17TH ANNUAL CONFERENCE OF THE BRITISH HIV ASSOCIATION (BHIVA)

British HIV Association BHIVA

Dr Richard Harrigan

British Columbia Centre for Excellence in HIV/AIDS,
Vancouver, Canada

6-8 April 2011, Bournemouth International Centre

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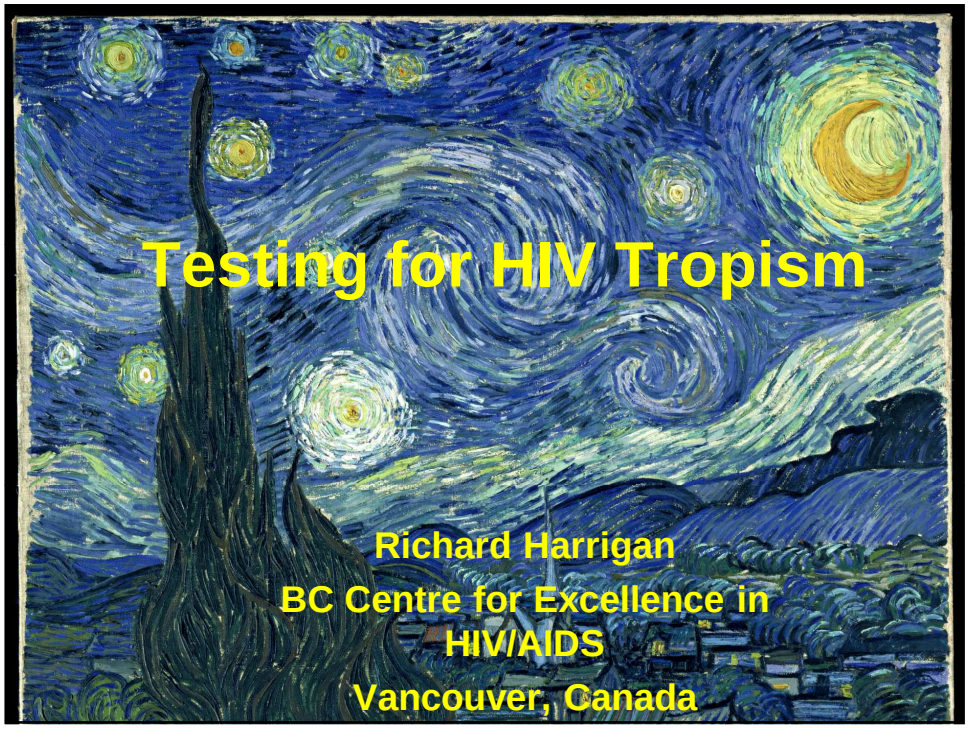
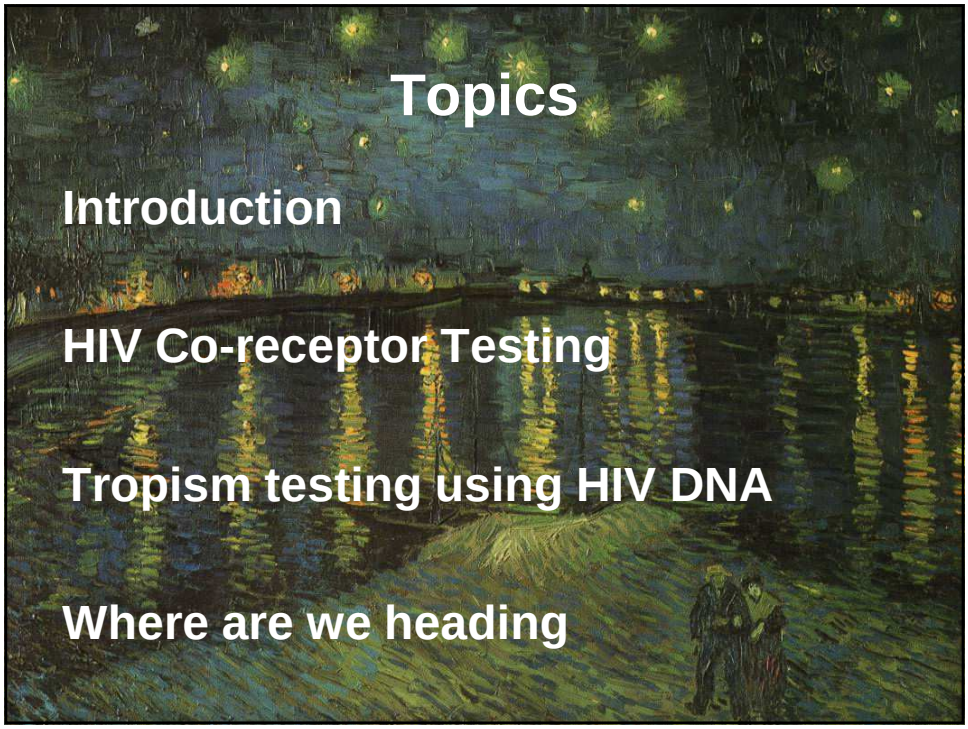
COMPETING INTEREST OF FINANCIAL VALUE ≥ £1,000:	
Speaker Name	**Statement**
Dr Richard Harrigan:	Dr Harrigan has acted in a consultancy capacity for ViiV Healthcare, Pfizer, Quest Diagnostics and Virco. He has also received grants for research from Abbott, GlaxoSmithKline, Merck, Pfizer and ViiV Healthcare. Dr Harrigan owns shares in Merck.
Date	1 April 2011

