Comprehensive assessment of anti-tumor PDL1 blockade effect in a sarcoma mouse model

Imane NAFIA1, Assia CHAIB1, Doriano BORTOLLOTTO1, Loïc CERF1, Manon CLEMENCEAULT1, Ariël SAVINNA, Antoine ITALIANO3, Alban BESSEDE1

1ExpliQyte Immuno-Oncology, Bordeaux, France; 2Institut Roche, Boulogne Biarritz, France; 3Institut Bergonié, Bordeaux, France

ABSTRACT

BACKGROUND: Development of novel immunotherapeutics in oncology is of crucial interest and their good testing at preclinical stage relies on 1) the features of the animal model used and 2) the application of an appropriate comprehensive strategy for the deep delineation of mechanisms underlying resistance/sensitivity to a drug.

METHODS: Using a syngeneic sarcoma mouse model, treated with anti-PDL1, we investigated by intratumoral microdialysis and flow cytometry the immunometabolic profile and immune landscape of the tumor, respectively. The anti-tumor effect of PDL1 blockade was assessed through tumor growth monitoring and tumoral biopsies were also collected for gene expression analysis. Finally, involvement of CD8 T cells in the anti-tumor PDL1 blockade-mediated activity was addressed using a specific depleting antibody-based strategy.

RESULTS: When compared to a non-tumor area, data obtained from tumor microdialyses highlighted i) a slight Kynurenine pathway activation, ii) a strong Arginase activity, and iii) a high Adenosine production. Interestingly, anti-PDL1 effect was associated with a decrease of the tumor Adenosine level thus arguing for an important role of the Adenosine axis in the control of the anti-tumor immune response. In addition, PDL1 blockade led to an intratumoral CD4+ T cell enrichment, with a higher abundance of lymphocytes also involved in tumoral macrophages and inflammatory profile of tumor immune cell infiltrate, and CD8-driven response. Interestingly, this multiparametric dataset evidences that while an anti-tumor immune-driven response can be evaluated through in vivo monitoring, profiling immune function in parallel by the mean of relevant models and immunological readouts permits for providing a deeper understanding of how a cancer therapy performs, and might thus serve to thoroughly explore novel immunotherapeutics.

CONCLUSIONS & PERSPECTIVES

This study on the syngeneic MCA205 sarcoma mouse model shows that this PDL1-responding model is featured of a specific suppressive immunometabolic profile, itself involved, at least partially, in the response to an anti-PDL1 antibody. Anti-tumor activity of PDL1 blockade is indeed shown to be underpinned by a great impact on the immune system, involving the development of a pro-inflammatory profile of tumor immune cell infiltrate, and CD8-driven response. Interestingly, this multiparametric dataset evidences that while an anti-tumor immune-driven response can be evaluated through in vivo monitoring, profiling immune function in parallel by the mean of relevant models and immunological readouts permits for providing a deeper understanding of how a cancer therapy performs, and might thus serve to thoroughly explore novel immunotherapeutics.

METHODS

Figure 1: After MCA205 sarcoma tumor inoculation in immunocompetent C57BL/6 mice, animals were allocated to the treatment groups and processed either for "efficacy" or as "satellites", for the respective experiments and reagents as mentioned above.

Figure 2: Individually (A) and mean (B) tumor volume (mm3), and Kaplan-Meier plot survival (C) of MCA205 tumor-bearing mice upon vehicle (n=10) and anti-PDL1 antibody (n=10) treatments.

Figure 3: Immunometabolic pathways profiling of MCA205 tumors on day 13 post-tumor inoculation (200nm average voxel) by intratumoral microdialysis and metabolite level determination by LC/MS.

Figure 4: Intratumoral gene expression analysis by mean of RNA-sequencing A) reveals a proper upregulated interferon signature and significant enrichment in genes belonging to Abrogate response, for the rechallenged vs vehicle, C) with mean FDR q value -2.6263227.

Figure 5: Multiparametric flow cytometry based analysis of the Lymphoid and Myeloid immune cell subsets in intratumoral tumors, on day 12 post-tumor inoculation.

Figure 6: Effect of CD8 depletion in vehicle and anti-PDL1 antibody-treated MCA205 tumors bearing mice. Individual (A) and mean (B) tumor volume (mm3), and (C) Kaplan-Meier plot survival (200nm average voxel) analysis revealed, in addition to an interferon signature, changes in genes from the myeloid / neutrophil subsets including Arg1 and key immunosuppressive CD11b/Gr1Low/Int cell subsets, in favor of an increase of the M1/M2 macrophages ratio. Interestingly, CD8 depletion fully abrogated anti-PDL1 anti-tumor effect thus showing the unequivocal role of this population. Finally, gene expression analysis revealed, in addition to an interferon signature, changes in genes from the myeloid / neutrophil subsets including Arg1 and key chemokines.

Figure 7: Blood immunoprofiling supports the effectiveness of CD8 depletion. Flow cytometry monitoring of CD3, CD4, and CD8 cells in peripheral blood samples. 1 day and 2 weeks after the 1st injection of CD8-depleting Ab, CD8-depleting Ab produced an efficient and stable CD8 depletion concomitantly with a higher proportion of CD4 T cells.