Clinical and Translational Results from a Phase 1b Study of the HDAC Inhibitor Mocetinostat with Ipilimumab and Nivolumab in Unresectable Stage III/IV Melanoma

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Abstract

Checkpoint immunotherapies (CPI) have improved clinical outcomes for patients with metastatic melanoma. Preclinical data suggest that histone deacetylase (HDAC) inhibition enhances antitumor immune activity and may augment the clinical benefit of CPI. In a phase Ib open-label, pilot trial (NCT03565406), patients with therapy-naive metastatic melanoma were treated with the class I/IV HDAC inhibitor mocetinostat at 70mg orally three times a week in combination with nivolumab (3mg/kg) and ipilimumab (1mg/kg) every three weeks for 12 weeks. This was followed by 12-week maintenance cycles of nivolumab every two weeks and mocetinostat at the same dose and schedule as induction. The endpoints of the trial were toxicity, definition of a recommended phase 2 dose, preliminary assessment of response, and correlative marker determination. Patient PBMC and serum samples collected at baseline and week seven on-treatment were assessed by flow cytometry and Luminex multiplex assays for immune correlates. Ten patients were treated, nine with 70mg and one with 50mg mocetinostat. In the 70mg cohort, eight patients had objective response (seven partial responses and one complete responses). The one patient in the 50mg cohort had an early progression of disease. All patients had grade 2 or higher toxicities, and six had grade 3-4 adverse events. Patient PBMC samples showed significant decreases in myeloid-derived suppressor cells and trends towards reduced M2 phenotypes. Patient serum samples showed significant upregulation of Granzyme A and TNF and trends towards increased Granzyme B and IFNγ. In vitro treatment of melanoma patient PBMC with mocetinostat recapitulated increases in inflammatory proteins and decreases in myeloid-derived suppressor cells and M2 phenotypes. Combination CPI and mocetinostat had favorable response rates but with high levels of toxicity. Assessment of immune correlates support an on-treatment shift away from immunosuppressive phenotypes towards enhanced immune responses.

Phase Ib pilot trial

NCT03565406
- Ten patients with therapy-naive metastatic melanoma
- Class I/IV HDAC inhibitor mocetinostat in combination with nivolumab (3mg/kg) and ipilimumab (1mg/kg) every three weeks for 12 weeks.
  - 12-week maintenance cycles of nivolumab every two weeks and mocetinostat at the same dose and schedule as induction.

Patient PBMC and serum samples
- baseline and week seven on-treatment
  - flow cytometry and Luminex multiplex assays.

Patient Demographics and Response

Patient ID	Age	Sex	Race	Disease & Stage	LDH Level (IU/L)	Mocetinostat Dose	Best Response	Toxicity Events (Grade)	Highest Toxicity Grade
001	65	M	ASIAN	Cutaneous; M1c	505	70mg	PR	Pruitis (2), Pneumonoitis (2), Multifocal Consolidation (2)	2
002	70	M	WHITE	Cutaneous; M1c	352	70mg	CR	Diarrhea (2), Colitis (3), Plueural effusion (2), Pneumonitis (2), Hypothyroidism (2)	3
003	57	F	WHITE	Cutaneous; M1c	414	70mg	PR	Adrenal Insufficiency (2)	2
004	75	M	NOT REPORTED	Cutaneous; M1c	362	70mg	PR	Pruitis (2), Pneumonoitis (2)	2
005	36	M	WHITE	Cutaneous; IIIc	417	70mg	PR	Fever (2)+, Headache (3), Vomiting (2), Meningitis (3), Increased ALT (2), Diarrhea (2)	3
006	70	M	WHITE	Cutaneous; M1b	491	70mg	PD	Dyspepsia (2), Vomiting (2), Diarrhea (2), Pruritis (2), Rash (2)+, Dehydration (3)	3
008	64	F	WHITE	Mucosal; M1c	452	70mg	PR	Rash (2)+, Rash (3), AST increase (2), ALT increase (2), Headache (3)	3
009	68	F	BLACK	Cutaneous; IIIc	432	70mg	PR	Dyspepsia (2), AST increase (3), ALT increase (3), AST increase (4), ALT increase (4), Bilod Billirubin increase (2)	4
010	73	F	WHITE	Cutaneous; M1c	784	70mg	PR	Pneumonitis (2)	2
011	72	F	WHITE	Cutaneous; M1c	1863	50mg	PD	Meningitis (3), Rash (3)	3

Table 1. Patient Demographics, Response and Toxicities
+ indicates multiple events of same type and grades.

