FHD-286, A POTENT AND SELECTIVE INHIBITOR OF BRG1/BRM (SMARCA4/2), SHIFTS METASTATIC UVEAL MELANOMA TUMOR TOWARDS A LESS IMMUNOSUPPRESSIVE STATE IN PATIENT SAMPLES

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Poster #A041
Abstract #34812

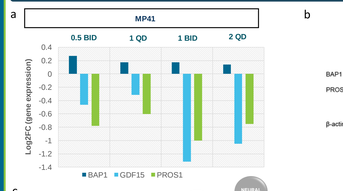
ABSTRACT

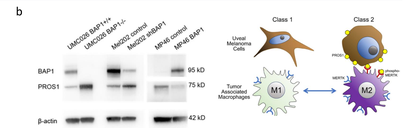
BRG1/BRM (SMARCA4/SMARCA2) are key components of the SWI/SNF complex that are critical in regulation of transcriptional programs that control cell fate and identity. Tumor cells aberrantly upregulate BRG1 levels, hijacking “stemness features”. Furthermore, BRG1 has been shown to impact immune cell polarization, driving programs associated with T cell exhaustion and an immunosuppressive cell state. Our group previously demonstrated that the combination of FHD-286 and anti-PD-1 antibody is synergistic in syngeneic mouse models from various lineages, and substantially shifted the tumor microenvironment (TME) towards a more tumor killing state (Ichikawa, K. 2022, SITC). FHD-286 was evaluated in a phase 1 dose escalation (FHD-286-001, NCT04879017), in metastatic uveal melanoma (mUM) , a tumor with low response rate to standard immune checkpoint therapy as well as high levels of immunosuppressive cell infiltration. Thus, we endeavored to determine if there was evidence of biological changes in peripheral blood and TME shifts in samples taken from our phase 1 dose escalation in patients with metastatic uveal melanoma.

Analysis of peripheral blood identified changes in expression of genes associated with a reduction in immunosuppressive signatures, particularly a dose dependent reduction of FOXP3 from baseline, as well as an increase in transcripts associated with immune activation. Patient pair biopsies analyzed using a multiplexed immunofluorescence panel (mIF) showed reduction in immunosuppressive cells of multiple lineages in on treatment samples, with 12/13 patients showing a reduction of one or more of the following: FOXP3+ Tregs (8/13), PD-L1+ Macrophages (8/13), markers of T cell exhaustion (PD1) on both CD4 (7/13) and CD8 T cells (8/13), as well as an increase in the M2 Macrophage to tumor cells distance (10/13), or an increase in CD8/Treg ratio (4/13). Taken together these results suggest FHD-286 may reduce the immunosuppressive “blockade”, priming mUM patients to response in combination with an immune checkpoint inhibitor.

BACKGROUND

FHD-286 IMPACT IN UM PRECLINICAL MODELS MAY SHIFT TUMORS TO A LESS AGGRESSIVE, AND LESS IMMUNOSUPPRESSIVE STATE





Kaler et al, 2022 Cancers (Basel)

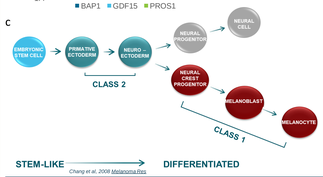


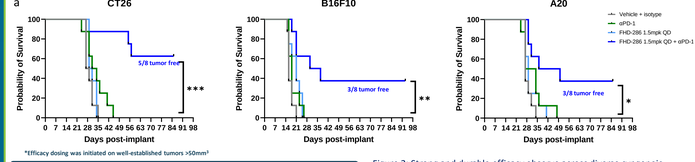
Figure 1: Loss of BAP-1 in mUM tumors correlates wot increased immunosuppressive state and increased M2 Macrophage polarization.

a) Previous publications (Wan et al. 2023) highlighted that FHD-286 inhibition in preclinical models of UM leads to a subtle increase in BAP1 expression and dose-dependent decrease in PROS1 and GDF15.

b) These two genes were identified as being upregulated with BAP1 loss (Kaler, et al 2022 *Cancers*) and linked to immunosuppressive macrophage polarization.

