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and Epidemiology



PATHOGEN GENOMICS

APPLICATION TO
PUBLIC HEALTH, AND
CLINICAL MEDICINE

Dr Ng Oon Tek

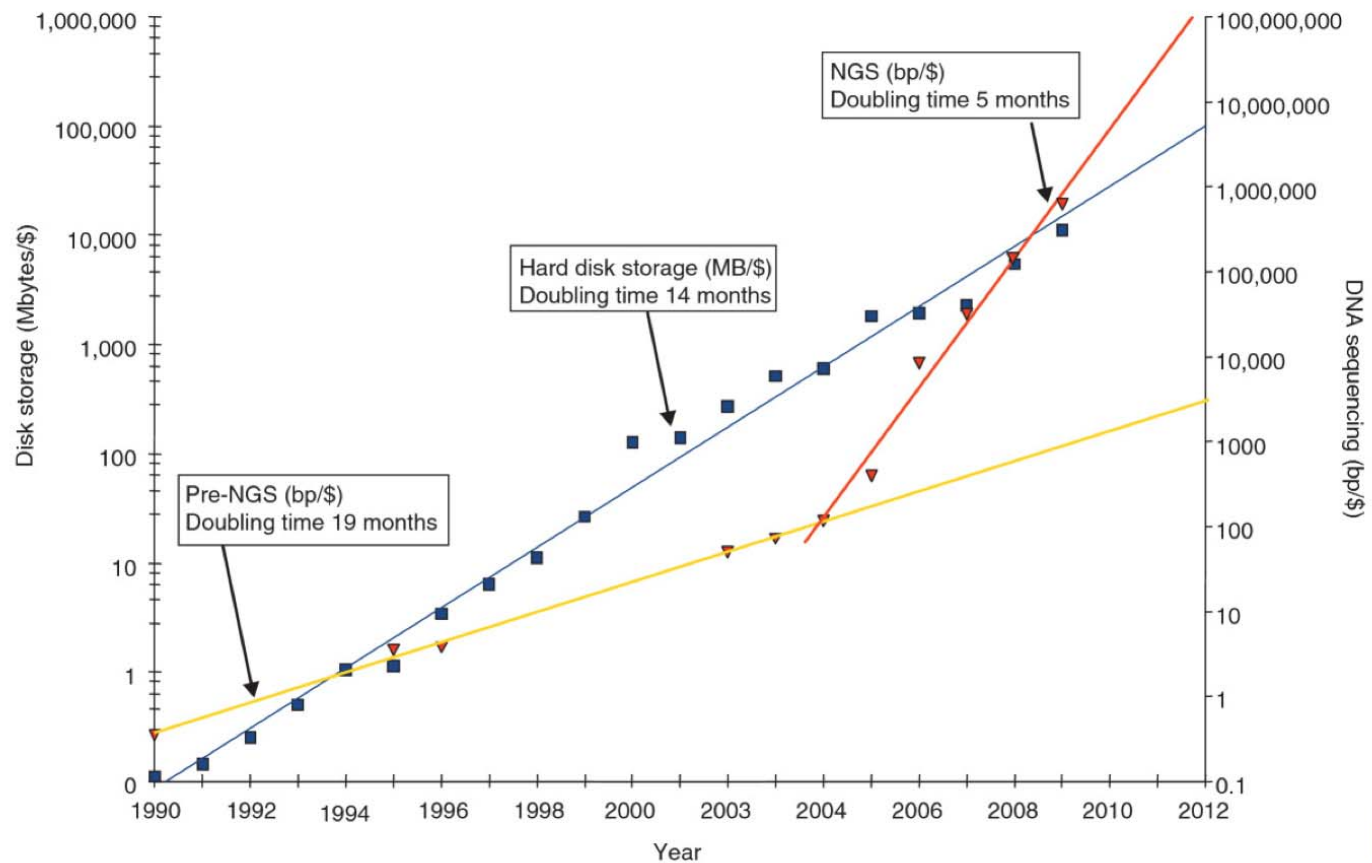
GENOMICS 101 – WHAT?

ATG-TCA-AAT-AAA-AGT-AAT-GAT-AAT-GGC-AGA-GCA-TAT-GAG-TTT-GCA-TTT-ATA-AAT-GAA-
TTA-GGA-CGC-ATT-GCA-ACT-CAA-AAT-CAG-AAT-ATA-AAT-ATC-GAA-AAG-AAT-TCT-AGC-TAT-
TAC-GTA-GTT-GAG-AAA-TCT-TGG-AGT-ACA-TTA-TCG-GAT-CTT-GAA-AAA-GAA-AAA-TAT-ACA-
AAA-AGT-GCA-ATT-GCT-GGT-ATC-AAT-CTT-ATA-ACA-AGC-TTA-GAG-CCA-ATA-ATA-GAA-GAT-
GGT-AAT-GGT-GTA-TTA-AAC-TTA-AAA-ATA-CAA-GCT-GAT-AAT-AAA-GGT-GAA-TTA-GGC-GAT-
ATT-AGA-GAT-ATT-TTA-ATT-CAA-AGA-GAA-AAT-ATT-AAT-TGG-GAA-ATT-GGT-TTA-AGT-TTA-
AAA-CAT-AAT-CAT-TTT-GCT-GTG-AAA-CAT-AGT-CGT-TTA-TCA-CAT-AAA-ATT-GAT-TTT-TCA-
GAA-AAA-TGG-TTC-CAA-TTA-CCT-TCT-TCT-CAA-AAT-TAT-TGG-GAT-AAT-ATA-CTC-CCT-ATT-
TTT-GAG-AAA-TTA-GAA-ATT-TAT-AAA-AAA-GAT-AAA-ATA-AAA-TGG-AGA-GAG-TTA-TCT-AAT-
AAA-GAA-GAT-TGC-ATT-TAT-TAT-CCC-ATA-CTT-AAA-TCA-TTT-ATA-GCA-GAA-ATT-AAA-GAA-
AAG-TAT-GAT-AAA-TAT-AAT-TCT-ATT-GTT-CCA-CAG-AGA-ATG-GTT-GAA-TAT-TTA-CTT-GGA-
TAT-TTT-GAT-TTC-TAT-AAA-ATC-ATA-AGT-CAA-GAT-AAT-AAG-AAA-CTA-ACA-TCT-ATT-CAA-
TCA-TTT-AAT-TTA-CGT-GGA-ACA-CTA-AAT-AAA-CCC-TCT-AAA-AAA-CGA-AAG-GCA-GAC-ATT-
TTT-ATA-CCT-GTA-GCT-AAT-TTA-CCA-ACT-AGA-ATC-ATT-GAT-ATA-GAT-TTT-AAG-CCA-AAT-
AGT-AAA-AAC-ACG-GTT-GAA-TTA-TAT-TTA-GAT-AAA-GGA-TGG-CAA-TTT-AGT-TTT-AGA-ATA-
CAT-AAT-GCT-TCT-ACT-ATT-ATT-GAA-CCG-AGC-TTG-AAA-TTT-GAT-ATA-AAA-CTT-ATT-GGT-
GTT-CCT-GCC-ACA-ATA-ATT-TGT-TTA-GAG-ACC-CCT-TGG-GAA-GAA-TGA



