIV	1983	-	25+
CMV	1960	-	48+
M. tuberculosis	1882	*	126+
Malaria	1880	-	128+

Global HIV Vaccine Enterprise

Promoting innovation and collaboration
to speed the search for an HIV vaccine

The Canadian HIV Vaccine Initiative (CHVI)

- Memorandum of Understanding signed in August 2006 formalized collaboration between the Government of Canada and the Gates Foundation to strengthen global efforts to accelerate the development of HIV vaccines and contribute to the achievement of the Global HIV Vaccine Enterprise's Scientific Strategic Plan.
- MOU identifies up to $111M from the Government of Canada ($85M in newly invested funds; $26M in reallocated funds) and up to $28M from the Gates Foundation to be invested over 5 years.
- The Foundation's main priority is the establishment of a pilot scale clinical trial lot manufacturing facility in Canada.
- CHVI was announced in February 2007 by the Prime Minister and Bill Gates.
- Government of Canada Horizontal Initiative involving the Canadian International Development Agency, the Public Health Agency of Canada, Industry Canada, the Canadian Institutes of Health Research and Health Canada.
- Goals:
  - Coordinate Canadian domestic and international contribution to Global HIV Vaccine Enterprise;
  - Accelerate development of a preventive HIV vaccine;
  - Collaborate with key global and domestic partners.

Immunity not Luck:
Comprehensive studies of Mechanisms of HIV Resistance in Highly Exposed Uninfected Women

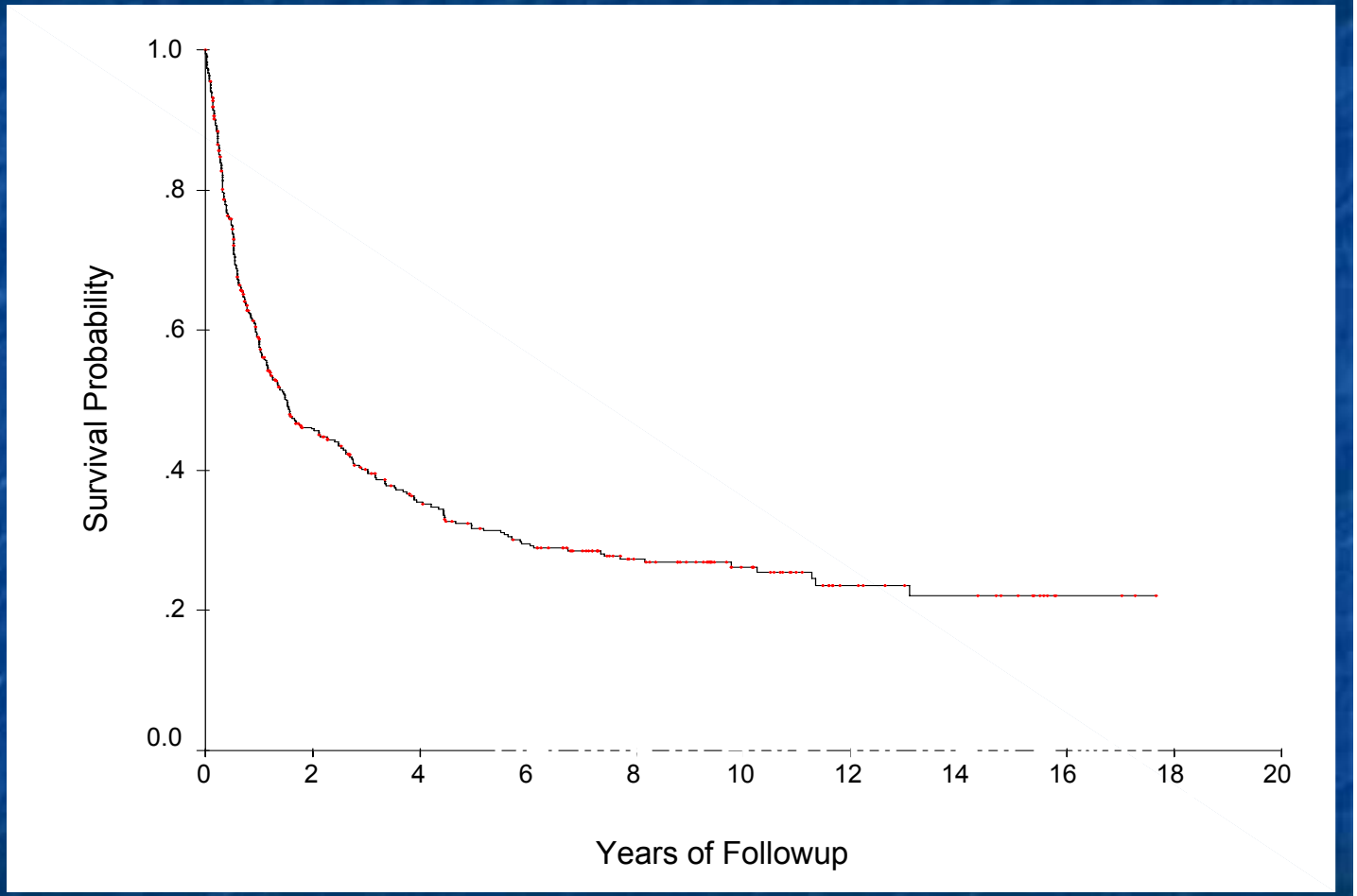
A Bill and Melinda Gates Foundation Grand Challenges in Global Health Project

Within a population there is heterogeneity in susceptibility to infection and disease due to infectious agents

Beginning with Jenner, most vaccines have been developed through at least a basic understanding of natural acquired immunity to infection

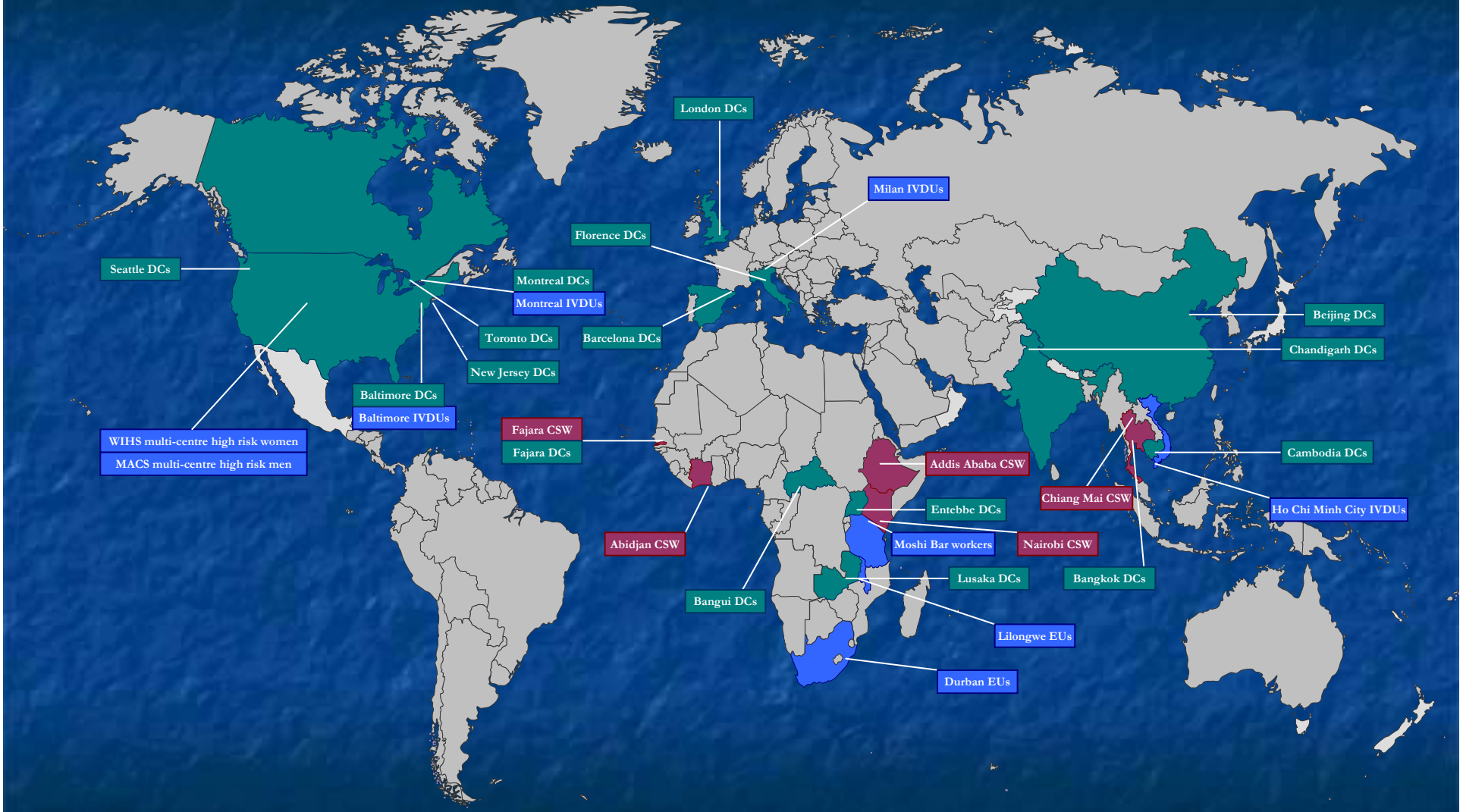
Resistance to HIV-1 Infection

- Highly-exposed persistently seronegative.
- 3 years follow, HIV-1 serology and PCR negative, Active in sex work.
- 10% of highly exposed sex workers resistant to HIV-1.