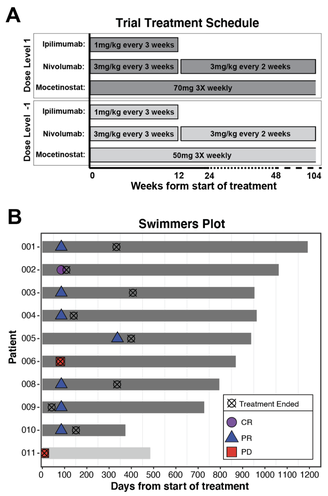

Figure 1. Clinical trial S17-00861 design and patient outcomes. (A) A schematic of treatment duration and dose schedules for patients enrolled in clinical trial S17-00861 is shown. **(B)** A swimmers plot of patient treatment durations and responses is shown. Patients receiving dose level 1 of mocetinostat are shown with dark grey bars (patients 001-010) and dose level -1 is shown in light grey (patient 011). The length of the bar corresponds to the number of days from start of treatment until last evaluation. The type and time of response is shown with colored shapes: complete response as purple circles, partial as blue triangles, and progressive disease as red squares. The time on treatment (i.e. when treatment stopped) is shown with circles containing an X.

Results

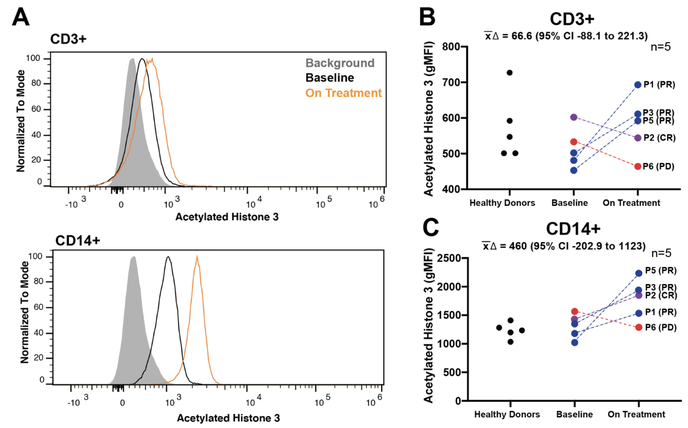

Figure 2: On-treatment changes in acetylated histone 3 levels in patient T-cells and monocytes. Patient PBMC were evaluated by intracellular flow cytometry for acetylated histone 3 levels. **(A)** Histograms of acetylated histone 3 geometric mean fluorescence intensity (gMFI) in CD3+ (top panel) and CD14+ (bottom panel) cells are shown from a representative patient. Background fluorescence is shown in grey, baseline patient sample levels in black and week seven of treatment in orange. **(B)** gMFI levels of acetylated histone 3 in treated paired patient T-cells and healthy donor T-cells are shown. Partial response patients are colored blue, the complete response patient is purple and a progressing patient is red. **(C)** Acetylated histone 3 levels in CD14+ cells are likewise shown. Corresponding means of intra-patient gMFI changes and 95% confidence intervals for all samples assessed are reported at the top of each panel.

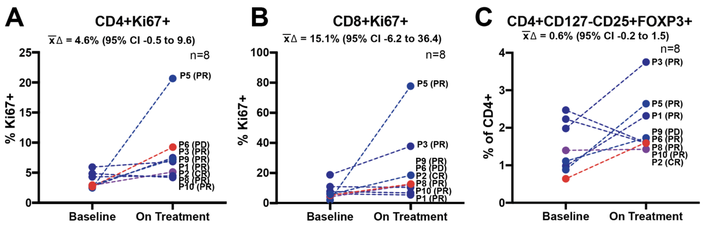

Figure 3. Mocetinostat does not impair proliferation of patient T-cells. Paired patient PBMC samples, baseline and week seven of treatment, were assessed by flow cytometry. **(A, B)** Ki67 expression in CD4+ and CD8+, as a marker of proliferation, was assessed. **(C)** The frequency of Tregs as a percent of the CD4+ population was also assessed. Partial response patients are colored blue, the complete response patient is purple and a progressing patient is red. Corresponding means of intra-patient changes and 95% confidence intervals for all samples assessed are reported at the top of each panel.