http://commons.wikimedia.org/wiki/File:HaeIII_DNA_code.gif
<http://computer.howstuffworks.com/cache.htm>

NextGen Sequencing a Game-Changer



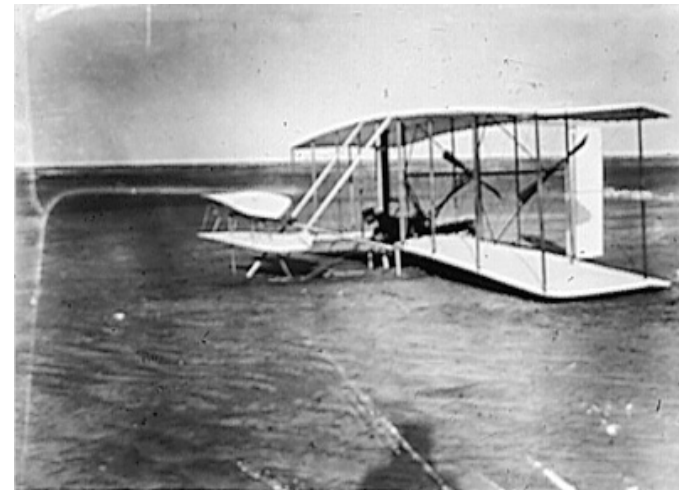


ADVANCE

Whole genomes

High speed

Low-cost



<http://www.wright-house.com/wright-brothers/wrights/1903.html>

<http://777boeing.com/>

GENOMICS 101 - SO WHAT?



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Done deal. Multiple examples in high-impact publications.

Opportunity:
Real-time application and intervention.



Applied mainly in human genome-wide studies and personalized medicine.

Opportunity:
Personalized pathogen medicine.



Tin Tock Seng
HOSPITAL

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APPLICATIONS

Transmission:

- 1. HIV**
- 2. Complex transmission – NDM-1**

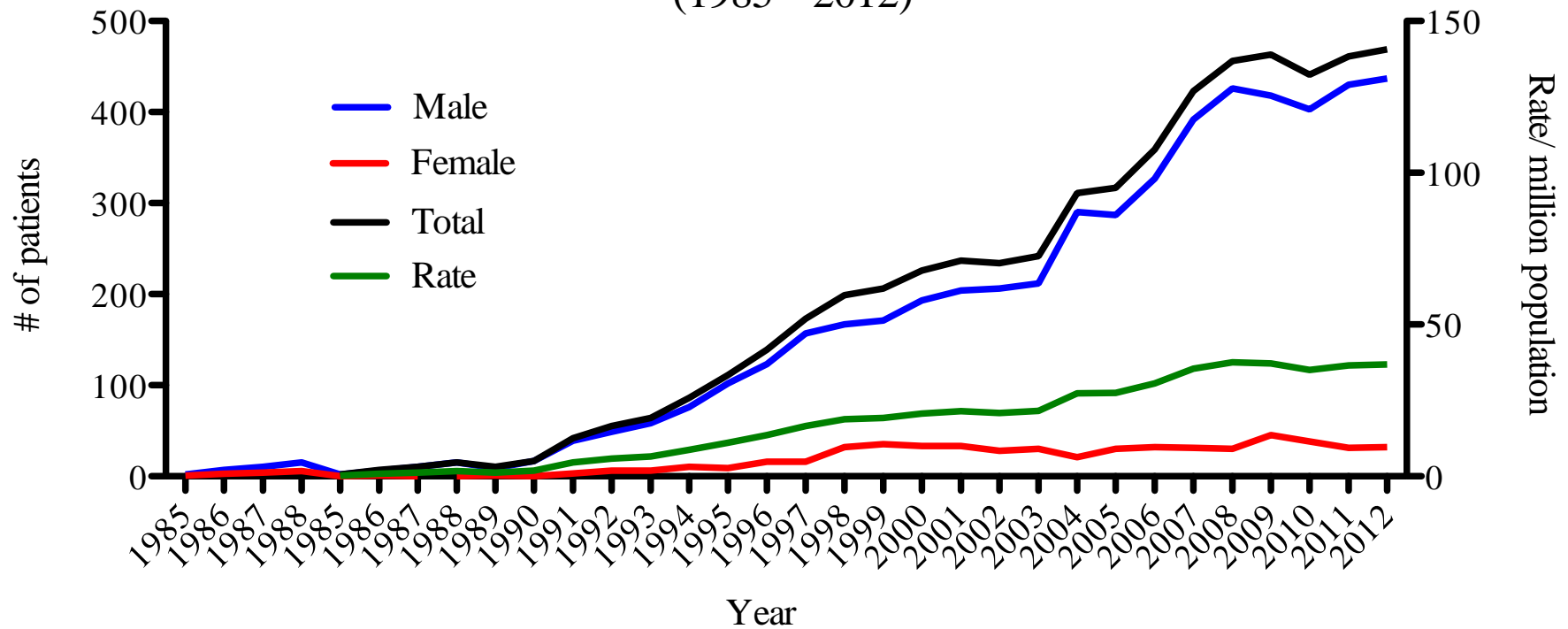
Therapeutics:

- 1. HIV-1 Co-receptor determination**

(to be published)

EPIDEMIOLOGY OF HIV IN SINGAPORE

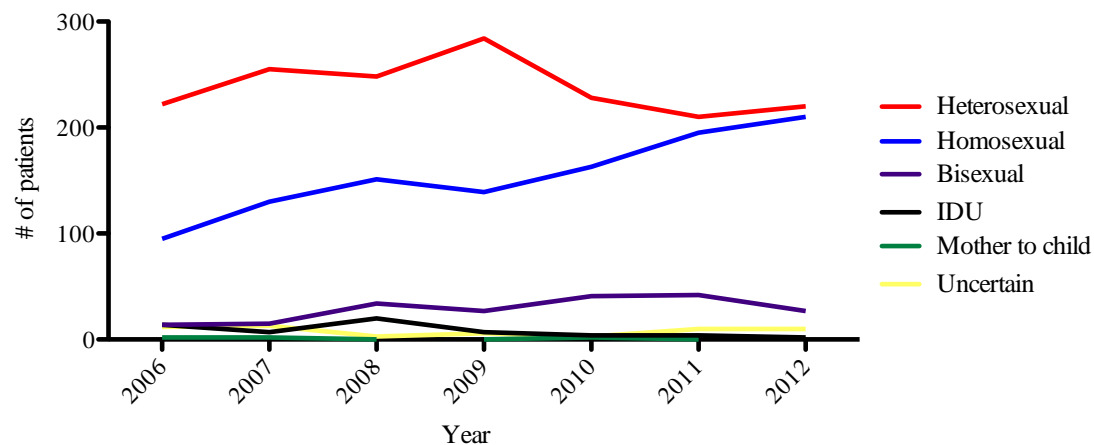
Number of Singapore Residents reported with HIV/AIDS
(1985 - 2012)



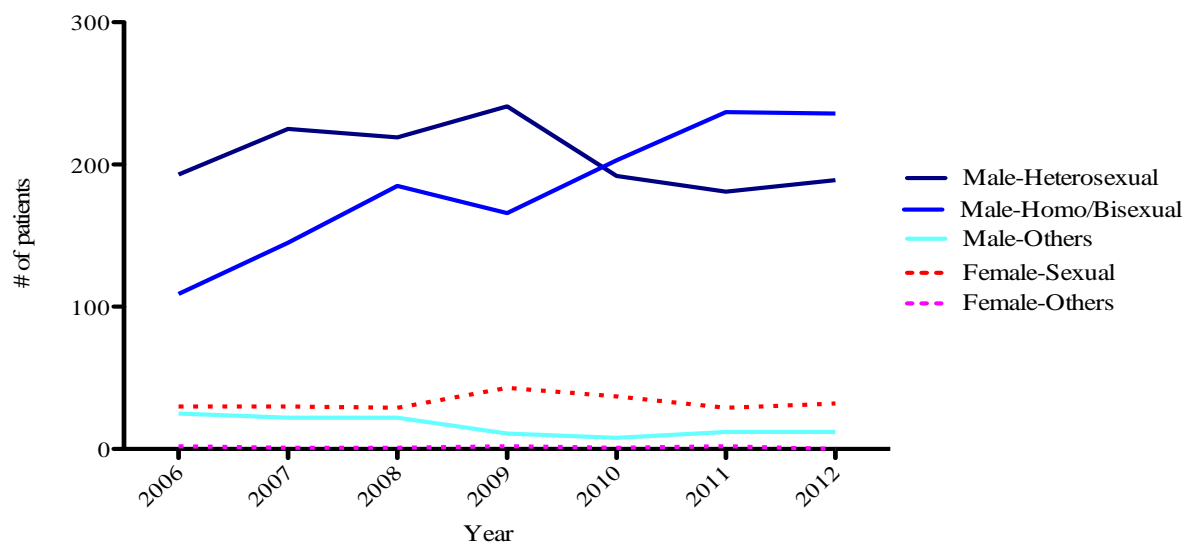
MODE OF TRANSMISSION



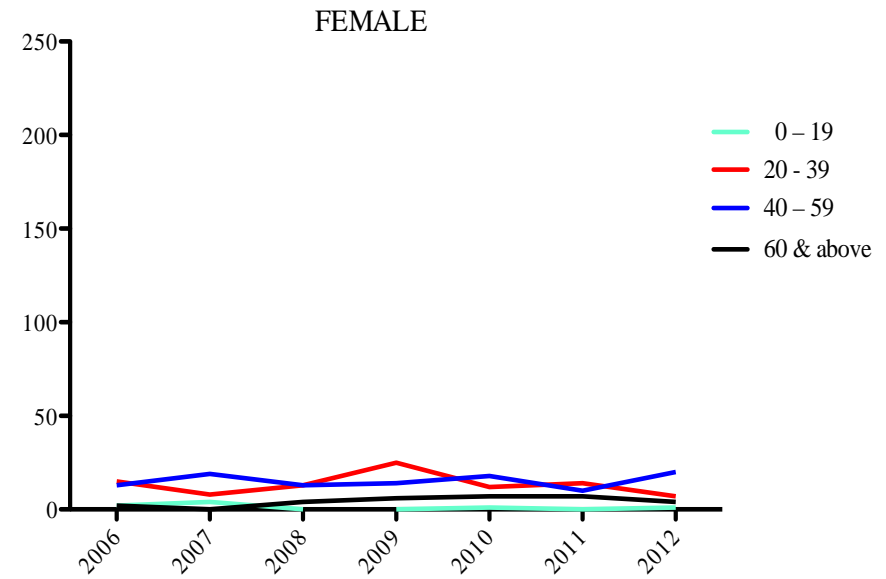
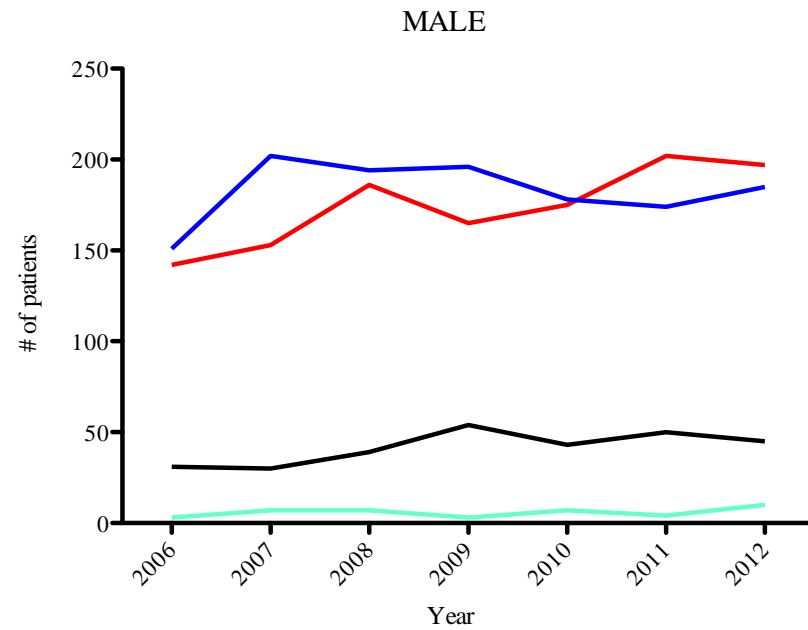
By Risk Behavior



