

Relevance of HIV Co-receptor Based Entry

- X4-usage is associated with poor outcome
 - Quicker progression to AIDS
 - Rapid CD4 decline
 - Reduced survival time in untreated individuals
- CCR5 antagonists
 - Screening for X4 usage
 - Resistance after failure

Current Implementations of V3 Genotyping Algorithms are Inadequate for the Prediction of X4 Co-receptor Usage in Clinical Isolates:

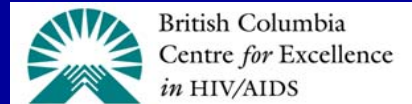
Approaches to Improved Predictions

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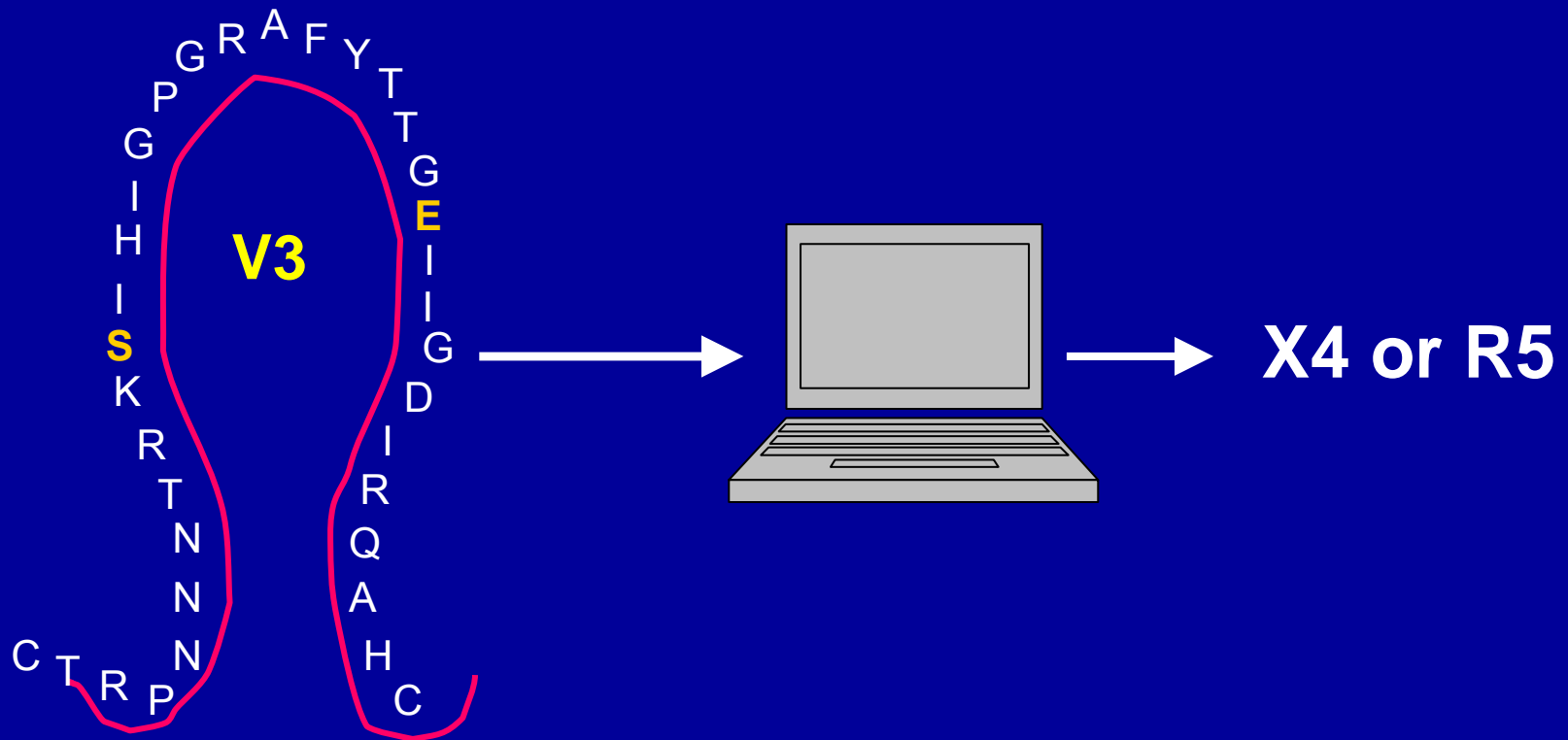


Determining Co-receptor Usage

- Monogram Recombinant Phenotype Assay
 - Uses all of patients envelope
 - Grows pseudotyped virus in cells expressing CCR5 or CXCR4
 - Output measures in Relative Light Units (RLUs)
 - Approximately 3 weeks lab time
 - Relatively costly (?)

