

Overcoming resistance to immune-checkpoint blockade due to deficient antigen presentation in melanoma using in vivo crispr screens

Alexandra Lakatos¹, Dorottya Pál¹, Anna Sára Lengyel, MD², Petra Péter¹, Botond Szabolcs¹, Elmar Lutz¹, David E. Fisher MD, PhD³, Lajos V. Kemény MD, PhD^{1,2,3}

¹HCEMM-SU Translational Dermatology Research Group, Department of Physiology, Semmelweis University, 37-47. Tüzoltó street 1094 Budapest, Hungary

²Department of Dermatology, Venerology and Dermatoooncology, Semmelweis University, 41. Mária street 1085 Budapest, Hungary

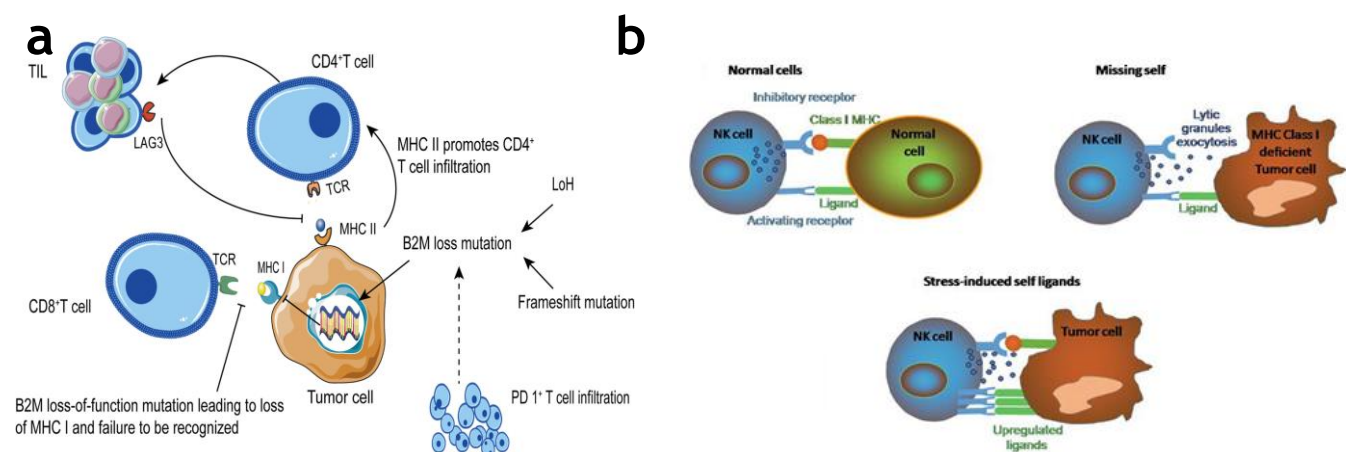
³Cutaneous Biology Research Center, Department of Dermatology and Cancer Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02114, USA.



Abstract

Despite recent developments in the field of immune-oncology by the introduction of immune-checkpoint blockade (ICB) in the management of melanoma, resistance to ICB therapies (aPD1 and aCTLA-4 therapies) still poses a tremendous problem. The most common resistance mechanism, the loss of antigen presentation, might offer novel vulnerabilities to antigen presentation-independent mechanisms. Here, we aim to identify novel therapeutic approaches to overcome resistance to ICB therapies. We plan to find regulators and novel therapeutic targets in melanoma models of antigen presentation deficiency by performing in vivo subgenome and genome-wide crispr screens. Our project may provide a better mechanistic understanding of melanoma-immune cell interactions and offers translational relevance.

