

Genome-scale neoantigen survey using ATLAS™ in a non-small cell lung cancer patient identifies unique vaccine candidates that are not predictable by algorithms

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- Despite the unprecedented efficacy of checkpoint blockade (CPB) therapy in treating some cancers, a large number of patients still fail to respond these interventions. Recent data indicate that the combination of CPB and neoantigen vaccines may prolong survival in some cancer patients.

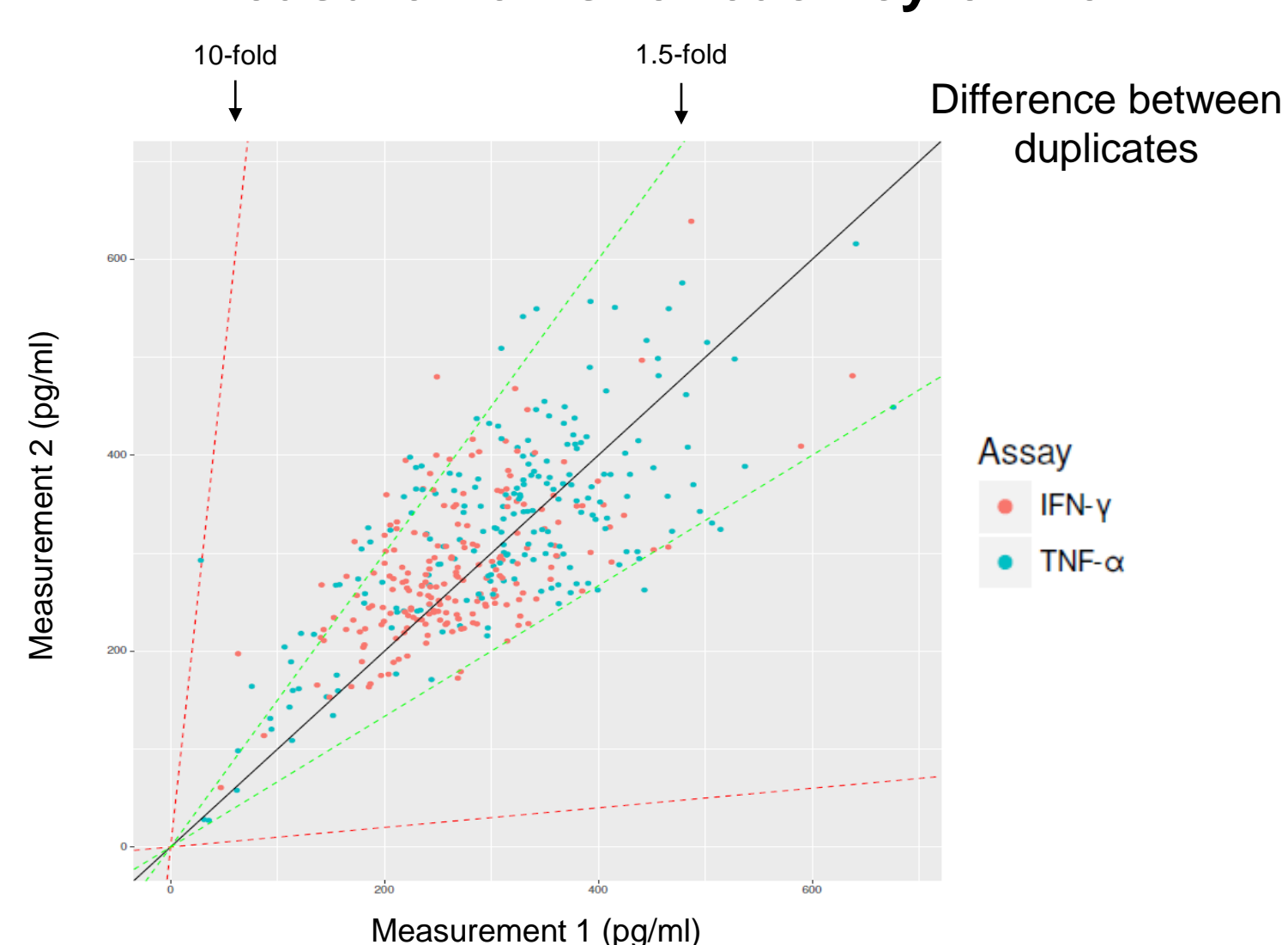
- Current approaches to neoantigen prioritization involve deep sequencing of tumor samples, followed by selection of epitopes based on prediction algorithms primarily examining MHC class I binding affinity and processivity. However this strategy has proved challenging with the majority of candidates lacking immunogenic activity in patients.

