

Expression of ATP-binding Cassette (ABC) Membrane Drug Efflux Transporters in Human Testicular Tissue

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Building Better Therapeutics

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**OHTN 2013
RESEARCH
CONFERENCE**

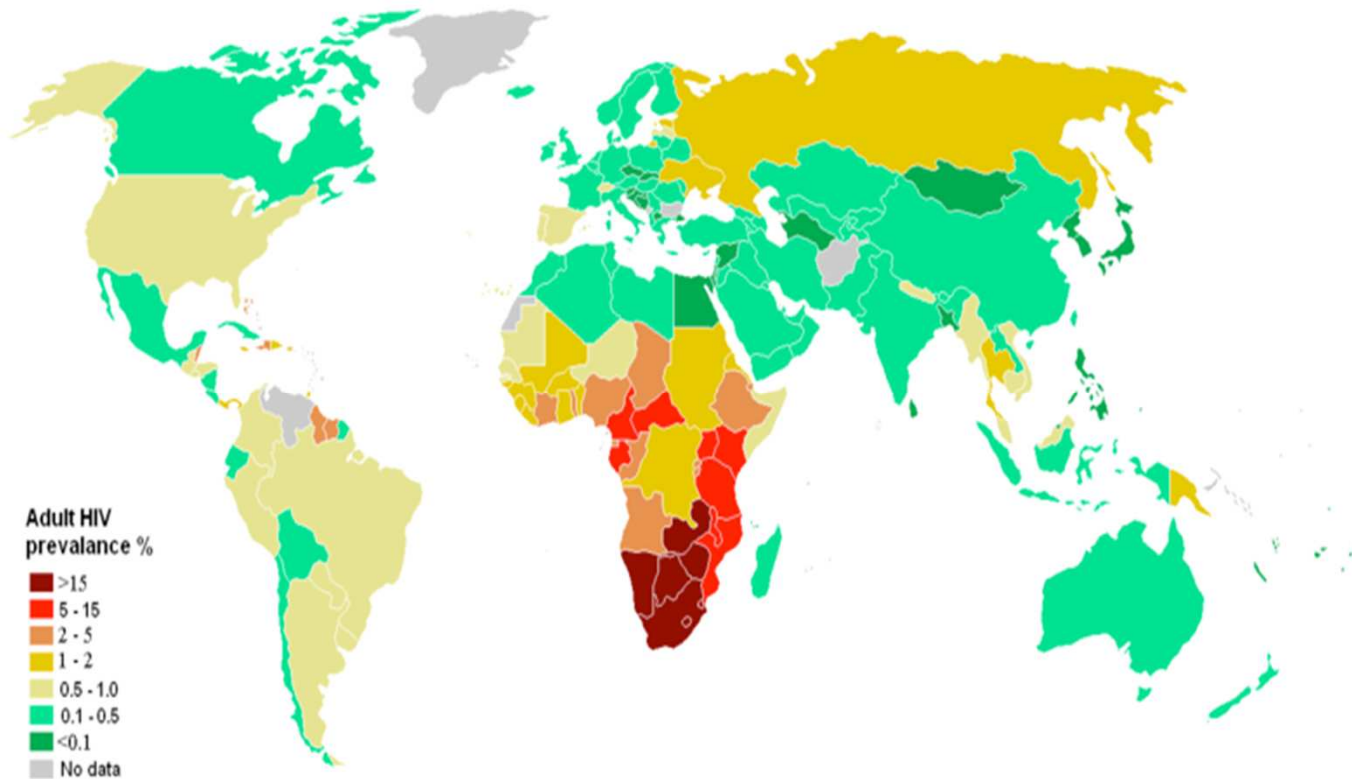
NOVEMBER 17-19, 2013

CHANGING THE COURSE OF THE
HIV PREVENTION, ENGAGEMENT AND
TREATMENT CASCADE

Outline

- Background
- Hypothesis
- Methods
