CA-4948 alters tumor associated macrophage and myeloid activity in the tumor microenvironment of murine models of melanoma metastases

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BACKGROUND

Melanoma remains a daunting clinical challenge. Immunotherapy remains the mainstay in treatment of metastatic disease. Yet nearly 50% of patients fail to reach a complete response to immunotherapy, due to multiple potential mechanisms of ICI resistance both acquired and innate [1]. One key mechanism of resistance is the suppressive nature of the tumor microenvironment (TME), driven in large part to the highly inflamed nature of melanoma metastases. Though beneficial to ICI efficacy, prolonged inflammation is co-opted by the tumor as a pro-growth factor and leads to localized immunosuppression [2.3]. This is most pronounced in the TME of melanoma brain metastases (MBM). Our group has previous shown that a key marker of this inflammation in MBM is the myddosomal pathway and specifically IRAK-4 activation [4]. This activation leads to downstream activation of both the MAPK and NF-KB pathways, leading to multiple protumorigenic features in melanoma [4]. We have previously shown that inhibition of IRAK-4 through the oral small molecule inhibitor CA-4948 in murine models of melanoma increased tumor infiltrating lymphocyte penetration into tumors and when given in combination with anti-PD-1 therapy improves overall survival over anti-PD-1 therapy alone [5]. In this current study we show this is driven in large part to changes in myeloid lineage phenotype and activation in the TME, with decreased PD-L1 expression and phenotypic skewing of both recruited and resident macrophage/myeloid lineage cells. We further show the combination efficacy in multiple metastatic models of murine MBM. These data support the combination of CA-4948 with ICI therapy as a novel strategy in overcoming ICI resistance.

METHODS

Human MBM samples (1, 2, 3) were obtained from the Florida Center for Brain Tumor Research (FCBTR) and subjected to cryosectioning and advanced IHC. Patient samples were then stained for activated IRAK1 expression, the final rate limiting step in myddosomal activation and counterstained for tumor cells and CD45+ cells. Results show increased expression in CD45+ myeloid cells within the TME. C57Bl/6 mice had B16F10 or B2905 cell implanted intracranial through stereotaxis and allowed to mature for 5 days prior to the initiation of therapy with either vehicle control, oral emavusertib (100mg/kg qD), anti-PD1 therapy 75mg/kg q72hrs) or combination therapy for 14 days and followed for overall survival. Combination emavusertib and anti-PD-1 therapy had a survival advantage in both aggressive MBM model systems. Tumors were extracted from a parallel cohort of B16F10 harboring mice at day 7 of treatment and analyzed via flow cytometry for expression of CD45+, CD11b+, and counter stained with Pd-l1 to differentiate microglia (CD45+low CD11B+high) and TAMs (CD45+highCD11B+low) for PD-L1 expression. Results indicate treatment with emavusertib reduces PD-L1 expression on both microglia and TAMs in MBM and corrects for the reflexive increase in PD-L1 seen with anti-PD-1 ab therapy.

Results

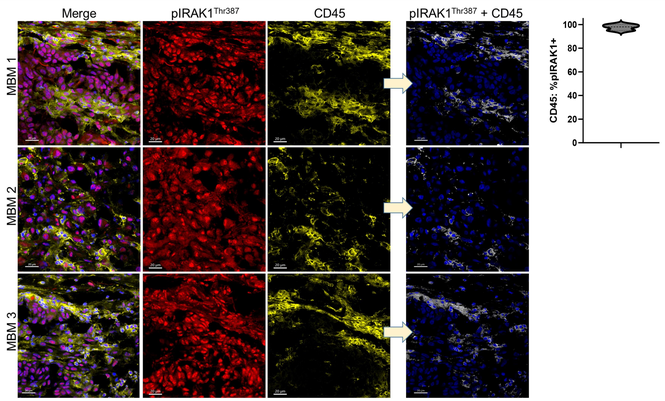

Fig 1. Degree of MYD88 activation in infiltrating leukocytes shown as pIRAK1+ CD45 cells in human MBM tissue. Representative analysis through Amaris software to quantify pIRAK1 expression shows near 100% correlation with CD45+ cells within the TME. Indicating myeloid cells as the key driver of inflammatory upregulation and not tumor expression levels.

