Background
Adoptive cell therapy (ACT) with ex-vivo expanded tumor infiltrating lymphocytes (TILs) has shown remarkable results in patients with metastatic melanoma (MM). There is a crucial need for noninvasive biomarkers for screening patients which could improve the selection of patients responsive to therapy. Most studies have until now investigated phenotype, numbers, and persistence of infused TILs, while the role of peripheral immune cell in this process is still poorly understood. Here, we sought to elucidate potential peripheral immune signatures before and early on-treatment to predict patients with clinical outcome of ACT-TIL therapy, as well as characterize its impact on circulating immune cells.

Methods
Here we retrospectively analyzed peripheral blood mononuclear cells (PBMCs) and serum on-treatment changes from 45 patients with MM treated at our cancer center in one of three clinical trials: Study I (NCT00937625), Study II (NCT02379195), and Study III (NCT02354690). Three multicolor flow cytometry panels were developed, two focused on T cells and one designed to measure all major mononuclear subsets and their characteristics. Data were evaluated by algorithm-based clustering and manual gate-setting of major immune cell subsets. Multiplex Luminex and ELISA was used to analyze serum samples to further reveal specific cytokine profiles of responsiveness to TIL therapy.

Results
Higher frequency of CD8+ effector memory (EM) T cells was the only subset characterizing a peripheral immune signature of responsiveness pre-TIL. However, post-TIL therapy multiple immune subsets were associated with clinical outcome. Non-responders were characterized by mobilization of monocyctic and NK subsets at the expense of CD4+ T cells and several subsets of dendritic cells (DCs). While major pro-inflammatory cytokines, IL-6 and IL-8, decreased post-TIL in responding patients they persisted in non-responding patients. In contrast, responders post-TIL therapy was characterized by higher frequency of both cDC2s and pDCs, CD4+ T cells and a subpopulation of CD4+ EM T cells expressing CD28, CD29, CD95, and PD-1.

Conclusions
While TIL therapy encompasses adoptive transfer of ex-vivo expanded preexisting TILs such therapy seems to modify both lymphoid and myeloid cell populations in peripheral blood. This indicates that early on-treatment peripheral immune signature of responsiveness to TIL therapy is characterized by higher frequency of CD4+ T cells and subsets of DCs, and a decrease in inflammatory cells and their mediators.

Trial Registration
NCT00937625, NCT02379195, NCT02354690

REFERENCES

Ethics Approval
The Ethical Committee of the Capital region of Denmark, the Danish Data Protection Agency and the Danish Medical Agencies approved Study I (NCT00937625: EudraCT no. 2008-008141-20), Study II (NCT02379195: EudraCT no. 2014-001420-29), and Study III (NCT02354690: EudraCT no. 2014-001419-38).


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