- Results
- Summary
- Future Work

Background



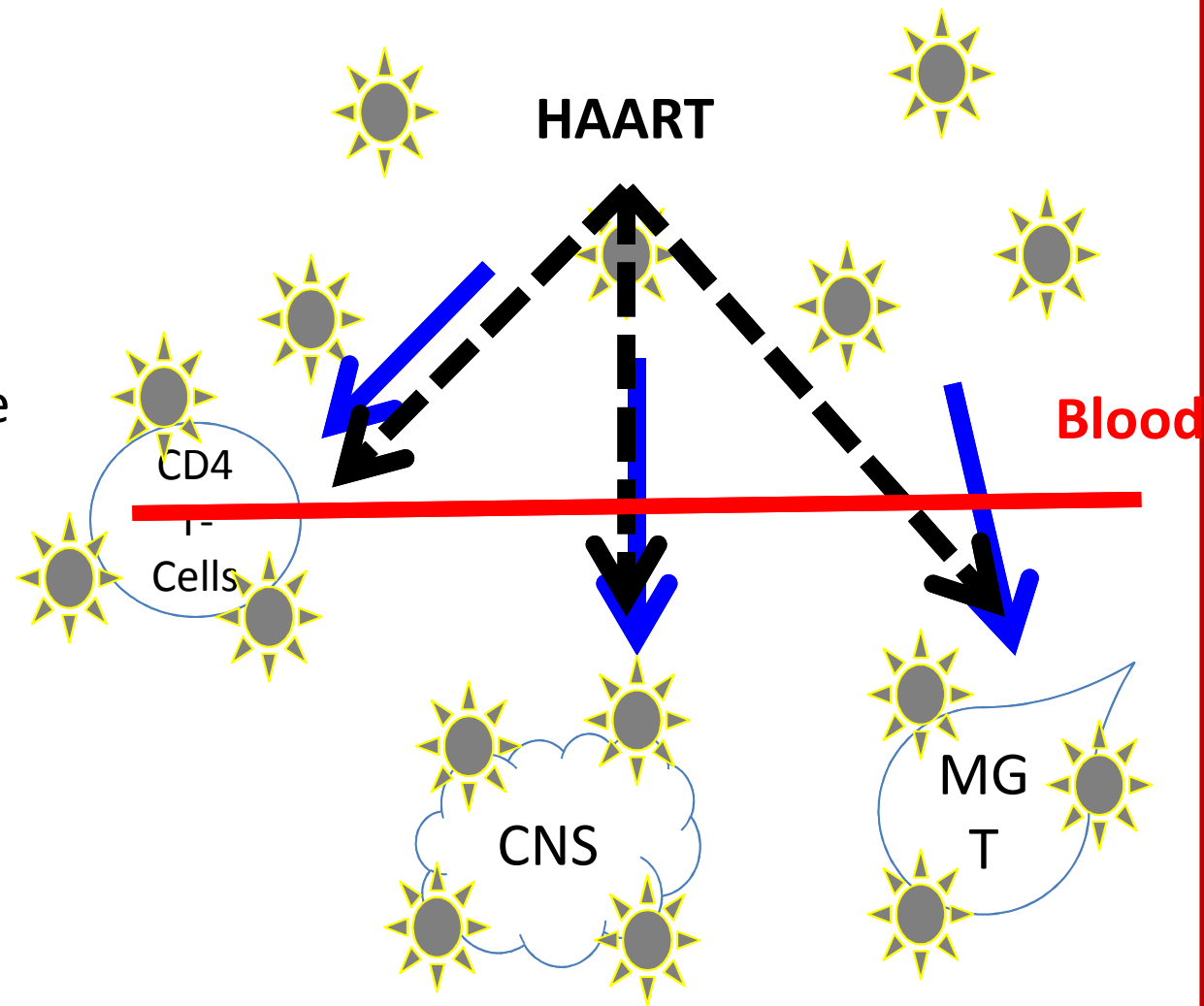
http://immunizebc.ca/sites/default/files/campaign/800px-hiv_epidem.png Accessed Aug 22, 2013

- More than 30 million deaths¹
- ~34 million people currently infected¹

1. UNAIDS. (2012). UNAIDS - Global Fact Sheet. World AIDS Day 2012 Global Fact Sheet. Retrieved August 09, 2013, from http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_FactSheet_Global_en.pdf

