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Review Article

Trends in Adoptive Tumor Infiltrating Lymphocytes (TILs) Therapy

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Abstract

Adoptive T cell therapy based on tumor infiltrating lymphocytes (TILs) is the most effective treatment for malignant melanoma. The TILs therapy has demonstrated high overall response rates, resulting in tumor regression and prolonged survival in comparison to IL-2 and ipilimumab treatments. Expansion of TILs under ex-vivo conditions using patients' own tumor tissue is a prerequisite for effective adoptive transfer immunotherapy. The process involves growing cells in the presence of IL-2, followed by identification of tumor reactive cells by immunoassays. The treatment itself is safe but preconditioning with chemo or radiotherapy prior to TILs infusion and IL-2 administration is associated with severe side effects. Chemotherapy depletes regulatory T cells and thus enhances efficacy of TILs. Studies have demonstrated that dendritic cell (DC) vaccine augments T cell activation and its infiltration to the primary tumor site. The combination of TILs and DC may circumvent the need for IL-2 injection. This hypothesis is still under investigation for further clinical evidences. This review focuses on the clinical developments of the TILs based adoptive T therapy and its possible combination with DC vaccine.

Key words: tumor infiltrating lymphocytes; Dendritic cells; Clinical trial; Cancer vaccines.

Introduction

Immunotherapy using adoptive T cells has emerged as one of the potent treatment options for metastatic tumors. Three different types of T cells have been reported, including autologus tumor infiltrating lymphocytes (TIL) [1], T cells transduced with chimeric antigen receptor (CAR) [2] and T cells transduced with high affinity T cells receptors (TCR) [3]. The latter two acquire anti-tumor specificity through genetic modifications. The genetically modified T cells are then expanded under *in vitro* conditions followed by adoptive transfer to patients. TILs, on the other hand, are isolated directly from tumors. TILs are activated and expanded under *ex vivo* conditions so that they respond to tumor cells specifically. The existence of lymphocytes in tumors is often associated with better clinical outcomes and reflects tumor-host interaction. Clinical studies have demonstrated higher percentage of activated T regulatory (Treg) cells and activated $CD8^+$ T cells in peripheral blood during the TILs treatment, compared to the untreated control subjects [4]. Extensive studies on the TILs therapy have shown that it is comparable to the established treatments for curing various cancers, especially metastatic melanoma [5]. In this review we summarize different aspects of the adoptive TILs therapy.

Methodology for TILs expansion

Tumor fragments are obtained from the patient by surgery (Figure 1, Step 1).

The basic protocol for TILs expansion involves directly incubating tumor fragments in culture plates in the complete media (RPMI 1640, 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mmol/L-glutamine, supplemented with 10% human serum) (Step 2a). A dense carpet of lymphocytes appears around the tumor fragment after 1-2 weeks of incubation. Alternatively, the tumor fragments are digested enzymatically by collagenase, hyaluronidase, and DNAse (Step 2b), followed by purification on a single step ficoll gradient (Step 3). The TILs fraction is separated and incubated in the complete medium (Step 4). These lymphocytes (young TILs) are expanded to a confluent growth. While the young TILs expand, they eliminate tumor cells by direct contact or by the secretion of cytokines. Each TILs culture from initial tumor fragment gives around 5×10^7 cells after 21-36 days of culture. IL-2 is commonly used in TILs expansion protocol as a T cell growth factor. It has also been reported that IL-15 and IL-21 have similar proliferative effects on TILs [6, 7]. IL-21 promotes the expansion of $CD8^+$ T cells from TILs with less differentiated "young" phenotype, superior cytotoxic effector characteristics, and low collateral Treg numbers [8]. Rapid expansion method involves TILs culture in which irradiated peripheral blood mononuclear cells (PBMCs) serve as feeder cells along with anti-CD3 antibody (Step 5) [10]. The expansion step increases the cell count by 3000 folds in about 14 days. This process requires a large quantity of cytokines