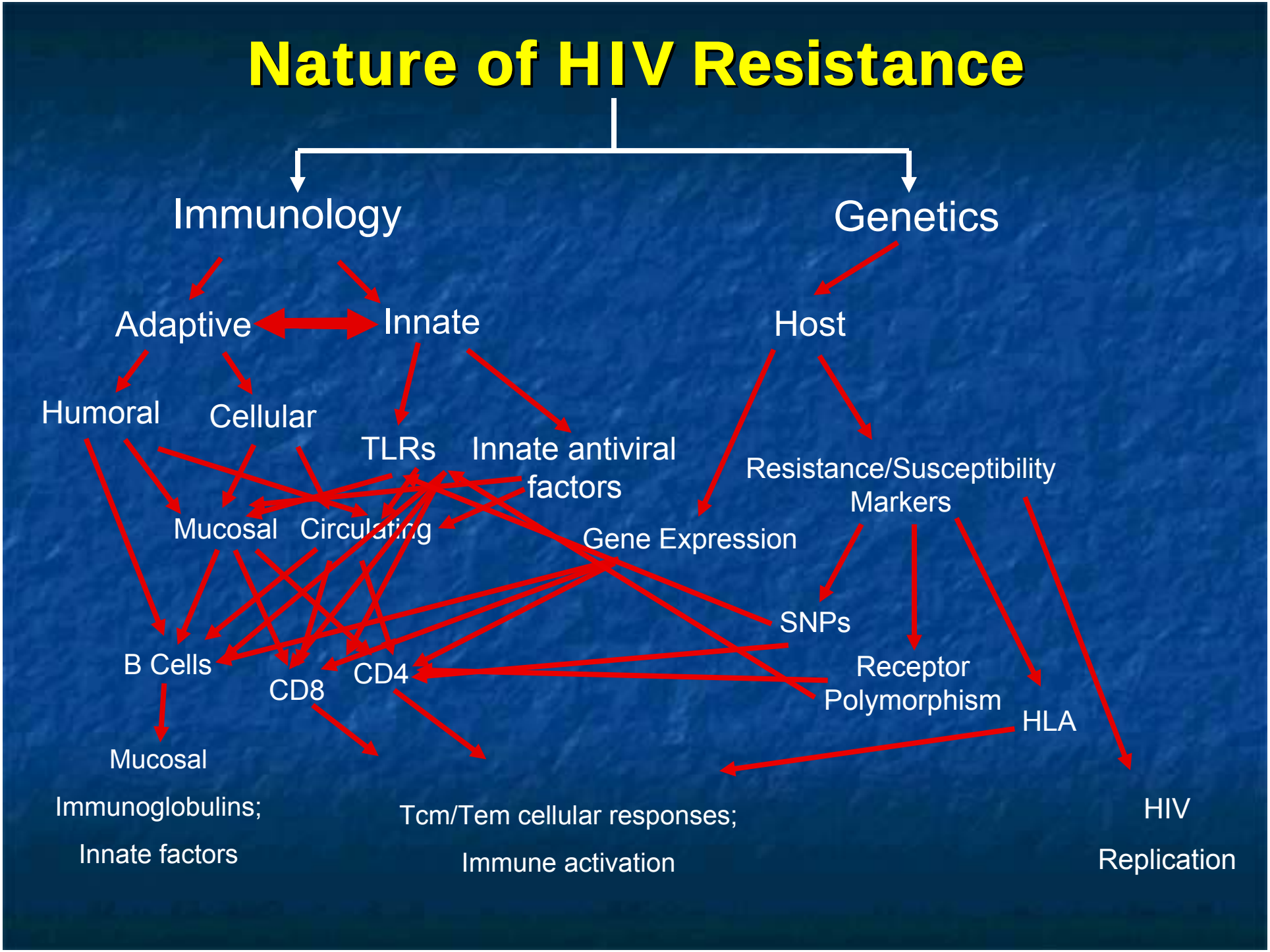


Adapted from Fowke et al Lancet 1996; 348: 1347–51

Altered susceptibility to HIV is not unique



Nature of HIV Resistance



- **Goal 1: To characterize correlates of protective immune responses to HIV in the systemic and mucosal compartments of resistant and susceptible women:**
- **Goal 2: To identify genetic and innate factors associated with resistance in resistant women and their families**
- **Goal 3: To determine how genotypes/phenotypes identified above will determine immune responsiveness to a model antigenic challenge of a live, attenuated mucosal vaccinogen.**
- **Goal 4: To determine if genotypes/phenotypes associated with resistance, or with a favourable response to the model vaccinogen protect against HIV infection in a prospective study of HIV seroconversion in sexworker and non-sexworker cohorts.**

Gene expression profiles of HIV Resistant sex workers

E M Songok PhD

- Goal 2: To identify genetic and innate factors associated with resistance in resistant women and their families

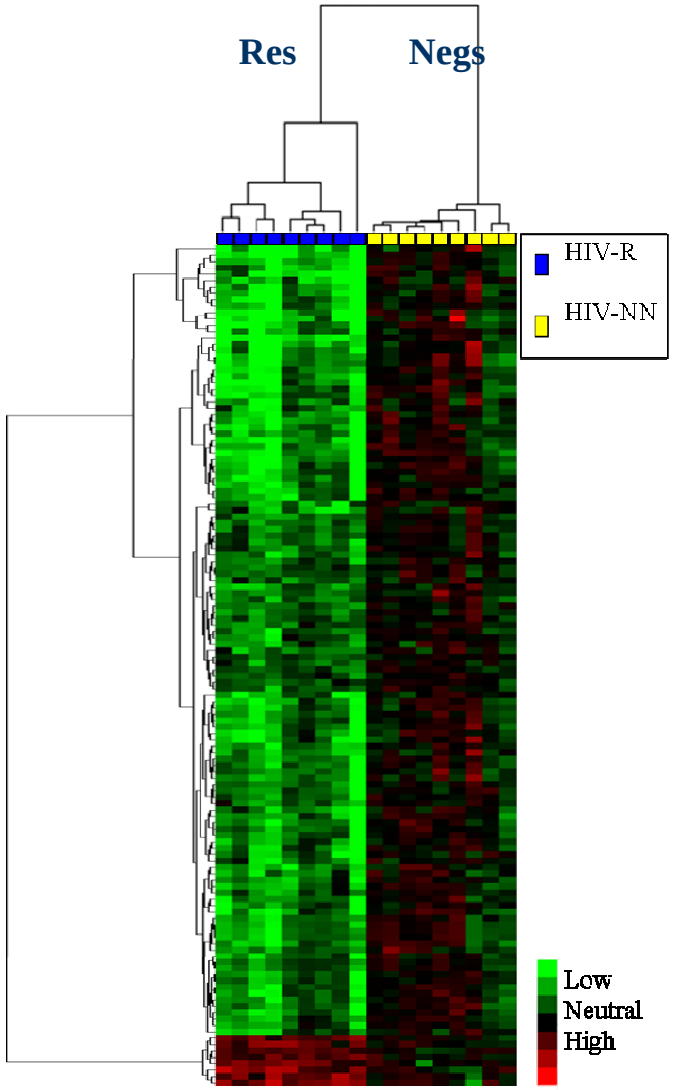
- 2.3: Determine by gene expression analysis if there are genes differentially expressed in resistant women and their families

Outcome

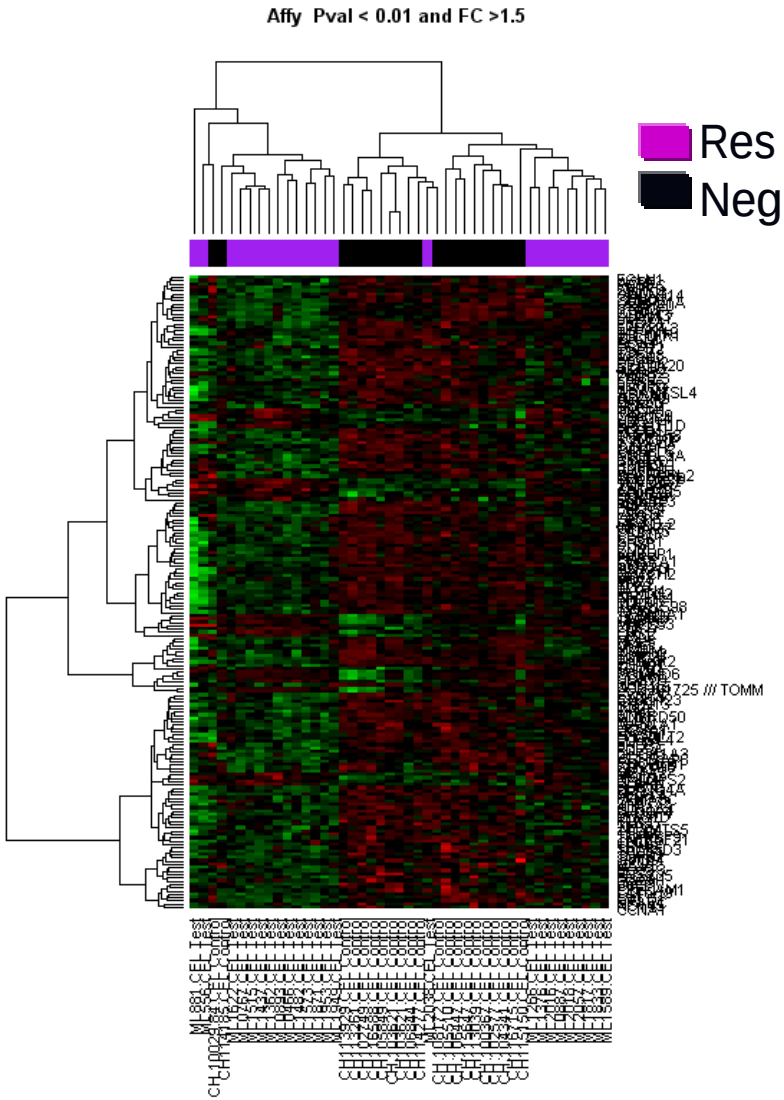
- 1474 genes: 819 down, 655 up (FC 1.3, p =0.0001)
- 1214 (79%) could be annotated by their Gene Ontology term (GO-TERM)
- 390 genes (25%) could be mapped to known signalling pathways (KEGG)

