Immune Modulation of Melanoma Brain Metastases by IRAK-4 Inhibition

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BACKGROUND

Melanoma brain metastases (MBM) remain the primary driver of melanoma associated mortality. With improved survival from current therapy, the rate of MBM is expected to rise and it is already estimated that up to 60% of patients with metastatic disease will develop MBM during the course of their disease [1]. With dual agent immunotherapy or dual BRAF/MEK targeted therapy, the intracranial response rate can reach 50% [2]. This leaves half of patients in a position of either partial, temporary, or no response to treatment in their area of highest risk disease. Additionally, these sites lose response to both immunotherapy and targeted therapy sooner than areas of peripheral disease [3]. Novel strategies are needed to improve the treatment of MBM patients. We propose IRAK-4 as a novel target in MBM and the use of oral CA-4948 to inhibit IRAK-4 expression in combination with anti-PD1 therapy. We have previously discussed the ability of CA-4948 to rapidly cross the BBB in tumor bearing and naïve mice and shown single agent activity of CA-4948 in murine MBM (manuscript in review). In this study, we show that inhibition of IRAK-4 has no detrimental effect on antigen processing, presentation, or T cell activation. We validate the homology between human and mouse CA-4948:IRAK-4 receptor interactions. Additionally, we show that CA-4948 + anti-PD1 therapy has the potential to increase CD8+ TILs in an aggressive murine model of checkpoint resistant MBM. Finally, we show that this interaction occurs in peripheral tumors and combination therapy confers a survival advantage in mice.

METHODS

- IRAK-4 homology modelling and docking study was performed to compare human and mouse interactions between IRAK-4 and CA-4948 using GlideXP [4,5] and DeepAtom [6,7] machine learning based scoring.
- MBM was then modeled in C57BL6 mice with B16.F10 and tumors implanted via stereotax. Tumors were allowed to grow for 5 days, and therapy was given for 7 days (excipient, CA-4948 100mg/kg qD, anti-PD1 200ug q72hr, or combination) prior to tumor resection and flow cytometry analysis. Tumors were dissected, enzymatically digested and debris removed prior to CD45+ cell isolation by CD45 MicroBeads (Miltenyi Biotec).
- Finally, C57BL6 mice had B16.F10 tumors implanted in the flank only and allowed to establish for 5 days prior to starting treatment (as above) for 14 days. Mice were then followed for survival analysis.

RESULTS

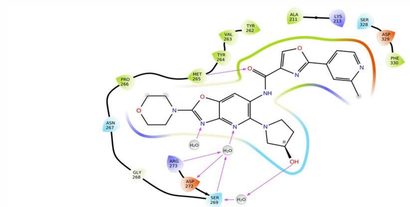


Figure 1: Ligand interaction diagram of CA-4948 bound to human IRAK4. Produced by Maestro. Hydrogen bonding shown with magenta arrows.

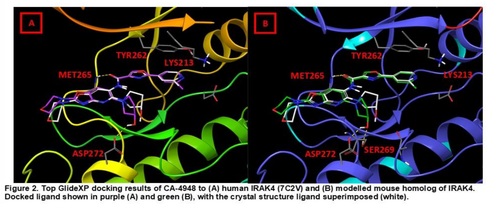
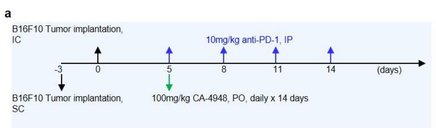


Figure 2. Top GlideXP docking results of CA-4948 to (A) human IRAK4 (7C2V) and (B) modelled mouse homolog of IRAK4. Docked ligand shown in purple (A) and green (B), with the crystal structure ligand superimposed (white).

Table 1. Docking Scores and RMSD for Top GlideXP Poses

Receptor	GlideXP Docking Score (kcal/mol)	RMSD to Crystal Structure Ligand Pose (Å)	DeepAtom Score (kcal/mol)
7C2V	-6.5	0.743	-11.1
Mouse Homolog	-8.9	0.870	-11.1



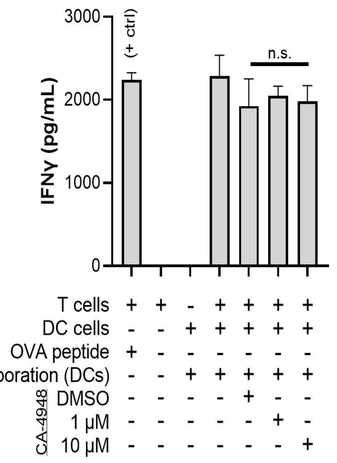


Figure 3. Effects of CA-4948 does not inhibit antigen processing, presentation, or T cell activation. Mouse dendritic cells harvested from splenic isolation and either cultured alone or subjected to electroporation with OVA mRNA. DCs were then cultured with OT-1 T cells alone or in the presence of vehicle (DMSO), CA-4948 1μM, or 10μM for 24hrs. Supernatant was then collected and analyzed for IFNγ expression via ELISA. OT-1 T cells pulses with OVA peptide were utilized as a positive control, T cells alone, and DC's pulses with OVA mRNA in the absence of T cells were utilized as negative controls. CA-4948 does not interfere with DC antigen processing and presentation or T cell activation. Experimental conducted in 96 well plate with all values in triplicate and representative of median IFNγ release.

