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Longitudinal immune monitoring of patients receiving intratumoral injection of a MART-1 T-cell receptor-transduced cell line (C-Cure 709)

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Background aims

Adoptive transfer of tumor-specific lymphocytes is a promising strategy in the treatment of cancer. We conducted intratumoral administration of an allogeneic irradiated continuous T-cell line (C-Cure 709) expressing an HLA-A2-restricted MART-1-specific T-cell receptor (TCR) into HLA-A2$^+$ melanoma patients. The C-Cure 709 cell line is cytotoxic against MART-1$^+$ HLA-A2$^+$ melanoma cell lines and secretes several immune stimulatory cytokines upon stimulation.

Methods

Anti-tumor immune responses against the commonly expressed tumor antigen (Ag) MART-1 were longitudinally analyzed in peripheral blood by fluorescence-activated cell sorting (FACS) before and after intratumoral injection of C-Cure 709.

Results

No treatment-induced increase in Ag-specific T-cell frequencies was observed in peripheral blood, and the phenotype of MART-1-specific T cells was very stable during the treatment. Interestingly, despite a very stable frequency of MART-1-specific T cells over the course of treatment, clonotype mapping revealed that the response was in fact highly diverse and dynamic, with new clonotypes emerging during treatment. Only a few clonotypes were recurrently detected in consecutive samples. One MART-1-specific T-cell clone disappearing from peripheral blood was later detected in a metastatic lesion.

Conclusions

Sequence analyzes of the CDR3 region revealed conserved structural characteristics in the MART-1-specific TCR used by T-cell clones.

Keywords

adoptive transfer, MART-1 antigen, melanoma, T cells.

Introduction

Adoptive transfer of tumor-reactive T cells represents a promising strategy for cancer therapy and it has been shown to be an effective immunotherapeutic approach for patients with metastatic melanoma [1]. Melanoma-specific tumor-infiltrating lymphocytes (TIL) can be found in melanoma metastases [2,3] and data from exploratory clinical trials have demonstrated that infusion of expanded autologous TIL with anti-melanoma reactivity is able to mediate tumor regression in a significant fraction of melanoma patients [4,5]. However, the use of autologous TIL is often limited by the availability of these cells in the tumor tissues; even more so considering non-melanoma cancers. In addition, treatment of early stage cancers, for example minimal residual disease, is in many cases not possible. Thus such a regimen has a limited possibility of becoming more broadly applicable.

To circumvent the problems of achieving sufficient autologous TIL, T-cell receptor (TCR)-transduced peripheral blood lymphocytes (PBL) have been used as a

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source of antigen (Ag)-specific T cells for adoptive transfer. Studies have demonstrated that this strategy is able to generate tumor-specific T cells efficiently [6–8]. Recently, transgenic $CD8^+$ T cells expressing the TCR specific for the tumor-associated Ag MART-1 were adoptively transferred into 15 melanoma patients [9]. Two patients with progressive disease showed sustained clinical responses and had detectable genetically engineered cytotoxic T lymphocytes (CTL) in peripheral blood up to 1 year after infusion. This therapy represents a potential regimen for the treatment of many different malignant diseases.

We aimed to generate a non-autologous adoptive transfer approach that could be available for all patients expressing HLA-A2. For this purpose we used an irradiated continuous T-cell line stably expressing a TCR specific for the HLA-A2-restricted MART-$1_{27–35}$ peptide (AAGIGILTV) (C-Cure 709), which was administrated intratumorally via ultra-sound guidance. After irradiation, this continuous T-cell line maintains cytotoxic potential and cytokine secretion for 1–2 days [10]. Upon initial intratumoral tumor-cell killing, such a T-cell line might be able to induce an inflammatory reaction leading to a systemic immune response directed against tumor cells. A phase I trial was carried out in 2002–2004 to test this hypothesis [10]. We investigated the impact of intratumoral vaccination with the continuous C-Cure 709 T-cell line on the generation of a peripheral anti-tumor response. PBL were analyzed at different time points during vaccination with C-Cure 709 for responses against the commonly recognized Ag MART-1. The MART-1-specific T cells were analyzed in more detail to determine the frequency, functionality, phenotype and clonal composition. Furthermore, tracking of T-cell clonotypes in biopsies from metastatic lesions was carried out to investigate the potential migration of tumor-specific T cells to tumor sites.

Methods

The C-Cure 709 T-cell line

The C-Cure 709 cell line was constructed by transduction of the immortal monoclonal T-cell line C-Cure 707 with a retroviral vector encoding an HLA-A2-restricted TCR specific for the MART-$1_{27–35}$ (AAGIGILTV) Ag [10]. The Ag specificity of the endogene TCR is unknown. As shown previously, irradiation of C-Cure 709 cells did not alter the tumor killing and cytokine production capabilities of C-Cure 709 cells [10].

Patients and treatment regime

Fifteen patients with metastatic melanoma were included in the phase I trial. Their median age was 54 years (range 24–63) [10]. They received irradiated (60 Gy) C-Cure 709 cells intratumorally on days 1, 4, 7, 10, 14 and 28 (one vaccination cycle). On day 38 the patients were evaluated and progression-free patients continued with a second and third vaccination cycle. During the first vaccination cycle blood samples were collected 1 day before vaccination start (day —1) and subsequently on days 7, 14, 28 and 38. In any following treatment cycles blood was collected on days 1 (day 43), 14 (day 57) and 38 (day 81). Biopsies from metastases used and not used for injection of C-Cure 709 were taken prior to intratumoral injection on days 1 and 14 of the first vaccination cycle, and before start up of any second (day 43) or third vaccination cycle (day 87). Timing of biopsies to treatment, along with the irradiation of C-Cure 709 T cells before injection, rendered it improbable that injected cells were still present in the metastases when biopsies were taken. Blood sample and biopsies were only taken upon informed written consent. In the present study, we used PBL from seven different patients (numbers 4, 5, 6, 9, 10, 13 and 15) and analyzed biopsies from four patients (numbers 6, 9, 10 and 13). We were not able to analyze biopsies from all time points because of the destruction of samples during transport. The following biopsies were analyzed: patient 6, one biopsy collected on day —1; patient 9, four biopsies from a metastatic lymphnode used for injection of C-Cure 709 collected on days —1, 14, 43 and 87; patient 10, two biopsies collected on day 14 (one was used for injection and one was not) and one biopsy from day 43 (not used for injection); patient 13, two biopsies collected on day —1 (one used for injection and one not used for injection) and day 14 (not used for injection).

Purification of peripheral blood mononuclear cells and TIL

Peripheral blood mononuclear cells (PBMC) were isolated by density-gradient centrifugation on Lymphoprep® (Nycomed Diagnostika, Oslo, Norway) and cryopreserved. TIL were isolated from core needle biopsies by tissue crushing and passive diffusion to T-cell medium. TIL were separated from the crushed tissue by single-cell separation filters.