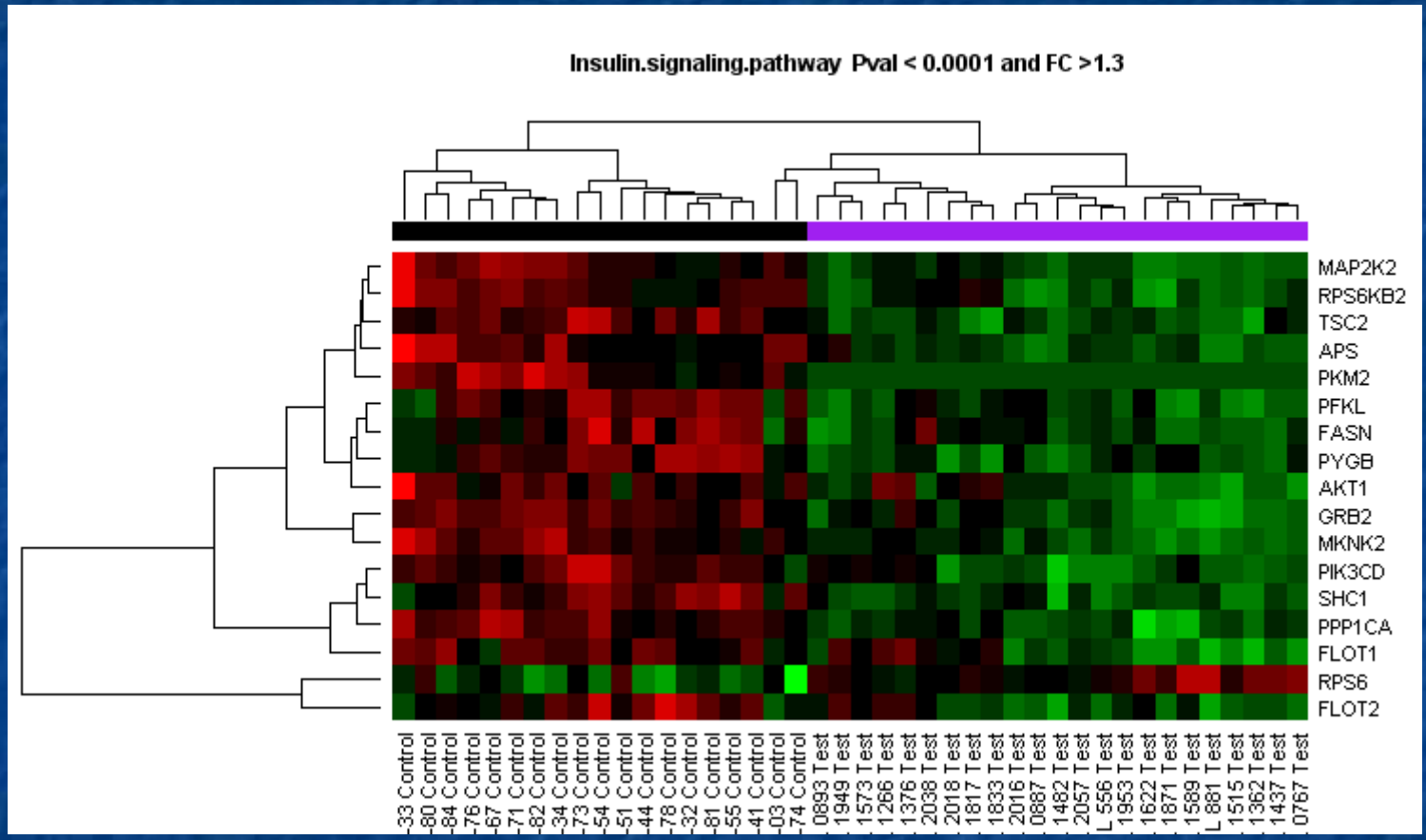
Gene expression profiling in HIV Resistants