Determining Co-receptor Usage

- Potential for Genotypic testing
 - Uses patient V3 loop only
 - Relatively fast (3-7 days)
 - Relatively inexpensive



Predictors from V3 genotypes are reasonably adequate for clonal samples

| Method | Sensitivity on Clonal Samples ⁶ (Specificity > 90%) |
|---|---|
| 11/25 charge rule ¹ | 58% |
| SVM _{genomic} ² | 67% |
| Neural Network ³ | 75% |
| PSSM _{SI/NSI} and PSSM _{X4/R5} ⁴ | 62% (SI/NSI) |
| SVM _{geno2pheno} ⁵ | - |

¹De Jong et. al. J. Virol. 1992

²Pillai et. al. Res. Hum. Retroviruses 2003

³Resch et. al. Virology 2001

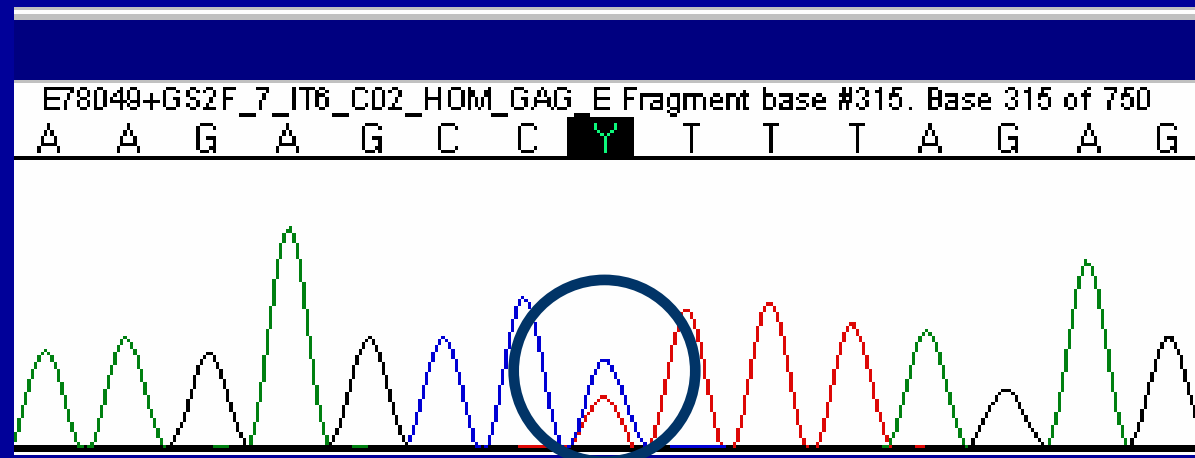
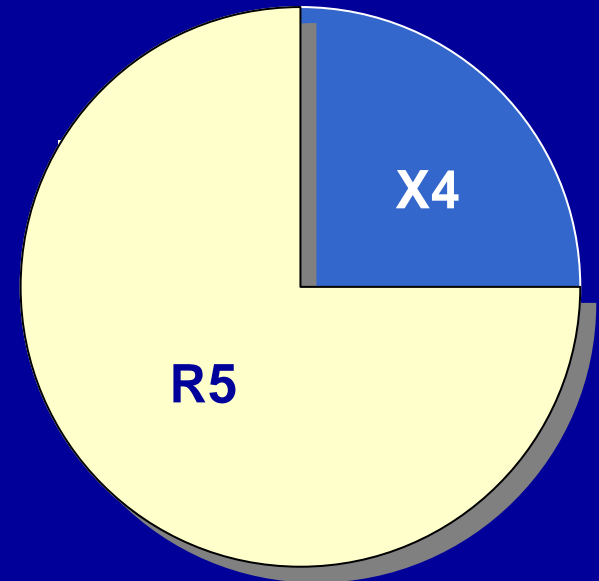
⁴Jensen et. al. J. Virol 2003

⁵Sing et. al. 1st International Workshop on targeting HIV Entry 2005 (Abstract 30)

⁶ Jensen et. al. AIDS Rev. 2003

How Do Known Predictors Compare on Clinically Derived Data?

