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Review

Tumor-infiltrating lymphocytes for the treatment of metastatic cancer☆

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ABSTRACT

Over the past few years melanoma incidence has been rising steadily, resulting in an increase in melanoma related mortality. Until recently, therapeutic options for metastatic melanoma were scarce. Chemotherapy and, in some countries, IL-2 were the only registered treatment modalities. In the last five years, treatment with immunotherapy (anti CTLA-4, anti PD-1, or the combination of these antibodies) has shown very promising results and was able to improve survival in patients with metastatic melanoma. Adoptive cell therapy using tumor-infiltrating lymphocytes is yet another, but highly promising, immunotherapeutic strategy for patients with metastatic melanoma. This review will discuss the development of TIL as a treatment option for melanoma, its mode of action and simplification over time, and the possibilities to expand this therapy to other types of cancer. Also, the future directions of TIL based therapies will be highlighted.

Abbreviations: ACT, adoptive cell transfer; APC, antigen presenting cell; CI, confidence interval; CR, complete remission; C/T, cancer/testis; CTLA-4, cytotoxic t-lymphocyte-associated protein-4; Cy, cyclophosphamide; DNA, deoxyribonucleic acid; DTIC, dacarbazine; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; FDA, Food and Drug Administration; Flu, fludarabine; gp100, glycoprotein 100; Gy, gray; HD-IL2, high dose interleukin-2; HPV, human papilloma virus; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; IU, international unit; i.v., intravenous; kg, kilogram; LAK, lymphokine-activated killer; MAGE, melanoma antigen; MAPK, mitogen-activated protein kinase; MDA, melanocyte differentiation antigens; MHC, major histocompatibility complex; mg, milligram; MIU, million international units; NCI, National Cancer Institute; NK, natural killer (cell); NKI, Netherlands Cancer Institute; NSCLC, non-small cell lung cancer; NMA, non-myeloablative; OR, objective response; ORR, objective response rate; PD, progressive disease; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression free survival; PR, partial response; RCC, renal cell carcinoma; RCT, randomized controlled trial; SAE, serious adverse events; SB, Surgery Branch of the National Institutes of Health; sBM, symptomatic brain metastases; s.c., subcutaneous; TBI, total body irradiation; TCR, T-Cell Receptor; t.i.d, ter in die; TIL, tumor-infiltrating lymphocytes; RECIST, response evaluation criteria in solid tumors; REP, rapid expansion protocol.

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1. Introduction

In 1863, Rudolf Virchow described the presence of lymphoid cells in neoplastic tissue and hypothesized a connection between inflammation and cancer (Virchow, 1863). Over the past two decades, clear correlations have been found between the presence of lymphocytic infiltrates within tumors and patients' clinical outcome in several tumor types, including metastatic melanoma, ovarian cancer, colorectal cancer and breast cancer subtypes (Clemente et al., 1996; Pages et al., 2010; Santoiemma and Powell, 2015; Tuthill et al., 2002; Zhang et al., 2003). The first attempts to isolate and characterize the lymphoid cells in cancerous tissue dates back to the 1970-ies and revealed that many tumor tissues contained lymphocytes (Blazar and Heppner, 1978; Zettergren et al., 1973). Pioneering work in this field of research has been performed by Dr. Steven Rosenberg from the Surgery Branch (SB) of the National Institutes of Health (NIH), Bethesda, Maryland. Rosenberg and colleagues started by growing tumor-infiltrating lymphocytes (TIL) from multiple murine tumors and demonstrated antitumor activity of these TILs *in vivo* (Spiess et al., 1987). In a murine sarcoma model, infusion of TIL in combination with T cell growth factor interleukin-2 (IL-2), appeared to be 50–100 times more effective in killing tumor cells than Lymphokine-Activated Killer (LAK) cells, that were generated by culturing peripheral blood lymphocytes in the presence of high concentrations of IL-2 (Rosenberg et al., 1986). Importantly, TIL cultured from human tumors were also able to lyse autologous but not allogeneic tumor cells in a major histocompatibility complex (MHC) dependent fashion in the majority of cases. This observation pointed towards some patient-specificity of this treatment, while this was lacking completely in LAK cell therapy (Rosenberg et al., 1985). In a first TIL pilot study, twelve patients with metastatic cancer were treated with TIL, with or without the chemotherapeutic agent cyclophosphamide and IL-2 (Topalian et al., 1988). Two partial responses were observed, one in a patient with melanoma and one in a patient with renal cell carcinoma. Both patients received cyclophosphamide prior to TIL infusion. This was the first indication that TIL therapy could induce clinical responses in patients with metastatic cancer and formed the basis for further studies, which will be discussed in this review.