By Gender



HIV-INFECTED RESIDENTS BY AGE



CHANGING EPIDEMIOLOGY



	1996 ¹	2002-2007 ²	2008-2009 ³
<u>MSM</u>	<u>n=19</u>	<u>n=27</u>	<u>n=73</u>
CRF01_AE	1 (5%)	11 (41%)	36 (49%)
B	<u>8 (95%)</u>	15 (59%)	30 (41%)
CRF51_01B	-	-	7 (10%)
<u>Heterosexual</u>	<u>n=26</u>	<u>n=17</u>	<u>n=88</u>
CRF01_AE	<u>23 (88%)</u>	<u>11 (65%)</u>	<u>73 (83%)</u>
B	3 (12%)	6 (35%)	12 (14%)
CRF51_01B	-	-	3 (3%)
<u>Bisexual</u>	<u>n=10</u>	<u>n=5</u>	<u>n=17</u>
CRF01_AE	5 (50%)	3 (60%)	6 (35%)
B	5 (50%)	2 (40%)	6 (35%)
CRF51_01B	-	-	5 (30%)

1. J Acquir Immune Defic Syndr 2005;38:5-13.

2. HIV Med 2009;10:370-7.

3. AIDS Res Hum Retroviruses. 2011 Jan 14. [Epub ahead of print].

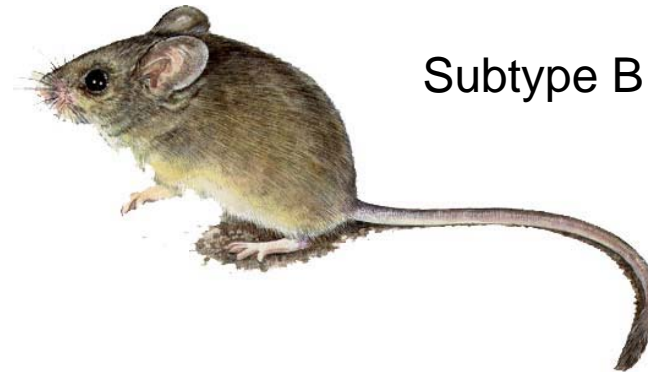
