H-1028

Co-receptor Tropism Predictions Based on V3 Loop Sequence in Antiretroviral-Experienced Patients are Specific but Insensitive for the Detection of CXCR4-using Variants.

E. STAWISKI, J. WHITCOMB, E. COAKLEY, S. FRANSEN, E. PAXINOS, J. TOMA, T. WRIN, W. HUANG, N.PARKIN, C. CHAPPEY, C.J. PETROPOULOS

Monogram Biosciences, South San Francisco CA USA.

Corresponding author:
Monogram Biosciences, Inc.
345 Oyster Point Blvd.
South San Francisco, CA 94080
estawiski@monogrambio.com

47th ICAAC
Chicago, IL
Sept. 17-20, 2007

BACKGROUND

- Interest in co-receptor tropism (CRT) assays is growing with the availability of CCR5 antagonists for treatment of HIV-1 infection.
- The Trofile™ CRT assay[1] is being used extensively to support the pre-clinical and clinical development of CRT antagonists.
- The performance of genotype assays in heavily treatment-experienced patients, which is the likely target population for CCR5 antagonists, is unknown.
- V3 region genotype based methods such as the modified 11/25 Charge Rule[2], Decision Trees[3], Position Specific Scoring Matrices (PSSM)[4], and Support Vector Machines (SVM)[3] have been primarily trained and tested on clonal sequences from subtype B sequences
- Previous studies have highlighted technical difficulties in obtaining high quality envelope sequence and limitations of interpretation systems.

METHODS

- Samples from triple-class (NRTI/NNRTI/PI) experienced patients were studied (N = 587).
- CRT was determined by the Trofile™ assay and reported as CCR5, CXCR4, or dual/mixed (DM).
- The data set consisted of 300 CCR5, 277 DM and 10 CXCR4 CRT viruses.
- Population V3 loop sequences were determined using conventional sequencing techniques.
- A total of 27 samples (4.6%) had V3 genotypes that were considered unreportable and not analyzed further
- CRT was predicted (CCR5 only or CXCR4 using) according to published algorithms, the SVM was trained on publicly available data from the Los Alamos National Laboratory HIV database.

CRT V3 Genotype Prediction Definitions:

To evaluate CRT genotype-based predictions, viruses are classified as CCR5-using and DM or CXCR4 -using. All CRT predictions were compared to the corresponding phenotypic CRT determined by Trofile.

Sensitivity = Number of correctly predicted CXCR4-using viruses ÷ Total number of CXCR4-using viruses
Specificity = Number of correctly predicted R5 viruses ÷ Total number of R5 viruses
Discordance = Total percentage of data set incorrectly predicted

Trofile as the Reference Phenotype Assay

- Recent clinical trials highlight the utility of the Trofile assay to determine tropism prior to CCR5 co-receptor tropism antagonist use.
- The ACTG 5211 and MOTIVATE trials compared the activity of a CCR5 co-receptor tropism antagonist versus placebo in treatment-experienced individuals with R5 HIV by Trofile and who also received an optimized background regimen.
  - In ACTG 5211 70%-73% of individuals receiving vicriviroc had week-24 HIV-1 RNA levels <400 copies/mL compared to 18% in the placebo arm[5].
  - In the MOTIVATE 1 and 2 trials 61% of individuals receiving twice daily maraviroc had week-24 HIV-1 RNA levels <400 copies/mL arms compared to 28% in the placebo arms[6,7,8].
- Conversely, the A4001029 study compared the activity of maraviroc versus placebo in treatment experienced individuals with D/M or X4 tropism who received an optimized background regimen[9]. In this study there were no significant differences in HIV-1 RNA outcomes in the maraviroc group compared to those receiving placebo, highlighting the limited antiviral activity of this drug class in the setting of non-R5 tropism.
- Estimation of the positive and negative predictive value (PPV, NPV) of the Trofile assay is hampered by the difficulty in accounting for differences in the optimized background therapy for individual patients.
- The PPV is largely impacted by the sensitivity to detect low level CXCR4-using variants, which is in turn influenced by disease stage and treatment history. A reasonable estimate of PPV can be based on the percentage of patients in a given population with CXCR4-using minor variants near the limit of detection of the Trofile assay:
  - ARV-naive patients: 95 - 97%
  - ARV-experienced patients: 90 - 95%
- NPV approaches 100%. This estimate is based on the observation from the Pfizer A4001029 trial that among individuals with D/M or X4 HIV the observed HIV RNA responses in the maraviroc and placebo groups were not different[9].

Figure 1: Phylogenetic Analysis of *env* V3 and Flanking Sequences

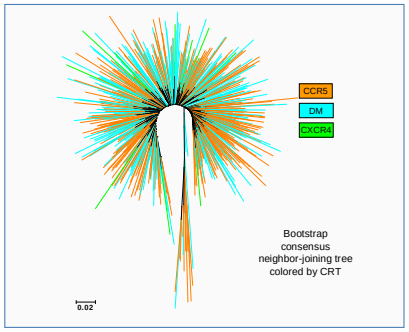


Figure 2: Genotyping can be Challenging due to the Large Number of Ambiguous Bases

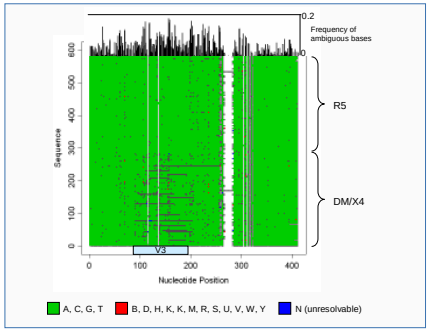
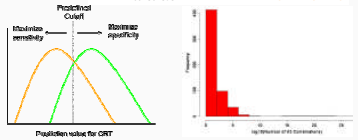


Figure 3: Considering All Possible Combinations of Amino Acid Mixtures

Due to sequence ambiguities that result in more than one amino acid at a single position, one strategy is to generate all possible combinations of V3 sequences and perform CRT genotype predictions on all sequences (example below). Other studies have used arbitrary cutoffs for the minimum percentage needed to have that particular CRT (≥25 %).