During the past decade, a much better understanding of the working mechanism of TIL therapy has been gained, especially regarding the role of lymphodepleting conditioning of the host, the role of interleukin-2 as a survival factor for the infused TIL, the optimal quality and quantity of the infused cells and their antigen recognition pattern. In addition, although growing TIL was for a long time only successful in metastatic melanoma, the current protocols of TIL outgrowth are now also being explored in other types of cancer as well. These aspects and future developments will be discussed here.

2. TIL therapy for metastatic melanoma

Since, the first clinical trial with TIL therapy by Rosenberg et al., a series of phase I/II clinical trials have shown that infusion of TIL combined with lymphodepleting preconditioning and followed by high dose bolus infusional IL-2 can mediate objective responses in patients with metastatic melanoma (Dudley et al., 2010, 2013, 2002, 2005, 2008; Rosenberg et al., 2011; Rosenberg et al., 1994). Originally, the protocol consisted of a metastasectomy of one or more melanoma lesions. A total size of around 3 cm in diameter was required to be able to successfully grow TIL from these lesions. These resected melanomas were subsequently fragmented into microcultures in the presence of IL-2. Once enough TIL were grown from these cultures, TIL were tested for recognition of autologous melanoma cells (usually melanoma cell lines or freshly frozen tumor digest), and if not available, reactivity to a panel of human leukocyte antigen (HLA) matched allogeneic melanoma cell lines. Readout was the measurement of interferon-γ (IFN) secreted in the medium using an IFN-γ enzyme-linked immunosorbent assay (ELISA). Only those cultures containing melanoma-reactive TIL were further propagated and rapidly expanded by stimulation with soluble anti-CD3 monoclonal antibody, high concentration of IL-2 (6000 IU/ml) and irradiated allogeneic or autologous feeder cells. Starting with approximately 50×10^6 TIL, these numbers were expanded in a 14-day time period to $1–20 \times 10^{10}$ $CD3^+$ TIL. After concentration of the cells to a 200–300 ml suspension, the product was ready for infusion. It was convincingly shown that TILs selected for reactivity towards autologous melanoma cells displayed high functional activity in metastatic melanoma patients, with ORR varying between 34% and 72% of treated patients some of whom developed a long-lasting complete remission, however, there were some important drawbacks associated with this elaborate TIL production protocol (Dudley et al., 2005, 2008; Rosenberg et al., 1994). First, the selection of TIL for reactivity against autologous melanoma required the presence of an autologous melanoma cell line. With a success rate for growing cell lines from patient material of less than 50%, the selection step on autologous tumor could not be done in at least half of the patients (Dudley et al., 2003). Secondly, as only a fraction of cultures contained tumor-reactive TILs, the total culture time to obtain enough cells for initiating rapid expansion (200×10^6 TIL) was long. The risk for these refractory melanoma patients to rapidly progress up to a stage that TIL therapy was no longer considered beneficial, increased with longer culture time. Thirdly, longer culture time also translated into obtaining TIL with a more terminally differentiated phenotype, decreasing their capacity to persist *in vivo* after infusion (Huang et al., 2005; Powell et al., 2005). Together with the inability to grow TIL from 20 to 25% of metastatic melanoma patients, the accumulative dropout rate amounted to 70% or more of patients that could not be treated with TIL in these early studies.

In their first clinical study with these so-called "selected TILs", Rosenberg et al. treated 86 metastatic melanoma patients, of whom 57 received a single dose of 25 mg/kg cyclophosphamide as a lymphodepleting regiment, followed by infusion of selected TIL and high-dose intravenous bolus IL-2 (Rosenberg et al., 1994). The overall ORR in this clinical trial was 34%. Significant differences in overall ORR were noted in patients who were treated with TIL from younger cultures ($p = 0.0001$), TIL with shorter doubling times ($p = 0.03$) and