RECOMBINATION



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CRF01_AE



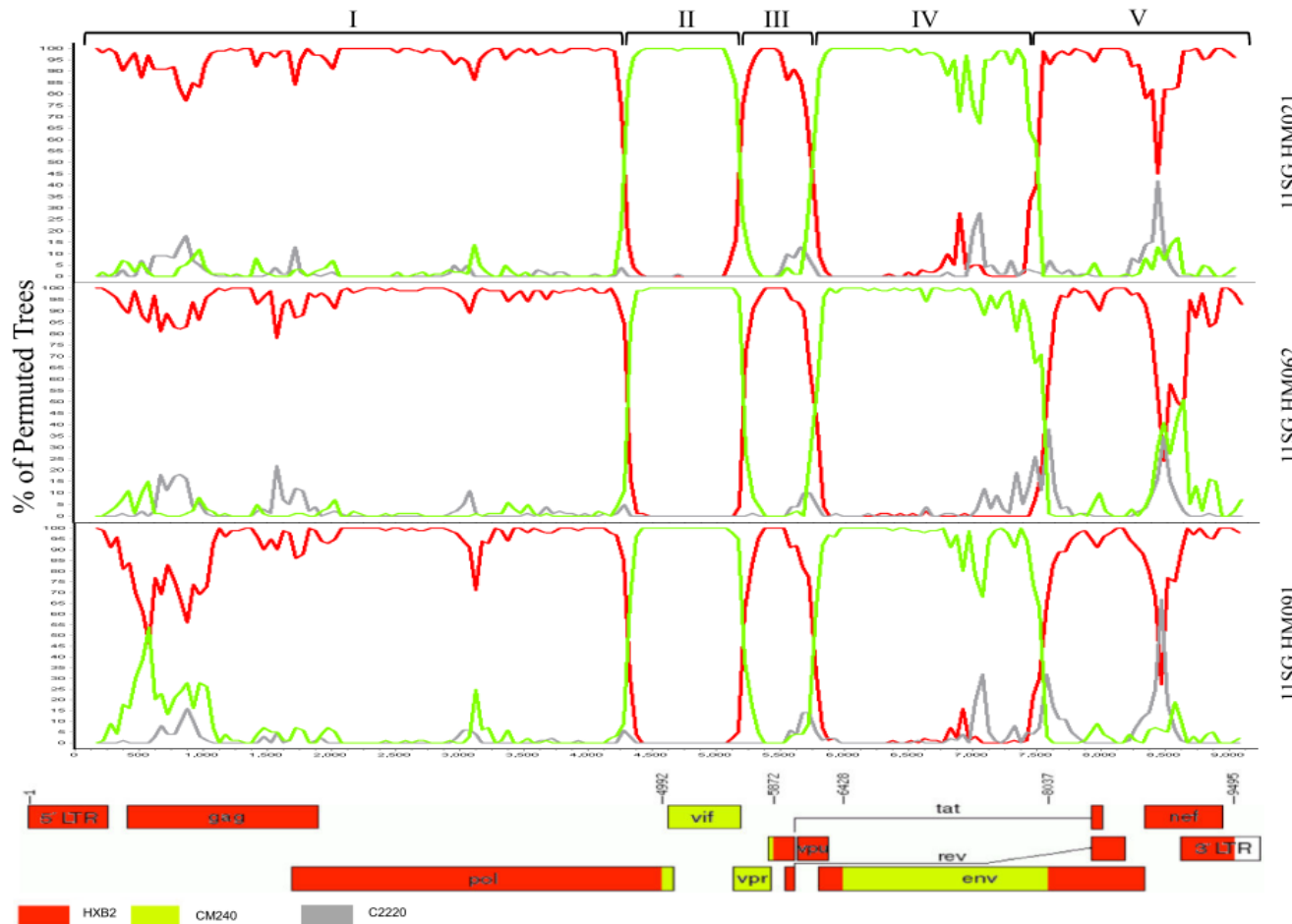
Subtype B



Recombinant
(CRF51_01B)

Kollewin.com; free-extras.com;
Skeletaldrawing.com/ext_photos/chimera.jpg

CRF51_01B

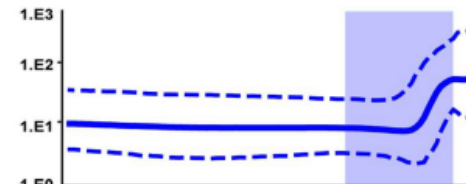
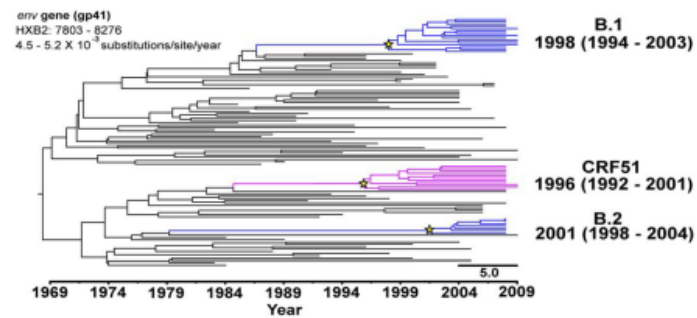
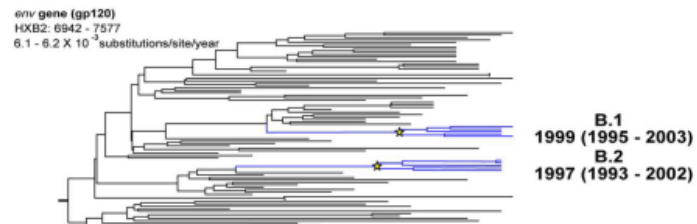
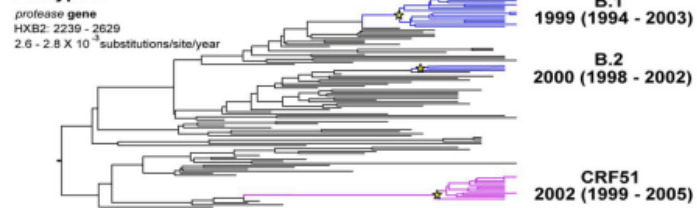


Timing Of Clusters



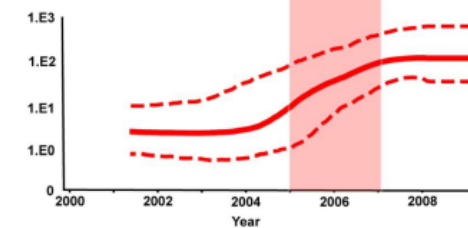
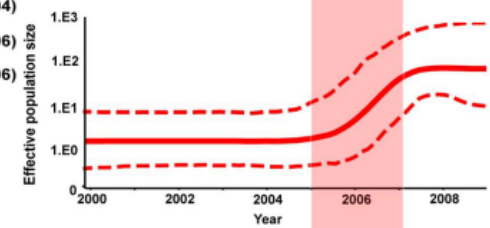
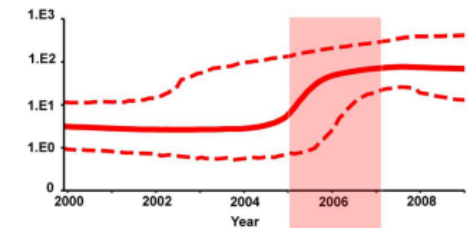
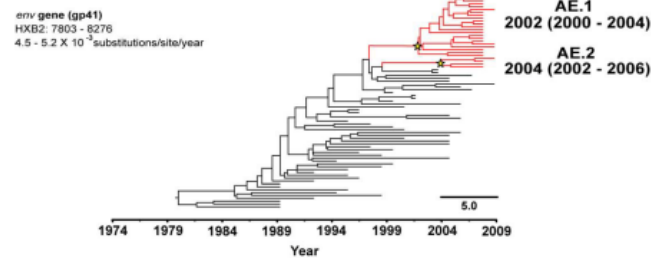
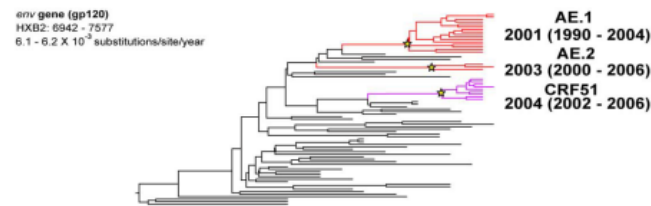
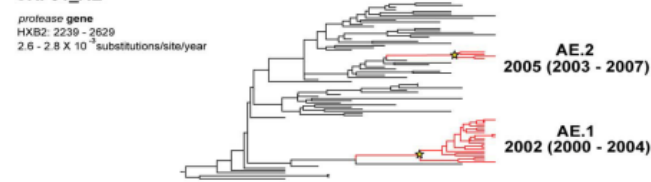
1a