Background



Resistance mechanisms to immune checkpoint blockade in melanoma due to loss of MHC-I expression.

a) MHC-I loss can occur via point mutation, which may be caused by loss of heterozygosity or frameshift mutation in B2M gene. Loss of B2M function is associated with resistance to ICB therapies in melanoma patients due to inefficient CD8+ T-cell mediated tumor clearance, however, so far overcoming resistance due to loss of MHC-I is unresolved. **Therefore, overcoming resistance to MHC-I loss is of great clinical interest.**

b) In the absence of MHC-I presentation, additional, CD8 independent mechanisms could be utilized in the management of melanoma. MHC-I is an inhibitor of NK cell function, therefore NK cells are promising assets in tumor elimination where MHC-I expression is diminished. Indeed, NK cells have been shown to be able to elicit increased cancer cell killing in vivo in the absence of tumor MHC-I expression, especially when stimulated by STING agonists. **Identifying novel regulators that can sensitize melanoma cells with low MHC-I expression to immune clearance or specifically to NK cell mediated clearance, might carry huge clinical benefit.**

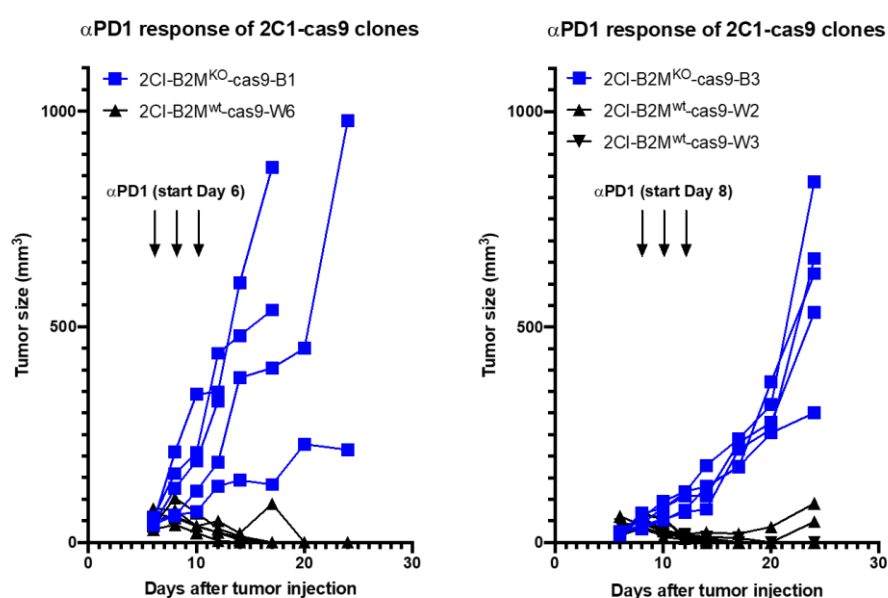


Figure 1. Creating a mouse model of melanoma with intrinsic resistance to ICB by genetic deletion of MHC-I.

First, to create a melanoma model where MHC-I independent melanoma-immune system interactions can be studied, we created a syngeneic mouse model of melanoma that is unable to present antigens. The cell line is derived from the D4M (C57Bl6J) background, but has a high mutation load due to prior chronic exposure to an alkylating agent. We ablated surface MHC-I expression with the deletion of the anchoring B2M subunit. We next engineered Cas9 stable expression in this cell line and isolated single cell clones that have high cutting efficiency (>80%) and have similar proliferation rate in vitro to control single cell clones with B2M expression. Validation experiments showed that the **absence of tumor specific B2M expression (and resulting loss of MHC-I expression) confers resistance to ICB therapy in our mouse model of melanoma** compared with B2M wild type clones, similar to resistance observed in human melanoma.