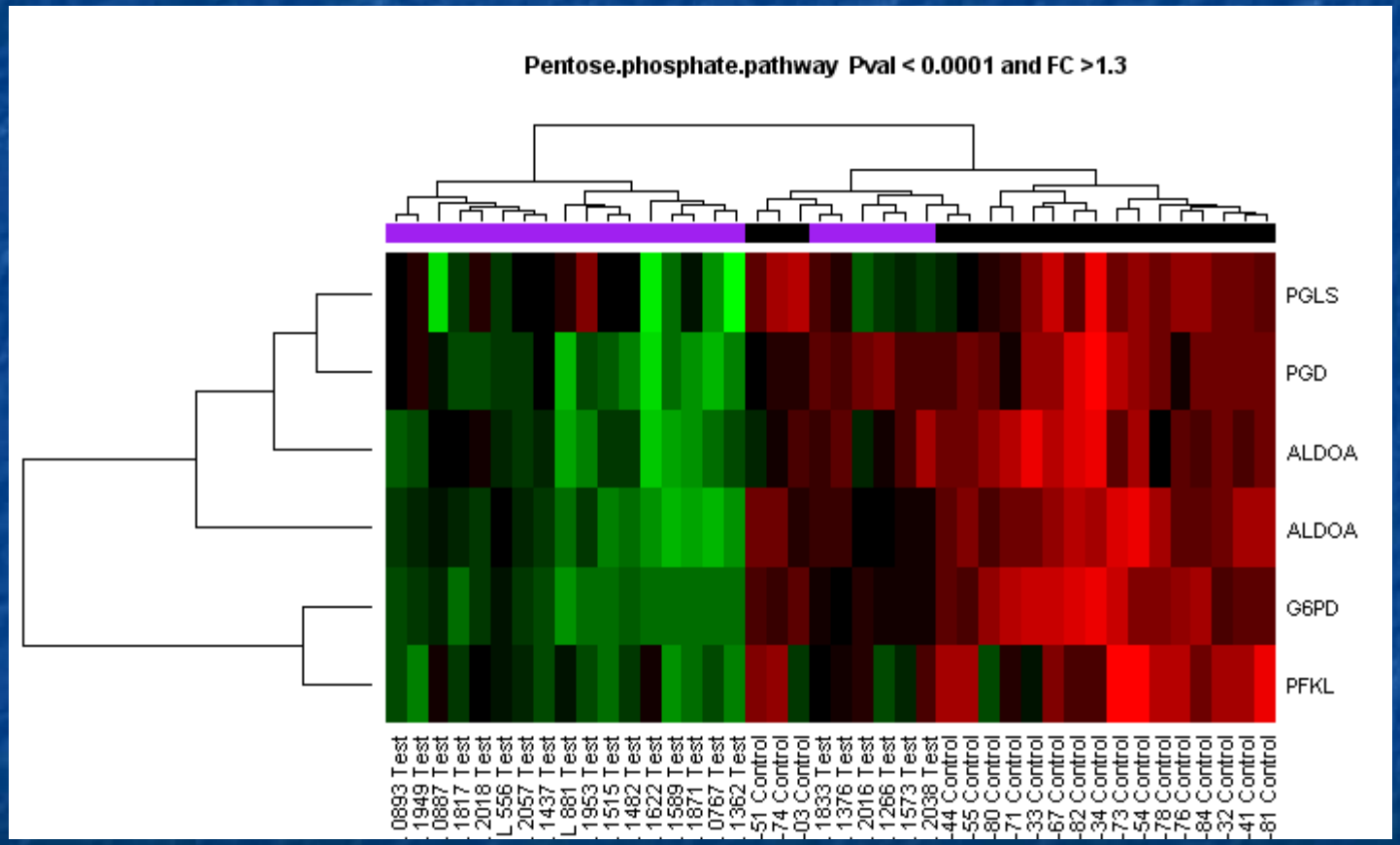
CD4 T cells



Whole Blood

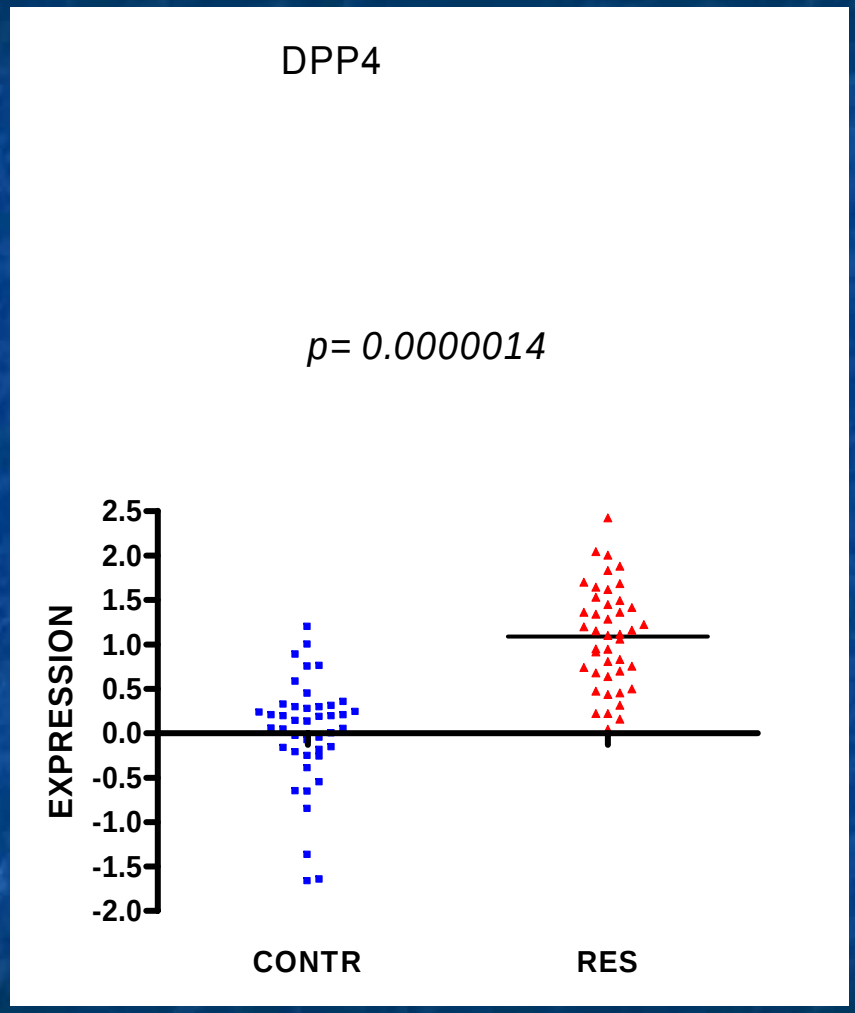


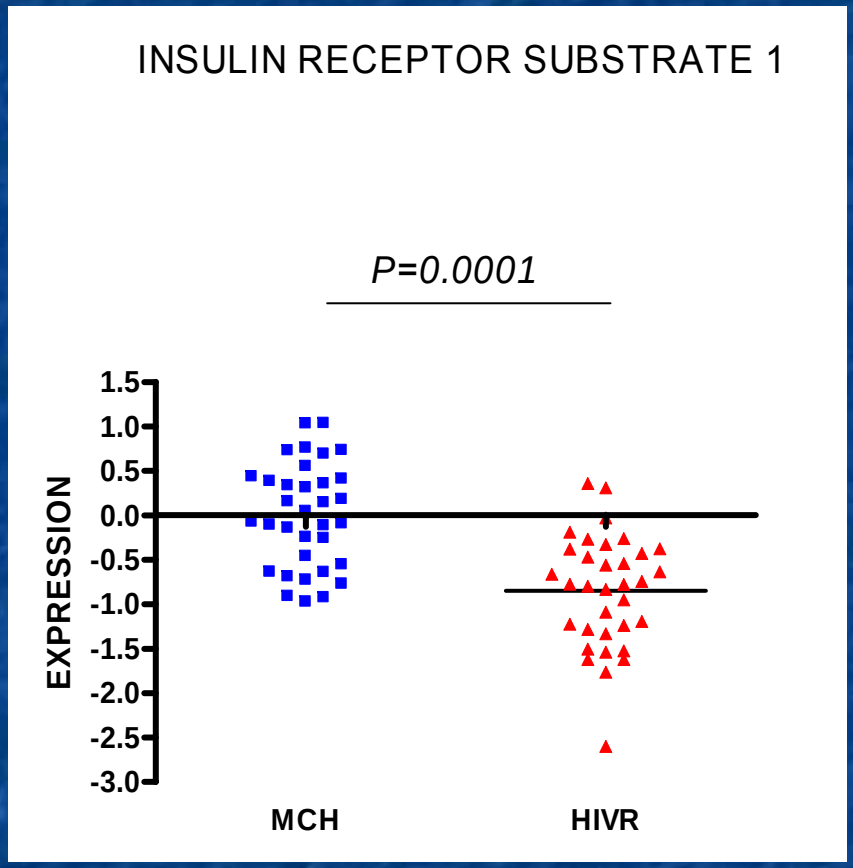

Insulin.signaling.pathway Pval < 0.0001 and FC >1.3
MAP2K2
RPS6KB2
TSC2
APS
PKM2
PFKL
FASN
PYGB
AKT1
GRB2
MKNK2
PIK3CD
SHC1
PPP1CA
FLOT1
RPS6
FLOT2
-33 Control
-80 Control
-84 Control
-76 Control
-67 Control
-71 Control
-82 Control
-34 Control
-73 Control
-54 Control
-51 Control
-44 Control
-78 Control
-32 Control
-81 Control
-55 Control
-41 Control
-03 Control
-74 Control
0893 Test
1949 Test
1573 Test
1266 Test
1376 Test
2038 Test
2018 Test
1817 Test
1833 Test
2016 Test
0887 Test
1482 Test
2057 Test
L 556 Test
1953 Test
1622 Test
1871 Test
1589 Test
L 881 Test
1515 Test
1362 Test
1437 Test
0767 Test


Pentose.phosphate.pathway Pval < 0.0001 and FC >1.3
PGLS
PGD
ALDOA
ALDOA
G6PD
PFKL
0893 Test
1949 Test
0887 Test
1817 Test
2018 Test
L 556 Test
2057 Test
1437 Test
L 881 Test
1953 Test
1515 Test
1482 Test
1622 Test
1589 Test
1871 Test
0767 Test
1362 Test
51 Control
74 Control
03 Control
1833 Test
1376 Test
2016 Test
1266 Test
1573 Test
2038 Test
44 Control
55 Control
80 Control
71 Control
33 Control
67 Control
82 Control
34 Control
73 Control
54 Control
78 Control
76 Control
84 Control
32 Control
41 Control
81 Control

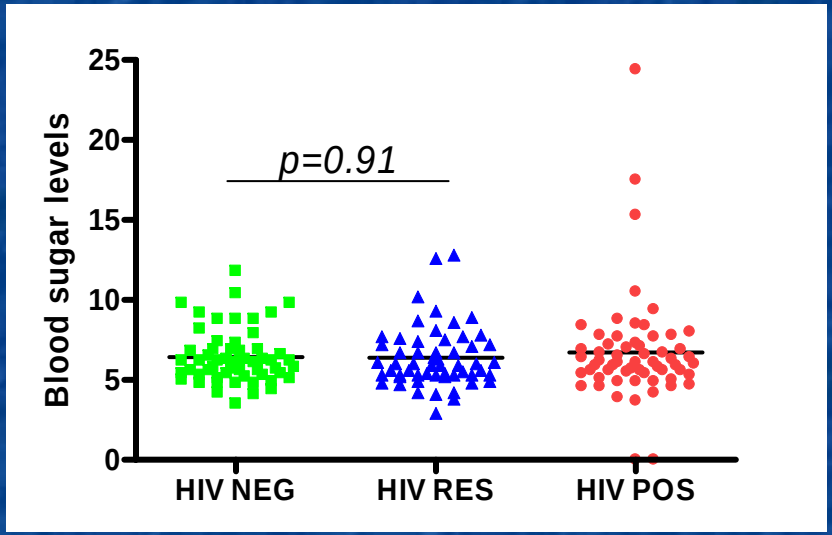
Key genes impacted insulin/glycolysis pathway suggest Type 2 diabetes

- DPP4
- IRS-1
- G6PDH
- GA3PDH
- PGM-1


DPP4
p= 0.0000014
EXPRESSION
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
CONTR
RES


INSULIN RECEPTOR SUBSTRATE 1
P=0.0001
EXPRESSION
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
MCH
HIVR

But..preliminary Random Blood glucose levels…



n=55 each

Observations

- A gene signature pattern on HIV resistance exists
- More than 50% of genes earlier identified by various workers found in our screening
- Evidence of association of HIV resistance with genetic traits of Type 2 diabetes