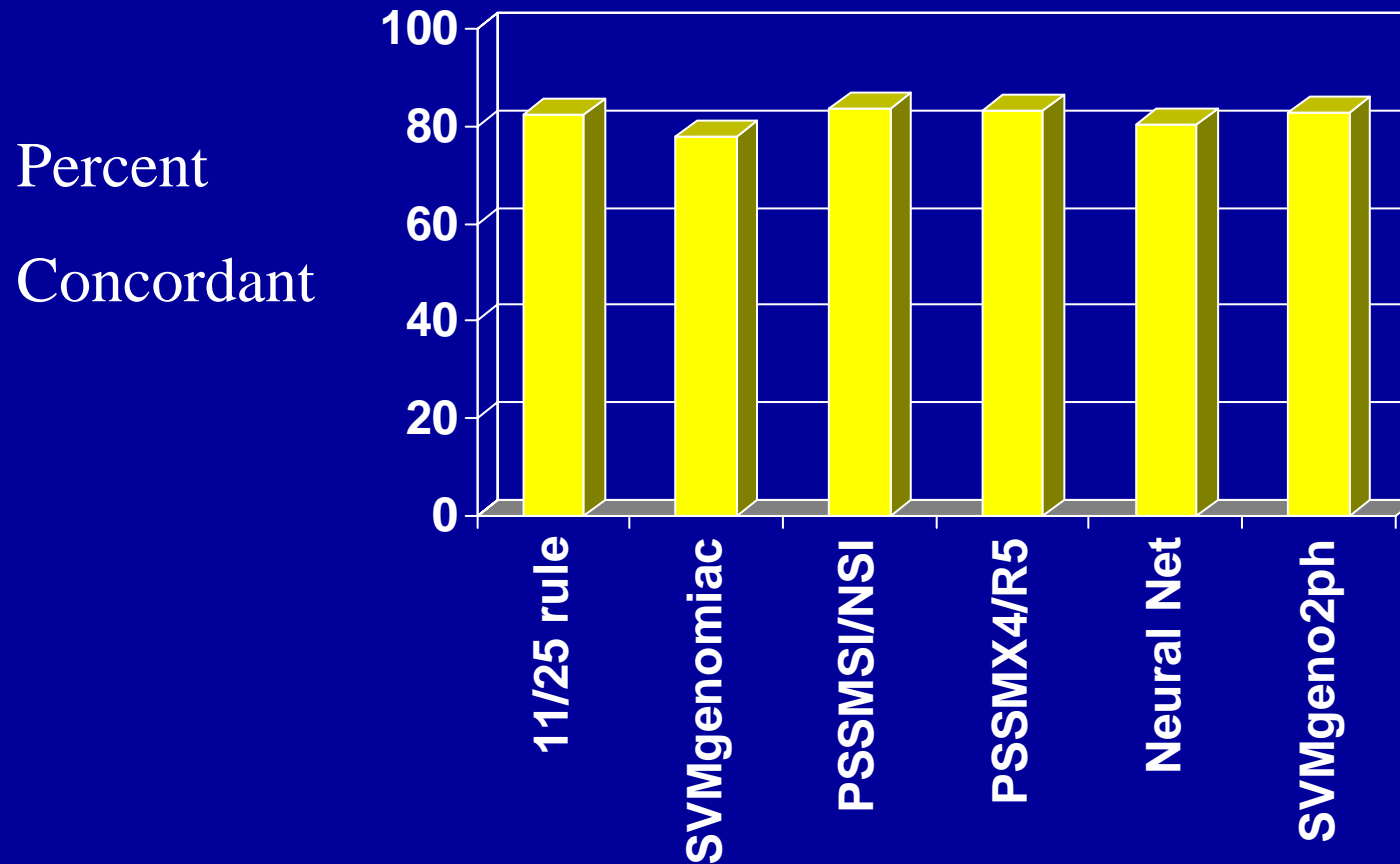
- Predictors trained on clones
- HIV exists as a heterogeneous swarm
 - X4 usually minority species
 - Results in nucleotide mixtures



