Predictive Biomarkers of Immunotherapy Toxicity in Metastatic Melanoma Patients

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Backround

• Immune checkpoint inhibitors (ICIs), anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab and pembrolizumab), have improved the overall survival and response rates in patients with metastatic melanoma6. More recently, the combination of anti-CTLA-4 and anti-PD-1 has been shown to yield superior clinical outcomes compared to single agent blockade.
• Despite this progress, a substantial proportion of patients receiving immune checkpoint inhibitors develops severe treatment-induced side effects, an issue magnified in patients receiving combined regimens. 28% of patients on combination therapy developed severe toxicity and 36% of patients required treatment termination due to toxicity.
• The mechanisms underlying ICi efficacy and toxicities have yet to be fully elucidated, however they are thought to result from T-cell dysfunction, which increases the anti-tumor adaptive immune response and subsequently reduces T-cell tolerance to antigens previously recognized as “self” leading to autoimmunity.1
• To date there is no biomarker test to predict immunotherapy-associated immune related adverse events (irAEs)2.

We hypothesize that a subset of melanoma patients have a baseline autoimmune susceptibility, characterized by a repertoire of specific, pre-existing autoantibodies, which predicts the development of irAEs following ICi therapy.

Study Cohort: Comprised of patients diagnosed with advanced melanoma from 2011-2016 and treated at NYU Langone Medical Center with immunotherapy. All patients included in the analysis consented to participation in the enrolled in the Immunotherapeutics Melanoma Cooperative Group (IMCG) Biospecimen database protocol.

Data Collection: Pre-treatment sera samples were prospectively collected from three treatment-based cohorts: a) anti-CTLA-4 (n=37) b) anti-PD-1 (n=27) and c) anti-CTLA-4+anti-PD-1 combination therapy (n=11). The anti-CTLA-4 cohort also had patient-matched post-treatment samples collected and run on the HuProt™ human proteome microarray (CDI laboratories), to profile the autoimmunity (autoantibody) intensity. Patients were stratified by therapy type and immunotherapy-induced toxicity outcomes, per Common Terminology Criteria for Adverse Events (CTAE) guidelines no (CTAE grade 0), mild (CTAE grade 1-2), and severe (CTAE grade 3-5). From these groupings we derived five toxicity comparisons: i) no vs. mild toxicity, ii) no vs. severe toxicity, iii) mild vs. severe toxicity, iv) no vs. mild and severe toxicity, and v) no and severe toxicity. As quality control, two identical sera samples were collected from 10 immunotherapy patients to assess the reproducibility of the HuProt™ microarray.

Statistical Methods: Fisher’s tests were used to assess differences in patient demographics and disease characteristics from each treatment cohort. A non-parametric test (Wilcoxon Rank Test) was performed to group to assess for significant differences in IgG3 MAb antibody between toxicity outcomes. A positive hit rate was defined as a) significant difference in antibody intensity (p<0.05) b) antibody intensity beyond the peak array intensity plus two standard deviation; c) an intense fold change of at least two between toxicity groups.

Array Reproducibility and Validation

Proteome microarray: consistent detection of autoantibody intensities in both inter- and intrapatient duplicates

Microarray validation: post-treatment increase in anti-CTLA-4 antibodies detected

Toxicity-associated autoantibodies enriched for nuclear localization and immunoregulatory pathways

Compared to entire HuProt™ array, toxicity-associated autoantibodies enriched for nuclear targets, suggestive of immunoregulatory phenotype

Toxicity-associated autoantibodies common to specific immunoregulatory pathways had significantly different levels based on toxicity outcomes

SVM Classifier Models

Panels of autoantibodies, derived from SVM analysis, suggest a predictive signature of ICi toxicity

Summary & Conclusions

• Toxicity-associated autoantibodies were identified in each treatment cohort, subsets were linked to site-specific toxicities and treatment termination.
• As seen in many autoimmune diseases, there was an enrichment for nuclear targets among the autoantibodies associated with toxicity.
• Autoantibodies common to several immunoregulatory pathways had significantly different levels based on toxicity outcome.
• SVM classifier panel identified features that could be used to develop biomarker panels to identify patients prone to irAEs following treatment with immune checkpoint inhibitors.
• In the context of this study, the autoantibodies could reflect B-cell exposure to mutant tumor antigens and/or baseline differences in cellular metabolism which may predispose a subset of patients to toxicity.
• Our study lays the groundwork for further analysis of the role of autoantibodies as predictors of ICi-induced toxicity in melanoma and suggests their potential utility in other cancers.

References