Poster # 934

“GPCR-ome” modulation upon PD1 / PDL1 axis blockade

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ABSTRACT

BACKGROUND: Immunotherapy represents a novel weapon within the cancer armamentarium with unprecedented response observed in several indications including among others metastatic melanoma and Lung cancer. However, the limited rate of response accounting for app. 30% requires further optimization and thus calls for interest in elucidating mechanisms underlying sensitivity to immune checkpoint inhibitors. G Protein–Coupled Receptors (GPCRs) are the largest gene family of cell membrane–associated molecules mediating signal transmission involved in several physiological functions including the shaping of the immune response, but their role in tumor biology and particularly in immune evasion and resistance to immunotherapy remains to be elucidated.

METHODS: Using a preclinical syngeneic mouse model of sarcoma based on the inoculation of MCA205 sarcoma cell line, we investigated - besides the evaluation of tumor growth and survival - a large set of GPCRs upon PDL1 or PD1 blockade. Gene expression analysis was performed on tumor samples by mean of RNA sequencing. Data were analyzed using computational methodology including CIBERSORT and GSEA algorithms to evaluate immune cell subset within the tumor and specific gene datasets enrichment, respectively. A large set of GPCRs (1412 in total) was then investigated through conventional Differential Gene Expression (DGE) analysis.

RESULTS: PDL1/PD1 axis blockade, using specific antibodies, exhibited a strong anti-tumor effect. As highlighted by CIBERSORT analysis, anti-PD1/PDL1 antibodies triggered a CD8 and activated NK cells infiltration within the tumor. The recruitment of such effector populations was associated with enrichment in genes from different hallmarks datasets including allograft rejection and interferon signaling thus confirming the induction of an efficient anti-tumor immune response. Concomitantly, this study revealed a significant up-regulation of several GPCRs already known to be involved in immune evasion - including Adenosine, Prostaglandins and chemokine receptors - but also highlighted the modulation of unexpected orphan or peptidic receptors.

METHODS

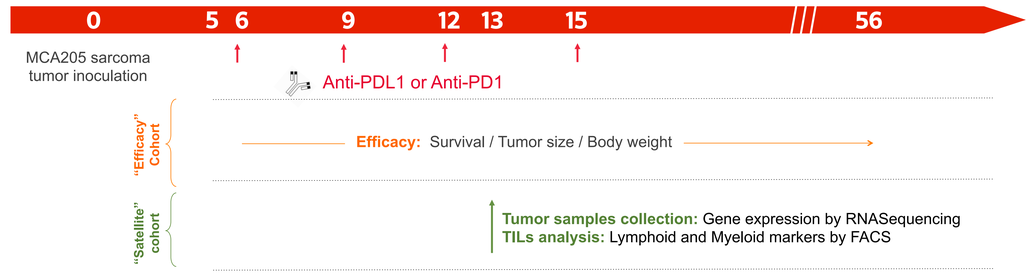

Figure 1*: After MCA205 sarcoma tumors were inoculated in immunocompetent C57BL/6J mice, animals were allocated to the different treatment groups and processed either for “efficacy” or as “satellites”, for the respective readouts as mentioned above.*

RESULTS

Anti-tumor effect of PD1/PDL1 axis blockade

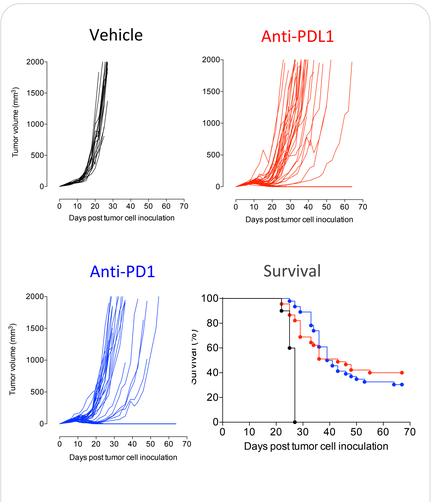

Figure 2*: Individual tumor volume (mm³) of MCA205 tumor-bearing mice upon vehicle (n=15), anti-PD1 (n=45) or anti-PDL1 (n=45) antibodies treatments. Survival is also represented as Kaplan-Meier curves.*

Anti-PD1/PDL1 antibodies modulate the intratumoral immune landscape

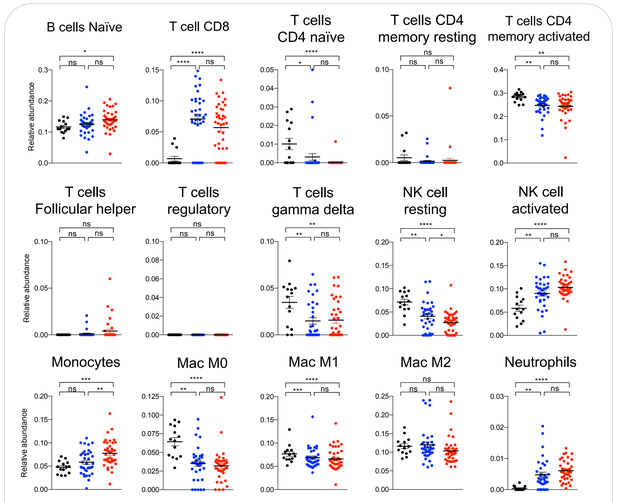

Figure 3: Assessment of the immune contexture in MCA205 tumors collected from mice treated with either vehicle (n=15), anti-PD1 (n=45) or anti-PDL1 (n=45) antibodies. Tumors were processed for RNASequencing and data were analyzed using the CIBERSORT algorithm to highlight 15 immune cell population. As depicted, anti-PD1/PDL1 antibodies induce a strong enrichment in effector populations including CD8+ T cells and activated NKs.