6-8 April 2011, Bournemouth International Centre

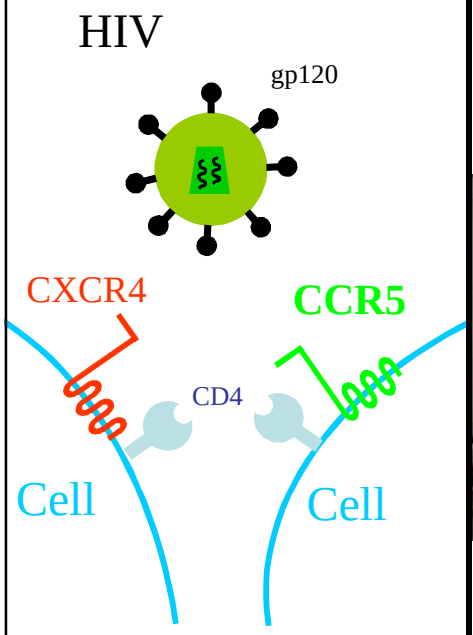

Testing for HIV Tropism
Richard Harrigan
BC Centre for Excellence in HIV/AIDS
Vancouver, Canada


Topics
Introduction
HIV Co-receptor Testing
Tropism testing using HIV DNA
Where are we heading

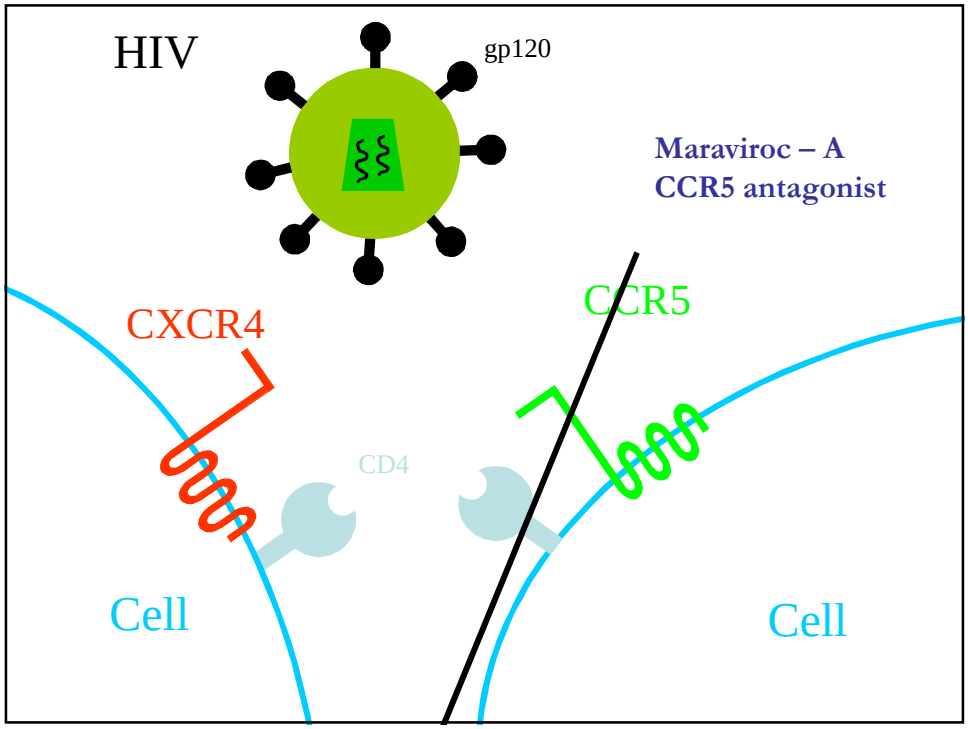
HIV Tropism

and

Co-receptor Antagonists

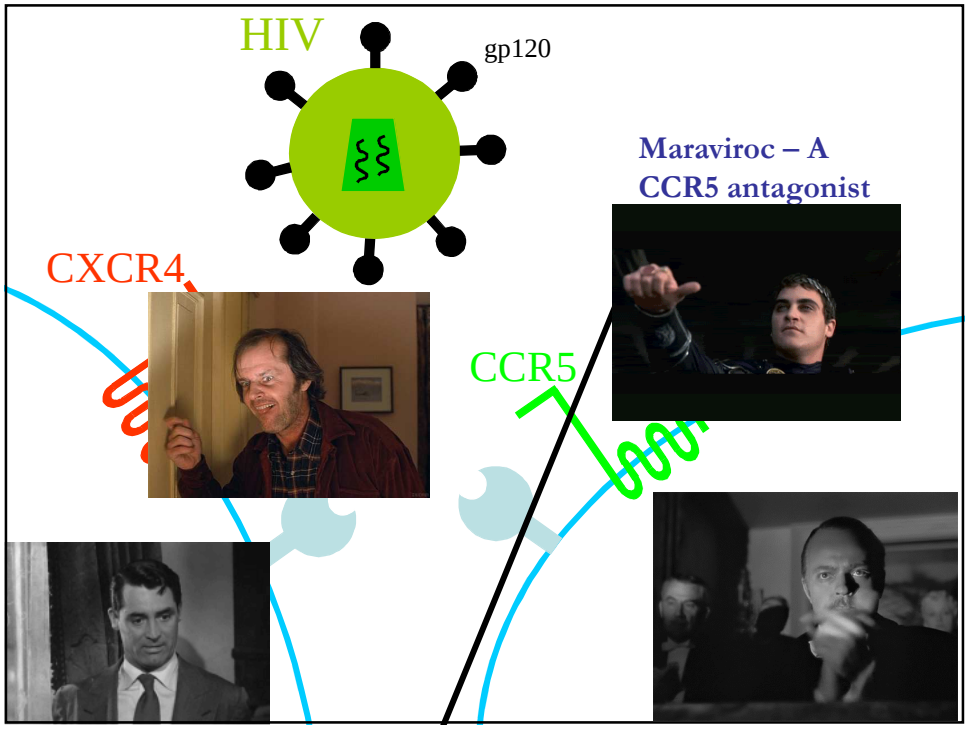


Alternative



Background

- MVC Trials (MOTIVATE/1029 and MERIT)
- Trofile – Original and ESTA test
- HIV RNA vs HIV DNA
- Genotype Testing



Practical Issues with Phenotypic Trofile Test

- Relatively slow
- Expensive
- Requires relatively large fresh blood draw with pVL >1000
- Performed only in S. San Francisco
- Relatively high failure rate

Why Genotype ?

- Determinants of tropism mainly (perhaps not exclusively) in V3 region
- Faster, cheaper and much more broadly available than other methods
- Already routine in many places for routine resistance testing
- New technology allows sensitive detection of minority species

Results and Algorithms (Simplified)