- ATLAS™ is a T cell antigen discovery platform in which putative antigens are expressed as individual clones that can be processed by any subject's antigen presenting cells (APCs) and presented as peptide epitopes in the context of their own MHC class I or II molecules.

- We identified both MHC class I and II neoantigens inducing T cell cytokine responses in a non-small cell lung cancer (NSCLC) patient. Approximately 50% of MHC class I neoantigens were not predicted by multiple *in silico* methods; MHC class II neoantigens can not be effectively predicted using current *in silico* methods.

Neoantigen Identification

Figure 4. Good alignment between replicate measurements for each cytokine



- Example data for CD8⁺ T cells post-treatment:
- Biological analysis: >3SD of mean of negative control
 - 7 antigens with significant biologically relevant IFN γ responses compared to negative controls – **most robust hits**
- Statistical analysis: Wilcoxon rank sum, $p < 0.05$
 - 57 antigens have IFN γ responses statistically different from negative control – **28% overall hit rate**

Conclusions

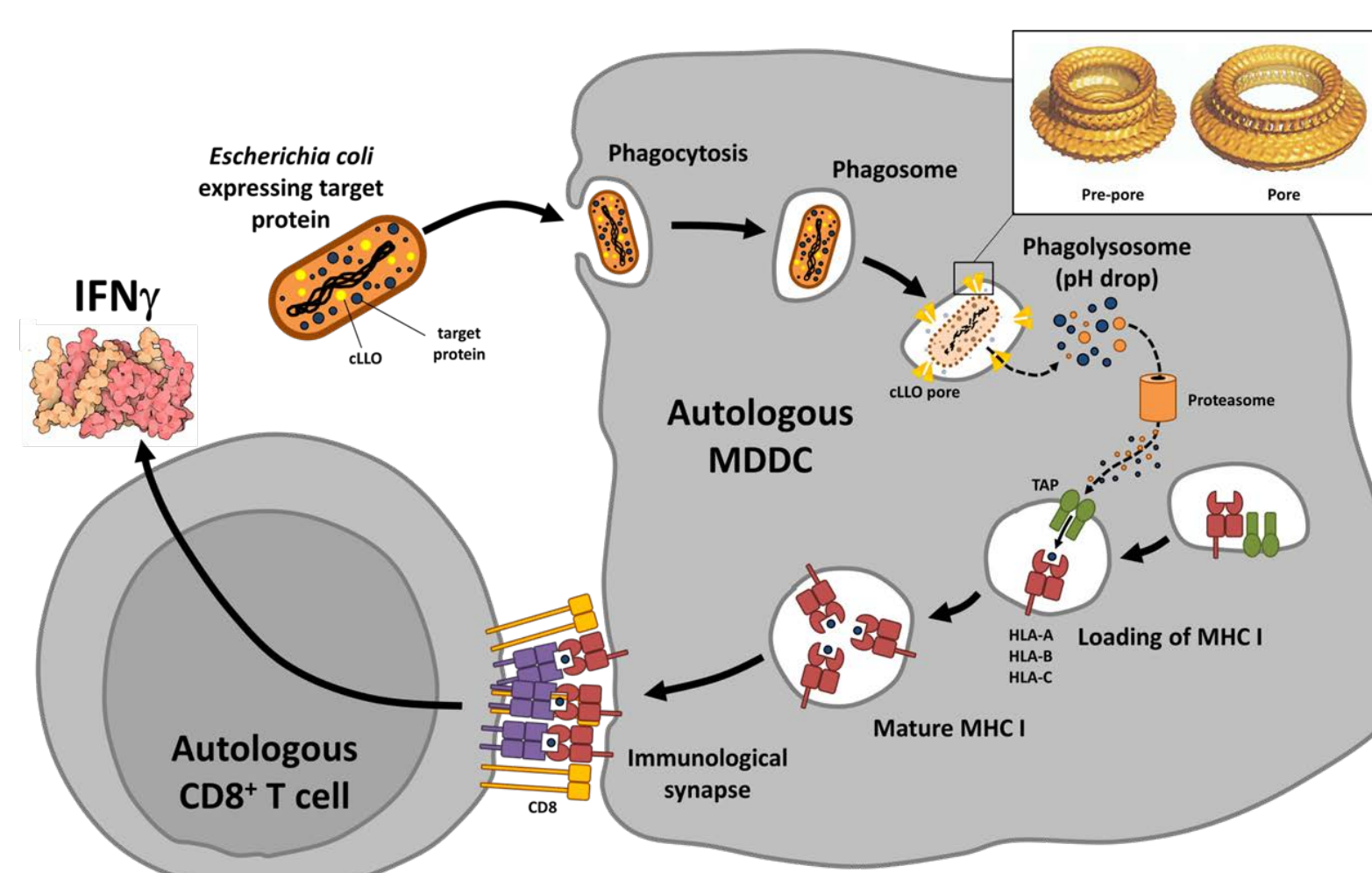
- ATLAS was able to detect neoantigen-specific immune responses in NSCLC patients both pre- and post-CPB therapy
- Based on ATLAS, not all T cell antigens are stimulatory; suppressive responses to T cell antigens were also observed; while these epitopes may emerge from predictive algorithms, their suppressive activity cannot be predicted
- CD4⁺ and CD8⁺ T cell antigens are mostly unique with only 3% of putative neoantigens eliciting responses from both T cell subsets
- Breadth of CD4⁺ T cell responses based on IFN γ secretion increased upon checkpoint therapy
- Current MHC class I epitope prediction algorithms have a high false positive rate, and missed a large number of relevant and suppressive antigens