Methods

- Total of 920 samples (Antiretroviral Naïve)
 - Matched V3 loop genotype and co-receptor phenotype (Trofile)
 - N=769 R5; N=151 X4/R5
 - Median CD4=270; pVL=5.1 log
- Test predicted tropism using 6 published V3 genotypic predictors vs measured phenotype

All methods had good concordance with phenotype...



... but all had very poor sensitivity for X4

| Method | Sensitivity | Specificity |
|---------------------------------|-------------|-------------|
| 11/25 rule | 30.5% | 93.4% |
| SVM_{genomic} | 21.8% | 89.6% |
| PSSM_{SI/NSI} | 33.8% | 95.3% |
| PSSM_{X4/R5} | 24.5% | 96.9% |
| Neural Network | 44.4% | 87.5% |
| SVM_{geno2pheno} | 44.7% | 90.6% |

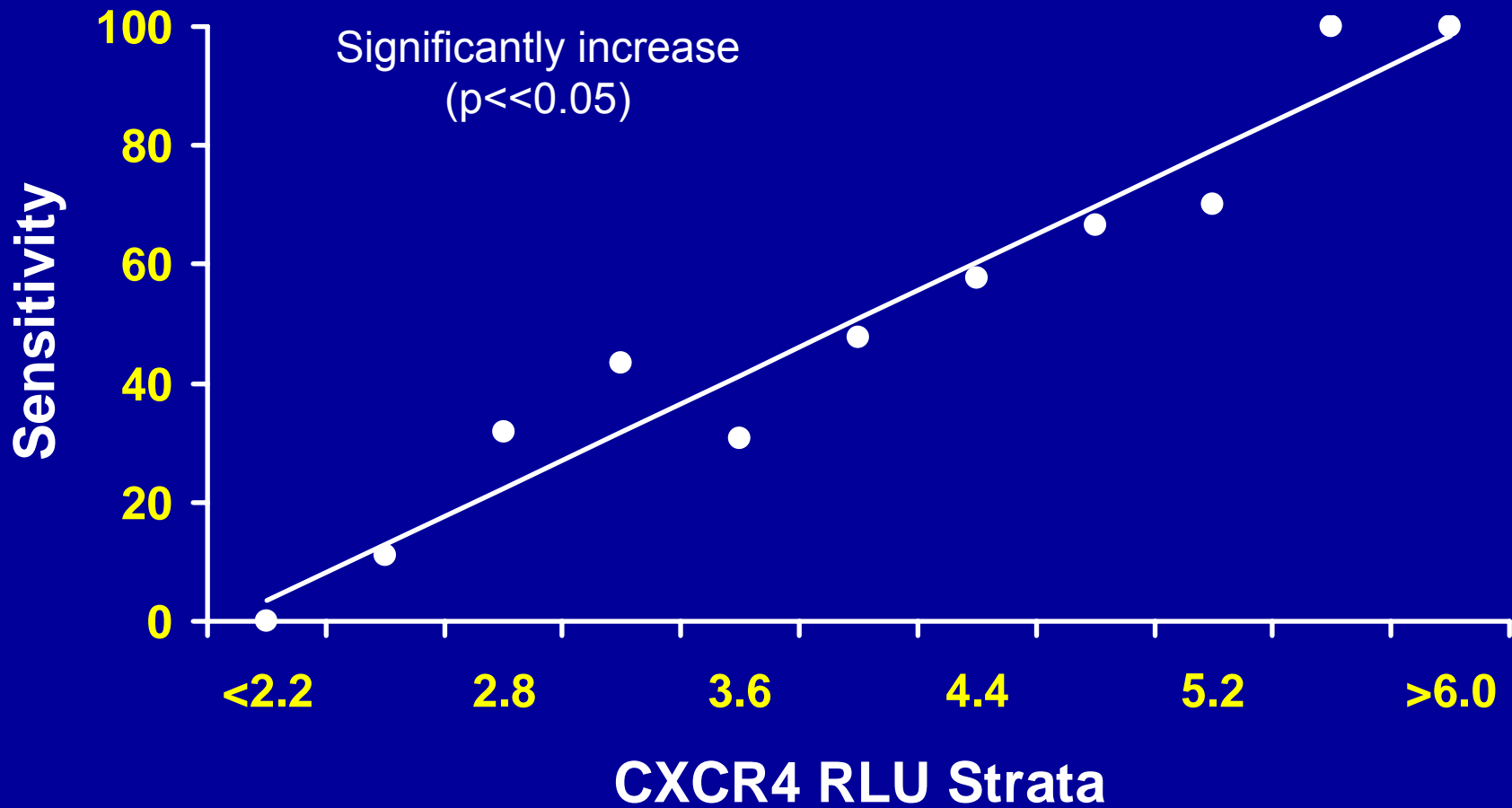
*For sequence ambiguities, classify sample as X4 if $\geq 25\%$ of permutations are classified as X4