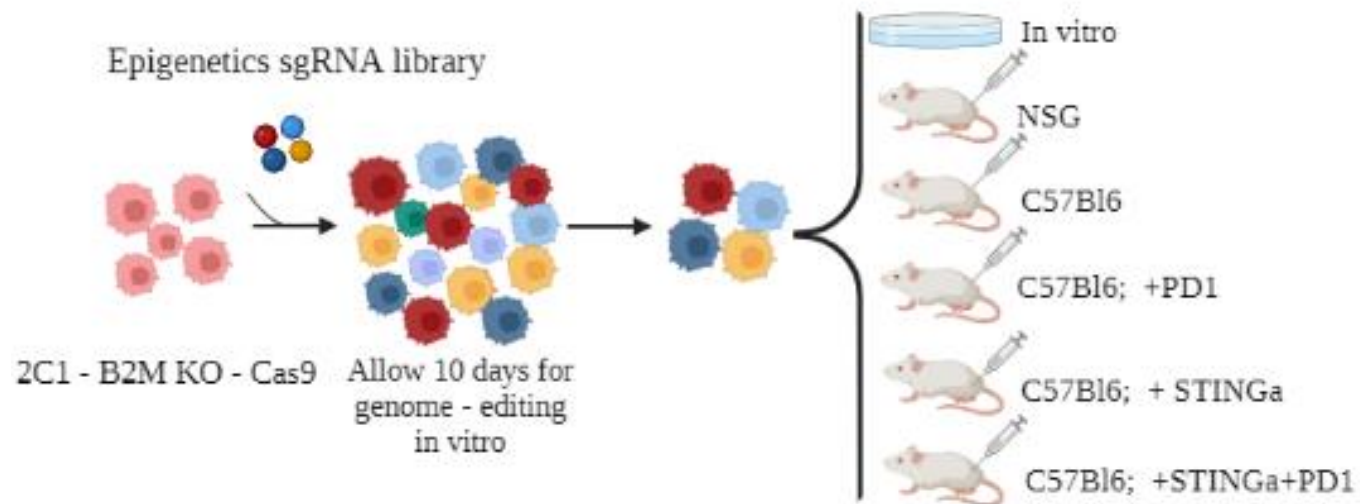


Figure 2. Experimental setup for crispr screening in 2C1-B2M KO-Cas9 melanoma cells.

First, to test the feasibility of our approach, we conducted a pilot experiment using the 2C1-B2M-KO-Cas9 cell line and infected the cells with an epigenetic domain-specific library of guideRNAs targeting 600 epigenetic genes with a library size of 10,000 guideRNAs. The infection was carried out using our optimized protocols ensuring that any individual cell was infected only with 1 gRNA. After culturing the cell lines in vitro for 10 days, cells were injected to immunocompromised NSG mice or in immunocompetent C57Bl6 mice. Separate groups of C57Bl6 mice were treated with ICB (aPD1) and/or with a STING agonist.

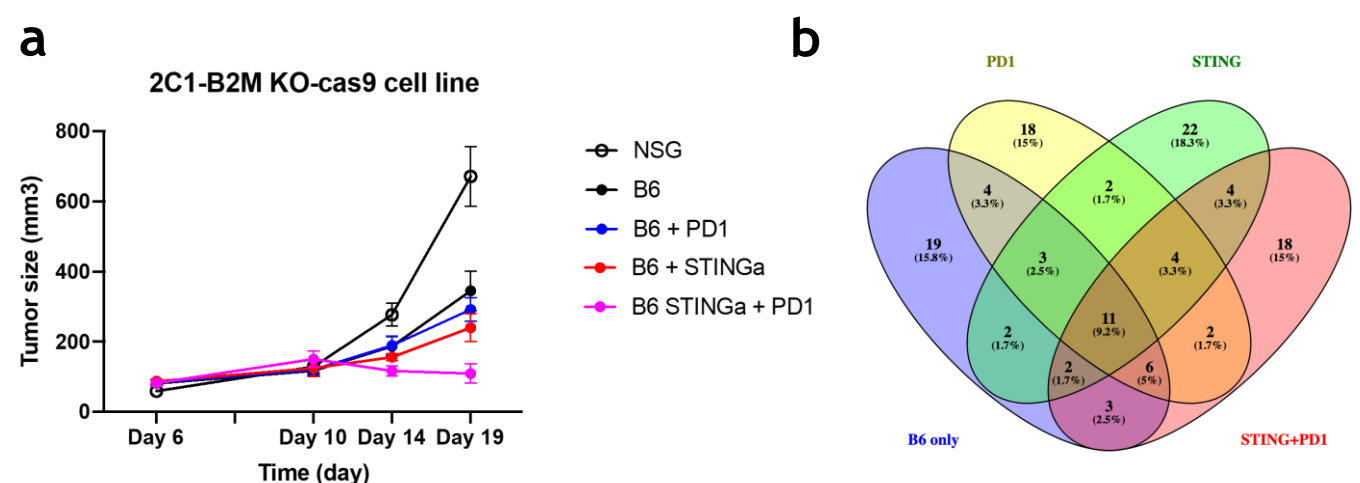


Figure 3. Preliminary results using in vivo crispr screen suggest multiple novel epigenetic targets to overcome resistance to ICB.

