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Analysis of T and NK cells immune response in Ipilimumab treated Melanoma patients

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Background

The most promising immunotherapeutics tested in metastatic melanoma patients are the monoclonal antibody blocking CTLA-4 (Ipilimumab), and those interfering with PD-1 and PD-L1. However, the lack of knowledge on predictive biomarkers that could assist the treatment remains a limiting factor. We speculate that, along with additional markers, the immunoscore [1] is fundamental as prognostic and predictive marker for response to immunotherapies in metastatic melanoma. Our previous data demonstrate that NK cells control the melanoma progression [2,3]. Therefore we have analysed both T cells and NK cells subsets frequencies and receptors repertoire in the peripheral blood of Ipilimumab treated patients with Stage IV metastatic melanoma.

Material and methods

Peripheral blood mononuclear cells (PBMCs) from 12 different patients with stage IV metastatic melanoma were collected and analyzed. Each patient received 4 infusions of Ipilimumab each 21 days. Before each infusion we collected patients' blood and isolated PBMCs to analyze the lymphocytes compartment.

We stained for the following antibodies: CD56 PE, CD3 Fitc, CD56 APC, CD4 PeCy7, CD8 APCCy7, CD152 (CTLA4) PE, CD279 (PD-1) PE, CCR7 PeCy7, CD158a/h (KIR2DL1/S1) PE, CD158b (KIR2DL2/DL3) PE, CD158e (KIR3DL1) PE, CD16 APCCy7, CD57 PE, CD69 PE, CD314 (NKG2D) PE, CD226 (DNAM-1) PE, CD337 (NKp30) PE, CD336 (NKp44) PE, CD335 (NKp46) PE (Miltenyi Biotech), CD192 (CCR2) AlexaFluor 647, CXCR2 (IL8RB) APC, 7-AADStaining Solution (BD Italia), TIM3 PE (ebioscience), NKG2C PE (R&D Systems). The analysis was performed with FACS CANTO II. Statistical analysis was performed with Anova and Student's t-test.

Results

Our data indicate that, after the first Ipilimumab treatment, an inversion of CD4/CD8 ratio occurs with a concomitant increase in the $CD56^{dim}$ population and a higher expression of TIM-3 and NKp46 molecules on the surface of NK cells. Moreover, the frequency of NK and T cells expressing KIRs and CCR7 is reduced, while the mean fluorescence intensity of CD16 and PD1 is upregulated on both $CD56^{bright}$ and $CD56^{dim}$ NK cells.

Conclusions

These preliminary data indicate that early during Ipilimumab treatment, cytotoxic lymphocytes $CD8^+$ T cells and $CD56^{dim}$ NK cells expand and become activated. NK cells seem to be polarized towards a $CD56^{dim}CD16^{bright}$ $KIRs^+NKp46^+TIM3^+$ phenotype. Ipilimumab treatment may induce NK cells maturation, which might in turn drive activation of $CD8^+$ T cells.

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