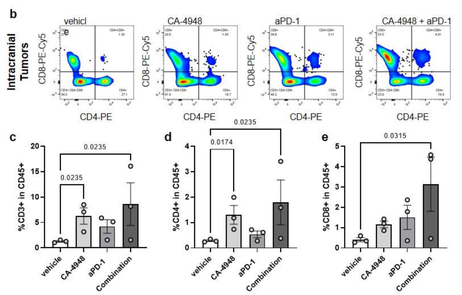


Figure 4. Combination CA-4948 with anti-PD-1 immune checkpoint blockade demonstrates improved survival in metastatic melanoma. (a) Treatment map for B16F10 preclinical assessment of combination CA-4948 with anti-PD-1 immunotherapy. (b) Flow cytometry analysis of tumor-infiltrating immune cells collected from intracranial tumors isolated on treatment day 7 (day 12 of study), with flow plots depicting CD4+ and CD8+ stratification of CD45+CD3+ lymphocytes. Quantitative analyses of tumor-infiltrating immune cells shown in (c) demonstrate elevated CD3+ lymphocytes present in all treatment groups, with combination therapy showing the most significant infiltration of these cells. Of the CD3+ lymphocytes present in tumors, further subset delineation shows significantly elevated numbers of both (d) CD4+ and (e) CD8+ lymphocytes in combination treated tumors. Treatment was well tolerated with no signs of toxicity noted. These data support that CA-4948 may sensitize tumors to anti-PD-1 immune checkpoint therapy, and is a promising anti-tumor treatment for metastatic melanoma and likely other solid tumors.

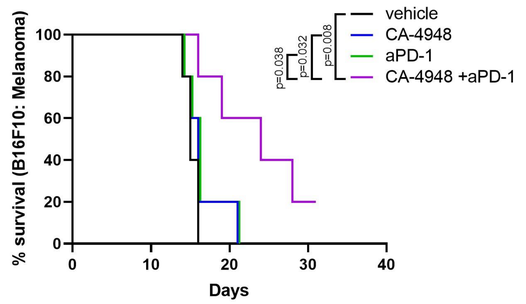


Figure 5. Combination CA-4948 with anti-PD-1 immune checkpoint blockade demonstrates improved survival in metastatic melanoma. C57BL6 mice implanted with B16.F10 tumors in the right posterior flank were allowed to establish for 5 days until digitally palpable on examination. Mice were then treated with either vehicle control, CA-4948 (100mg/kg qD), aPD-1 (200um q72hrs), or combination for 14 days and mouse were observed for tumor growth and overall survival. Mice were euthanized when tumors research size greater than 1.5cm in any dimension or if greater than 1.0cm with significant ulceration as per IACUC approved human endpoint. Overall survival response of B16F10 tumor-bearing syngeneic mice shows improved survival in combination treated animals as compared to all other treatment groups (p =0.008, by log rank test).

CONCLUSIONS

- There is direct homology between murine and human CA-4948:IRAK-4 receptor interactions making murine modeling a tangible corollary for human disease.
- CA-4948 shows no inhibitory effects on antigen processing, presentation, or T cell activation
- Combination CA-4948 + anti-PD1 therapy significantly increases CD8+ and CD4+ TILs in an aggressive checkpoint resistant MBM mouse model.
- Combination CA-4948 + anti-PD1 therapy additionally shows activity and provides a survival advantage in an aggressive checkpoint resistant cutaneous melanoma model.

REFERENCES

1. Liu, H., et al., Predictive value of a nomogram for melanomas with brain metastases at initial diagnosis. Cancer Med, 2019. 8(18): p. 7577-7585.
2. Acharya, S., et al., Distant intracranial failure in melanoma brain metastases treated with stereotactic radiosurgery in the era of immunotherapy and targeted agents. Adv Radiat Oncol, 2017. 2(4): p. 572-580.
3. Sloot, S., et al., Improved survival of patients with melanoma brain metastases in the era of targeted BRAF and immune checkpoint therapies. Cancer, 2018. 124(2): p. 297-305.
4. Schrödinger, LLC. (2021). Schrödinger Release 2022-3. New York, New York 2021.
5. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein−Ligand Complexes. J. Med. Chem. 2006, 49 (21), 6177–6196. https://doi.org/10.1021/jm051256o.
6. Rezaei, M. A.; Li, Y.; Li, X.; Li, C. Improving the Accuracy of Protein-Ligand Binding Affinity Prediction by Deep Learning Models: Benchmark and Model. ChemRxiv 2019. https://doi.org/10.26434/chemrxiv.9866912.v1
7. Li, Y.; Rezaei, M. A.; Li, C.; Li, X.; Wu, D. DeepAtom: A Framework for Protein-Ligand Binding Affinity Prediction. IEEE Int. Conf. Bioinforma. Biomed. 2019. https://doi.org/https://doi.org/10.48550/arXiv.1912.00318.

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