CTRPSNNTRRGIHIGPGRAFYTTGEIIGDIRQAHC

CTRPSNNQRKRIYIGPGRAFYTTGRIIGDIRQAHC

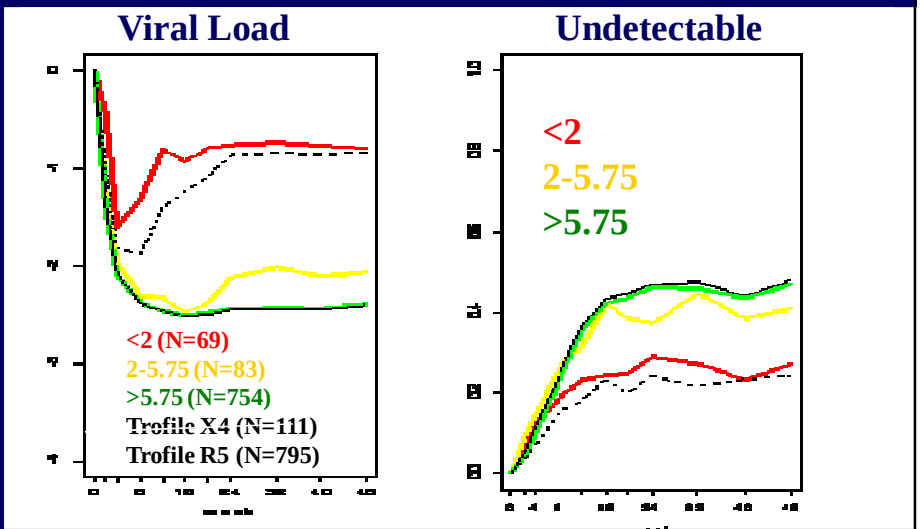
Sequence put through an algorithm called "g2P" (Geno2Pheno)

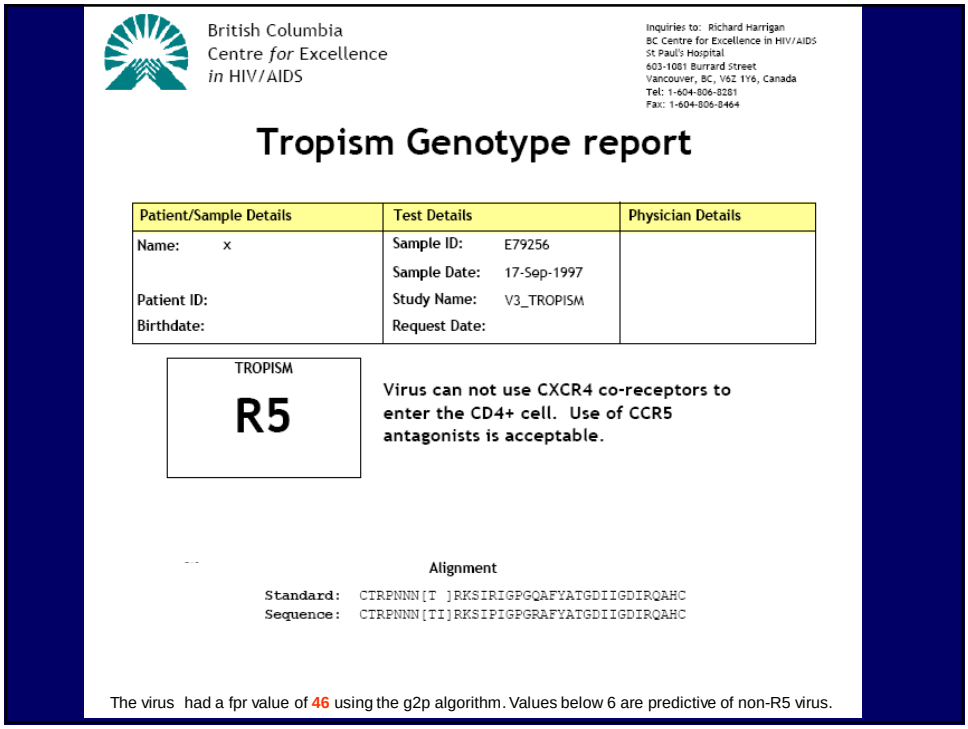
Interpretation

- The **"false positive rate"** is the estimated probability that a sample in incorrectly called X4
- The lower the g2P fpr, the more certain we are that the sample is X4

fpr cut-offs for interpreting genotype

Motivate&1029 Studies





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Tel: 1-604-806-8281
Fax: 1-604-806-8464

Tropism Genotype report

Patient/Sample Details	Test Details	Physician Details
Name: x	Sample ID: E79256	
	Sample Date: 17-Sep-1997	
Patient ID:	Study Name: V3_TROPISM	
Birthdate:	Request Date:	