Acknowledgements

- Thank you to the patient and their family who consented to participate in this study

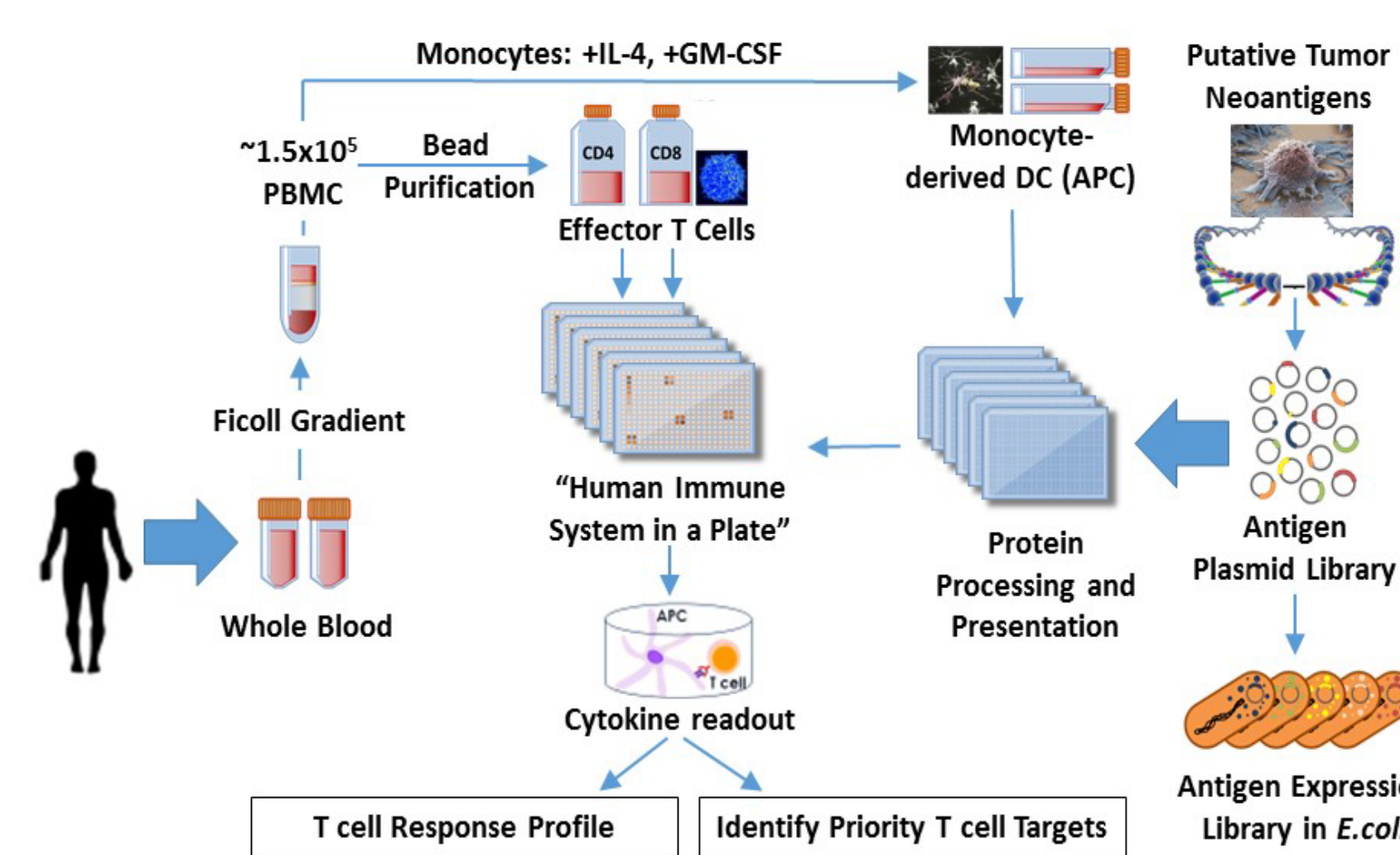
Methods: The ATLAS™ Platform

Figure 1. Lysteriolysin O (cLLO) facilitates MHC class I presentation by MDDC



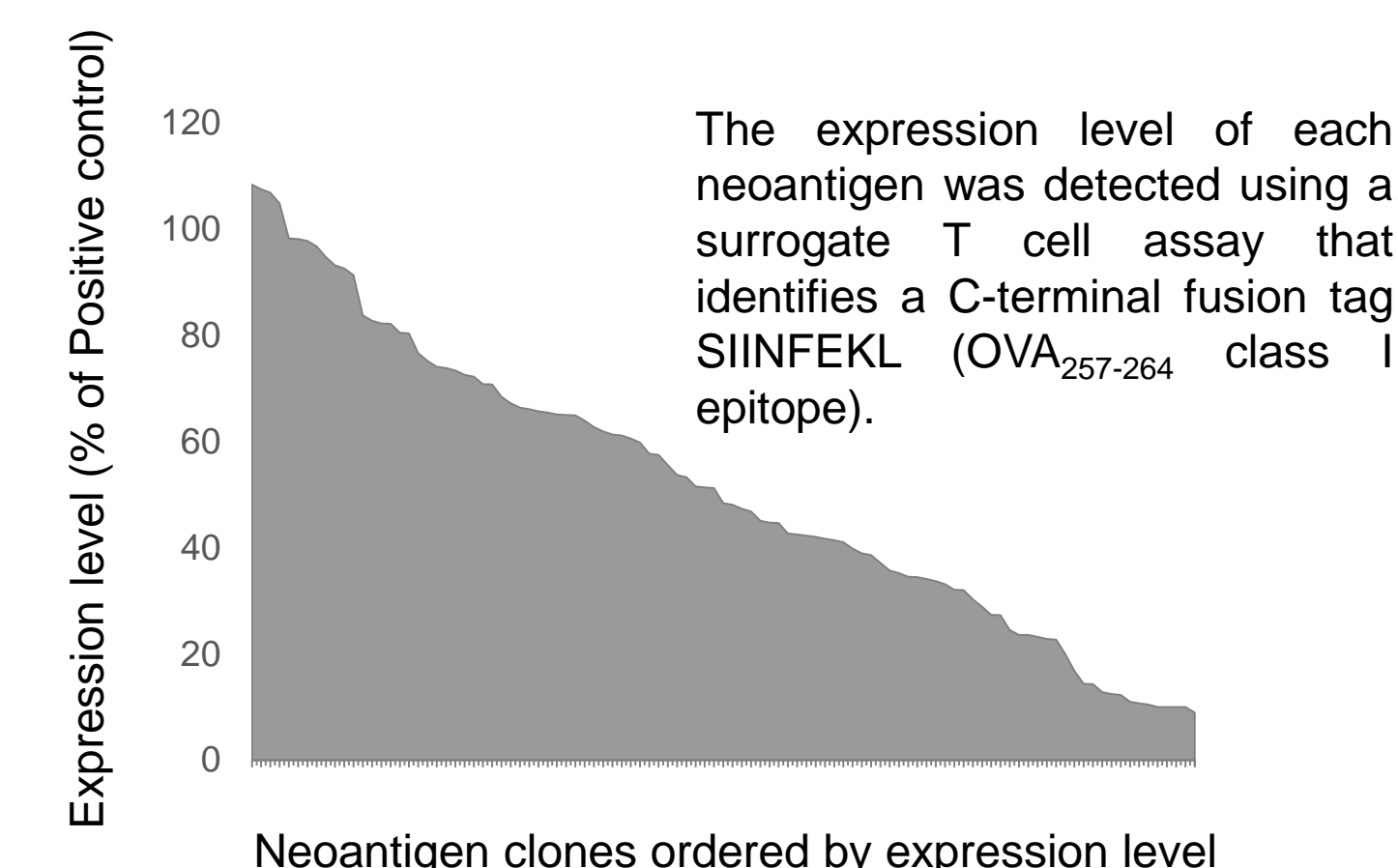
- MHC class II presentation to CD4⁺ T cells facilitated through conventional endocytic route of processing of *E. coli* not co-expressing cLLO².
- Whole exome sequencing of a tumor biopsy from a NSCLC patient identified 202 somatic mutations. Each somatic mutation was recombinantly expressed in *E. coli* to create a screening library.