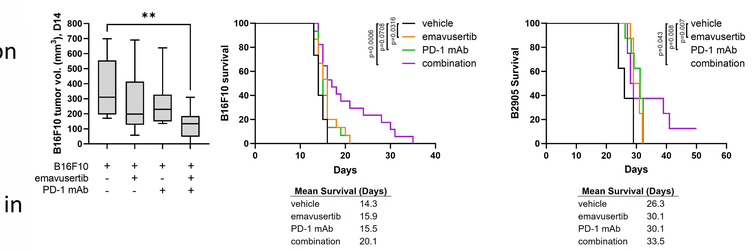

Fig 2. Combination emavusertib plus anti-PD1 therapy results in significant survival advantage in both B16F10 and B2905 murine models of MBM. C57BL6 mice were implanted with either B16F10 or B2905 cells intracranially through stereotaxis and allowed to mature for 5 days. Mice were then treated with either vehicle control plus IgG ab, emavusertib 100mg/kg qD, anti-PD-1 therapy 75mg/kg q72hrs, or combination emavusertib + anti-PD-1 therapy for 14 days and then monitored for overall survival. (N= 10 mice per group).

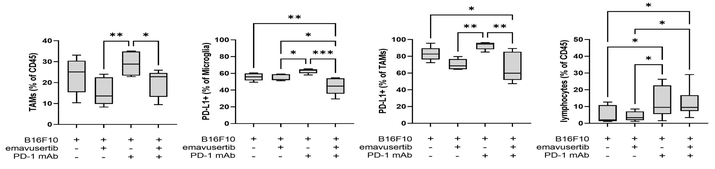

Fig 3. Combination emavusertib plus anti-PD1 therapy results in reduction in PD-L1 expression in both microglia and TAMs in murine MBM models. C57BL6 mice were implanted with either B16F10 intracranially through stereotaxis and allowed to mature for 5 days. Mice were then treated with either vehicle control plus IgG ab, emavusertib 100mg/kg qD, anti-PD-1 therapy 75mg/kg q72hrs, or combination emavusertib + anti-PD-1 therapy for 7 days and then sacrificed for primary endpoint. (N= 10 mice per group). MBM were extracted and half-hemispherei cell isolates were generated from the tumor bearing hemisphere. Cell isolates were subjected to flow cytometry for analysis of CD45 expression (High vs Low), CD11B expression (High vs Low), and PD-L1 expression. Microglia were defined by Low expression of CD45 and High expression of CD11B, TAMs were defined by High expression of CD45 and Low expression of CD11B. CD8+ lymphocytes were sorted by CD3+CD8+ expression. Results show treatment with emavusertib significantly decreases expression of TAMs in the TME and overcomes the reflexive increase in this population triggered by anti-PD-1 therapy. Further reduction is seen in both PD-L1 expression on resident microglia and TAMs in the TME of MBM samples analyzed. The combination of this effect results in a significant increase in CD8+ T cell infiltration with in the TME.

Conclusion

1. Co-localization of IRAK1 signaling and Myddosomal activation in CD45+ cells in Human MBM samples implies a strong role for resident and recruited myeloid cells in response to inflammation
2. Inhibition of IRAK4 through oral emavusertib (IRAK-4 blockade) results in decreased recruitment of PD-L1+ resident microglia and recruited tumor associated macrophages, thereby enhancing CD8+ t cell function and reduced hyper-exhaustion
3. Combination IRAK4 inhibition with emavusertib in combination with anti-PD-1 therapy shows a significant survival advantage in two murine melanoma models of brain metastases, B16F10 and B2905 with some long-term responders
4. This data supports the use of IRAK4 inhibition as a strategy to improve anti-PD-1 therapy in MBM and is currently the centerpiece of a first in human clinical trial

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