Subtype B



1b

CRF01_AE

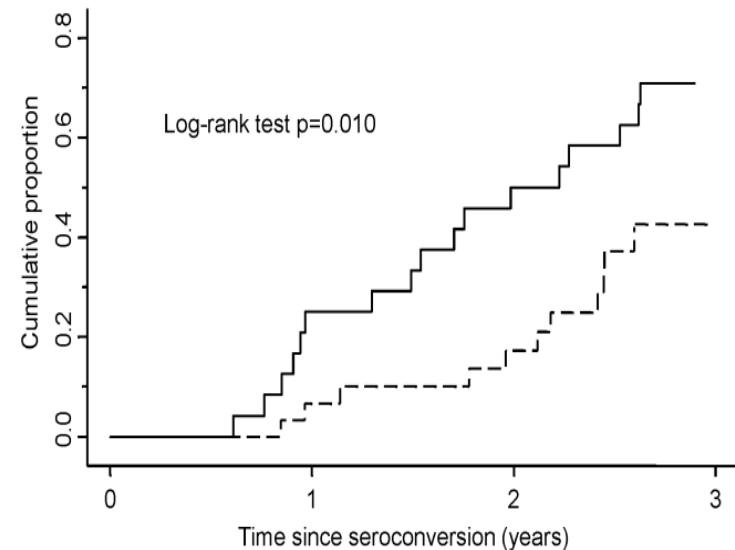


Clinical Implication (Inter-subtype)

■ Subtype CRF01_AE infected patients experienced a **58 cell/mm³/year** greater loss of CD4 T-cells compared to non-CRF01_AE infected patients.

■ Subtype CRF01_AE infected patients were treated **1.8 years** faster compared to non-CRF01_AE infected patients.

HR: 2.5 times more for treatment for AE



No. at risk (events):

Subtype AE	24	(6)	18	(6)	12	(5)	4
Non-AE subtypes	30	(2)	28	(3)	23	(6)	11

— Subtype AE - - - - Non-AE subtypes

Clinical Implication

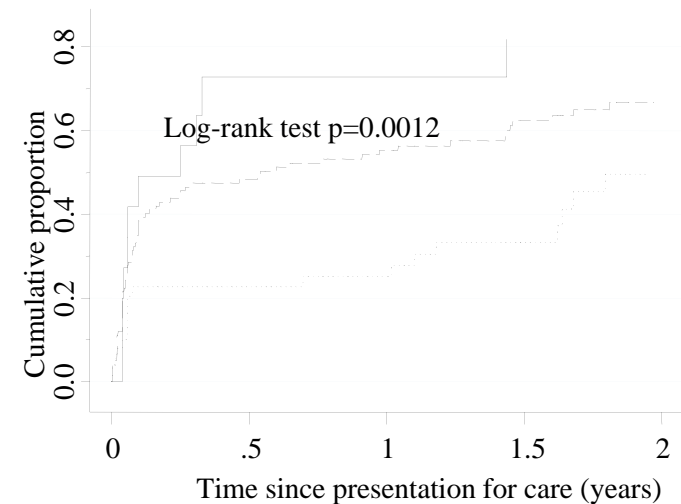
(Inter-subtype, CRF51_01B)



■ Subtype CRF51_01B infected patients experienced a 105 cell/mm³/year greater loss of CD4 T-cells compared to subtype B infected patients.

■ Subtype CRF51_01B infected patients were treated 3 years faster compared to subtype B infected patients.

Markedly faster treatment for CRF51_01B



Number at risk

CRF51_01B	15	(10)	3	(0)	3	(1)	2	(0)	2
CRF01_AE	118	(55)	55	(7)	43	(6)	30	(3)	16
Subtype B	50	(11)	34	(1)	28	(3)	21	(4)	9