Background

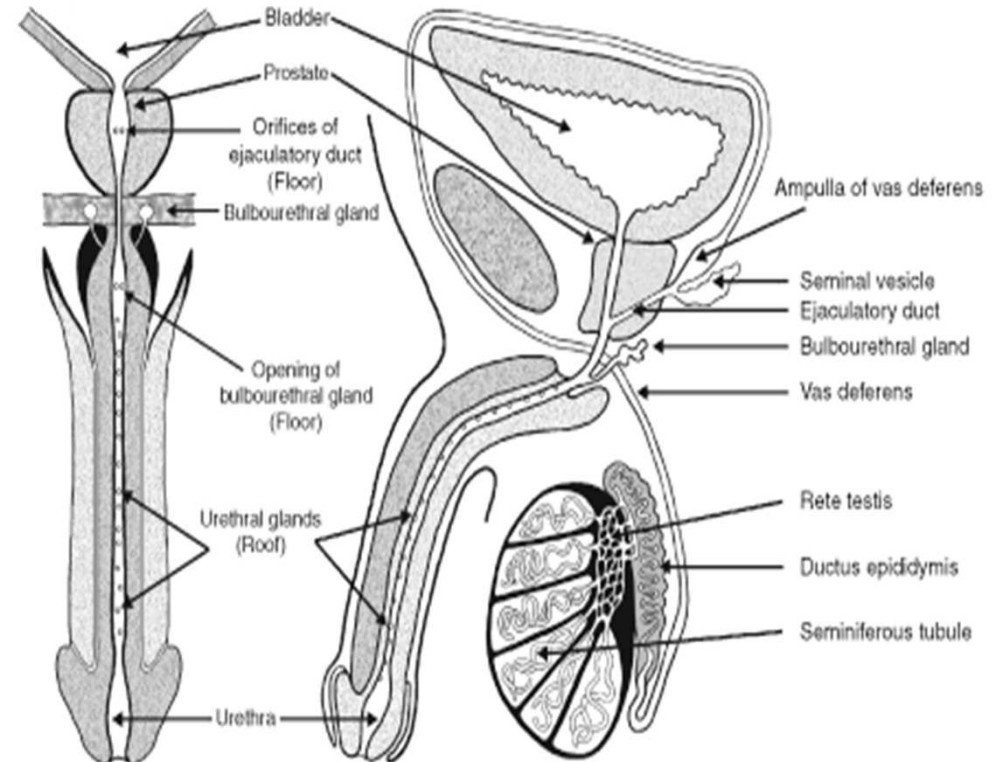
- Significant reduction of mortality thanks to HAART
- But still unable to eliminate latent infections in viral reservoirs
- Reservoirs defined as sites where viral replication persists with more stable kinetics than main pool of virus¹



1. Dahl, V., Josefsson, L., & Palmer, S. (2010). HIV reservoirs, latency, and reactivation: prospects for eradication. *Antiviral research*, 85(1), 286–94. doi:10.1016/j.antiviral.2009.09.016

Background

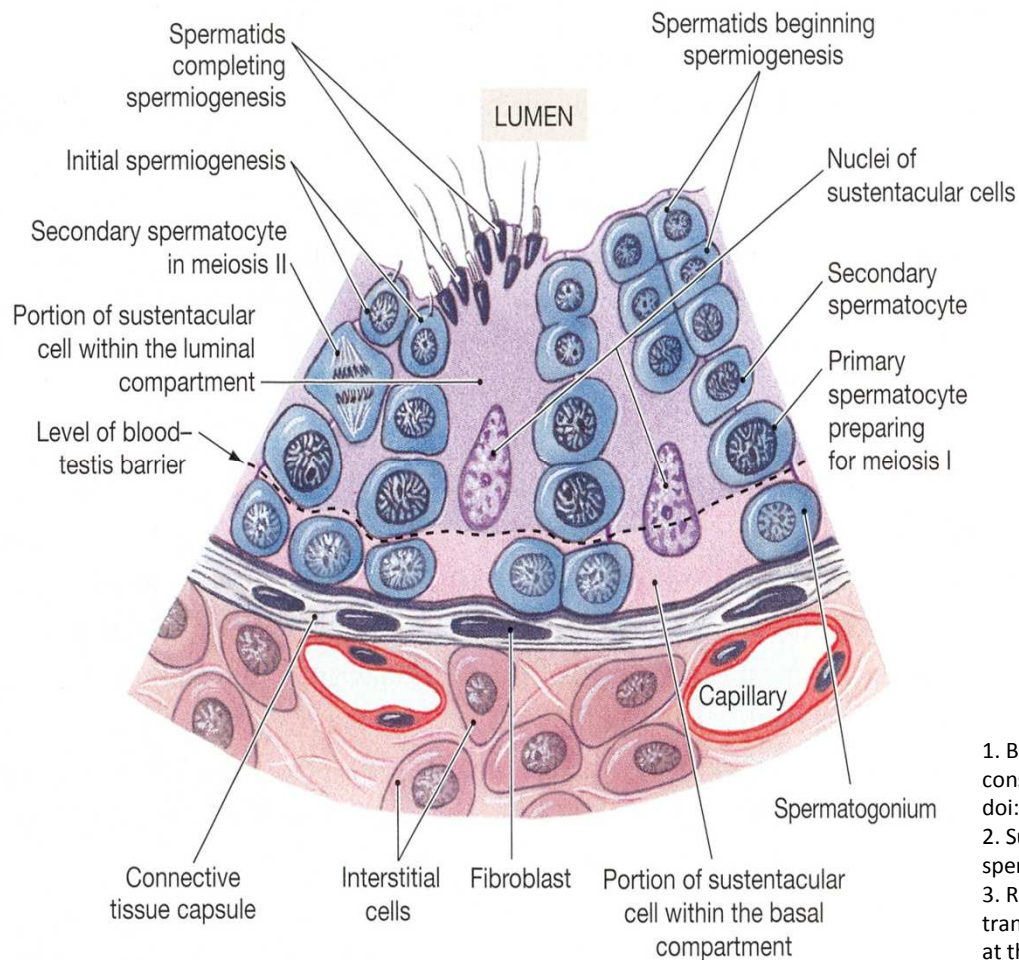
- Testis believed to be latent reservoir
- SIV primarily infects lymphocytes in macaque testis²
- HIV receptors present in models of human testis³
 - CXCR4, CCR5, CD4, DC-SIGN
- Variants in testis distinct from those in plasma⁴



