Phenotypic characterization of infused tumor-infiltrating lymphocytes correlates with response to adoptive cellular therapy in patients with metastatic melanoma

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INTRODUCTION

Adaptive cellular therapy (ACT) with tumor-infiltrating lymphocytes (TIL) is a highly personalized immunotherapeutic strategy with promising results in metastatic melanoma (MM). The positive outcome of a randomized, multicenter phase 3 clinical trial evaluating TIL in MM patients (NCT02271487) was recently announced. In this trial, the objective response rate (ORR) for TIL-treated patients was 49% with 20% obtaining a complete response (CR) according to RECIST 1.1. However, there are currently no validated biomarkers that correlate with response to therapy (Flow Panel 2).

METHODS

Cryopreserved samples of TIL infusion products from all patients with stage III-IV/MM treated with TIL in the phase 3 trial were analyzed using multiparametric flow cytometry. The phenotypic characterization of the infusion product and OR to therapy according to RECIST 1.1 were investigated with manual gating and with dimensionally reduction (SNE) and machine learning algorithms (PCA). This preclinical TIL characterization was used to select suitable TILs for clinical use in ongoing adoptive therapy trials.

CONCLUSION

In this study, we present an extensive characterization of the melanoma TIL infusion product and identify several cellular subsets that correlate with response to therapy. A higher number of infused TIL was associated with response to therapy. In addition, single marker analysis revealed several lymphocyte subsets, including CD8+, CD69-, BTLA- and CD103- TILs, significantly correlated with response to therapy. Supervised clustering analysis showed that effector memory CD8+ TILs with a CD28-, HLA-DR- and a CD27-, CD29-, CD39- phenotype correlated with response to therapy (Flow Panel 2). In addition, TILs with a CD8+, CD28+ and CD95Low phenotype was associated with response to therapy (Flow Panel 2).

RESULTS

Table 1: Baseline characteristics of TIL-treated patients. In total, 60 patients received TIL, with 39 responders (46%) (complete and partial responses) and 21 non-responders (26%) (stable and progressive disease). For two patients, the response was unassessable due to rapid clinical progression or death. None of the clinical baseline characteristics were significantly associated with response to therapy.

Figure 1: Composition of TIL melanoma infusions per patient. Subsets of TILs were identified using unsupervised neighbor embedding plots of four, singlet cells of infused TILs from all TIL-treated patients (Flow Panel 1).

Figure 2: Phenotypic characterization of TIL correlates with response to therapy. (A-D) Boxplots showing the total number of infused cells (A) and the total number of CD4+ and CD8+ TILs (both live- and dead-cells) (D). (B-D) Boxplots showing the total number of CD8+ TIL, with a (B) negative or (C) positive expression of each marker in Flow Panel 2. (E-F) Boxplots showing the total number of CD8+ TIL, with an (E) negative or (F) positive expression of each marker in Flow Panel 3. Only a significant, apparent p-values after correction for multiple comparisons are shown (p-value 0.001, 0.01, 0.022). A higher number and percentage of patients expressing each marker were observed in responders compared to non-responders in Flow Panel 1 and Flow Panel 2.

Figure 3: Superimposed clustering of CD3+CD8+ TIL reveals different subsets associated with response to therapy (Flow Panel 2). (A) SNE plots of five CD3+CD8+ cell clusters of infusions products from all TIL-treated patients. (B) Heatmap of scaled marker expression (columns) per each cluster (rows). Red arrows indicate clusters with a significant difference between responding and non-responding patients. Cluster analysis showed that effector memory CD8+CD103+ TILs from CD8+, HLA-DR- and CD27- phenotype was associated with response to therapy. Only clusters that were significantly associated with response to therapy are shown.

Figure 4: Superimposed clustering of CD3+CD8+ TIL reveals different subsets associated with response to therapy (Flow Panel 3). (A) SNE plots of five CD3+CD8+ cell clusters of infusions products from all TIL-treated patients. Clustering of scaled marker expression (columns) per each cluster (rows). Red arrows indicate clusters with a significant difference between responding and non-responding patients. (B) Boxplot shows each cluster’s absolute number of cells for responding and non-responding patients. P-values are not adjusted for multiple comparisons. Cluster analysis showed that especially CD8+ TILs with a CD69-, CD57- and CD150Low phenotype was associated with response to therapy.

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References


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