TROPISM

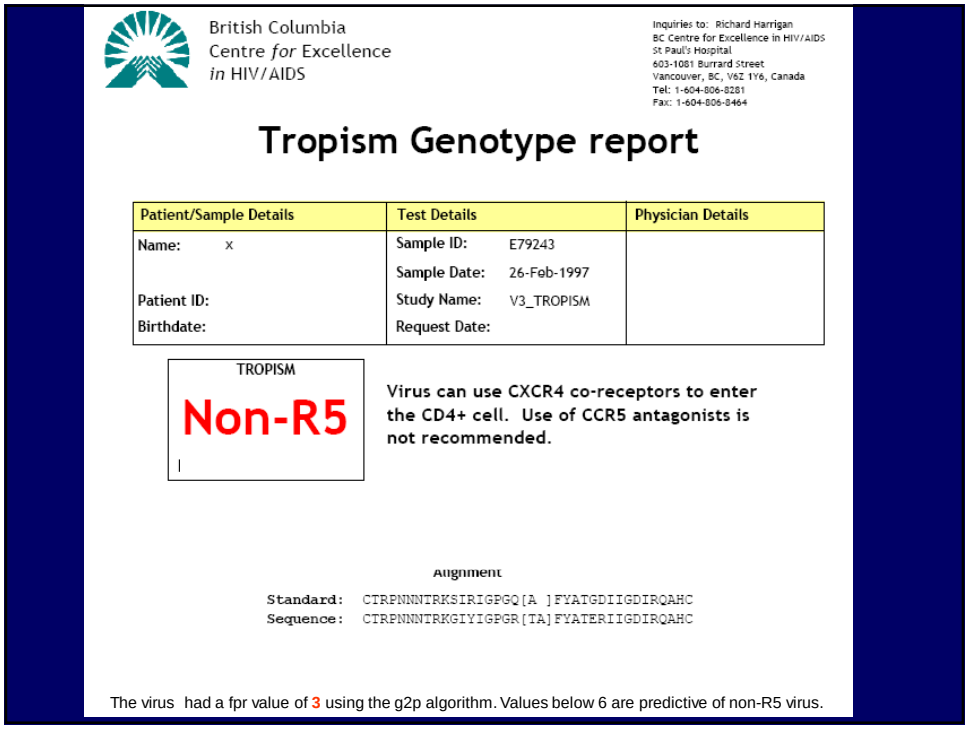
R5

Virus can not use CXCR4 co-receptors to enter the CD4+ cell. Use of CCR5 antagonists is acceptable.

Alignment

```
Standard:  CTRPNNN[T ]RKSIRIGPGQAFYATGDIIGDIRQAHC
Sequence:  CTRPNNN[TI]RKSIPIGPGRAFYATGDIIGDIRQAHC
```

The virus had a fpr value of **46** using the g2p algorithm. Values below 6 are predictive of non-R5 virus.



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Tropism Genotype report

Patient/Sample Details	Test Details	Physician Details
Name: x	Sample ID: E79243	
	Sample Date: 26-Feb-1997	
Patient ID:	Study Name: V3_TROPISM	
Birthdate:	Request Date:	

TROPISM

Non-R5

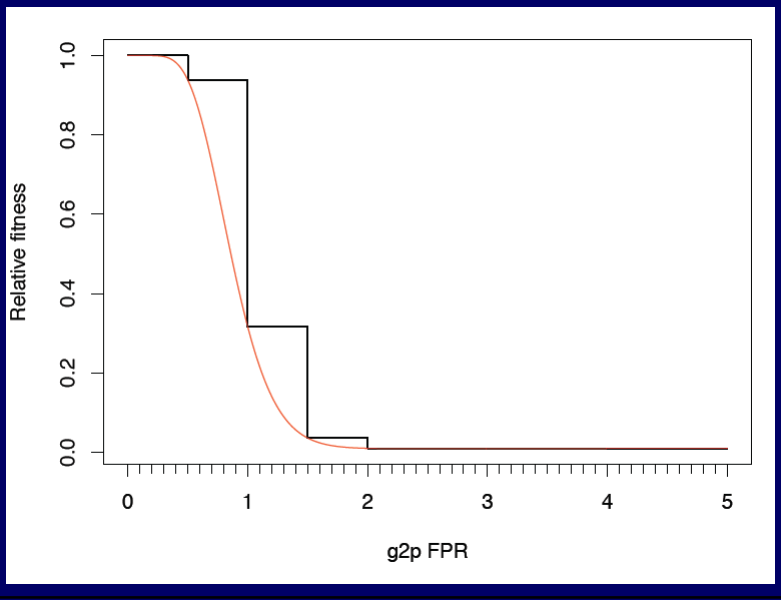
Virus can use CXCR4 co-receptors to enter the CD4+ cell. Use of CCR5 antagonists is not recommended.