<http://www.nature.com/clpt/journal/v83/n3/images/6100342f1.gif> Accessed on Oct 27, 2013

1. Le Tortorec, A., Le Grand, R., Denis, H., Satie, A.-P., Mannioui, K., Roques, P., ... Dejuqc-Rainsford, N. (2008). Infection of semen-producing organs by SIV during the acute and chronic stages of the disease. *PloS one*, 3(3), e1792. doi:10.1371/journal.pone.0001792
2. Roulet, V., Satie, A.-P., Ruffault, A., Tortorec, A. Le, Denis, H., Guist'hau, O., ... Dejuqc-Rainsford, N. (2006). Susceptibility of Human Testis to Human Immunodeficiency Virus-1 Infection in Situ and in Vitro. *The American Journal of Pathology*, 169(6), 2094–2103. doi:10.2353/ajpath.2006.060191
3. Paranjpe, S., Craigo, J., Patterson, B., Ding, M., Barroso, P., Harrison, L., ... Gupta, P. (2002). Subcompartmentalization of HIV-1 quasispecies between seminal cells and seminal plasma indicates their origin in distinct genital tissues. *AIDS research and human retroviruses*, 18(17), 1271–80. doi:10.1089/08892202320886316

Background



- Low ARV permeability in testis possibly due to blood-testis barrier
- BTB made of Sertoli cells¹
- Drug efflux transporters expressed at barrier regulate entry of xenobiotics^{2,3}
- Drug uptake transporters and metabolic enzymes also affect permeability

1. Bronson, R. (2011). Biology of the male reproductive tract: its cellular and morphological considerations. *American journal of reproductive immunology* (New York, N.Y. : 1989), 65(3), 212–9. doi:10.1111/j.1600-0897.2010.00944.x
2. Su, L., Mruk, D. D., & Cheng, C. Y. (2011). Drug transporters, the blood-testis barrier, and spermatogenesis. *The Journal of endocrinology*, 208(3), 207–23. doi:10.1677/JOE-10-0363
3. Robillard, K. R., Hoque, T., & Bendayan, R. (2012). Expression of ATP-binding cassette membrane transporters in rodent and human sertoli cells: relevance to the permeability of antiretroviral therapy at the blood-testis barrier. *The Journal of pharmacology and experimental therapeutics*, 340(1), 96–108. doi:10.1124/jpet.111.186916

<http://os1.amc.nl/celbiologie/20102011/auc/mannelijk/graphics/c0000001.jpg> Accessed on Oct 25, 2013

Background

- 49 members in ABC superfamily discovered to date
- ATP-dependent transmembrane proteins
- Actively effluxes wide variety of drugs, drug conjugates and drug metabolites against concentration gradient

