44P PD-L1 blockade during ex vivo expansion of virus-specific T cells for the treatment of infections after allogeneic hematopoietic stem cell transplantation modulates the phenotype and functional activity of T cells

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Background: Viral infection is a major cause of disease and mortality after allogeneic hematopoietic stem cell transplantation (AHSCT) in hematologic malignancies. It has been shown that adoptive transfer of expanded virus-specific T cells (VSTs) was capable of treating infections that are resistant to conventional therapies. Recently, it has been shown that PD-L1 expression by activated T cells plays a major role on their survival and activity. Here, we investigated PD-L1 expression during VSTs expansion and the effect of PD-L1 blockade on the phenotype and functional activity of these VSTs.

Methods: VSTs were generated from healthy donors PBMCs with or without the addition of PD-L1 antibody after stimulation in G-Rex-10 flasks with a pool of 11 overlapping peptides libraries spanning the 5 most immunodominant viral antigens. All VSTs

were collected at day 14. We used ELISpot assay to measure IFN-γ production by VSTs and flow cytometry analysis for phenotyping.

Results: Blockade of PD-L1 has induced 1.8 folds higher proliferation rate and 2 folds increase in IFN-γ production against the viral antigens. After 6 days of expansion, 78% of CD4+ and 60% of CD8+ VST subsets expressed the PD-L1 molecule. At the end of expansion, CD4+ PD-L1+ VSTs were reduced to 6.8% whereas the CD8+ PD-L1+ VSTs were increased to 87.6%. Interestingly, PD-L1 blockade resulted in a further reduction in the CD4+ PD-L1+ population (2.2%), without affecting the CD8+ PD-L1+ VSTs (89.5%). After PD-L1 treatment, the cytotoxicity marker CD107 was slightly increased (1.2 folds) in the CD4+ cells but decreased by 2 folds in the CD8+ VSTs and CD4+ CD45RO+ memory T cells were decreased from 46.1% to 33%.

Conclusions: We have standardized an ex vivo protocol for rapid expansion of VSTs against major viruses causing infection after AHSCT. These VSTs were shown to highly express PD-L1. Blocking of PD-L1 improved the expansion, the anti-viral specificity and the cytotoxicity of these VSTs. This should promote a long-lasting antiviral activity of VSTs in patients undergoing AHSCT. Further investigations are being carried out to confirm these results.

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