Alignment

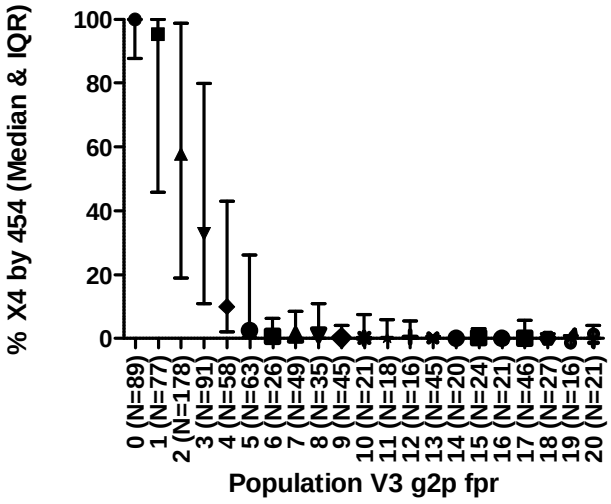
```
Standard:  CTRPNNNTRKSIRIGPGQ[A ]FYATGDIIGDIRQAHC
Sequence:  CTRPNNNTRKGIYIGPGR[TA]FYATERIIGDIRQAHC
```

The virus had a fpr value of **3** using the g2p algorithm. Values below 6 are predictive of non-R5 virus.

Estimation of Relative Fitness in Presence of MVC



Relationship between Population g2P score and %X4 by quantitative "deep" sequencing



Comparisons of Assays – MERIT Primary Outcome*

	MVC arm	EFV	Difference*	LCB 97.5%
Trofile	235/360 65%	250/361 69%	-4.20	-10.9**
ESTA	213/311 68%	207/303 68%	-0.17	-7.41
Population-based V3	212/318 67%	214/315 68%	-1.27	-8.87
"Deep" Sequencing	210/312 67%	217/316 69%	-1.36	-8.67

*MVC not approved in EU in drug naïve patients

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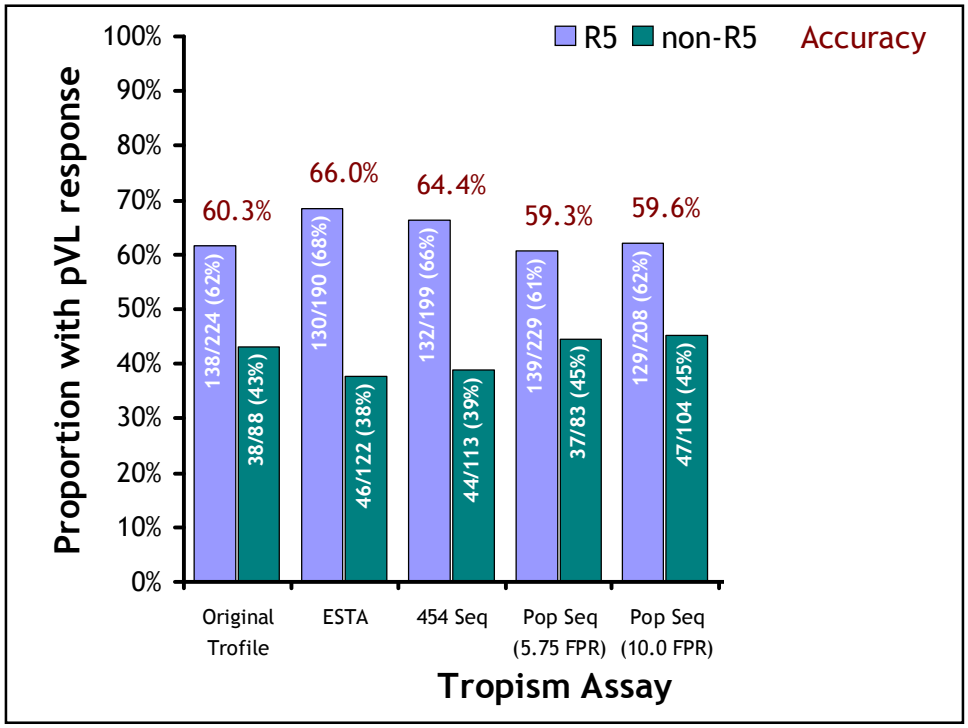
(Almost) Unbiased Estimation of Tropism Prediction from therapy experienced patients (MOTIVATE/1029)