T cell Function in HIV Resistance: The Role of Immune Quiescence

Keith Fowke
Universities of Manitoba and Nairobi

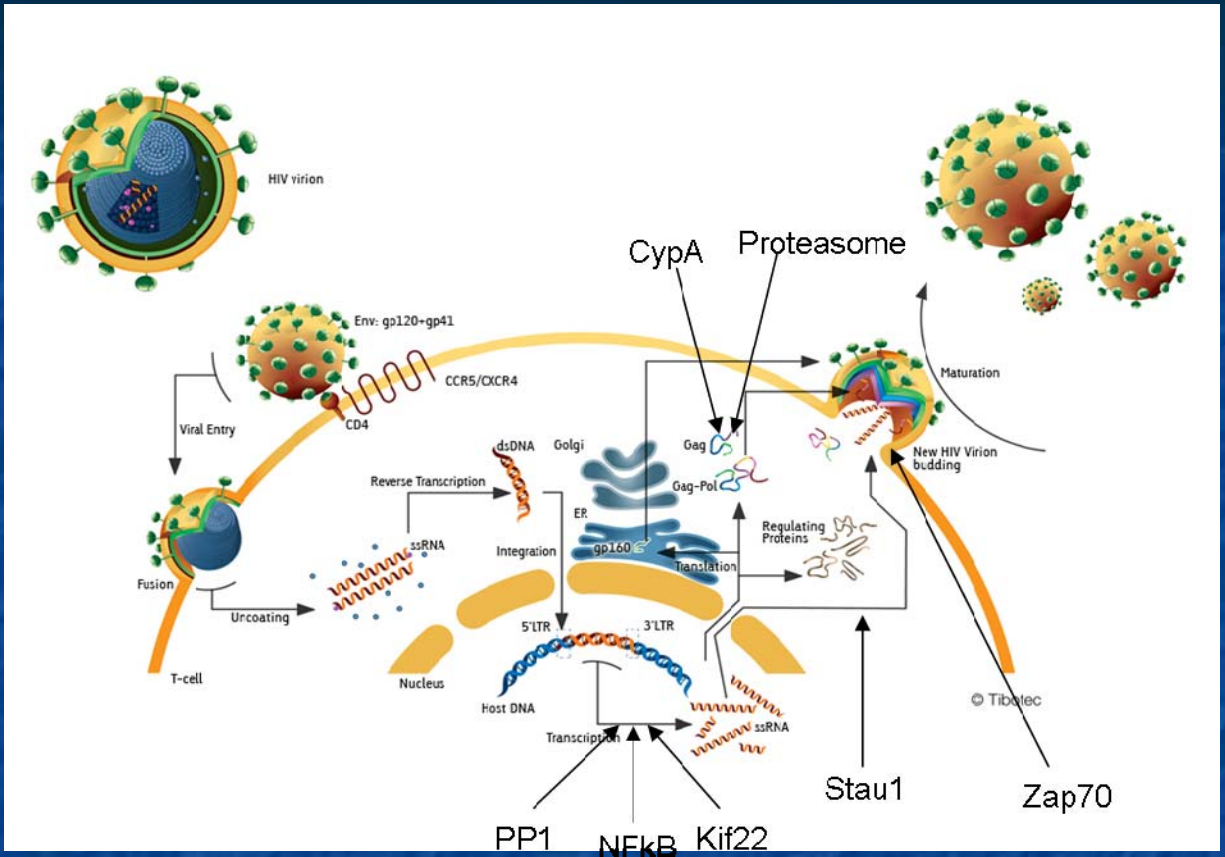
Assessment of T cell Function

- Gene expression analysis
  - Purified CD4+ T cells – Paul McLaren
    - 9 Res, 9 High-risk negatives
  - Whole Blood – Martim Songok
    - 23 Res, 19 Low-risk negatives
  - Used Affymetrix U133 Plus 2.0
- T cell functional assays
  - Cytokine production
  - Cellular activation markers
  - Regulatory T cells

HIV-Dependent Host Factors in CD4 cells

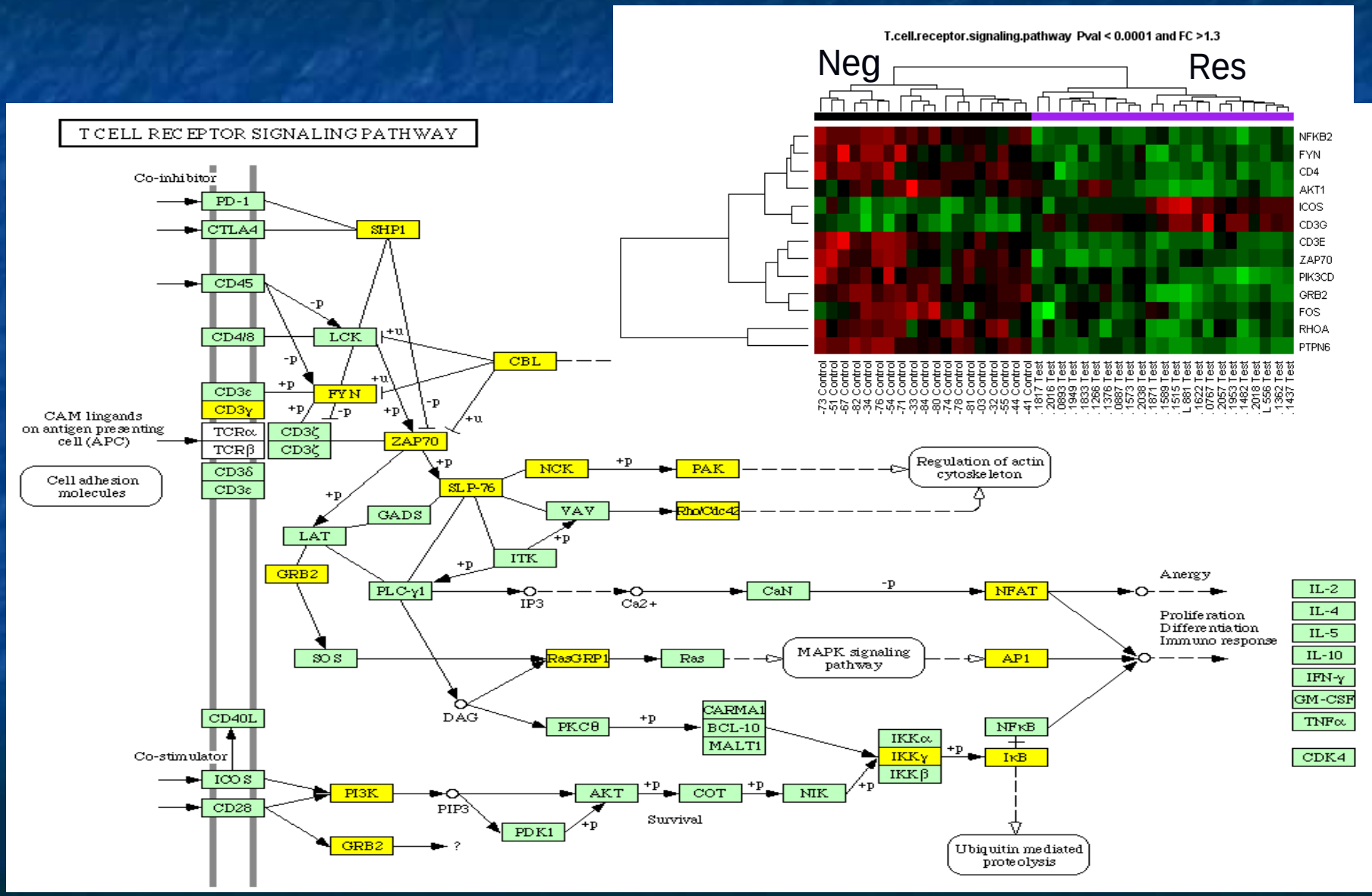
Gene Symbol	Fold	P Value	Description
PPP1CA	-2.19	0.00001	protein phosphatase 1
STAU	-1.67	0.00002	staufen, RNA binding protein
KIF22	-1.88	0.00005	kinesin family member 22
RELA	-1.40	0.0009	NFkB subunit (p65)
ITGA5	-1.79	0.004	integrin, alpha 5
CYPA	-1.43	0.009	peptidylprolyl isomerase A (cyclophilin A)
NFKB1	-1.71	0.01	NFkB subunit (p50)

Down Regulated Genes involved in HIV replication

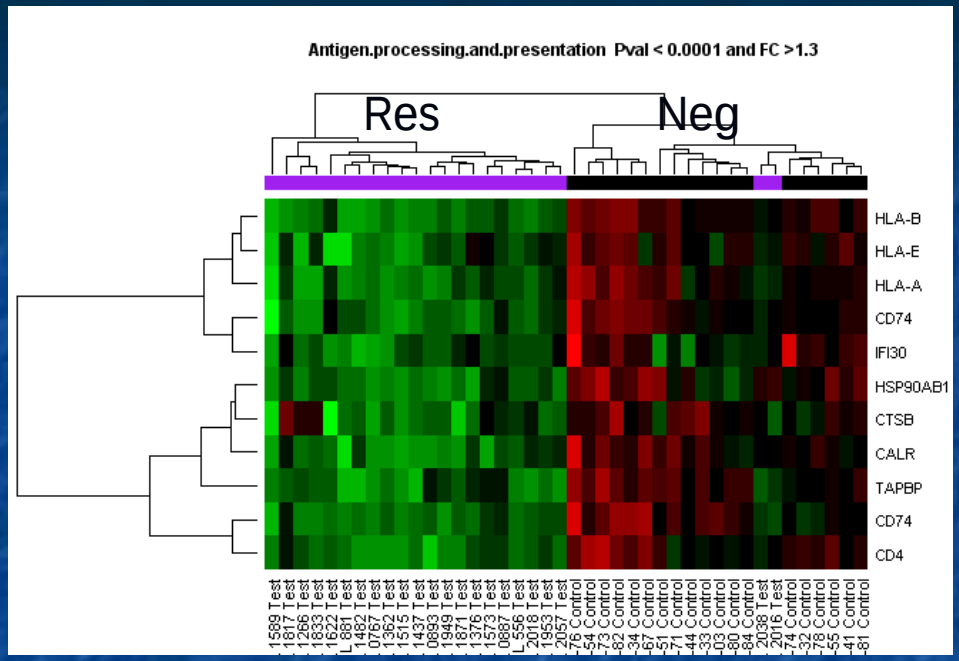


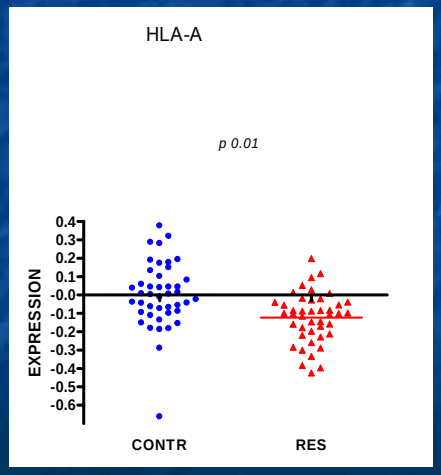
- Brass et al (Science 2008) used siRNA functional analyses, 260 HIV-dependent Host Factors
- In CD4 T cell array – 45 were shared, 42 lower expression in Res
- In Whole Blood array - 109 (42%) were present, most lower expression
- Cellular machinery does not favour HIV replication