Figure 2. ATLAS™ technology workflow



- Protein coding sequences were synthesized covering ~100 amino acids with the mutation centrally located, cloned into the ATLAS™ expression vector, and sequence verified. Pre and post-treatment peripheral blood mononuclear cells (PBMC) were collected from one patient treated with, and responding to, anti-PD-1 therapy (pembrolizumab).

Figure 3. Expression verification



- The expression level of each neoantigen was detected using a surrogate T cell assay that identifies a C-terminal fusion tag SIINFEKL (OVA₂₅₇₋₂₆₄ class I epitope).
- PBMC were enriched by density gradient centrifugation. CD4⁺ and CD8⁺ T cells were sorted and non-specifically expanded, and CD14⁺ monocytes were differentiated into dendritic cells (MDDC).
- Library clones were screened in duplicate using 2,000 MDDC and 80,000 T cells per well, at an *E. coli*:MDDC ratio of 250:1; 20 replicates of *E. coli* expressing Neon Green were included as negative controls.
- Assay supernatants were harvested at 24hr and cytokines levels analyzed using a Meso Scale Discovery custom kit, for detection of IFN γ and TNF α .

CD8⁺ T cell responses

Figure 5. Multiple neoantigens were identified through CD8⁺ T cell responses pre- and post-checkpoint blockade therapy