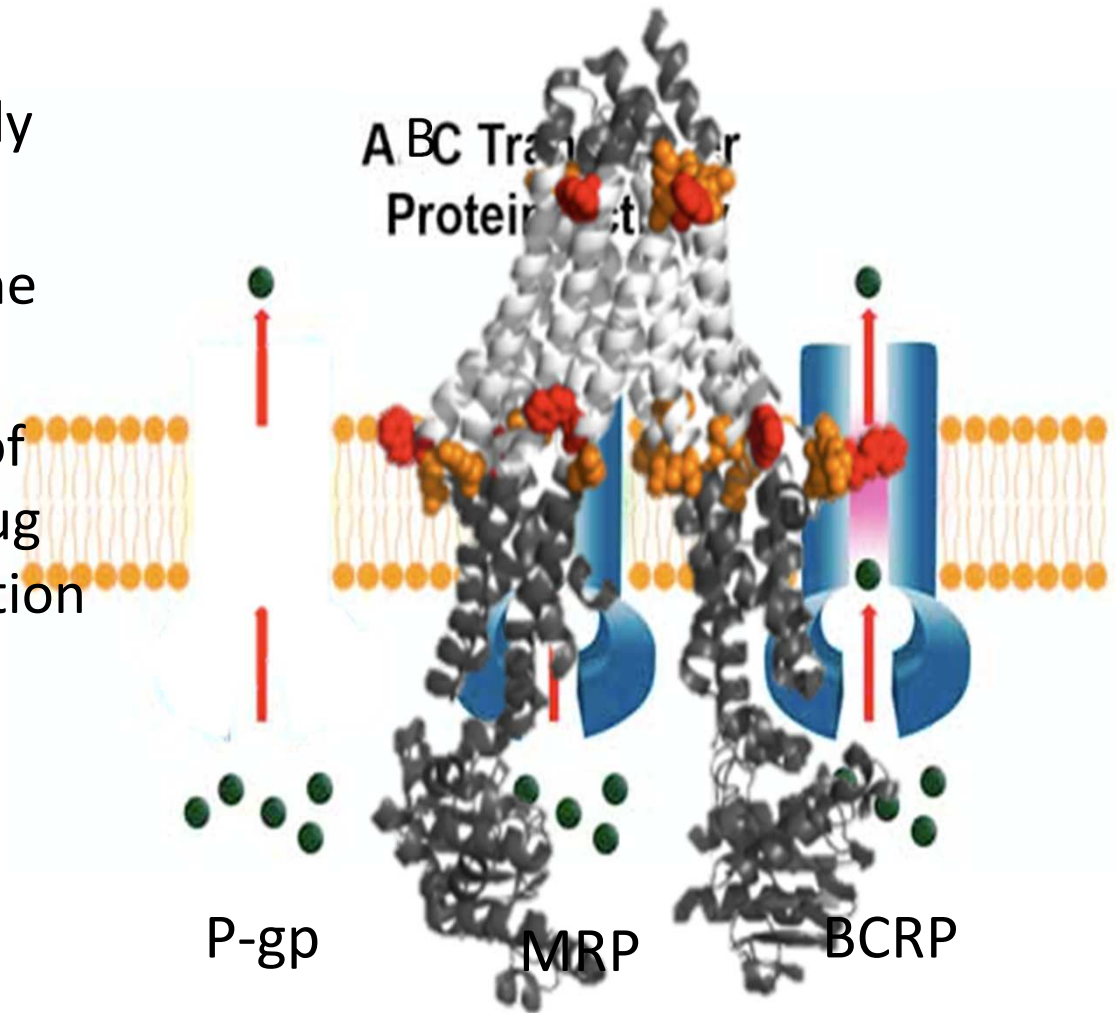
