Reliable Genotypic Tropism Tests for the Major HIV-1 Subtypes

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ABSTRACT

Background:

Major obstacles to widespread use of the CCR5-antagonist **maraviroc** (MVC) in ART are;

i. Pre-treatment prognostic phenotypic tropism tests are expensive and time consuming.

ii. Cheaper and more rapid genotypic tropism tests have been developed primarily for HIV-1 subtype B, which account for <10% of infections worldwide.

In response, we developed PhenoSeq; a suite of reliable genotypic tropism tests specific for HIV-1 subtypes A, B, C, D, CRF01_AE and CRF02_AG, which together account for ~95% of infections worldwide.

Methods:

PhenoSeq algorithm prediction criteria were informed by analysis of every previously published HIV-1 subtype A, B, C, D, CRF01_AE and CRF02_AG Env **V3 sequence.**

	HIV Subtype					
	A	B	C	D	AE	AG
CXCR4-using (n=)	40	185	80	57	50	42
CCR5-using (n=)	118	538	429	80	128	54

PhenoSeq algorithms were validated against independent V3 sequences from patients enrolled in Pfizer clinical trials (A4001064 and MERIT), relative to results of phenotypic tropism tests; original Trofile assay (OTA), enhanced sensitivity Trofile assay (ESTA).

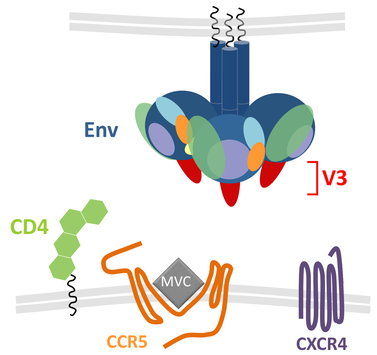
Results:

PhenoSeq algorithms exhibited more favourable sensitivity/specificity profiles and AUROC scores for establishing the tropism of HIV-1 subtypes A, B, C, D, CRF01_AE and CRF02_AG than current alternatives (including geno2pheno and WebPSSM).

Conclusions:

As the only platform of algorithms that can reliably infer the tropism of ***all*** the major global HIV-1 subtypes, PhenoSeq has the potential to inform the appropriate use of maraviroc and future CCR5 blocking drugs in regions of the world where non-B HIV-1 predominates, and which are burdened the most by the HIV-1 pandemic.

BACKGROUND/METHODS



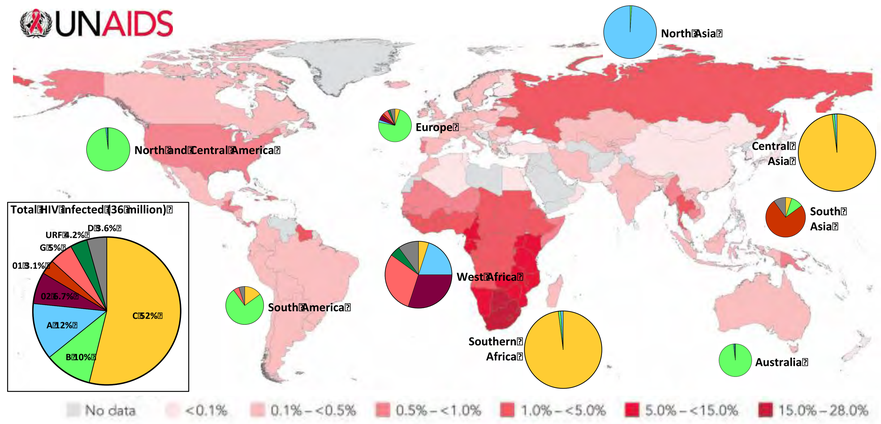
HIV infects targets cells via binding of **Env** to;