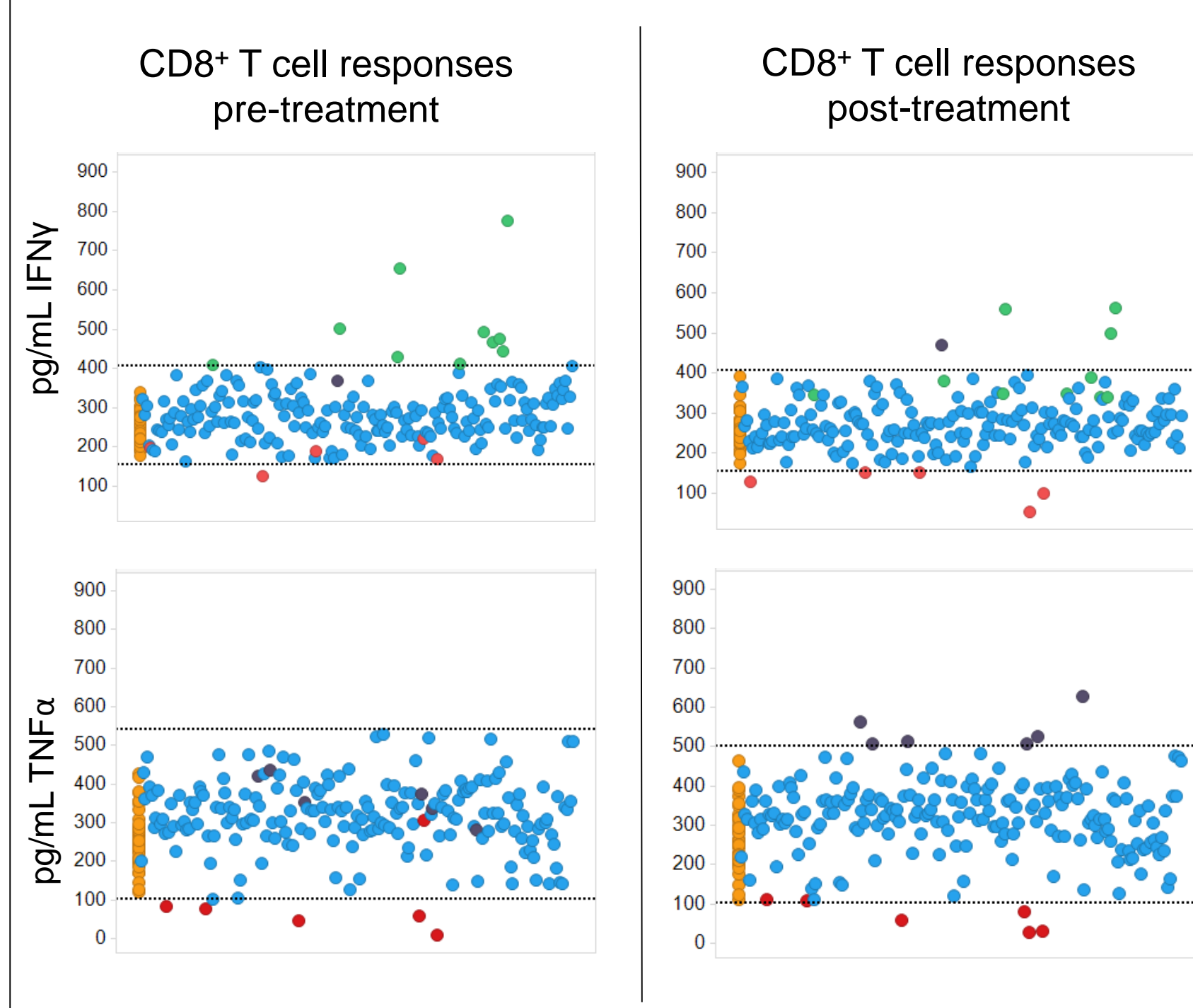
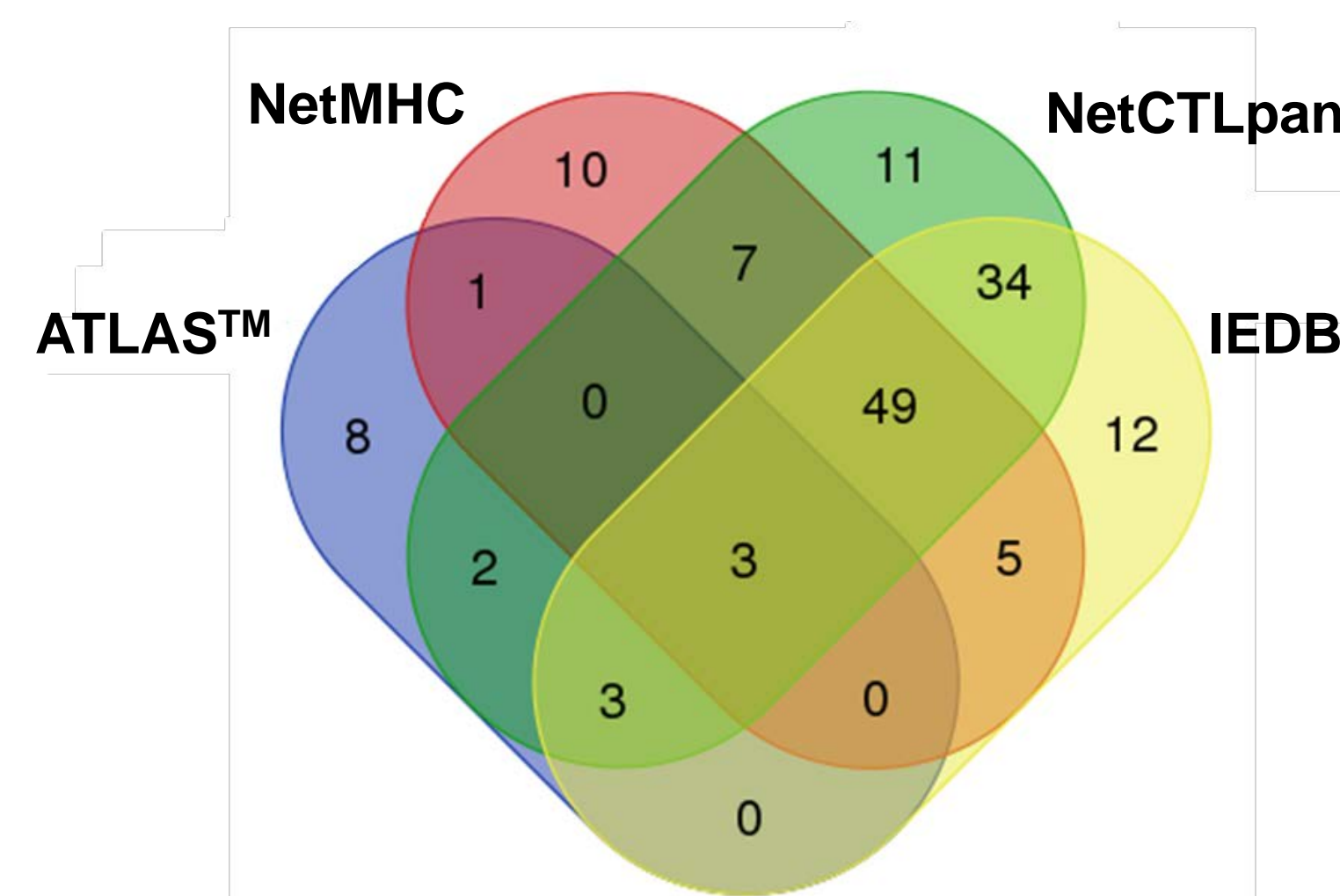