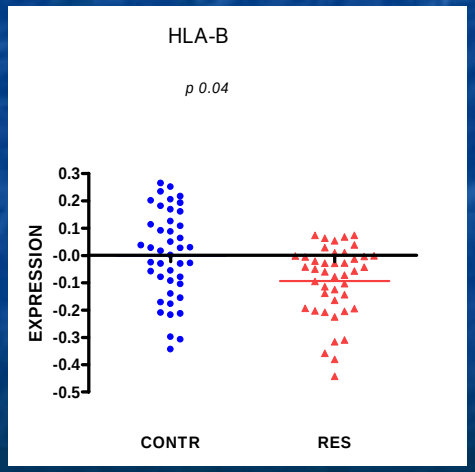
T cell receptor signalling in Whole Blood

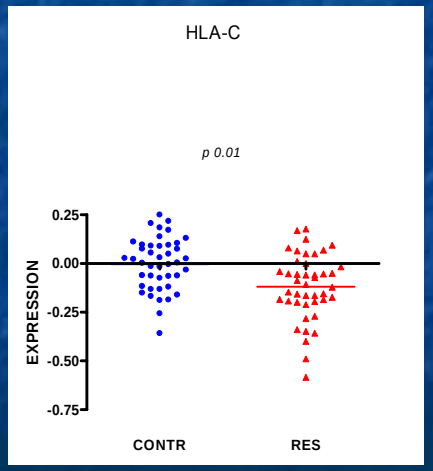


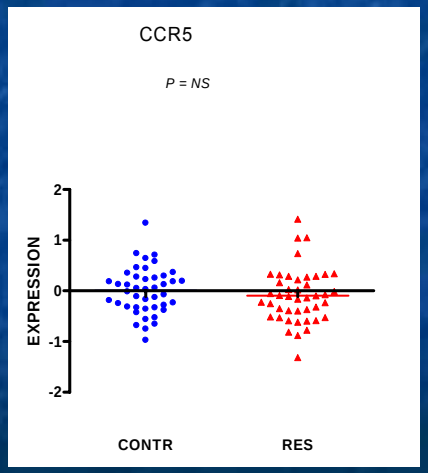
Antigen Presentation Pathway in Whole Blood of HIV-RES











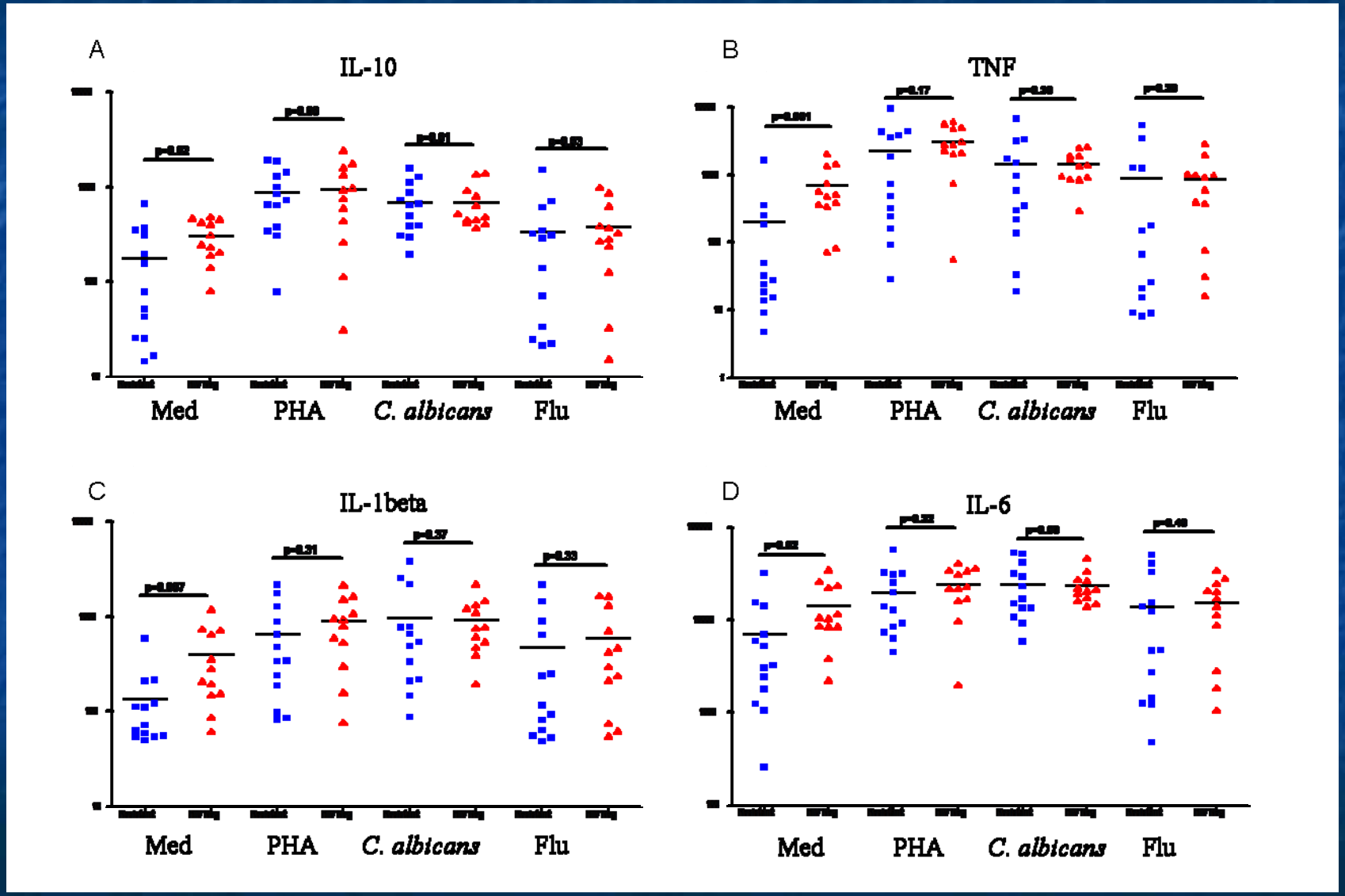
Similar finding for NK receptors (KIR), TLRs (7 and 8), IRFs (1 and 7)

Assessment of T cell Function

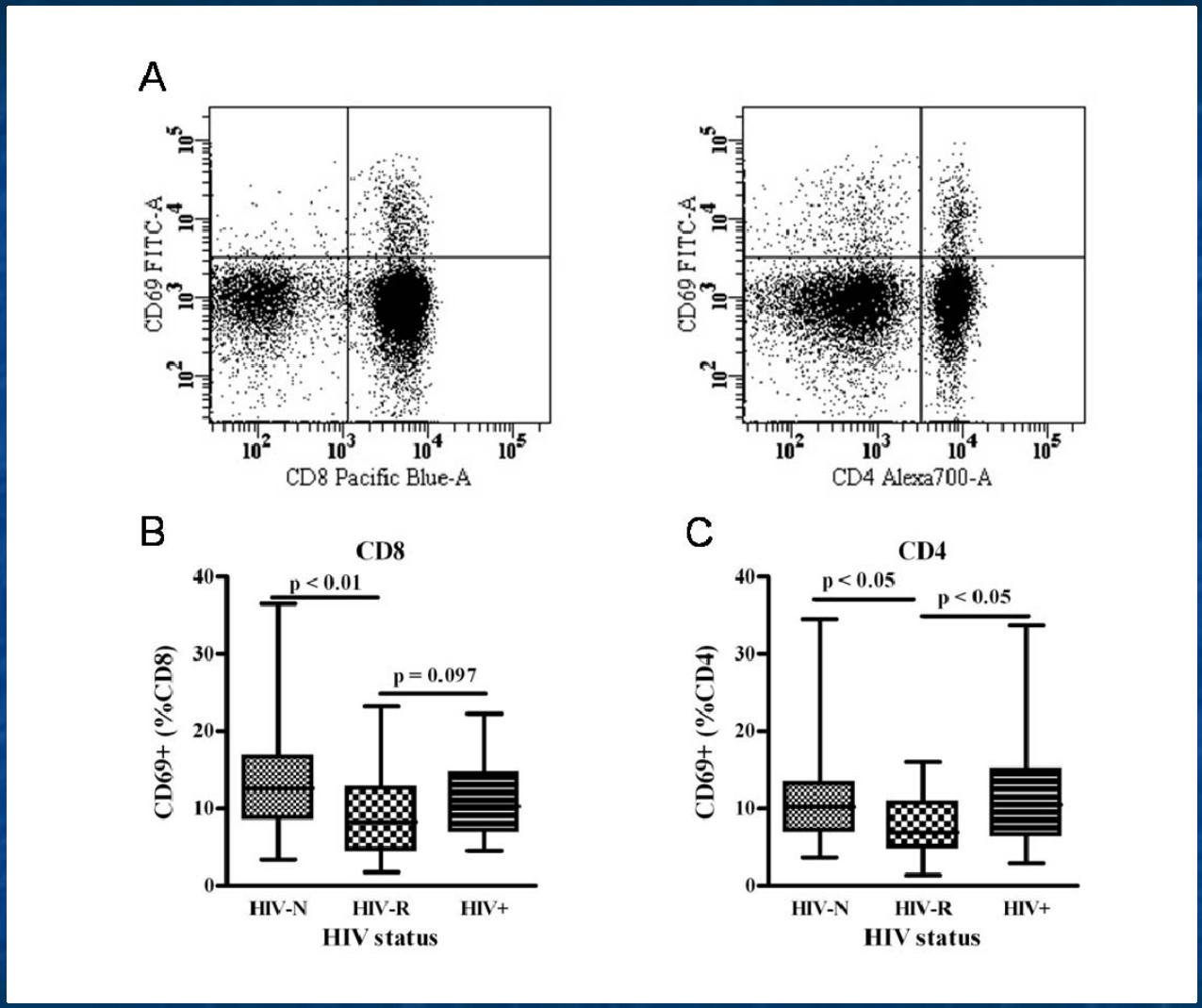
- Gene expression analysis
  - Purified CD4+ T cells
    - 9 Res, 9 High-risk negatives
  - Whole Blood
    - 23 Res, 19 Low-risk negatives
  - Used Affymetrix U133 Plus 2.0