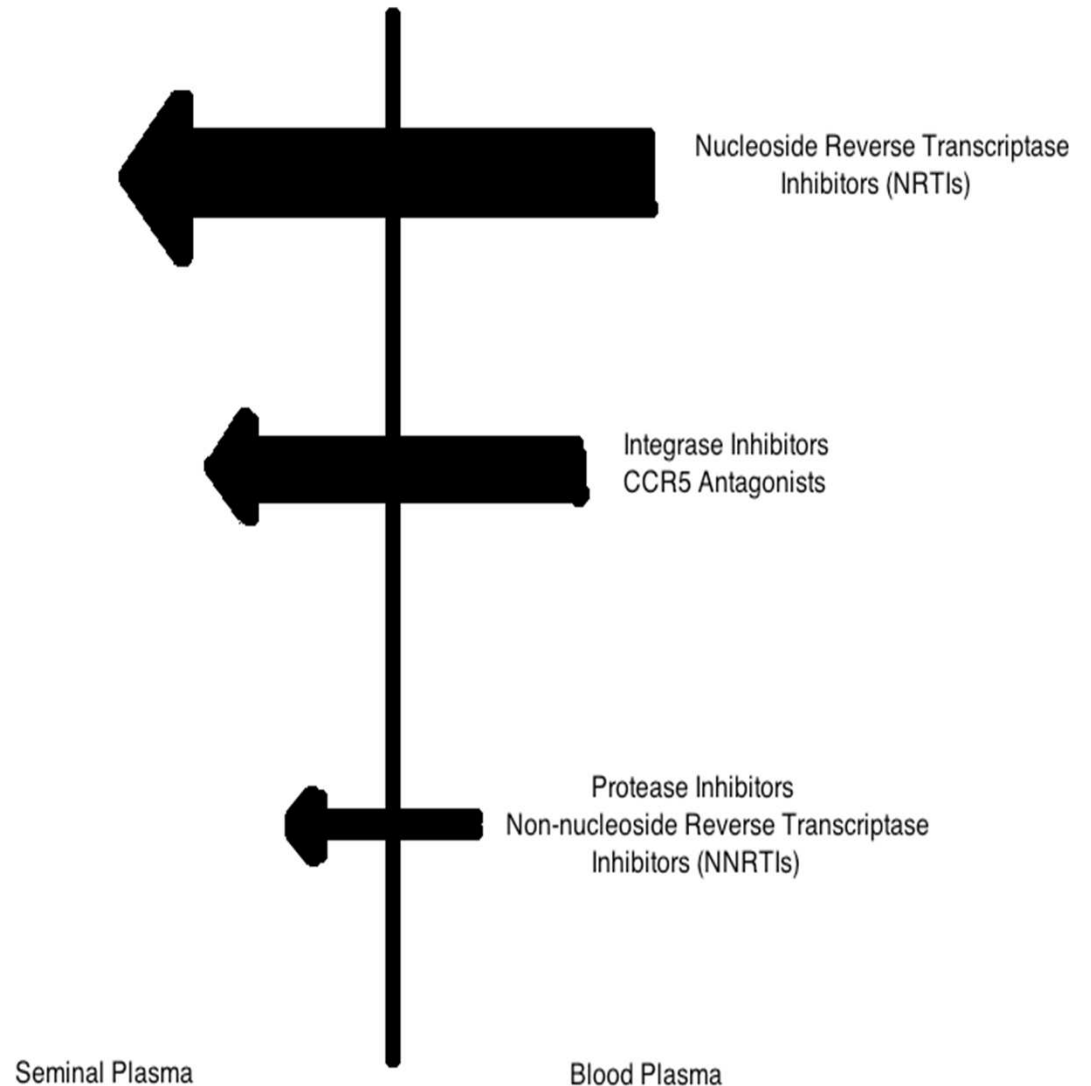