Figure 7. Limited overlap between CD8⁺-specific T cell neoantigens identified by ATLAS™ and epitope prediction algorithms



Results

Non-specifically expanded CD4⁺ or CD8⁺ T cells were screened in duplicate against autologous MDDC individually pulsed with *E. coli* expressing each of 195 or 201 somatic mutations, respectively. Clones that induced mean IFN γ or TNF α responses that were statistically different from background (Wilcoxon Rank Sum, $p < 0.05$) and exceeded 3 standard deviations (SD) of the mean of the negative control Neon Green clones (N=20) were considered antigens (indicated by horizontal dotted line).

- NeoAg pre-treatment
- NeoAg post-treatment
- Suppressive NeoAg
- Negative controls (NG)

Table 1. Mutations identified as neoantigens based on IFN γ and TNF α responses

| T cell responses | Pre-treatment | Post-treatment | Both |
|------------------|---------------|----------------|------|
| CD8 ⁺ | 5% | 5% | 1% |
| CD4 ⁺ | 10% | 17% | 5% |

MHC class I epitopes were predicted for all screened neoantigens using three commonly used algorithms: NetMHC, NetCTLpan and IEDB, and using patient-specific haplotypes HLA-A*02:01/*32:01, HLA-B*40:01:02/*45:01:01, HLA-C*06:02/*03:04¹.

All of the unique epitopes identified using these algorithms carry the tumor somatic mutation.

References

- Rizvi, *et al.* Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer (2015) *Science*. 348(6230): 124-8.
- Hu, *et al.* Escherichia coli expressing recombinant antigen and listeriolysin O stimulate class I-restricted CD8⁺ T cells following uptake by human APC (2004) *J Immunol* 172(3): 1595-601.