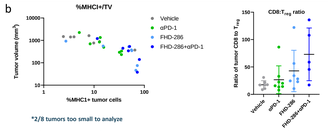
c) **Impact on these genes may potentially transition the cells from a less differentiated class 2 profile to a more differentiated class 1, and help prevent immune escape (See Wan et al. Poster # C016)**

FHD-286 SHOWS STRONG EFFICACY AND TME IMPACT IN PRECLINICAL SYNGENEIC MODELS



*Efficacy dosing was initiated on well-established tumors >50mm³

MECHANISTIC DATA GENERATED IN THE B16F10 MODEL*



*2/8 tumors too small to analyze

Figure 2: Strong and durable efficacy observe across diverse syngeneic models. Further mechanistic insights support role for FHD-286 in altering TME towards a more tumor killing state.

a) Ichikawa, SITC 2022 reported strong and durable responses to FHD-286 in combination with anti-PD-1 observed in 3/3 syngeneic models (CT26 (colorectal), B16F10 (cutaneous melanoma), A20 (lymphoma)) with well-established tumors.

b) Further in depth immunophenotyping mechanistic studies in B16F10 show impact on both tumor and immune cells offering multiple avenues to show combination benefit.

These results supported the need to explore what if any changes in were occurring in the TME of patients from FHD-286-001 Ph1 trial in metastatic Uveal Melanoma

METHODS

Subjects were enrolled in FHD-286-001 (NCT04879017) on a daily dosing regimen ranging from 2.5 to 10 mg or an intermittent regimen of 1-week on/1-week-off ranging from 10 to 22.5 mg in 73 patients. RNA sequencing was performed in 46 patient blood samples collected in Paxgene tubes during dose escalation (2.5 mg- 15mg, C1D1, C1D15, C3D1 and other intermediate timepoints) and analyzed for transcriptional changes associated with shifts in immune polarization and activation. In addition, ctDNA was measured in serial plasma samples using a targeted NGS panel.

Optional tumor biopsies were collected at screening and either Cycle 3 or end of treatment. Biopsies were collected from 16 patients, 13 of which had samples from screening and on-treatment. Biomarker changes in the tumor were quantified using multiplex IF (mIF) panel (Neogenomics-TIL and Myeloid Panel #2-19 markers) and analyzed by Neogenomics to identify 8 cell types of interest via co-localization of 20 different immune related markers. Of note, not all markers were expressed in patient samples, thus we focused our analysis on the immune cell types with highest abundance and strongest differential expression from screening and on treatment samples. Further analysis to determine the spatial orientation of cell types via nearest neighbor analysis as well as and location of cells inside or outside of the tumor. Additionally, variation or absence of specific immune cell presence in baseline and/or post treatment biopsies precluded normalization output for cell types in certain patients. Transcriptional changes were assessed using RNA sequencing of paired biopsies in 9 patients.

RESULTS

FHD-286 TREATMENT INDUCES DECREASE IN T$_{REG}$, MARKERS OF T CELL EXHAUSTION, AND PDL1+ TAM CONSISTENT WITH REDUCTION OF IMMUNOSUPPRESSIVE EFFECTS ON TME

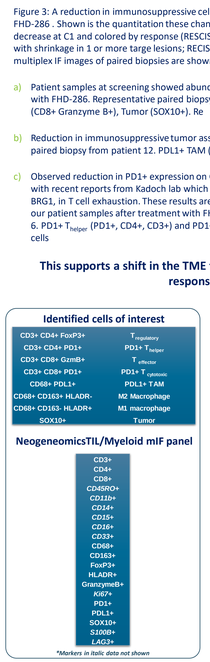
Figure 3: A reduction in immunosuppressive cell types was observed following treatment with FHD-286 . Shown is the quantitation these changes grouped by patients with observed ctDNA decrease at C1 and colored by response (RESCIST Responder=SD or better; Mixed Responder= PD with shrinkage in 1 or more targe lesions; RECIST Non-responder= PD) Additionally, representative multiplex IF images of paired biopsies are shown for each cell type of interest.*

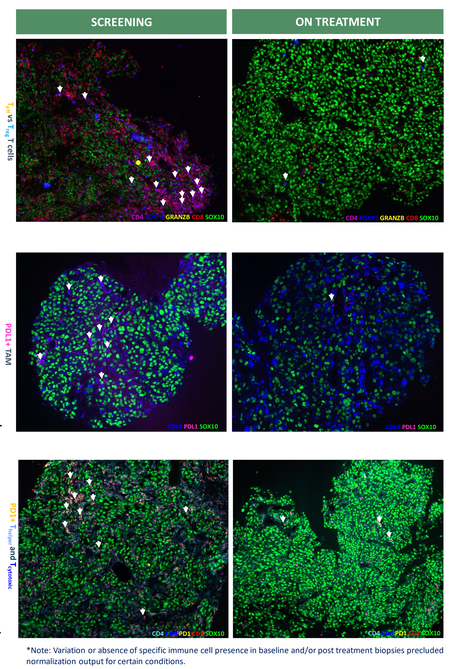
a) Patient samples at screening showed abundance of T$_{regs}$ which decreased following treatment with FHD-286. Representative paired biopsy from patient 3. T$_{regs}$ (CD4+, FOXP3+), T$_{effector}$ (CD8+ Granzyme B+), Tumor (SOX10+). Re