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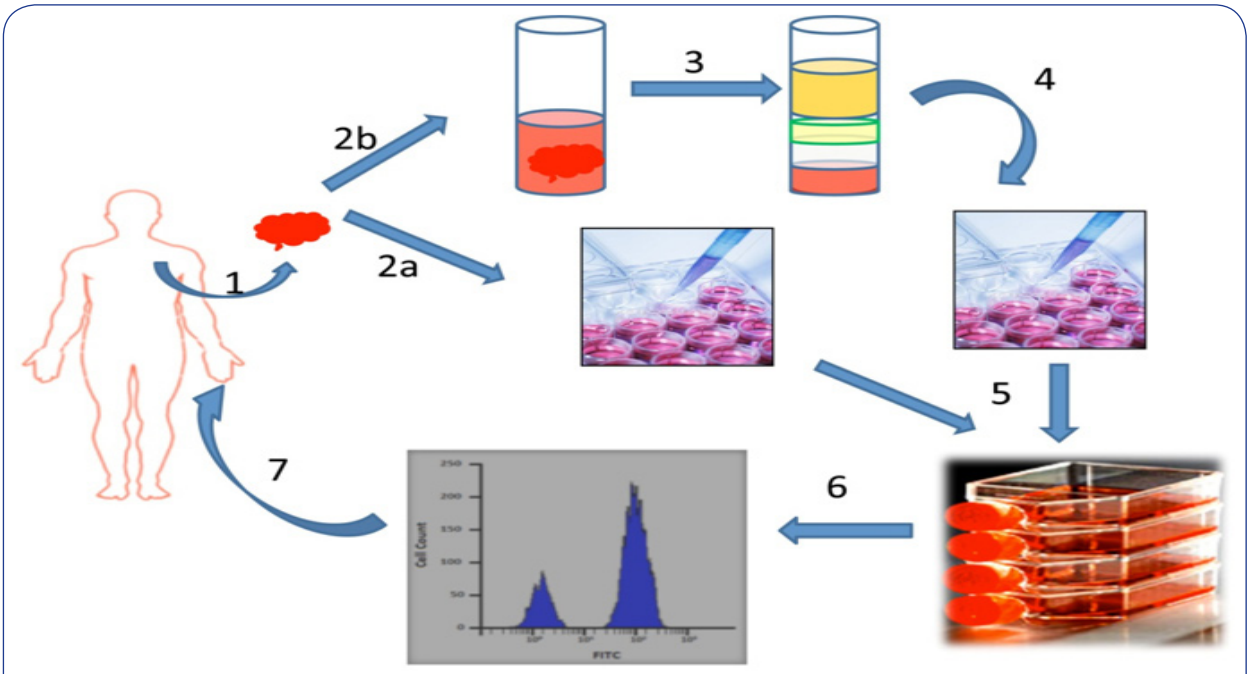

Figure 1: Schematic representation of initiation and expansion of tumor infiltration lymphocytes *ex vivo*

1. Surgical removal of cancer tissues from the patient

2a. Tumor fragments of approximate 1-2 mm dimension are placed in tissue culture plates

2b. Tumor fragments are immersed in a mixture of enzymes (collagenase, hyaluronidase, and DNAse) and incubated overnight

3. Ficoll gradient for lymphocyte separation

4. Lymphocytes are allowed to grow in culture plates with the complete medium

5. TILs are expanded in presence of anti-CD3 antibody and IL-2 in culture flask/bag

6. FACS analysis

7. Intravenous administration of TILs to the patient

and growth factors. Such consumptions can be reduced by using optimized conditions in bioreactors and gas permeable flasks [9]. The expanded cells are assayed for CD3, CD4, CD8, CD27, CD28, CD56, CD62L and CCR7 by flow cytometry analysis (Step 6). TILs' activity is estimated by measuring the IFN-gamma secretion [10]. Joseph et al (2011) found that the initial rate of TILs expansion is not affected by pathologic features of tumor, its location, subtype, or mutation status [11]. Independent initiation and expansion of diverse TILs cultures from a small melanoma specimen could improve the frequency of generating tumor-reactive cultures [11]. In addition, studies indicate that 'young TILs' are more tumor specific than standard TILs [7].

Clinical developments

The first clinical pilot study using TILs was reported in 1988 for metastatic melanoma [12]. The study was performed on 12 patients who were treated with variable doses and combination of TIL, IL-2 and cyclophosphamide. The result demonstrated partial response in 2 patients and partial regression in 1 patient. Tumor specific cytolytic activity was observed in 5 patients. The study demonstrated the possibility of treating the patients with a combined approach. In another report published in same year, 20 melanoma patients were treated with TILs and IL-2 preconditioned with a single dose of cyclophosphamide [13]. Objective response was observed in 9 patients who did not receive IL-2 and 2 patients in whom previous IL-2 therapy failed. These initial reports indicated that TILs treatment regimen may provide higher response rate in melanoma patients. In continuation of the above studies Rosenberg et al (1994) treated 86 patients of metastatic melanoma with autologus TILs and high dose of IL-2 [14]. Overall objective response rate of 35% was observed in patients who received cyclophosphamide and 31% in those who did not. In another study by Rosenberg et al (2011) three sequential clinical trials were performed in which 93 patients (metastatic melanoma) were treated with lympho depleting preparative regimen (chemotherapy alone or chemo with 2Gy or 12Gy irradiation), autologous TILs and IL2 [15]. Objective response rates by RECIST criteria in the three trials were 49%, 52% and 72%, respectively. Study showed that 22% of all patients

achieved complete tumor regression and 19% of the patients were disease-free for more than three years.