- T cell functional assays
  - Cytokine production
  - Cellular activation markers
  - Regulatory T cells

HIV Resistants have Normal Recall Responses



Activation (CD69) is lower in HIV resistant subjects



- HIV does not replicate efficiently in quiescent cells
- Fewer HIV target cells

Immune Quiescence in HIV Resistants

- Lower overall gene expression, CD4+ T cells and whole blood
- Lower gene expression in HIV and T cell receptor pathways
- Lower resting PBMC cytokine production
- Lower level of cellular activation on T cells

- Normal Antigen recall function – not immune suppression

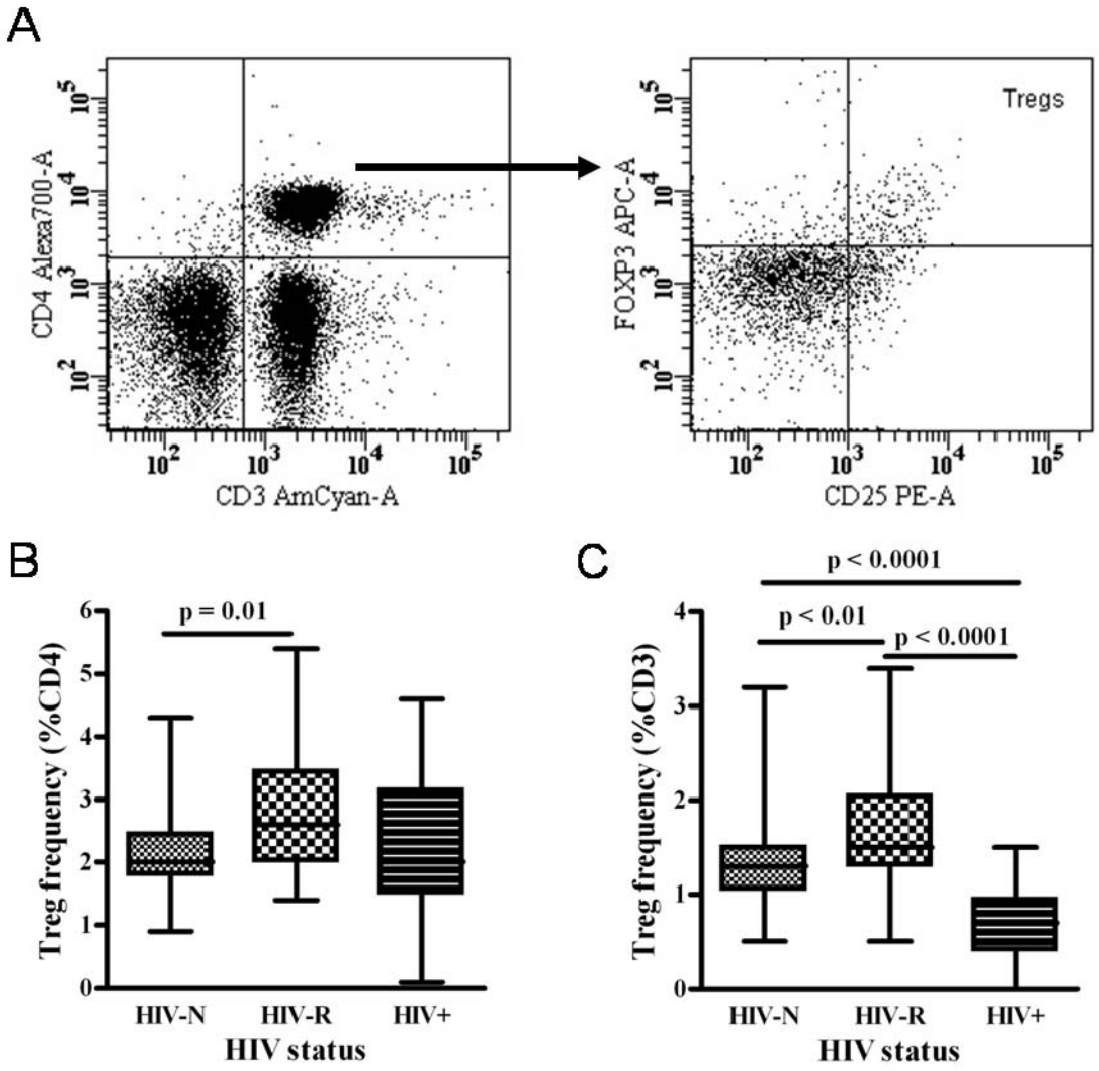
- OVERALL immune cells seem to be resting or quiescent

- We have termed this phenotype **Immune Quiescence**

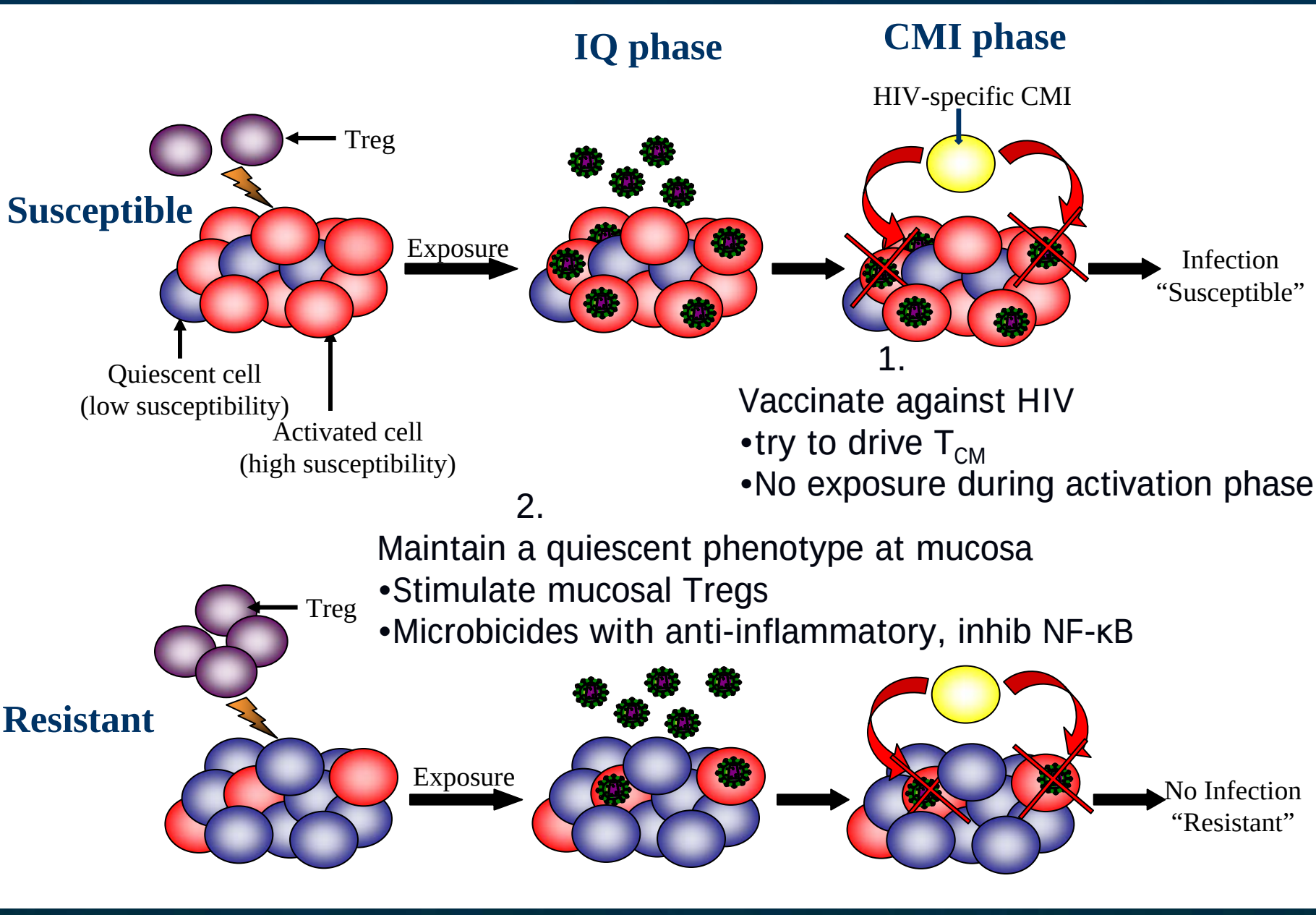
What is driving Immune Quiescence?