CRF51_01B ———
Subtype B CRF01_AE - - - -

Fig. 1. Cumulative proportion starting ART by subtype status

COMPARATIVE GENOMICS - PATHOGENESIS

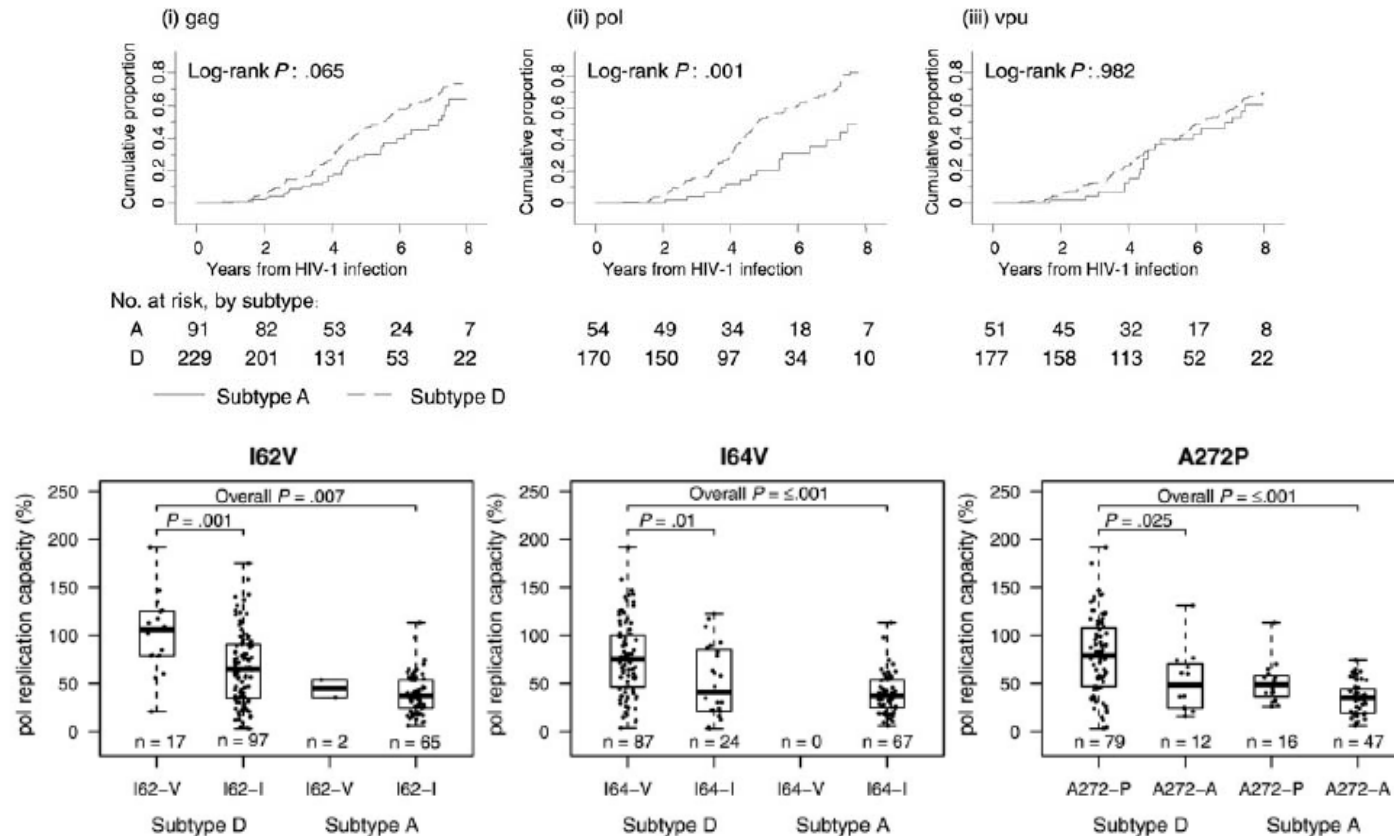
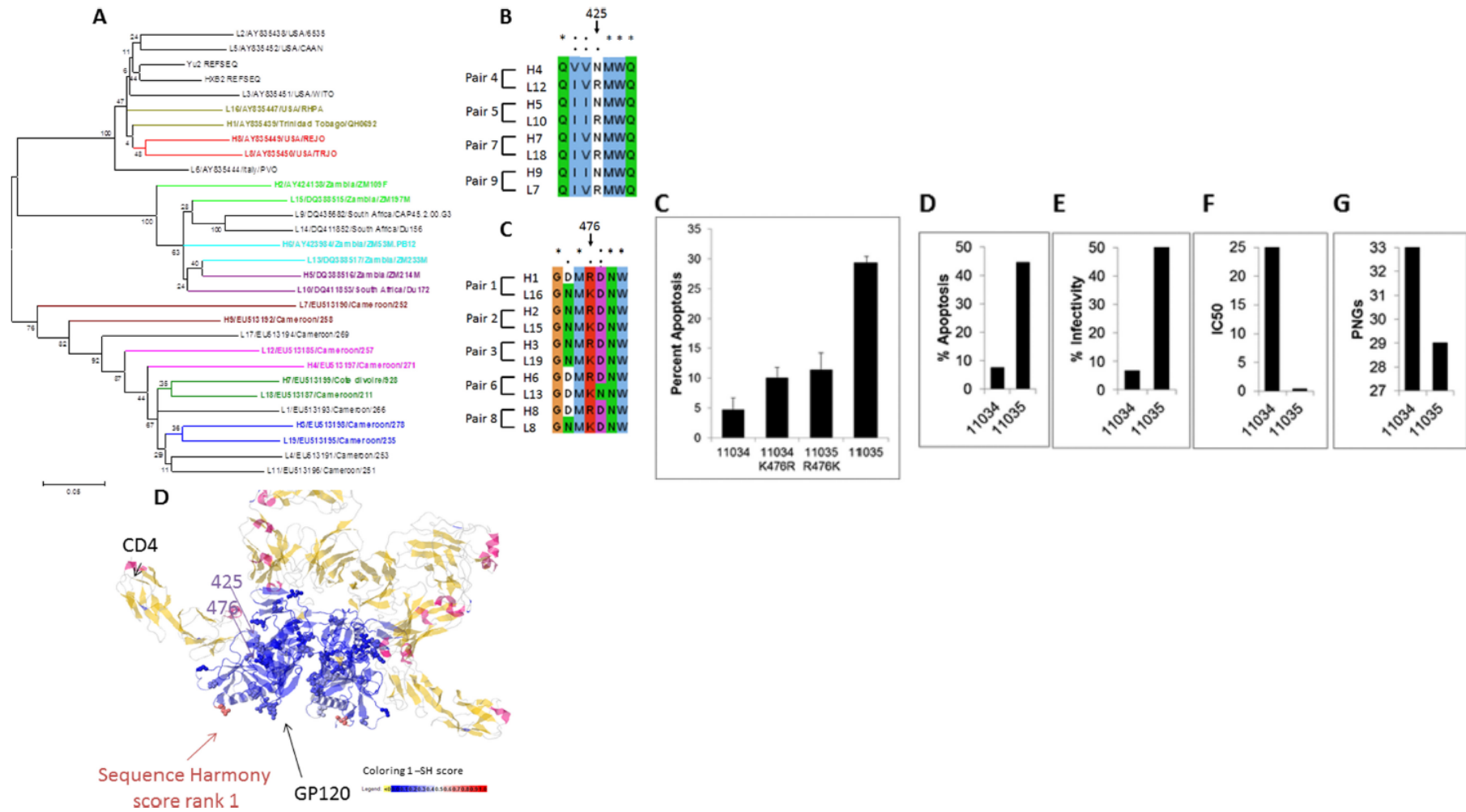


Figure 3. Box plots of pol replication capacities (RC), by amino acid polymorphisms and subtype. Results for the 3 amino acid positions (protease polymorphisms I62V and I64V and reverse transcriptase polymorphisms A272P) with significant interresidue differences in median pol RC are shown. The bars represent the highest and lowest pol RC of each category. P values for the first-stage comparison are Bonferroni corrected.