... even at relaxed specificity

| Method | Sensitivity | Specificity | |
|---------------------------------|-------------|-------------|-------------------|
| 11/25 rule | 30.5% | 93.4% | |
| SVM_{genomic} | 21.8% | 89.6% | Specificity = 90% |
| PSSM_{SI/NSI} | 33.8% | 95.3% | → 43.7% |
| PSSM_{X4/R5} | 24.5% | 96.9% | → 43.7% |
| Neural Network | 44.4% | 87.5% | |
| SVM_{geno2pheno} | 44.7% | 90.6% | |

*For sequence ambiguities, classify sample as X4 if $\geq 25\%$ of permutations are classified as X4

Sensitivity vs. Strength of Phenotype Response



Clonal Analysis shows minority X4 species not detected

| Sample ID | Observed Phenotype ₁ | 'Bulk' Genotype ₂ | Number 11/25- Clones ₂ | Number 11/25+ Clones ₂ | Percent X4 |
|-----------|---------------------------------|------------------------------|-----------------------------------|-----------------------------------|------------|
| 1 | R5 | - | 47 | 0 | 0.0% |
| 2 | R5 | - | 41 | 0 | 0.0 % |
| 3 | R5/X4 | + | 0 | 46 | 100.0 % |
| 4 | R5/X4 | + | 0 | 44 | 100.0 % |
| 5 | R5/X4 | + | 26 | 8 | 23.5 % |
| 6 | R5/X4 | - | 35 | 10 | 22.2 % |
| 7 | R5/X4 | - | 33 | 9 | 21.4 % |
| 8 | R5/X4 | - | 41 | 4 | 8.9 % |