GSEA-based identification of pathways engaged upon anti-PD1/PDL1 mAb treatment

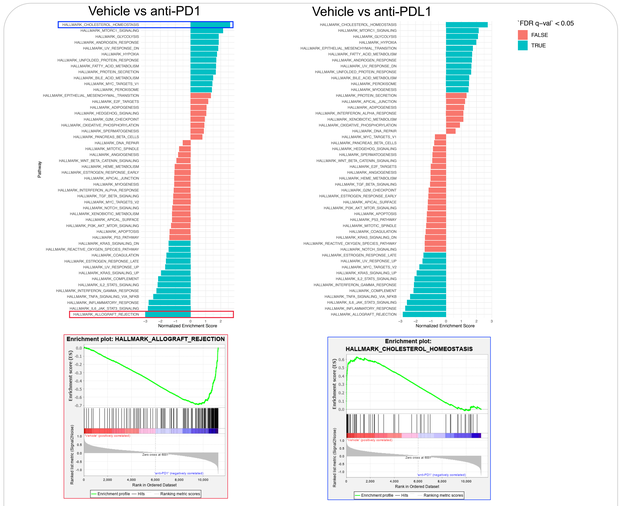

Figure 4: *RNASequencing data of MCA205 tumors - collected either from vehicle, or anti-PD1 and anti-PDL1 antibodies-treated mice - were processed for Gene Set Enrichment Analysis (GSEA) and revealed common features between both antibodies. As illustrated, anti-PD1/PDL1 antibodies induce a strong enrichment in genes from the « Allograft Rejection hallmark » gene set while an enrichment in « Cholesterol homeostasis » gene set is observed in Vehicle-treated mice.*

GPCR-ome modulation upon anti-PD1/PDL1 mAb treatment – novel resistance mechanisms ?

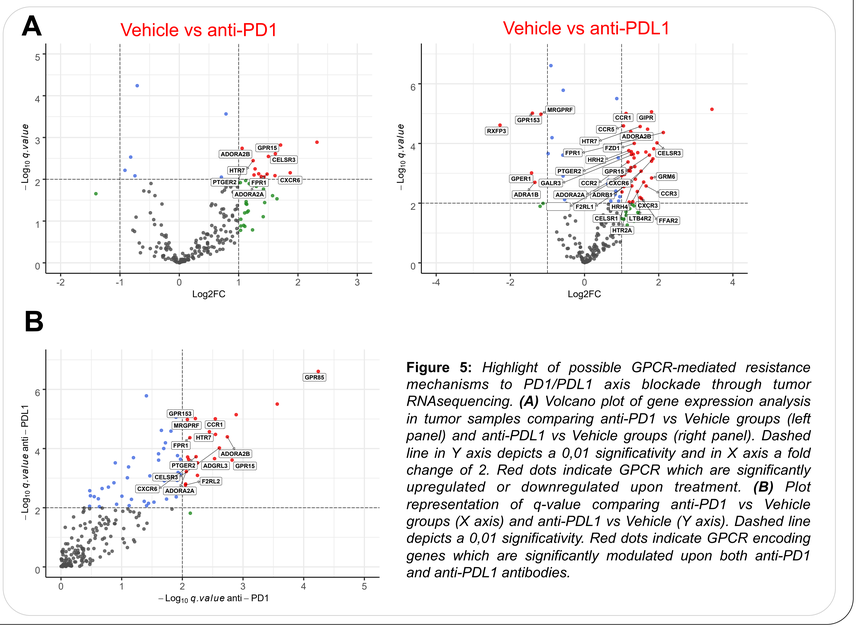

Figure 5: *Highlight of possible GPCR-mediated resistance mechanisms to PD1/PDL1 axis blockade through tumor RNAsequencing.* ***(A)*** *Volcano plot of gene expression analysis in tumor samples comparing anti-PD1 vs Vehicle groups (left panel) and anti-PDL1 vs Vehicle groups (right panel). Dashed line in Y axis depicts a 0,01 significativity and in X axis a fold change of 2. Red dots indicate GPCR which are significantly upregulated or downregulated upon treatment.* ***(B)*** *Plot representation of q-value comparing anti-PD1 vs Vehicle groups (X axis) and anti-PDL1 vs Vehicle (Y axis). Dashed line depicts a 0,01 significativity. Red dots indicate GPCR encoding genes which are significantly modulated upon both anti-PD1 and anti-PDL1 antibodies.*

CONCLUSIONS & PERSPECTIVES

Altogether, this novel dataset confirms the induction of an effective anti-tumor immune response upon PD1 / PDL1 blockade which is highlighted through CIBERSORT analysis and increase in both CD8 T cells and activated NK cell associated markers. It’s also revealed through GSEA that evidence a strong enrichment − in anti-PD1 / anti-PDL1 treated groups - in genes associated to the Allograft Rejection as well as the inflammatory response hallmarks. Finally, this study reveals the modulation of several GPCRs − some of them being specific to PD1 or PDL1 blockade and thus suggesting differential mechanisms of action of both antibodies. Interestingly, several GPCRs currently investigated as novel targets in cancer immunotherapy are found in the top list and include Adenosine (Adora2a/b) or Prostaglandins (Ptger2) receptors. Besides these targets, several other GPCRs are also depicted and warrant further investigation to delineate their exact participation in resistance / sensitivity to approved cancer immunotherapies.