Combination of PD-1/PD-L1 checkpoint blockade and dendritic cell therapy

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Introduction
The immune response as well as the efficacy of immunotherapy with MoDC is controlled via immune checkpoints, among them the PD-1/PDL-1 pathway. PD-L1, expressed on dendritic cells and on tumor cells, delivers an inhibitory signal to T-cells upon binding to PD-1, expressed on activated T-cells. This could lead to inactivation of the activated T-cells, which are going into apoptosis. An effective immune response against tumors requires the inhibition of such inhibitory signals as well as the activation of antigen specific T-cell response. Blocking of PD-L1 on MoDC as well as blocking of PD-1 on activated T-cells by systemic treatment with antibodies against PD-1 may thus lead to improved efficacy of DC therapy.

Material and Methods
MoDC from patients with advanced pancreatic cancer, who failed standard chemotherapy, were generated from patient’s monocytes by using standard protocols and further matured by activation of Toll-like receptor 3. MoDC were harvested on day 7 and analyzed by flow cytometry. PD-L1 blockade on MoDC was performed by adding anti-PD-L1 or soluble CD80 to MoDC on day 7. The effect of PD-L1 blockade on MoDC as well as the effect of checkpoint blockade with anti-PD-1 antibody Nivolumab on cytokine release and T-cell activity was measured using an allogeneic mixed lymphocyte culture (MLC, ratio MoDC : lymphocytes 1:10) and standard ELISA techniques.

Results
1. Cytokine release upon PD-L1 blockade on MoDC
Cytokine measurement using allogeneic MLC shows a change in the IFN-γ release when PD-L1 on DC is blocked by adding PD-L1 antibody or soluble CD80 to the culture. This effect is higher when anti-PD-L1 is added to the culture (Fig. 1 a). TNF-α is also increased but to a lower extent when anti-PD-L1 is added to the culture as compared to soluble CD80 (Figure 1b).

![Fig. 1a-c: Release of Th1 cytokines IFN-γ and TNF-α and Th-2 cytokine IL-5. MoDC from 6 patients were co-cultured with allogeneic lymphocytes. PD-L1 on MoDC was blocked by adding anti-PD-L1 or soluble CD80 to MoDC on day 7. The release of the TH-2 cytokine IL-5 is decreased by adding anti-PD-L1 or soluble CD80 to the culture as compared to the control (Figure 1c).](image)

2. Cytokine release upon PD-1 blockade on lymphocytes; TH-1 polarization (Th-1/Th-2 ratio) of the immune reaction caused by Nivolumab
By adding Nivolumab to the cultures IFN-γ release as well as TNF-α release are statistically significant (Wilcoxon Test) increased as compared to the control without Nivolumab (Figure 2 a + b). This effect is independent of the type of MoDC generation and maturation. The release of the TH-2 cytokine IL-5 is also increased by blocking PD-1 on lymphocytes but to a lesser extent (Figure 2c).

![Fig. 2a-c: Release of Th1 cytokines IFN-γ (a) and TNF-α (b) and TH-2 cytokine IL-5. MoDC from 6 patients were co-cultured with allogeneic lymphocytes. PD-1 was blocked by adding Nivolumab (100 µg/ml) to the culture. After 6 days the supernatant were harvested and cytokine release was analyzed by standard ELISA techniques.](image)

3. Influence of anti-PD-1 antibody Nivolumab on expression of Granzyme B in CD8+ T-cells
The authors would like to thank all technical assistants from the involved Institutes. A special thank is directed to the technical assistants Andrea Struck and Christina Vogel for their continued commitment in the field of the in vitro experiments. Furthermore the authors thank all patients and their relatives involved in this investigation.

Conclusion
Anti-PD-1 therapy has shown promising results in many solid tumors. However, an effective immune response against tumors requires an inhibition of inhibitory signals by for example anti-PD-1 checkpoint blockade as well as the activation of antigen specific T-cell response inducible by for example vaccine strategies. Here we show that systemic treatment with anti PD-1 antibody Nivolumab using a reduced dosage of 1 mg/kg body weight in combination with dendritic cell therapy can have therapeutic efficacy in patients with advanced pancreatic cancer. PD-1 blockade on lymphocytes triggers a remarkable TH-1 immune profile. Furthermore, CD8+ T-cells show an increased expression of Granzyme B upon Nivolumab treatment, which is necessary for the cytotoxic activity of T-cells. Thus, therapy efficacy may be improved by combination of checkpoint blockades and immunotherapies like dendritic cells to activate antigen specific effector cells trafficking into the tumor site.

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The authors would like to thank all technical assistants from the involved Institutes. A special thank is directed to the technical assistants Andrea Struck and Christina Vogel for their continued commitment in the field of the in vitro experiments. Furthermore the authors thank all patients and their relatives involved in this investigation.

Table 1: Clinical efficacy of DC therapy in combination with a reduced dosage of systemic treatment with Nivolumab

<table>
<thead>
<tr>
<th>Pat</th>
<th>Disease stage primary diagnosis</th>
<th>Disease stage secondary therapy</th>
<th>Surgery of the primary</th>
<th>survival (months) after onset of DC therapy</th>
<th>therapy</th>
<th>Nivolumab</th>
<th>systemic treatment</th>
<th>Survival (months) after primary diagnosis</th>
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<td>IV iv cr</td>
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<td>5 (remission)</td>
<td>11</td>
<td>No</td>
<td>IV iv cr</td>
<td>17 (remission)</td>
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<tr>
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<td>5 (PD)</td>
<td>15</td>
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<tr>
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<td>IV iv cr</td>
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<td>IV iv cr</td>
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<tr>
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<td>8</td>
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</table>

Fig. 3: TH-1/Th-2 ratio measured by the ratio of IFN-γ and IL-5 production.