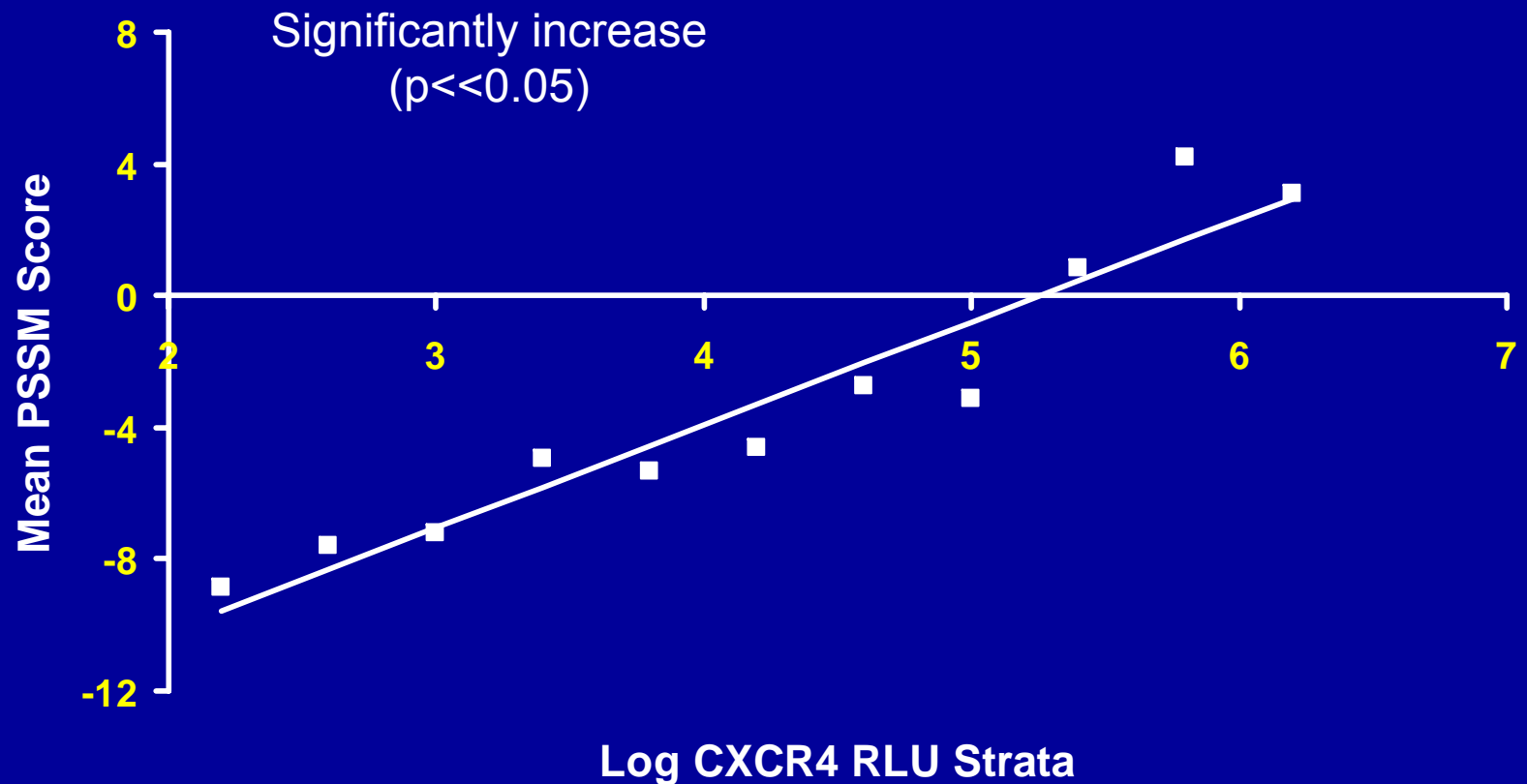
¹Using the Monogram Trofile Assay

²Using the '11/25' rule

Detection of V3 Amino Acid Mixtures Associated with X4-usage

| | <u>X4/R5</u> | <u>R5</u> | (p<<0.01) |
|----------------|--------------|------------|-----------|
| <u>Mixture</u> | 112 | 399 | |
| No Mixture | 39 | 370 | |
| | 74.2% | 51.8% | |

Mean PSSM Score vs. CXCR4 RLU Strata for Samples With No Mixtures



Conclusion

- Current implementations of population V3 genotype-based predictors are inadequate for usage on clinically derived samples due to poor sensitivity

Areas for Improvement (1)

- Improving genotyping sensitivity
 - More aggressive base calling (?)
 - Computational optimization of chromatogram signals
 - new methods to improve genotypic sensitivity (e.g. 454 sequencing) (?)
- Optimizing sensitivity for detecting CXCR4-using virus (not overall concordance)
- Use clinical/structural parameters to improve sensitivity

Areas for Improvement (2)

- Incorporate sequences outside the V3-loop (?)
- train predictors on training sets which match the test sets used
 - Clonal or clinically derived samples
 - Same phenotypic assay
 - Same clade makeup

Acknowledgements

BC Centre for Excellence in HIV/AIDS

- Andrew Low
- Richard Harrigan
- Dennison Chan
- Winnie Dong
- Theresa Mo

University of Georgia

- Mark Jensen

University of North Carolina

- Ronald Swanstrom

Pfizer

Max Planck Institute of Bioinformatics

- Tobias Sing
- Thomas Lengauer
- Oliver Sander

UCSD

- Satish Pillai
- Benjamin Good

MS Research

- David Heckerman
- Jennifer Listgarten

Monogram Biosciences