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Original Research

The proportion of circulating CD45RO$^+$CD8$^+$ memory T cells is correlated with clinical response in melanoma patients treated with ipilimumab

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Abstract ***Background:*** Immune checkpoint blockade (ICB) has been a breakthrough in the treatment of metastatic melanoma. But with only about 20–40% long-term responders and severe side-effects in about 12–17%, finding predictive markers for treatment response is of great interest.

Methods: We prospectively assessed clinical data, haematologic parameters and freshly isolated peripheral blood mononuclear cells of 30 patients treated with ipilimumab (n = 21) and pembrolizumab (n = 9) prior to the first 4 cycles with ICB and before the first tumour assessment.

Results: We discovered that the baseline levels of CD45RO$^+$CD8$^+$ T cells significantly differed among the patients. Thirteen (43%) of our patients had normal baseline levels of CD45RO$^+$CD8$^+$ T cells, whereas 17 (57%) patients were low on CD45RO$^+$CD8$^+$ T cells. The baseline levels of CD45RO$^+$CD8$^+$ T cells correlated significantly with the response to ipilimumab but not pembrolizumab. Patients with baseline levels of lower/equal 25% of CD45RO$^+$CD8$^+$ T cells did not respond to treatment with ipilimumab. Phenotyping the CD8$^+$ T cells in patients treated with ipilimumab revealed an activated HLA-DR$^+$CD25$^-$ phenotype, implying antigen non-specific stimulation. The levels of the HLA-DR$^+$CD25$^-$ CD8$^+$ T cells were significantly higher in patients with a normal baseline of CD45RO$^+$CD8$^+$ T cells and even increased significantly during treatment. Furthermore, proliferation of melanoma antigen recognized by T cells 1 (MART-1)-specific CD8$^+$ T cells was not observed.

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Patients with normal baseline levels of $CD45RO^+CD8^+$ T cells showed a significant longer overall survival when treated with ipilimumab but not pembrolizumab.
Conclusion: Patients with normal baseline levels of $CD45RO^+CD8^+$ T cells respond significantly more frequently to treatment with ipilimumab and the $CD8^+$ T cells appear to be antigen non-specifically activated. The baseline level of $CD45RO^+CD8^+$ T cells represents a promising factor as biomarker for the prediction of the response to ipilimumab.

1. Introduction

The treatment of metastatic melanoma has changed dramatically with the clinical approval of BRAF and MEK inhibitors [1–4] and immune checkpoint blockade (ICB) [5–9]. Ipilimumab is a non-activating antibody against the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) expressed mainly on activated $CD4^+$ and $CD8^+$ T cells and constitutively on regulatory T cells (Tregs) [10]. The interaction with its ligands CD80 and CD86 results in decreased proliferation and tolerance without the death of T cells [11]. Treatment with ipilimumab overcomes this inhibitory effect and leads to an enhanced immune response [12,13], which may lead to long-lasting responses in about 20% of the patients [5,14].

Programmed cell death protein 1 (PD-1) is expressed on T cells, B cells, Tregs and natural killer (NK) cells upon activation [15]. Binding of PD-1 to its ligand reduces the activity of effector T cells and may even lead to their apoptosis. By blocking the interaction, the T cell function may be restored [16–18]. The anti-PD-1 antibodies nivolumab and pembrolizumab block PD-1 and are approved for treatment of metastatic melanoma. Regarding the response rates of 30–40% in patient with metastatic melanoma, both have been shown to be even more effective than ipilimumab [8,9,19,20]. Thus, antibodies against both CTLA-4 and PD-1 lead to activation of T cells resulting at best in an enhanced antitumour response.

So far, some biomarkers for treatment response have been described: increase in the number of $CD8^+$ T cells [21] as well as higher numbers of $CD4^+ICOS^+$ T cells (inducible costimulator) have been associated with a favourable clinical outcome [22]. Another group showed an association of low $KI67^+EOMES^+CD8^+$ T cells with relapse [23]. Antibodies against NY-ESO-1 detected pre- or post-treatment may be associated with clinical efficacy [24]. High serum CTLA-4 levels [25], as well as the increase in absolute lymphocytes and circulating $CD4^+$ and $CD8^+$ T cells have been associated with a higher response rate [26]. However, a reliable marker to distinguish non-responders from responders has not yet been established for ipilimumab. Expression of PD-1-ligand in melanoma has been correlated with better response rates to the treatment with anti-PD-1 antibodies and therefore may be useful as predictive marker in future [6]. In this study, we analysed baseline factors including blood counts, clinical factors and subpopulations of peripheral blood mononuclear cells (PBMCs) of 30 patients before and during treatment with ipilimumab and pembrolizumab, delineated the phenotype of the relevant cells and correlated it with clinical response.

2. Patients and methods

2.1. Patient population and clinical evaluation

Thirty patients with advanced melanoma who were treated at the dermato-oncology unit of the University Hospital of Munich Ludwig Maximilian University (LMU), Germany, were included in the study (Table 1). The study protocol and the written informed consent for the patients were approved by the institutional ethics committee of the LMU. All patients were informed in detail about the study and informed consent was obtained from all individual participants included in the study.

We evaluated the response of the peripheral metastases to ICB on the basis of computed tomography scans. Fresh blood samples were taken one to two days prior to each treatment cycle and one to two day prior to the first tumour assessment. The blood was processed and the lymphocytes stained within hours after the blood draw. The haematologic parameters were analysed in the clinical routine.

2.2. Flow cytometry and antibodies

For separation of the PBMCs we used Lymphoprep© (Axis-Shield, Heidelberg, Germany) following the manual instruction. For cell surface staining, $1–2 \times 10^6$ PBMCs in 50 µl staining buffer consisting of Dulbecco's phosphate-buffered saline (Gibco), 5% foetal bovine serum (Gemini Bio-Products, Sacramento, CA) and 0.02% azide were stained with fluorochrome-conjugated antibodies against cell surface markers prepared as a master-mix solution for 30 min at 4 °C. Cells were acquired the same or the next day. The anti-human-antibodies CD4-FITC, CD3-PE, CD45RO-PeCy5, NKG2D-PeCy7, PD-1-FITC, CD56-APC, CD25-APC, HLA-DR-PeCy7, CCR7-PeCy7, CD25-PeCy7, CD8-FITC and the isotype controls for the staining were obtained from BioLegend (San Diego, CA). Anti-