Figure 1. Mouse P-gp crystal structure (Li, Jaimes and Aller, Protein Science. 2007)

Background

- Drug permeability in MGT usually reported as seminal plasma concentration¹
- But semen is a complex mixture of secretions from several glands
- Need to evaluate contribution of each MGT component to semen



Schematic of drug penetration between seminal plasma and blood plasma

1. Else, L. J., Taylor, S., Back, D. J., & Khoo, S. H. (2011). Pharmacokinetics of antiretroviral drugs in anatomical sanctuary sites: the male and female genital tract. *Antiviral therapy*, 16(8), 1149–67. doi:10.3851/IMP1919

Hypothesis

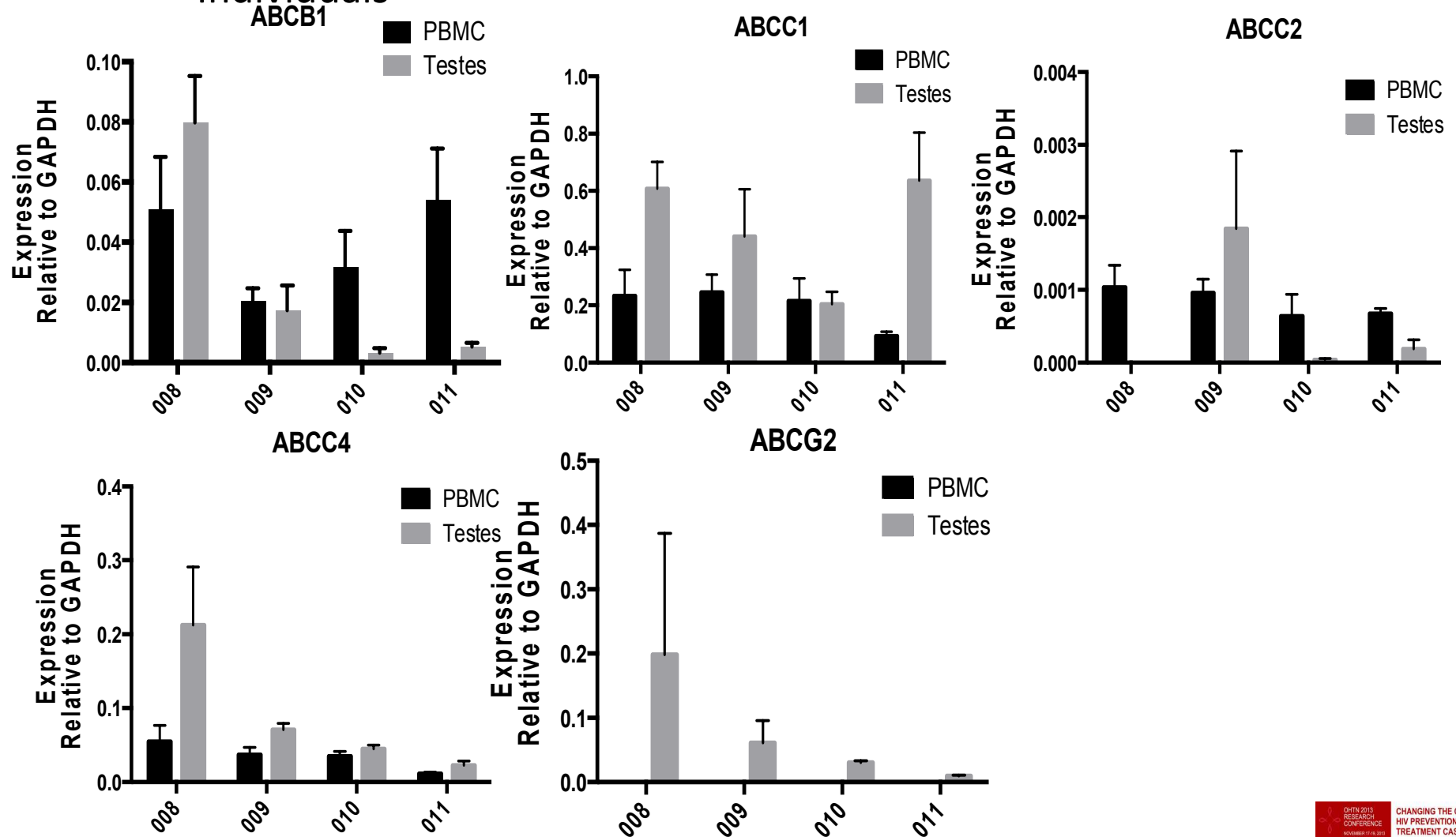
- Low ARV drug permeability in human testis is associated with the expression of drug transporters and drug metabolic enzymes

Methods

- Collaboration with Dr. Jean-Pierre Routy at the McGill University Health Centre and Dr. Pierre Brassard and Dr. Maud Belanger at the Metropolitan Centre of Plastic Surgery in Montreal
 - Testicular tissue and blood samples currently being collected from uninfected and HIV-infected patients on HAART
- Assess mRNA and protein expression of major drug transporters and drug metabolic enzymes using qPCR and western blot
- Quantify drug concentration in testis and blood
 - Data from collaborator Nancy Sheehan at McGill
- Determine localization of drug transporters using immunofluorescence imaging