MUTAGENESIS



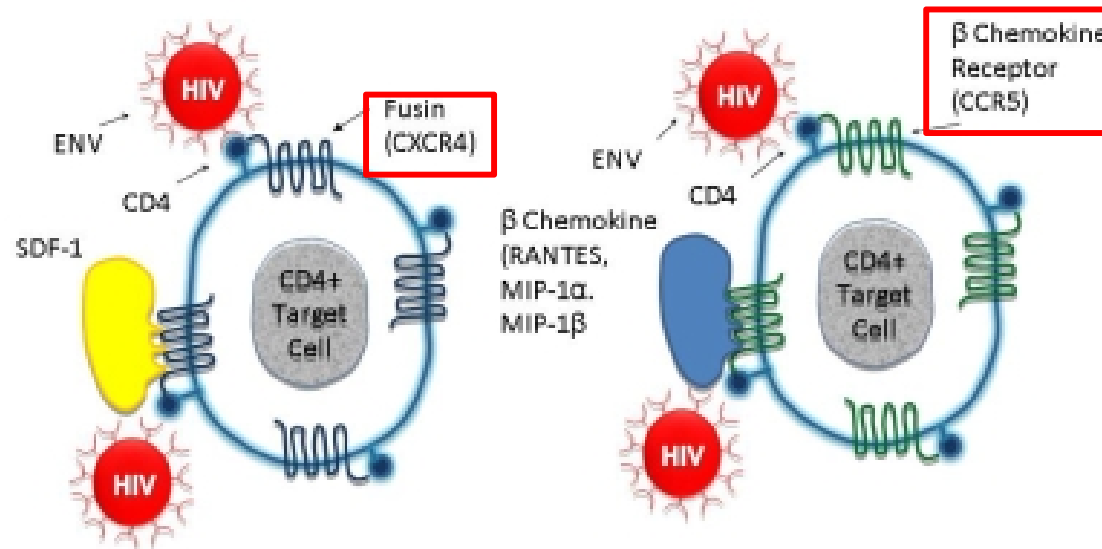
HIV Envelope-mediated Apoptosis



DOES SEQUENCE HELP US TREAT PATIENTS?



HIV receptor + coreceptors



Tale of two co-receptors:
R5 – can use drug
X4 – cannot use drug

Gene sequencing can tell us if HIV-1 is:
R5-using; OR
X4-using

R5-using HIV Virus Is Target For Drugs, Gene Therapy And Cure Strategies



BRIEF REPORT

Long-Term Control of HIV by CCR5 Delta32/ Delta32 Stem-Cell Transplantation

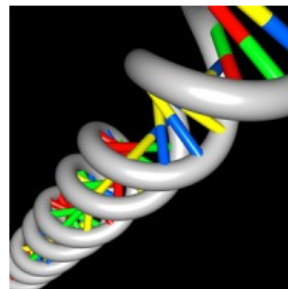
Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S.,
Susanne Ganepola, M.D., Arne Müßig, M.D., Kristina Allers, Ph.D.,
Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D.,
Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hofmann, M.D.,
and Eckhard Thiel, M.D.

CROI 2013: Zinc Finger Gene Therapy Leads to Durable T-Cell Recovery, Sangamo Says

Details Category: [Search for a Cure](#) Published on Monday,
11 March 2013 00:00 Written by Liz Highleyman



Modification of CD4 T-cells using zinc finger technology designed to render cells resistant to HIV led to durable immune reconstitution, while a different gene therapy technique may make cells less susceptible to viral infection, according to 2 studies presented at the 20th Conference on Retroviruses and Opportunistic Infections (CROI 2013) last week in Atlanta.



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Sangamo BioSciences is evaluating a technique in which T-cells are collected from a patient's blood via apheresis, a zinc finger nuclease is used to disrupt CCR5 gene expression, and the altered cells -- known as SB-728-T -- are allowed to multiply and infused back into the body.

ORIGINAL ARTICLE

Blockade of Lymphocyte Chemotaxis in Visceral Graft-versus-Host Disease

Ran Reshef, M.D., Selina M. Luger, M.D., Elizabeth O. Hexner, M.D.,
Alison W. Loren, M.D., Noelle V. Frey, M.D., Sunita D. Nasta, M.D.,
Steven C. Goldstein, M.D., Edward A. Stadtmauer, M.D.,
Jacqueline Smith, C.R.N.P., Sarah Bailey, B.A., Rosemarie Mick, M.S.,
Daniel F. Heitjan, Ph.D., Stephen G. Emerson, M.D., Ph.D.,
James A. Hoxie, M.D., Robert H. Vonderheide, M.D., D.Phil.,
and David L. Porter, M.D.

NB: Does not work with X4-using virus

Comparison Of Subtype Specific Predictions Using Common Co-R Prediction Tools

	sensitivity (TPR)	specificity (TNR)		sensitivity (TPR)	specificity (TNR)
Subtype A (n=27, X4:2 R5:25)			Subtype D (n=35, X4:6 R5:29)		
geno2pheno (5FPR)	100%	100%	WebPSSM (B x4r5)	100%	48.3%
WebPSSM (B x4r5)	100%	100%	geno2pheno (5FPR)	100%	72.4%
WebPSSM (B sinisi)	100%	100%	WebPSSM (B sinisi)	100%	69%
WebPSSM (C sinisi)	100%	72%	WebPSSM (C sinisi)	100%	0
Subtype B (n=204, X4:2 R5:201)			CRF01_AE (n=65, X4:0 R5:65)		
geno2pheno (5FPR)	100%	85%	geno2pheno (5FPR)	NA	81.5%
WebPSSM (B x4r5)	100%	98%	WebPSSM (B x4r5)	NA	80%
WebPSSM (B sinisi)	100%	96%	WebPSSM (B sinisi)	NA	84.6%
WebPSSM (C sinisi)	100%	4%	WebPSSM (C sinisi)	NA	10.8%
subtypeC (n=118, X4:0 R5:118)			CRF 51_01B (n=7, X4=0 R5:7)		
geno2pheno (5FPR)	NA	100	WebPSSM (B x4r5)	NA	0
WebPSSM (B x4r5)	NA	95.8	geno2pheno (5FPR)	NA	0
WebPSSM (B sinisi)	NA	100	WebPSSM (B sinisi)	NA	71.4%
WebPSSM (C sinisi)	NA	95.8	WebPSSM (C sinisi)	NA	0

Unpublished Data Generated from 449 Sequences Extracted Los Alamos HIV Database



CONCLUSION

NGS has revolutionized transmission studies.

- Next step may be real-time interventions.

NGS is waiting to find more bedside applications.

- Clinicians should apply.



CREATING GROWTH. ENHANCING LIVES.



DISCUSSION OR QUESTIONS



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