i. **CD4**, and then either

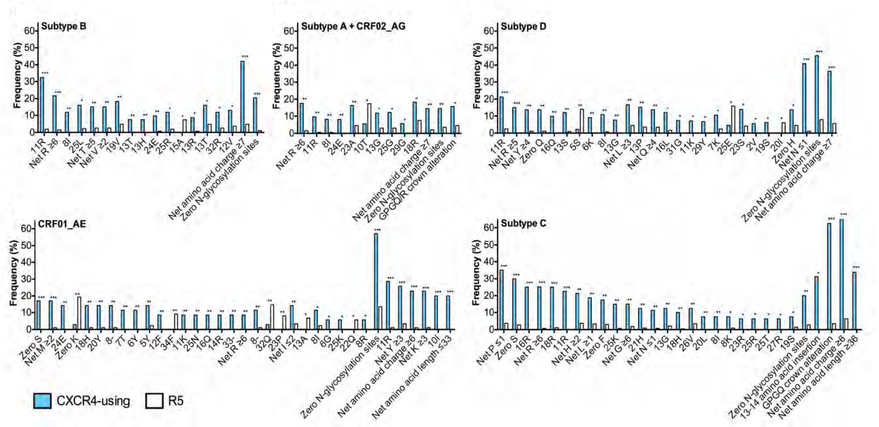
ii. **CCR5** or **CXCR4** cell surface receptors.

Maraviroc (**MVC**) is a CCR5 antagonist HIV-1 entry inhibitor. Since MVC has no effect on CXCR4-using viruses, MVC is only administered to patients harbouring R5 viruses.

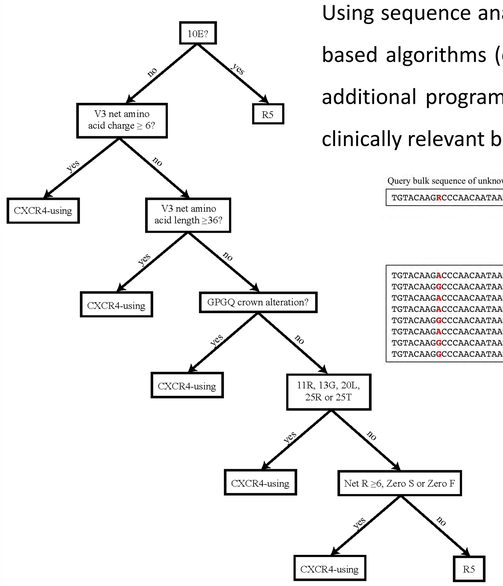
V3 sequence-based genotypic tropism tests are a rapid, cheap and effective way of determining HIV-1 coreceptor usage. However, the majority of genotypic tropism tests have been developed for HIV-1 subtype B. As such, they have poor accuracy against other HIV-1 subtypes.



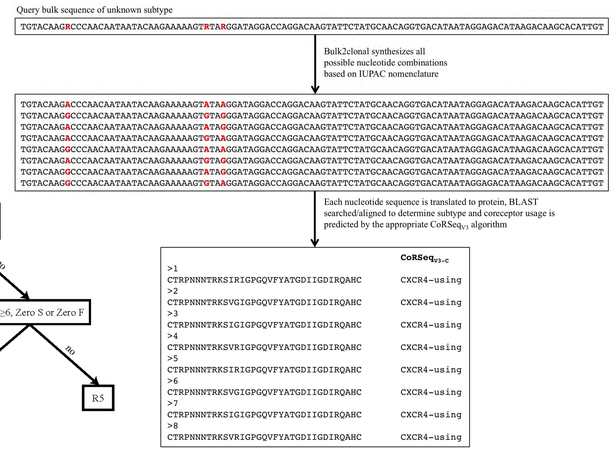
We developed PhenoSeq; a suite of genotypic tropism tests for each of the major HIV-1 subtypes, A, B, C, D, CRF01_AE and CRF02_AG. PhenoSeq prediction parameters were based on subtype-specific V3 mutations that distinguish CXCR4-using from R5 viruses;



RESULTS



Using sequence analysis information, we developed a suite of rule-based algorithms (e.g subtype C PhenoSeq algorithm, left) and an additional program called "**bulk2clonal**" that allows processing of clinically relevant bulk V3 sequences (shown below).



When PhenoSeq(+bulk2clonal) was tested against independent bulk V3 sequences -- including those from patients previously enrolled in Pfizer trials A4001064 and MERIT -- it exhibited predictive accuracy greater than any alternative, including in-use geno2heno and WebPSSM.