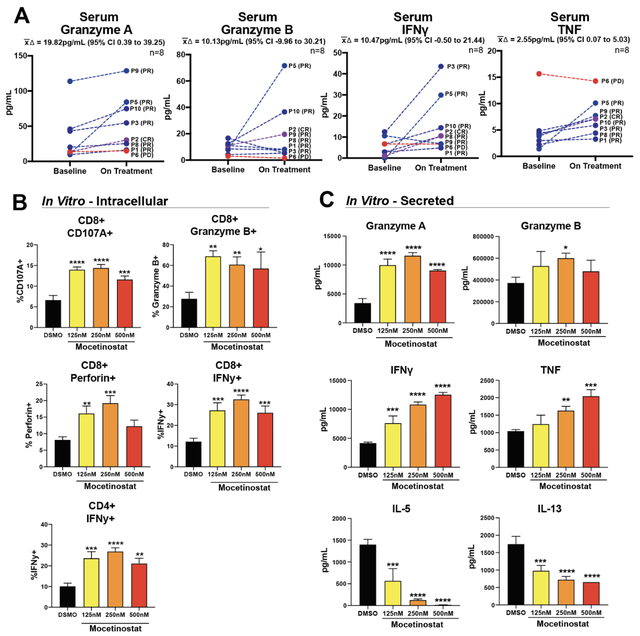

Figure 4. Mocetinostat enhances cytolytic/inflammatory protein levels. (A) Paired baseline and week seven of treatment serum samples from patients were assessed by multiplex Luminex assays. Partial response patients are colored blue, the complete response patient is purple, and a progressing patient is red. Corresponding means of intra-patient changes and 95% confidence intervals for all samples assessed are reported at the top of each panel. **(B, C)** T-cells isolated from melanoma patient PBMC were activated via CD3/CD28 antibody stimulation and simultaneously treated in vitro with indicated concentrations of mocetionstat or DMSO vehicle control. **(B)** After 48 hours, T-cells were assessed by intracellular flow cytometry for indicated cytolytic proteins. **(C)** Supernatants from the assay were assessed by multiplex Luminex for secreted concentrations of indicated cytokines and cytolytic proteins. Treatments were done in triplicate. Significance was assessed by One-Way ANOVAs with a Dunnet post hoc test. Indicated significance is for comparisons to DMSO controls. P-values are indicated as follows: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Results (cont)

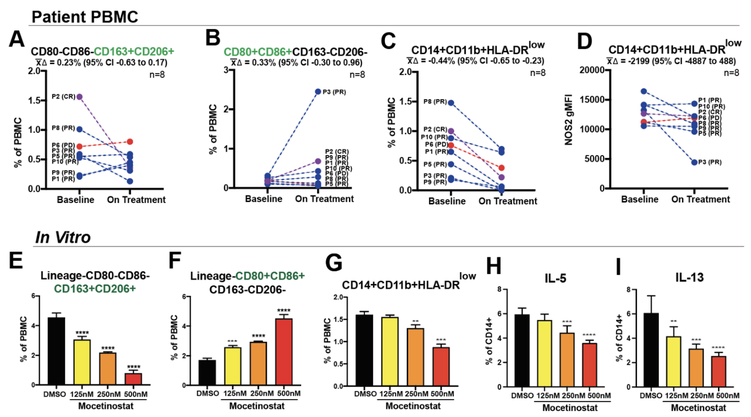

Figure 5. Mocetinostat decreases suppressive phenotypes. (A-D) Paired patient PBMC samples, baseline and week 7 of treatment, were assessed by flow cytometry. **(A)** Lineage negative (i.e. CD3-CD19-CD56-) leukocytes co-expressing CD163 and CD206 and lacking expression of CD80 and CD86 were assessed as a percentage of total live PBMC. **(B)** Lineage negative leukocytes co-expressing CD80 and CD86 and lacking expression of CD163 and CD206 were likewise assessed. **(C)** The percentage of MDSCs, defined by the indicated phenotype, was evaluated. **(D)** The gMFI of NOS2 expression in MDSC population was also evaluated. Partial response patients are colored blue, the complete response patient is purple and a progressing patient is red. Corresponding means of intra-patient changes and 95% confidence intervals for all samples assessed are reported at the top of each panel. **(E-I)** Melanoma patient PBMC were treated *in vitro* with indicated concentrations of mocetinostat for 24 (H and I) or 48 hours (E-G) and then evaluated by flow cytometry. **(E)** Lineage negative (i.e. CD3-CD19-CD56-) leukocytes co-expressing CD163 and CD206 and lacking expression of CD80 and CD86 were assessed as a percentage of total live PBMC. **(F)** Lineage negative leukocytes co-expressing CD80 and CD86 and lacking expression of CD163 and CD206 were also assessed. **(G)** The percentage of MDSCs, defined by the indicated phenotypes, were likewise evaluated. **(H,I)** Intracellular expression of IL-5 and IL-13 was assessed in CD14+ cells. Treatments were done in triplicate. Significance was assessed by One-Way ANOVAs with a Dunnet *post hoc* test. Indicated significance is for comparisons to DMSO controls. P-values are indicated as follows: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions

Findings:
All patients had grade 2 or higher toxicities, and six had grade 3-4 adverse events. Patient PBMC samples showed significant decreases in myeloid-derived suppressor cells and trends towards reduced M2 phenotypes. Patient serum samples showed significant upregulation of Granzyme A and TNF and trends towards increased Granzyme B and IFNγ. In vitro treatment of melanoma patient PBMC with mocetinostat recapitulated increases in inflammatory proteins and decreases in myeloid-derived suppressor cells and M2 phenotypes.

Limitations:
All the immune correlate assays in this study use patient PBMC samples. Assessment of patient tumor infiltrates during therapy was not possible given that paired baseline and on-treatment tumor biopsies were not obtained in this trial.

Conclusions:
Combination CPI and mocetinostat had favorable response rates but with high levels of toxicity. Assessment of immune correlates support an on-treatment shift away from immunosuppressive phenotypes towards enhanced immune responses.

Supporting Information

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Trial Registration: ClinicalTrials.gov NCT03565406

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