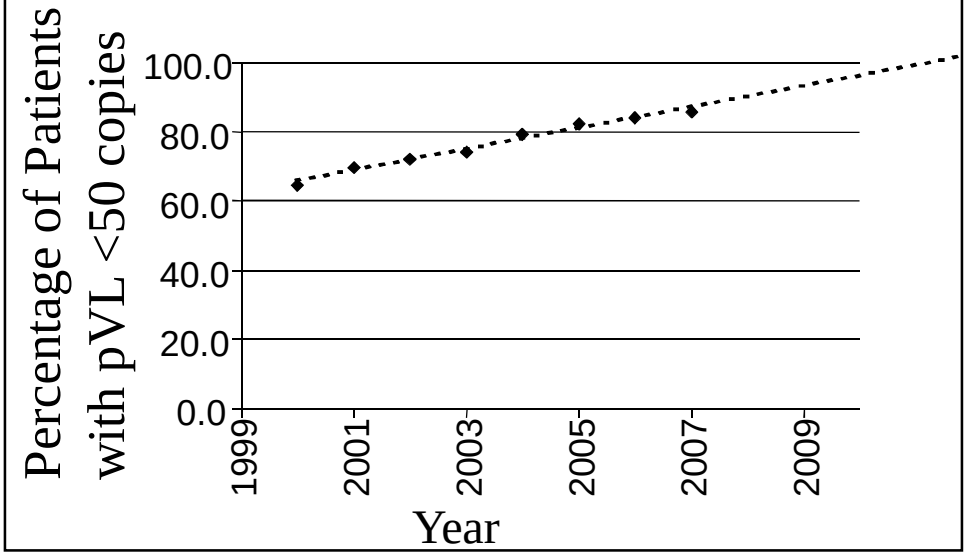


Testing HIV DNA for Tropism

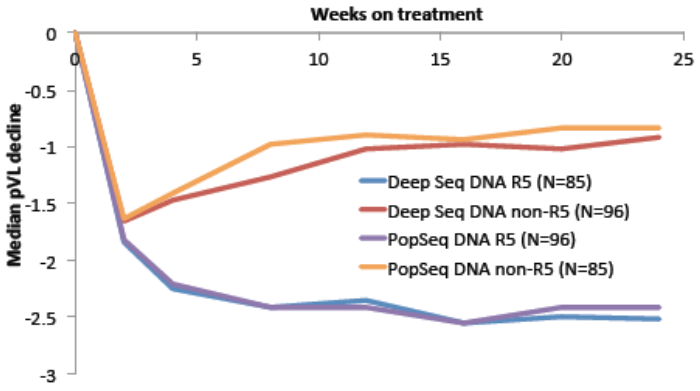
Why DNA ?

- Vast majority of patients now have HIV RNA pVL below 50 copies/mL
- HIV DNA levels not affected very much by therapy
- Could also test last detectable viral load sample (if available)

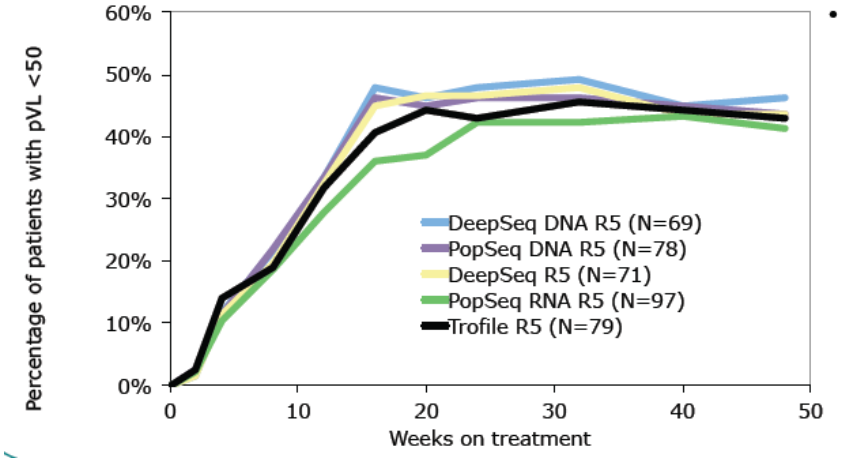
Trends in Viral Load Suppression



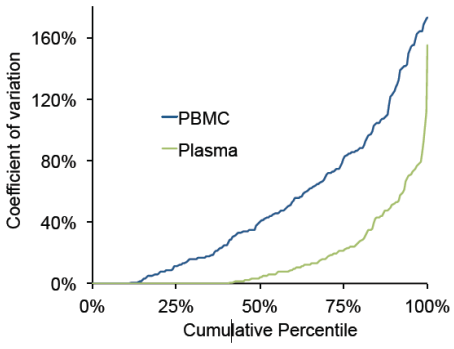
HIV DNA-based methods were good predictors of pVL response to MVC



