Background Though FDA-approved for use after surgery for stage III/IV melanoma in the adjuvant setting, ipilimumab (anti–CTLA-4) and interferon-α are not typically used because of the improved toxicity profile and relapse-free survival (RFS) benefits seen with anti–PD-1–based regimens in this patient population. However, it is important to study samples derived from anti-CTLA-4 treated patients from the mechanistic perspective to understand its contribution to ipilimumab/nivolumab therapy and to study single agent CTLA4 therapy in the adjuvant cases of BRAF wild-type patients who were re-resected and previously received anti-PD-1 therapy. Recently published data from our group indicated that nitric oxide (NO) increased in immune effector cells among patients treated with longer RFS, whereas NO also increased in immune suppressor cells among patients with shorter RFS. Given this unexpected dichotomy, we measured the posttranslational modification in the immune cells that is a direct result of increased NO levels on the interferon response protein STAT1 important for response to anti-CTLA-4 therapy/interferon responsiveness. Nitration is a stable post-translational modification of NO metabolism and STAT1 is nitrated at the same position (Y701) as it is phosphorylated.

Methods Peripheral blood mononuclear cells (PBMCs) collected from 35 patients at day 0 and day 155 of ipilimumab therapy were analyzed for phosphorylated STAT1 (pSTAT1; using flow cytometry) and nitrated STAT1 at position 701 (nSTAT1; using liquid chromatography with tandem mass spectrometry). Patients were divided into groups based on RFS post ipilimumab therapy.

Results Pre- and post-therapy PBMCs were available from 35 patients with stage IIIC/IV melanoma. The median patient age was 58 years (range, 21-78 years), and 63% of patients were male. pSTAT1 levels had the least variability in the tercile of patients with the longest RFS (P=0.002). Higher pSTAT1 levels in PBMCs were associated with worse RFS before (P = 0.007) and after therapy (P = 0.036) with ipilimumab. Expectedly, pSTAT1 levels in PBMCs increased following ipilimumab therapy. Patients whose STAT1 remained nitrated had decreased RFS, but intriguingly, increases in nitration of STAT1 following ipilimumab therapy was associated with favorable outcomes (P=0.01).

Conclusions These data suggest that interferon responsiveness is regulated by changes in nitration of STAT1. Nitration of Y701 on STAT1 may regulate excessive interferon responses by limiting pSTAT1 phosphorylation for successful ipilimumab-based therapy in melanoma and deserves further study in the adjuvant and metastatic settings.

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Trial Registration NCT00084656

Ethics Approval The study was approved by Moffitt Cancer Center Scientific Review Committee. The University of South Florida IRB determined that the study was non human subjects research as the PBMC specimens were deidentified prior to this research activity.