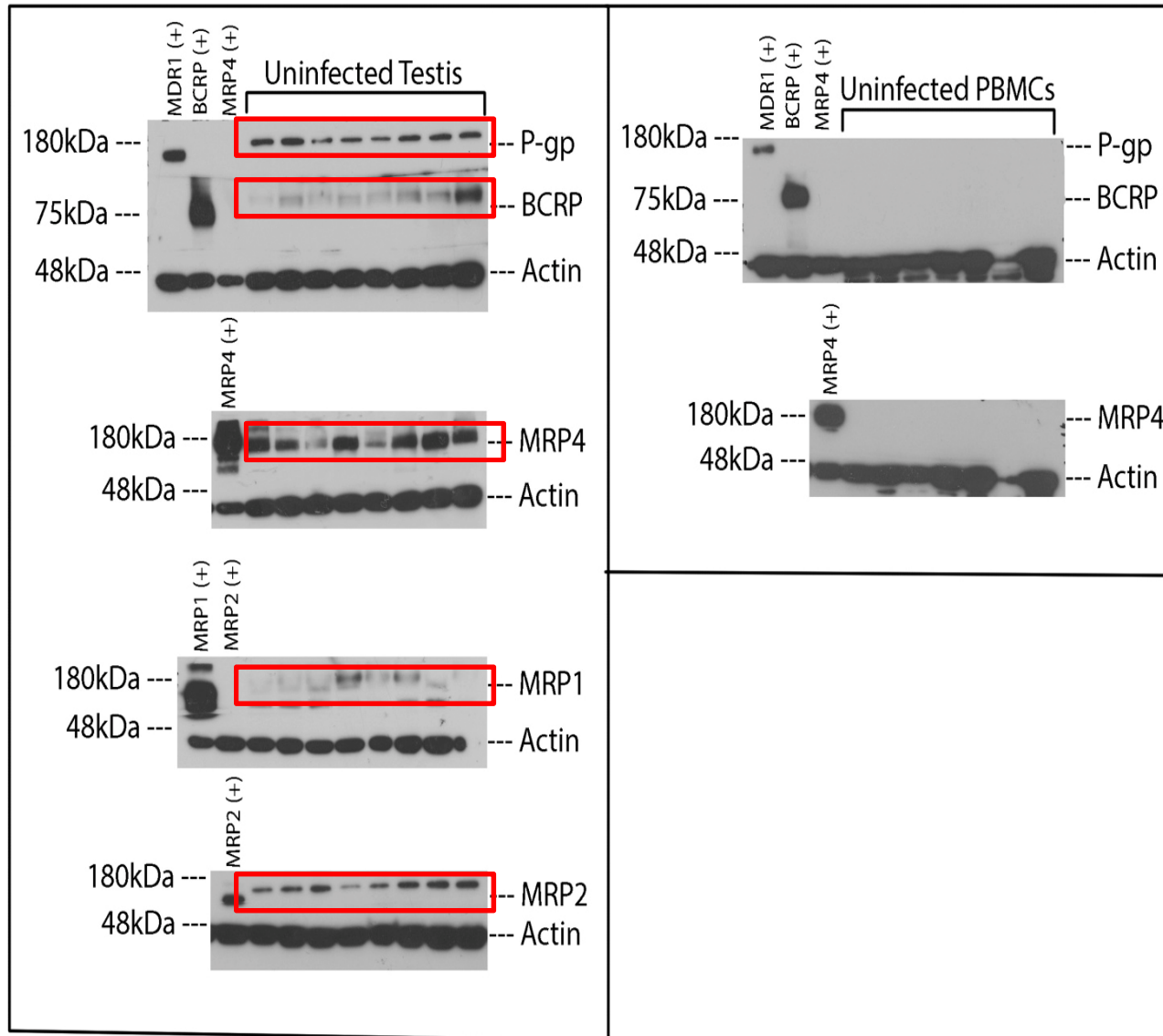
Results -

qPCR qPCR Data for Drug Efflux Transporters from Uninfected Individuals



Results – Western Blot

Western Blots of ABC Efflux Transporters



Summary

- mRNA expression of drug transporters varies in both testis and PBMCs between uninfected individuals
 - ABCC4 and ABCG2 show higher expression in testis
 - Previous studies on mRNA expression profiles suggest both interindividual variation and variation between methods of quantification
- Protein expression of drug transporters detected in testicular tissue but absent in PBMCs
- Discrepancy between qPCR and western blot data in PBMCs could be result of post-transcriptional modifications
- Overall our initial data suggest a potential for drug transporter proteins to interact with antiretroviral drugs in human testis

Future Work

- Continue to collect testicular tissues from uninfected individuals and HIV-infected patients on HAART
 - Examine drug transporter and drug metabolic enzyme localization using immunofluorescence
- Try to find and collect frozen testicular tissue, cell lysate or cDNA from HIV-infected HAART-naïve patients
- Gather ARV concentration data

Acknowledgement

- Dr. Md. Tozammel Hoque, Olena Kis and all the members of Dr. Reina Bendayan's lab
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- Dr. Pierre Brassard and Dr. Maud Belanger – Metropolitan Centre of Plastic Surgery
- Nancy Sheehan – Montreal Chest Institute



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(Dr. JP Routy, PI)



Ontario

MINISTRY OF TRAINING, COLLEGES AND UNIVERSITIES

Ontario Graduate Scholarship
(B. H.)



Ontario HIV
Treatment Network

OHTN Career Scientist Award
(Dr. R. Bendayan)