Humanized PD-1 knock-in mice as a model system for combination therapies with human specific PD-1 therapeutics

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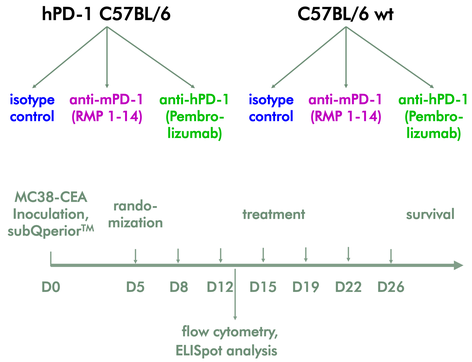
Introduction

Checkpoint inhibitor treatment has become a common therapy for various cancer types. As an escape mechanism, tumor cells express PD-L1 on their surface, as a ligand for PD-1 with players of the immune system (such as T cells), aimed at preventing the immune system from exerting its anti-tumour activities. Antibodies blocking the PD-L1/PD-1 interaction have emerged as front-line treatments for various oncological indications. Several anti-PD-L1 and anti-PD-1 therapeutics are already approved and in clinical use. For preclinical testing of such human specific antibodies, syngeneic tumour models are of limited use due to the limited cross-specificity of such antibodies, giving rise to the development of humanized mouse models in which combination therapies testing new drugs with already clinically approved human checkpoint inhibitors addressing human specific targets can be evaluated.



Study design for the MC38-CEA tumor model in hPD-1 C57/BL6 and C57/BL6 wt mice

Results

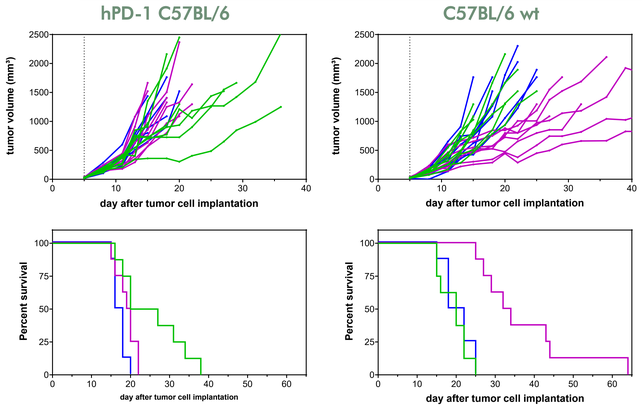


Fig. 1: MC38-CEA cells were inoculated into the mammary fat pad (subQperior™). After randomization at Day 5 the animals were treated with isotype control, anti-mPD-1 (RMP1-14) or anti-hPD-1 (Pembrolizumab).

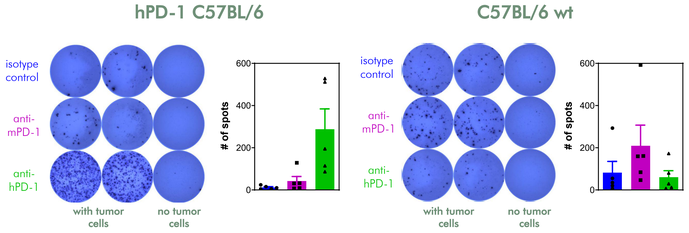


Fig. 2: Splenocytes (500.000 cells/well) from the different groups were isolated and incubated with MC38-CEA cells (50.000 cells/well) for two days on mouse IFN-γ ELISpot kit plates (R&D systems, EL485). After two days of incubation at 37 °C the ELISpot ELISA was developed following the manufacturer's instructions. The animals were treated with isotype control, anti-mPD-1 (RMP1-14) or anti-hPD-1 (Pembrolizumab).

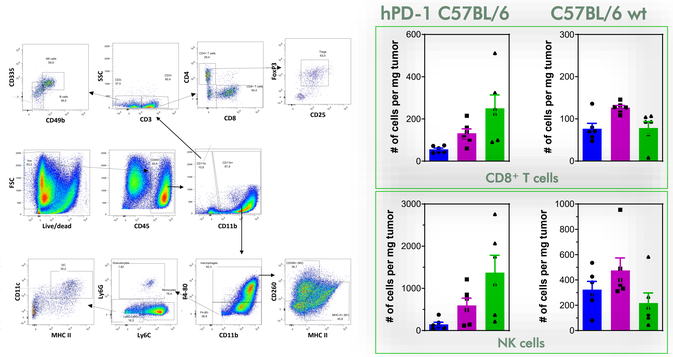


Fig. 3: Eight days after treatment start, MC38-CEA tumors were harvested and processed for flow cytometry analysis. Following, the single cell suspension were stained with a 17 marker panel. The gating strategy is depicted on the left. The animals were treated with isotype control, anti-mPD-1 (RMP1-14) or anti-hPD-1 (Pembrolizumab).

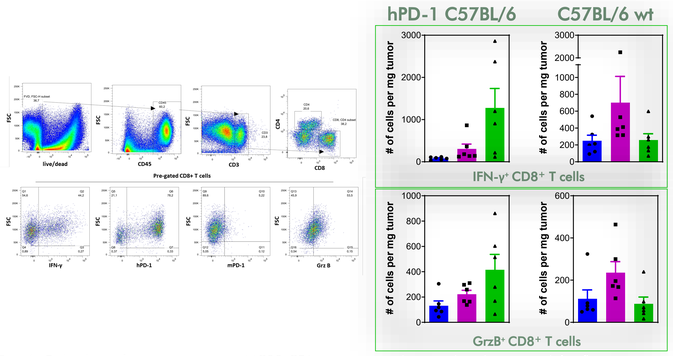


Fig. 4: Eight days after treatment start, MC38-CEA tumors were harvested and processed for flow cytometry analysis. The single cell suspension were stimulated with PMA/Ionomycin/Golgistop for 4h and followed by staining. The gating strategy is depicted on the left. The animals were treated with isotype control, anti-mPD-1 (RMP1-14) or anti-hPD-1 (Pembrolizumab).

Conclusion

- Tumor growth delay was observed for Pembrolizumab in hPD-1 C57/BL6 mice and for RMP1-14 in C57/BL6 mice only.
- T and NK cells are increased when treated with Pembrolizumab in hPD-1 C57/BL6 mice and RMP1-14 in C57/BL6 mice
- IFN-γ⁺ and Granzyme B⁺ CD8⁺ T cells are more frequent in hPD-1 C57/BL6 mice treated with Pembrolizumab and in C57/BL6 mice treated with RMP1-14
- ELISpot analysis reveals a higher number of IFN-γ⁺ secreting cells in hPD-1 C57/BL6 mice treated with Pembrolizumab and in C57/BL6 mice treated with RMP1-14

→ The humanized subQperior™ platform using hPD-1 C57BL/6 mice is a suitable tool to evaluate novel cancer therapies in combination with human specific anti-PD-1 therapeutics

References

1. Dovedi et. al., Cancer Discov. 2021; 11(5):1100-1117.
2. Acúrcio et. al., J Immunother Cancer. 2022; 10(7):e004695.

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