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**INFECTIOUS DISEASE**

**CASE STUDY**

# Immunosequencing identifies signatures of cytomegalovirus exposure history and HLA-mediated effects on the T-cell repertoire

Emerson RO, *et al.* (2017) *Nature Genetics* (49):659-665
**APRIL 2017**

## WHY IMMUNOSEQ?

Allows rapid, accurate identification and quantification of millions of sequences from hundreds of samples at high throughput

Data output easily incorporates into a variety of analytic tools for complex analysis

Accurate quantitation allows identification of index value to determine CMV status

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## BACKGROUND

The T-cell repertoire of an individual is large and vastly diverse with about $10^7$ unique sequences; this diversity is necessary to effectively recognize and respond to myriad antigens. It's much more common than would be mathematically expected for clones to be shared among different individuals. These public clones may allow for the determination of pathogen exposure status through immunosequencing.

## AIM

- Identify public clones significantly associated with Cytomegalovirus (CMV)
- Determine MHC I HLA restriction of these clones
- Develop an algorithm to successfully classify patients as CMV+ or CMV- and predict HLA type for MHC class I molecules using the immunoSEQ® Assay

## METHODS

1. **Cohort 1:** PBMCs from 666 bone marrow donors with known CMV status and HLA type → extract gDNA → **immunoSEQ (TCRB)**
2. Computationally identify sequences associated with CMV+ status and stratify these by HLA type
3. **Cohort 2:** PBMCs from separate 120 bone marrow donors → extract gDNA → **immunoSEQ (TCRB)**
4. Validate CMV status prediction on cohort 2

## RESULTS

- Identified 164 CMV associated clones in cohort 1 (figure 1)
- Calculated incidence of CMV associated clones predicts CMV status (figure 2) and confirmed with cross validation in cohort 1 and independent testing in cohort 2 (figure 3)
- Predicted overall CMV status with a sensitivity of 0.9 and specificity of 0.89
- Inferred HLA-A and B type with high sensitivity and specificity for the common alleles, with both metrics decreasing for the less common alleles (HLA-A in figure 4)

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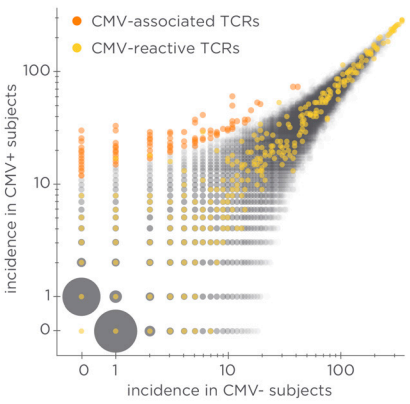


**Figure 1.** Comparison of all sequences in CMV+ and CMV- subjects. Clones reactive to CMV in green, clones associated with CMV in orange.

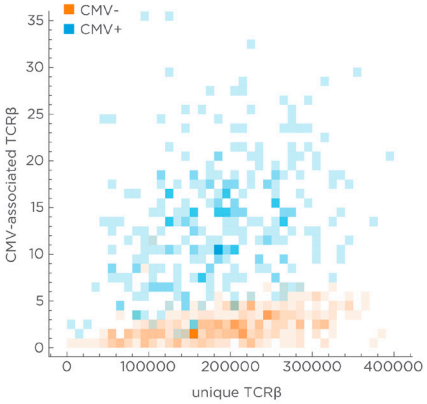


**Figure 2.** Plotting the number of CMV associated clones versus the number of unique clones per subject reveals an incidence that is predictive of serostatus.

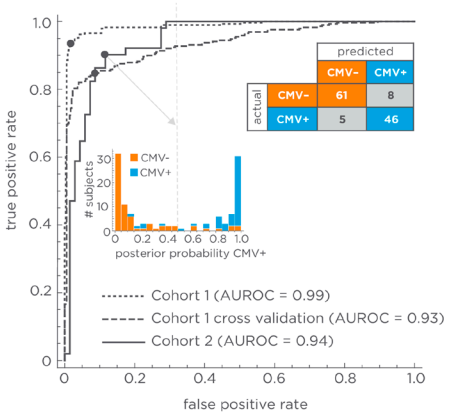


**Figure 3.** ROC curves for cohort 1, cross validation and cohort 2. Inset is confusion matrix for cohort 2 showing sensitivity of 0.90 and specificity of 0.89.

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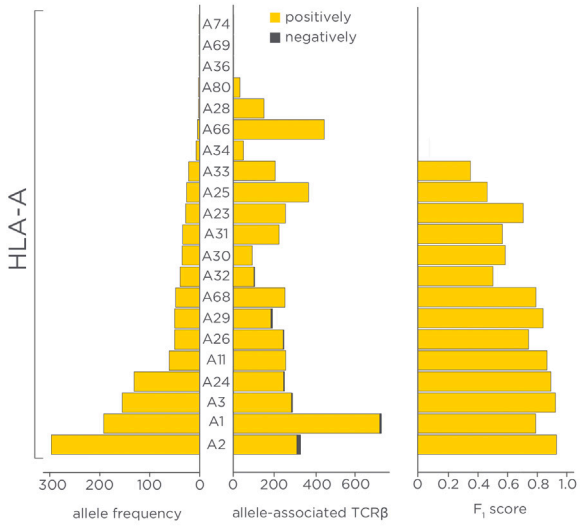


**Figure 4.** An example of HLA-A allele frequency (left) number of allele-associated clones (middle) and prediction accuracy score (right).

## CONCLUSIONS

- Data suggests immunologic phenotypes (CMV status and HLA type) shape the TCRB repertoire and can be inferred using the immunoSEQ Assay
  - Sensitivity and specificity will only grow as additional data is acquired
- The presence of the CMV-associated public TCRs defined in this study could be sufficient to serve as a diagnostic predictor for CMV status

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