CD4⁺ T cell responses

Figure 6. Increased breadth of CD4⁺ T cell IFN γ responses to potential neoantigens post checkpoint blockade therapy

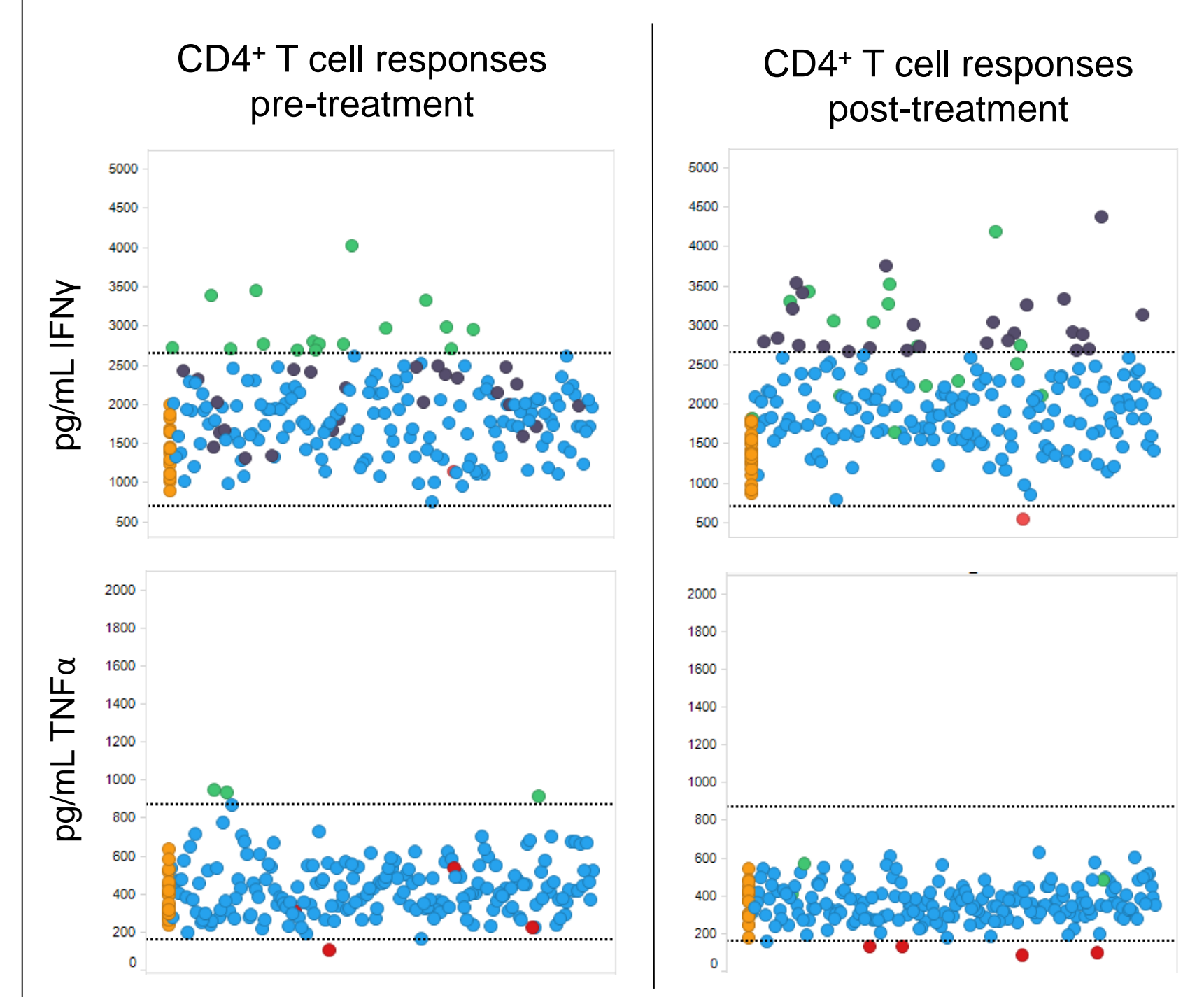


Figure 8. Epitope predictions had a high false positive rate and missed relevant and inhibitory antigens