…with similar rates of response for R5s whether testing plasma RNA or DNA



Note that Genotype scores were much more variable in the PBMC compartment



- Compared to plasma, PBMC samples gave more variable geno2pheno values across the triplicate amplifications performed for PopSeq
  - i.e., the three g2p values were more likely to differ in the PBMC vs the plasma
- Differences between the compartments may arise from different input copy number (which is unknown for PBMCs)

Where are we heading? Automation and Deep Sequencing

Assertions – How to do the test

- For genotype, prefer the g2p model
- The default settings on the g2p website (“German Recommendations”) are probably way too conservative
- BUT think about clinical parameters when interpreting any results
- Replicate PCR reactions should be done if you are testing low viral load samples (<5000 copies?) or proviral DNA samples
- Labs should participate in external sample exchange

Assertions – How to use the drugs

Earlier use of CCR5 antagonists is preferable

– Natural history suggests increased X4 prevalence with very low CD4 count (below 25 or 50)

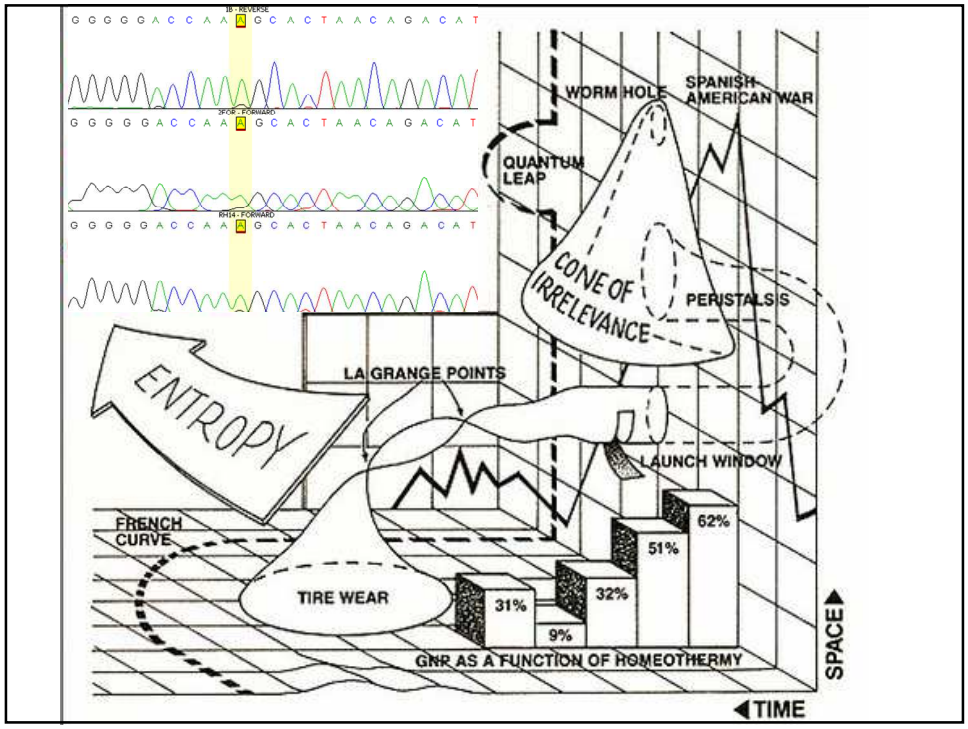
– More likely to waste time & money (a test result of X4 is relatively rare above 50 CD4 cells)

– Response is reduced below CD4 of ~25-50 regardless of the test method or results

Conclusions

- Tropism testing by V3 genotyping is an attractive method of determining HIV tropism
- DNA testing works, but perhaps not quite as well as testing HIV RNA
- Attention to the PATIENT not the test!

Acknowledgements


WORM HOLE
SPANISH AMERICAN WAR
QUANTUM LEAP
CONE OF IRRELEVANCE
PERISTALSIS
LA GRANGE POINTS
ENTROPY
LAUNCH WINDOW
FRENCH CURVE
TIRE WEAR
62%
51%
32%
31%
9%
GNR AS A FUNCTION OF HOMEOTHERMY
SPACE
TIME