		SVM	PSSM	11/25	DT
RS_Pat1	CTRPSNTRRSVSIGPGRAFYTTGDVIGDIRQAHC				
RS_Pat1_0	CTRPSNTRRSVSIGPGRAFYTTGDVIGDTRQAHC	R5	R5	R5	R5
RS_Pat1_1	CTRPSNTRRSVSIGPGRAFYTTGDVIGDTRQAHC	R5	R5	R5	R5
RS_Pat1_2	CTRPSNTRRSVSIGPGRAFYATGDVIGDTRQAHC	R5	R5	R5	R5
RS_Pat1_3	CTRPSNTRRSVSIGPGRAFYTGDVIGDTRQAHC	X4	R5	R5	R5
RS_Pat1_4	CTRPSNTRRSVSIGPGRAFYTTGDVIGDARQAHC	X4	R5	R5	R5
RS_Pat1_5	CTRPSNTRRSVSIGPGRAFYTTGDVIGDIRQAHC	X4	X4	R5	R5
RS_Pat1_6	CTRPSNTRRSVSIGPGRAFYATGDVIGDIRQAHC	X4	X4	R5	R5
RS_Pat1_7	CTRPSNTRRSVSIGPGRAFYATGDVIGDTRQAHC	X4	R5	R5	R5
Consensus:		X4	R5	R5	R5



Cutoffs for continuous predictors of CRT (PSSM and SVM) have a trade off between sensitivity and specificity depending where the cutoff for CRT is. We used default cutoffs which were determined to be optimal cutoffs on the clonal data in which it was trained.

Frequency distribution of the log10-transformed number of possible V3 amino acid combinations. Over 6 amino acid mixtures, amino acid combinations become computationally challenging to calculate.

Figure 4: V3 Loop Sequence-based CRT Prediction Results

For computational efficiency, sequences with more than 100 possible V3 combinations were dismissed (N=146, 26%). This resulted in CRT prediction on 414 sequences. Any instance of an CXCR4 call was predicted as an CXCR4 sequence (≥ 1%).

Method	CXCR4 Sensitivity	CXCR4 Specificity	Overall Discordance
11/25	39%	97%	22%
Decision Tree	24%	98%	27%
PSSM	33%	99%	23%
Support Vector Machine	56%	81%	28%

By these estimates 2 or 3 out of every 4 treatment-experienced individuals with X4 or D/M HIV would be misidentified as R5 depending on the genotypic algorithm

RESULTS

- Based on Trofile™ results, approximately half of the samples were defined as CXCR4-using
- CRT predictions used a combinatorial strategy which allowed V3 sequences with mixtures and up to 100 possible combinations to be included
- However V3 sequences containing very large numbers of mixtures, i.e., >100 possible combinations, could not be analyzed and were excluded, with loss of 26% of the dataset
- Among the remaining analyzable sequences sensitivity for CXCR4-using HIV was poor (24-56%)
- CRT prediction specificities were generally high (81-99%)
- Overall discordance within a given dataset ranged from 22-28%

CONCLUSIONS

- In treatment-experienced patients, envelope sequencing interpretation and tropism prediction is commonly hampered by extreme diversity in variable subpopulations of viruses.
- Currently, state of the art interpretation algorithms significantly under-report the presence of CXCR4-using virus.

ACKNOWLEDGEMENTS

We are grateful to the Monogram Biosciences Clinical Reference Laboratory for performance of all phenotype and genotype assays.

REFERENCES

1. Whitcomb J.M., Huang W., Fransen S., Limoli K., Toma J., Wrin T., Chappey C., Kiss L.D., Paxinos E.E., Petropoulos C.J. Antimicrob Agents Chemother. **51**:566-75, 2007.
2. Hoffman, N.G., Seillier-Moiseiwitsch F., Ahn J., Walker J. M., Swanstrom R. J. Virol. **76**:3852-3864, 2002.
3. Pillai S., Good B., Richman D., Corbeil J. AIDS Res Hum Retroviruses. **19**:145-9, 2003.
4. Jensen, M.A., Li F.-S., van 't Wout A.B., Nickle D.C., et al. J. Virol. **77**:13376-13388, 2003..
5. Gulick R.M., Su Z., Flexner C. J. Infect. Dis. **196**:304–31, 2007.
6. Lalezari J, Goodrich J, DeJesus E, et al. Abstract 104bLB, 14th Conference on Retroviruses and Opportunistic Infections; February 25-28, 2007; Los Angeles.
7. Nelson M, Fätkenheuer G, Konourina I, et al. Abstract 104aLB, 14th Conference on Retroviruses and Opportunistic Infections; February 25-28, 2007; Los Angeles.
8. Van der Ryst E, Cooper D, Konourina I, et al. Abstract WEPEB115LB, 4th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention; July 22-25, 2007; Sydney, Australia
9. Mayer H, van der Ryst E, Saag M, et al. Abstract THLB0215, XVI International AIDS Conference; August 13-18, 2006; Toronto, Canada.