Table 2 | Sensitivity, specificity and AUROC of genotypic algorithms for predicting coreceptor usage of test set V3 sequences

		PhenoSeq	g2p FPR 5.75%	g2p FPR 10%	WebPSSM$_{X4R5}$	WebPSSM$_{SI/NSI}$*
PhenoSeq-B Test Set 1	**Sens/Spec**	100/87.8	100/97.6	100/90.2	41.7/95.1	16.7/87.8
Mulinge *et al*	**AUROC**	0.94	0.99 (p = 0.17)	0.95 (p = 0.44)	0.68 (***p < 0.01***)	0.52 (***p < 0.01***)
12 CXCR4-using, 41 R5						
PhenoSeq-B Test Set 2	**Sens/Spec**	78.4/80.3	70.7/92.2	72.8/84	55.4/96.3	62/94.1
Los Alamos HIV Database	**AUROC**	0.79	0.82 (p = 0.32)	0.78 (p = 0.41)	0.76 (p = 0.24)	0.78 (p = 0.41)
92 CXCR4-using, 269 R5						
PhenoSeq-C Test Set 1	**Sens/Spec**	83.6/92.5	78.2/95	81.8/95	-	85.5/77.5
A4001064	**AUROC**	0.88	0.87 (p = 0.22)	0.88 (p = 0.30)	-	0.82 (p = 0.22)
55 CXCR4-using, 40 R5						
PhenoSeq-C Test Set 2	**Sens/Spec**	77.8/75.4	11.1/93	50/82.4	-	61.1/81.3
MERIT	**AUROC**	0.77	0.52 (***p < 0.01***)	0.66 (p = 0.15)	-	0.71 (p = 0.29)
18 CXCR4-using, 187 R5						
PhenoSeq-D Test Set	**Sens/Spec**	80.5/77.3	81.4/72.7	88.4/61.4	81.4/65.9	69.8/63.6
A4001064	**AUROC**	0.79	0.77 (p = 0.40)	0.75 (p = 0.29)	0.74 (p = 0.24)	0.67 (p = 0.05)
43 CXCR4-using, 44 R5						
PhenoSeq-AE Test Set	**Sens/Spec**	85.7/96	78.6/68	85.7/56	85.7/72	85.7/76
A4001064	**AUROC**	0.91	0.73 (***p = 0.046***)	0.71 (***p = 0.03***)	0.79 (p = 0.11)	0.81 (p = 0.15)
14 CXCR4-using, 25 R5						
PhenoSeq-A/AG Test Set 1	**Sens/Spec**	88.9/76.8	44.4/97.1	55.6/94.2	44.4/87	66.7/84
A4001064	**AUROC**	0.83	0.71 (p = 0.18)	0.75 (p = 0.27)	0.66 (p = 0.10)	0.75 (p = 0.28)
9 CXCR4-using, 69 R5						
PhenoSeq-A/AG Test Set 2	**Sens/Spec**	62.5/97.2	50/94.4	62.5/80.6	37.5/88.9	37.5/91.7
Mulinge *et al*	**AUROC**	0.80	0.72 (p = 0.30)	0.72 (p = 0.29)	0.63 (p = 0.14)	0.65 (p = 0.16)
8 CXCR4-using, 36 R5						

Sens (% sensitivity) and Spec (% specificity) was calculated as described in the Table 1 legend. P-values (two-tailed) for comparisons of area under the receiver operator characteristic curve (AUROC) were performed according to Hanley *et al*[31]. P-values < 0.05 were considered significant and are highlighted in bold italicized text. FPR, false positive rate.
*The subtype C specific WebPSSM$_{SI/NSI}$ algorithm was used for subtype C HIV-1 predictions.

CONCLUSION

Extensive clinical validation of PhenoSeq against MVC treatment outcomes of patients enrolled in Pfizer MVC trials is ongoing. PhenoSeq may enhance access to MVC and future CCR5 inhibitors, particularly in regions where non-B-HIV predominates such as southern Africa, central Asia and southeast Asia where the HIV pandemic is worst. PhenoSeq is freely available at;

www.burnet.edu.au/phenoseq

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