- Regulatory T cells
- T cells CD4+, CD25+, FoxP3+
- Function to suppress activation of immune cells

Tregs are higher in PBMC of HIV resistant subj



Two phase model of HIV resistance



Mucosal Proteomics of HIV-1 Resistance

Overview

- Goal of project:
  - Identify innate factors in cervicovaginal mucosa differentially expressed in HIV-resistant and HIV-susceptible women by proteomic techniques

Cervical Mucosal Protein Profiling

- "Unbiased" approaches to examine differential protein expression in cervical lavage fluid

- Proteomic platforms being used:
  - Traditional gel-based technique
    - 2D-DIGE
  - Mass spectrometry based techniques
    - SELDI-TOF
    - 2D LC FTICR-MS

2D-DIGE platform results

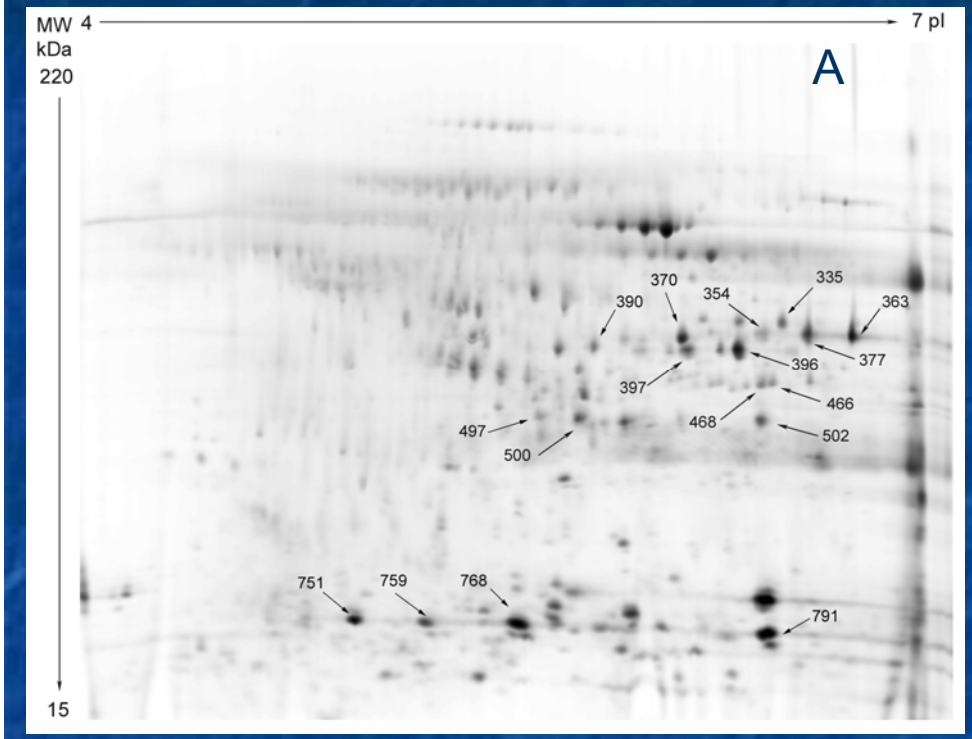
"*Identification of differentially expressed proteins in cervical mucosa of HIV-1-resistant Kenyan sex workers"*

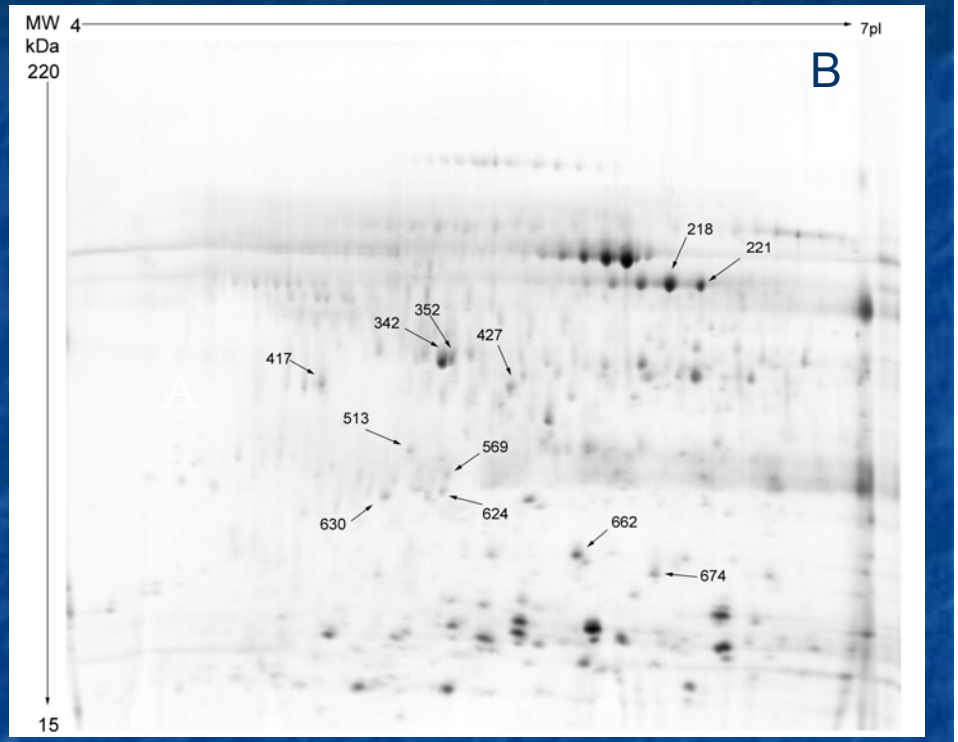
A. Burgener, J. Boutilier, C. Wachihi, J. Kimani, M. Carpenter, T. Ball, F. Plummer. J. Proteome Research. August 2008.

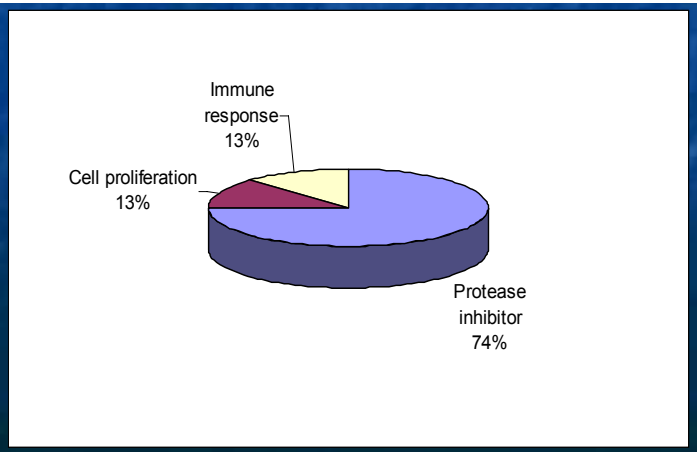
Differentially expressed protein spots in HIV-Resistant CVL fluid

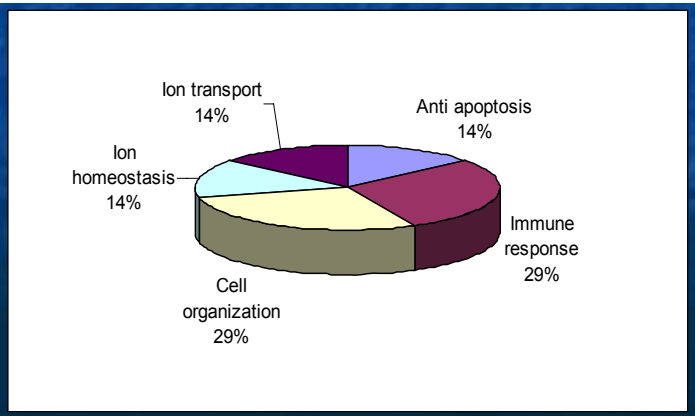
Overexpressed

Underexpressed









Majority of upregulated proteins are antiproteases

- **Cystatin A** – cysteine protease inhibitor
  - Known Anti-HIV-1 factor:
    - Blocks viral processing?
    - Blocks epithelial-lymphocyte interactions
    - Inhibition of Vif function? (essential for HIV-1 replication)
- **Serpins B1, B4, B13**
  - inhibitors of Cathepsin G, an inflammatory protease
    - Chemoattractant for monocytes/neutrophils, stimulates T-cells, enhances HIV-1 infectivity, degrades RANTES
- **Serpin B1 and A2ML1**
  - Inhibitors of human neutrophil elastase (HNE)
  - May antagonize HNE function and help maintain epithelial layer
- Overall these could contribute to an anti-inflammatory environment -> protective against HIV-1 infection?

Many downregulated proteins involved with HIV-1

- Complement component 3:
  - chemoattractant involved in immune response
- Rho dissociation inhibitor:
  - mediates HIV-1-infected cell migration though tight junctions
- Beta-actin:
  - binds HIV-1 reverse transcriptase and may be involved in HIV-1 secretion
- Apolipoprotein A:
  - surprisingly is known to bind gp41 of HIV-1 and act as a competitive inhibitor

Ultimate Goal: Inform HIV Vaccine/Therapeutics

- How will an understanding of HIV resistance contribute to a new vaccine/treatment?
  - New epitopes or targets for vaccine (Th, CTL)
  - Microbicide (HIV inhibitory molecules at the FGT or systemic)
  - Novel approaches to immunization – Treg vaccine?
  - Adjuvants/immunoregulation (alternate gene expression?)

Conclusions

- HIV vaccine research is at a scientific watershed
- The field is moving to where we have been for some time – we are at the forefront
- We are advancing paradigm shifting findings and hypotheses
- Also identifying potential microbicide candidates
- The chances to make a real difference have never been greater

Collaborators

- **Blake Ball, Joshua Kimani, Ma Luo**, (U Manitoba)
  - Genomics, Proteomics, Clinical
- **Keith Fowke** (U Manitoba)
  - T cell Immunology
- **Walter Jaoko, Benson Estamble** (U Nairobi)
  - Clinical; Challenge experiment
- **Rupert Kaul** (U Toronto)
  - Mucosal Immunology
- **Ken Rosenthal** (McMaster University)
  - Innate Immunology
- **Rafick Sekaly** (U Montreal)
  - Tetramers, TCR-Vb, 'omics'