a) 2C1-B2M KO-Cas9 cells containing the epigenetic library have displayed altered tumor growth in vivo in mice under immunological selection pressure. There was a significant difference between the tumor burden of NSG and immunocompetent mice and between immunocompetent mice treated with a STING agonist compared with mice receiving no treatment or receiving ICB (aPD1). **This suggested that an immunological selection pressure exists between the different groups, making our model ideal for in vivo crispr screening that requires a strong selection pressure between conditions.**

b) **Bioinformatic analyses suggest novel therapeutic targets in B2M deficient melanoma.** Multiple genes were associated with resistance to immune clearance but also identified genes that are associated with increased susceptibility to MHC-I independent cytotoxicity. **We have found 11 genes, that were negatively depleted in all immuno-competent mice compared with immuno-deficient NSG mice.** These results suggest that multiple genes could be targeted to enhance CD8-independent melanoma killing in vivo.

Discussion

In order to identify novel therapeutic approaches of immunoresistance to ICB by overcoming MHC-I deficiency, **we created an MHC-I deficient melanoma mouse model expressing cas9 for in vivo crispr screen experiments.** Initially, we have utilized a targeted epigenetic library for in vivo crispr screening. After optimizing gRNA library infection protocols, we have performed the screen in 5 different experimental conditions. Bioinformatic analyses provided gRNAs that are over- or underrepresented in tumors of immunocompetent mice compared to those in immunodeficient NSG mice, suggesting genes that mediate resistance or sensitivity to CD8 independent immune clearance, respectively. **Next, our goals are to validate the above discussed results using the epigenetic library, and more importantly, to extend our investigations by performing in vivo genome-wide crispr screens using custom-made gRNA libraries targeting all expressed genes in the 2C1-B2M KO-cas9 melanoma model.**

Acknowledgments



HCEMM Grant 2022-27

OTKA KFI grant 2021-25

Bolyai Scholarship 2021-24



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 739593.

References

- Lynda Addou-Klouche. NK Cells in Cancer Immunotherapy. *Natural Killer Cells*. 2017. doi:10.5772/intechopen.71217
- Vinay Kumar and Megan E. McNeerney. A new self: MHC-class-I-independent natural-killer-cell self-tolerance. *Nature Reviews Immunology*. 2005;5(5):363-374. doi:10.1038/nri1603
- Christopher J. Nicolai, Natalie Wolf, I-Chang Chang, Georgia Kim, Assaf Marcus, Chudi O. Ndubaku, Sarah M. McWhirter David H. Raulet. NK cells mediate clearance of CD8+ T cell-resistant tumors in response to STING agonists. *Physiology and Behavior*. 2017;176(12):139-148. doi:10.1126/sciimmunol.aaz2738.NK
- Daixi Ren, Yuzhe Hua, Boyao Yu, Xin Ye, Ziheng He, Chunwei Li, Jie Wang, Yongzhen Mo, Xiaoxu Wei, Yunhua Chen, Yujuan Zhou, Qianjin Liao, Hui Wang, Bo Xiang, Ming Zhou, Xiaoling Li, Guiyuan Li, Yong Li, Zhaoyang Zeng and Wei Xiong. Predictive biomarkers and mechanisms underlying resistance to PD1/PD-L1 blockade cancer immunotherapy. *Molecular Cancer*. 2020;19(1):1-19. doi:10.1186/s12943-020-1144-6
- Robert T. Manguso, Hans W. Pope, Margaret D. Zimmer, Flavian D. Brown, Kathleen B. Yates, Brian C. Miller, Natalie B. Collins, Kevin Bi, Martin W. LaFleur, Vikram R. Juneja, Sarah A. Weiss, Jennifer Lo, David E. Fisher, Diana Miao, Eliezer Van Allen, David E. Root, Arlene H. Sharpe, John G. Doench and W. Nicholas Haining. In vivo CRISPR screening identifies Ptpn22 as a cancer immunotherapy target. *Nature*. 2016;5(6):1-8. doi:10.1038/nature23270.