b) Reduction in immunosuppressive tumor associated macrophages (TAMs). Representative paired biopsy from patient 12. PDL1+ TAM (CD68+, PDL1+), Tumor (SOX10+)

c) Observed reduction in PD1+ expression on CD4 and CD8 T cells. These results are consistent with recent reports from Kadoch lab which highlighted the role of BAF complex, in particular BRG1, in T cell exhaustion. These results are supportive of reversion of T cell exhaustion in our patient samples after treatment with FHD-286. Representative paired biopsy from patient 6. PD1+ T$_{helper}$ (PD1+, CD4+, CD3+) and PD1+ T$_{cytotoxic}$ (PD1+, CD8+, CD3+), Tumor (SOX10+) cells

This supports a shift in the TME towards a state primed to be more responsive to ICIs.





Identified cells of interest

CD3+ CD4+ FoxP3+	T$_{regulatory}$
CD3+ CD4+ PD1+	PD1+ T$_{helper}$
CD3+ CD8+ GzmB+	T$_{effector}$
CD3+ CD8+ PD1+	PD1+ T$_{cytotoxic}$
CD68+ PDL1+	PDL1+ TAM
CD68+ CD163+ HLADR-	M2 Macrophage
CD68+ CD163- HLADR+	M1 macrophage
SOX10+	Tumor

NeogeneomicsTIL/Myeloid mIF panel

CD3+
CD4+
CD8+
CD45RO+
CD11b+
CD14+
CD15+
CD16+
CD33+
CD68+
CD163+
FoxP3+
HLADR+
GranzymeB+
Ki67+
PD1+
PDL1+
SOX10+
S100B+
LAG3+

*Markers in italic data not shown

Poster Legend

- RECIST Non-Responder
- RECIST Responder
- Mixed Responder

More immunosuppressive
Less immunosuppressive

M2 MACROPHAGES MOVE AWAY FROM TUMOR

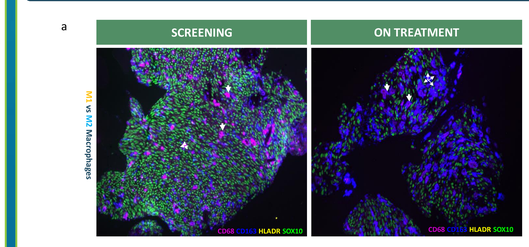


Figure 4: Give the previously reported connection between UM biology and M2 macrophages, We quantitated the presence location of M2 macrophages in the paired biopsies. M1 Macrophage (CD68+ CD163-, HLADR+), M2 Macrophage (CD68+ CD163+ HLADR+), Tumor (SOX10+).

a) Representative mIF image (patient 8) showing abundance of M2 macrophages “inside” tumor and close M2->Tumor cell distance which is altered following treatment with FHD-286

b) M2 macrophages shifted from “inside” the tumor area to “outside” the tumor, as defined by Neogenomics analysis.

c) Nearest neighbor analysis showed increase in the M2 macrophage to tumor cell distance.

FHD-286 SHIFTS THE TME OF UM PATIENTS

Patient	Visit	Treg Decrease?	M2 Shift to outside?	M2 to Tumor distance increase?	CD4+ PD1 Decrease?	CD8+ PD1+ Decrease?	CD68+ PDL1 Decrease?	Increase in CD8 GRANZYMB+/Treg ratio	Increase in CD8/CD4 ratio?
1	C3D1	Y			N	N	N	UNK	Y
1	EOT	N	N	N	N	N	N	Y	N
2	C3D1	N	Y	Y	Y	Y	Y	N	Y
3	C3D1	Y	Y	Y	Y	Y	Y	Y	N
4	C3D1	N	Y	Y	N	N	N	N	Y
5	C3D1	N	N	N	N	Y	Y	N	N
6	C3D1	Y	Y	N	Y	Y	Y	N	N
7	C3D1	N	N	N	N	N	N	N	Y
8	C3D1	Y	Y	Y	Y	Y	Y	N	Y
9	C3D1	Y	Y	Y	N	N	UNK	Y	Y
10	C3D1	UNK	Y	Y	Y	N	UNK	UNK	UNK
11	EOT	N	N	Y	N	N	Y	Y	N
12	EOT	Y	N	Y	Y	Y	Y	N	N
13	C3D7	Y	Y	Y	Y	Y	Y	N	Y

Table 1: Per patient changes summarizing cell type increase, decrease, or unknown due to lack of quantifiable number of the cells. Boxes are colored blue, indicating a reduction in immunosuppressive cells, red no change or increase in immunosuppressive cells, or white if change is unable to be determined. Patients with UNK change had no evidence of that cell type in screening or treatment sample.

