LONGITUDINAL SCRNASEQ PROFILING OF PBMCS FROM MELANOMA PATIENTS TREATED WITH ANTI-CTLA4 OR ANTI-PD1/CTLA4 COMBINATION ELUCIDATES MECHANISMS OF IMMUNOTHERAPY RESISTANCE

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Background Immunotherapy (IO) has changed the treatment paradigm for patients with metastatic melanoma. IO combination therapy such as anti-PD1 nivolumab (NIVO), anti-CTLA4 ipilimumab (IPI) and anti-LAG3 relatlimab (RELA) have replaced chemotherapy as first line treatment. Although candidate biomarkers such as interferon gamma gene expression, BRAF mutation, PD-L1 tumor expression, high tumor mutational burden and CD8 T-cell infiltration have been explored for their predictive value, no definitive biomarkers for IO response have been identified. Our study explores novel exploratory biomarkers that can help us understand which metastatic melanoma patients will have an increased benefit from either anti-CTLA4 or anti-CTLA4/PD1 treatment.

Methods ScRNAseq profiling of peripheral blood mononuclear cells (PBMCs) was performed on a subset of CheckMate-069 clinical trial (NCT01927419) samples, which included 52 patients with metastatic melanoma treated with either IPI (n=26) or NIVO + IPI (n=26), using the 5 prime 10x Genomics platform (total n=52, samples=104). PBMCs from patients were profiled at Baseline (C1D1) and after 2 cycles of therapy (C3D1) and separated as responders (R, n=20) or non-responders (NR, n=32). We deployed Seurat R package for cell phenotype determination including Tregs and interrogated drug response in R vs. NR, mechanisms of drug resistance/action, pharmacodynamic (PD) markers, and biomarker of response.

Results ScRNAseq analysis revealed that peripheral regulatory T-cells (Tregs) did not decrease post IO regardless of treatment type (IPI or NIVO+IPI) or response. In fact, the proportion of Treg cells increased post IO regardless of treatment or response, despite an observed decrease in their cell of origin proportion (CD4+ T-cells) (figure 1A). Although Tregs do not decrease, their ‘suppressive’ phenotype was down-regulated preferentially in responders as indicated by the selective downregulation of EZH2 and FOXP3 expression in responders (figure 1B). A lower Treg cell proportion at Baseline and higher CD8+ central-memory cell proportion at C3D1 served as a predictive response biomarker of IPI and NIVO+IPI respectively. The ratio of CD8+ effecter memory to Treg also predicts outcome benefit to anti-CTLA4 and may serve as a potential predictive biomarker (figure 2).

Conclusions Our analysis serves to elucidate a missing piece of anti-CTLA4 therapy mechanism of action and identifies potential new biomarkers that can be applied to inform stratification for combination therapy trials. Although Tregs were not depleted, EZH2 was selectively upregulated in T-regs of non-responders upon IPI treatment indicating that a combination therapy of EZH2i+anti-CTLA4 may increase response rates. Additionally, CD8+ effector memory/Treg cells ratio may serve as a new biomarker predictive of anti-CTLA4 benefit.

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Trial Registration NCT01927419

Study of Nivolumab (BMS-936558) Plus Ipilimumab Compared With Ipilimumab Alone in the Treatment of Previously Untreated, Unresectable, or Metastatic Melanoma (CheckMate 069)

Ethics Approval All subjects consented to this research.

Consent Written informed consent was obtained by BMS from the patient for this exploratory research and publication of the results.
Abstract 624 Figure 2  A higher CD8 effector memory cell to T-reg ratio at baseline predicts better outcome to Ipi

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