High Parameter Flow Cytometry and A New Computational Platform Reveal Unique Cell Phenotypes That Predict Melanoma Outcomes

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For patients with metastatic melanoma, immunotherapies have revolutionized treatment, but many patients fail to respond, and mechanisms of resistance remain unclear. Moreover, many immunotherapy modalities are in clinical trials, but there are few biomarkers known to predict response. We believe a major reason is the lack of in-depth immune profiling, and a lack of tools that completely and comprehensively examine datasets. The work presented here represents a new approach to biomarker research, which combines 30-parameter flow cytometry (HPFlow) with CytoBrute, a rapid computation platform that performs high parameter Boolean analysis that otherwise might be intractable.

We tested PBMC from a clinical trial in which metastatic melanoma patients received different sequences of nivolumab (anti-PD1) and ipilumamab (anti-CTLA4). One sequence of drugs was more effective, but both cohorts had treatment responders and disease progressors. Using new HPFlow, we tested patients (n=35) at baseline, week 13 (between drugs), and week 25. We designed five 22+ color panels, which measured immune checkpoints, checkpoint ligands, T-cell maturity, transcription factors, and activation. In lieu of dimension-reduction (like t-sne) to analyze the data, we designed CytoBrute to compute the %cells expressing every possible combinations of markers, from single markers, to combinations of two, three, and beyond. CytoBrute is fast; it reduces a process that takes 4 hours per file using R down to just 7 seconds.

We found several phenotypes whose frequency at baseline was associated with response to each drug sequence. These included simple ones, like CD4+ CD95+ T-cells, which were higher at baseline in people destined to progress ($p=0.0048$). We also found various resting memory CD4+ T-cells (defined by simple sets of two markers) were elevated in individuals destined to progress, and this relationship was unique to one of the drug sequences. The fact that different markers for similar cells, shared this relationship supported our interpretation. We also found a complex phenotype (defined by 14 markers) which, when present at low frequencies, predicted a favorable survival profile (50% of patients were alive beyond 40 months after diagnosis, $p=0.00095$). In contrast, 50% of patients with high levels perished in 10 months. We also found a new subset of regulatory T-cells that was important in responses, expressing suppressive markers like GARP and CD39, but not classical regulatory markers like CD25 and FoxP3.
Finally, we integrated CytoBrute into the machine learning algorithm LASSO, identifying specific PD1+ subsets that were completely abrogated with one regimen, but not the other. This result provided the first insight into specific subsets that might be preferentially lost with that immunotherapy regimen. The machine learning algorithms generated models that could predict outcomes with area-under-the-curve values from 0.7 to 0.8, rivaling biomarkers currently used in immune-oncology. Finally, we discriminated patients and healthy controls with great specificity and sensitivity (AUC = 0.96), establishing very clear differences in immune composition even in the peripheral blood of melanoma patients. This supports the feasibility of blood-based immune monitoring (rather than invasive tumor-based analysis). Collectively, we have demonstrated a novel and powerful approach to interrogating complex immunophenotypes, which has great potential for biomarker research.