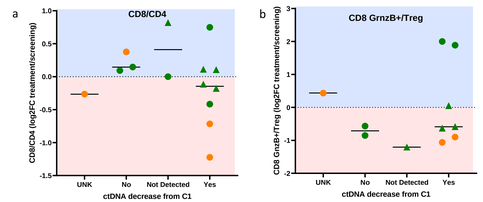


Figure 5: Changes in ratio of inflammatory to repressive T cells supports shift in TME towards a more tumor killing state.

a) 7/13 showed increase in over all CD8/CD4 ratio

b) 4/13 patients showed an increase in CD8+GrznB+/Treg ratio

RESULTS

DOSE DEPENDENT DOWNREGULATION OF FOXP3, AND INCREASE IN INFLAMMATORY TRANSCRIPTS IN PATIENT PBMCs

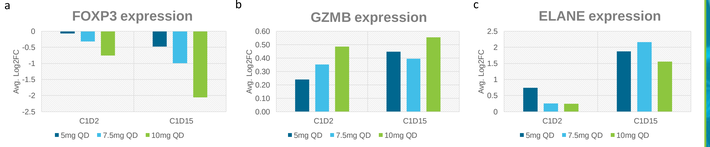


Figure 6: RNA seq obtained from the peripheral blood mononuclear cells (PBMCs) of patients in FHD-286-001 dose escalation (5 mg QD, 7.5mg QD, 10 mg QD) showed changes in transcripts associated with reduction in immunosuppressive effects.

a) Dose dependent decrease in FOXP3 mRNA expression from baseline on C1D2 following treatment with FHD-286, which deepens at steady state on C1D15

b) Dose dependent increase in Granzyme B mRNA expression from baseline on C1D2 following treatment with FHD-286, which appears to increase and plateau at steady state, showing less evidence of dose dependent effect.

c) Non- dose dependent increase in expression of ELANE, a marker of neutrophil activation, at C1D2 which increases at steady state on C1D15.

mUM RESPONDERS UPREGULATE NEUTROPHIL-ASSOCIATED GENES

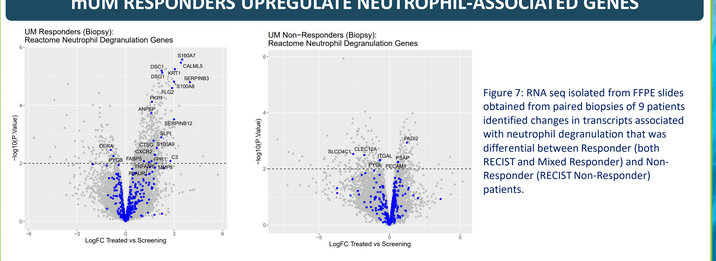


Figure 7: RNA seq isolated from FFPE slides obtained from paired biopsies of 9 patients identified changes in transcripts associated with neutrophil degranulation that was differential between Responder (both RECIST and Mixed Responder) and Non-Responder (RECIST Non-Responder) patients.

CONCLUSIONS

- Patients show changes in TME post FHD-286 treatment consistent with reduction of immunosuppressive impact
  - Reduction in number of Tregs
  - Reduction in marker of T cell exhaustion, PD-1, consistent with literature reports of BAF in regulation of T cell exhaustion
  - Reduction in immune suppressive PDL1+ TAMS
  - Shifting of repressive M2 macrophages away from tumor cells
- Patients showed transcriptional changes in PBMCs consistent with reduced immunosuppressive and increased inflammatory transcripts
  - Dose dependent down regulation FOXP3 transcript (Treg)
  - Increase in Granzyme B (Teff) transcript
  - Increase in transcripts associated with active neutrophil degranulation (e.g. ELANE)
- Transcriptional changes from paired biopsies showed increase in neutrophil degranulation genes following FHD-286 treatment in Responder and Putative responder patients
- Taken together these results suggest FHD-286 may reduce the immunosuppressive “blockade”, priming mUM patients to response in combination with an immune checkpoint inhibitor.

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- Dr. Michael Dougan

We wish to thank the patients, clinicians and investigators for their participation in the Phase 1 clinical trial of FHD-286-001