In a phase II metastatic melanoma study, clinical responses were evaluated using short-term cultured TILs with lympho-depleting chemotherapy [16]. The study showed that lympho-depleting chemotherapy followed by transfer of short-term cultured TILs could mediate tumor regression in 50% of the patients with minimum toxicity [16]. Goff et al (2010) found that TILs could mediate objective tumor regression in 49% to 72% of the patients with many long-term durable responses [17]. Further, this study established that active and specific TILs could be generated from tumors with larger size and higher lymphocytes percentage count.

The clinical usage of TILs has been demonstrated in melanoma associated metastasis and other malignancies both at therapeutic and prognostic level. Hong et al. (2010) investigated the response to the treatment of TIL, IL-2 and preconditioned lymphodepletion in melanoma brain metastasis patients [18]. About 41% of the patients who received the therapy demonstrated complete response whereas 35% achieved partial response. The results are significant as other systemic treatments for melanoma with brain metastasis are limited and largely ineffective. Further, it addresses the response at both intracranial and extra cranial sites simultaneously. The functional reactivity of TILs against tumor antigens in colorectal cancer patients was demonstrated by Koch et al (2006) [19]. In head and neck squamous cell carcinoma, patients were assessed for number of tumor infiltrating $CD3^+$ and $CD8^+$ cells. It was concluded that the ratio of $CD3^+$ and $CD8^+$ cells could serve as a biomarker to identify benefit to the patients from chemo-radiotherapy [20]. Sato et al (2005) performed immunohistochemical analysis for TILs and cancer testis antigens in 117 cases of epithelial ovarian cancer [21]. The study showed that intraepithelial $CD8^+$ TILs and a high $CD8^+$/Treg ratio were associated with favorable prognosis.

Galon et al (2006) studied tumor-infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and in situ immunohistochemical staining [22]. The data analysis suggested that immunological parameters were better predictors of patient survival than the histopathological methods. Furthermore, *in situ* data analysis suggested that TILs might act as a valuable prognostic tool in the treatment of colorectal cancer. Pages et al (2009) evaluated the treatments of cytotoxic (CD8) and memory (CD45RO) T cells in patients with early-stage colorectal cancer [23]. The results gave valuable information regarding tumor recurrence and survival in patients with early-stage colorectal cancer.

Gingras et al (2015) critically reviewed the relationship between immunity and breast carcinoma [24]. Mahmoud et al (2011) studied clinical outcome of the TILs treatment in breast cancer patients [25]. The results suggested that tumor-infiltrating $CD8^+$ T lymphocytes had antitumor activity and could potentially be exploited in the treatment of breast cancer. Since there has been an increase in the use of molecular fingerprint analysis in the field of cancer research, standard prognostic, predictive and diagnostic parameters are ever changing. Continued research in TILs can further our understanding in tumor immunotherapy and improve effectiveness of therapies.

Combination approach for effective antitumor response

Effective antitumor responses in patients with malignancies depend on the presence and function of immune cells that can recognize and eliminate tumor cells [26, 27, 28, 29]. Among the immune cells, DCs are the antigen presenting cells (APCs) that play major role in activating the immune system against cancer cells. The application of *ex vivo* generated DCs emerged in an effort to improve the therapeutic efficacy in cancer patients with dysfunctional endogenous DCs. In the last decade, a large number of clinical trials were carried out to establish the therapeutic efficacy of DC vaccines. Among the over 200 DC based clinical trials so far, melanoma was the most common cancer treated which established the feasibility and safety of DC vaccines [30].

The advantages of the DC vaccines include low toxicity (grade 3 and 4 level toxicities are rare), less possibilities of immunotherapy induced autoimmunity, and induction of immunogenicity even in advanced malignancies [31, 32]. On the other hand, there is still a lot to improve in the clinical efficacy. One approach to achieve that is the combinatorial therapy of DC Vaccines and TILs (Table 1). Park et al (2007) carried out preclinical studies on murine colorectal tumor by combining the two immunotherapeutic approaches [33]. The results demonstrated that an optimal time interval between adoptive T cell transfer and DC vaccination could induce a potent antitumor response. In a similar study with 4T1 mammary carcinoma mice model, DCs were fused with 4T1 tumor cells at 1:1 ratio to produce DC-tumor hybrid vaccines [34]. The hybrid vaccines and lymph node derived immune T cells were used for treatment. This strategy dramatically increased T cells in lungs and spleen, resulting in prolonged survival. In another melanoma murine model study, injection of DC vaccine along with adoptively transferred T cells improved the antitumor response in comparison to either treatment alone [35]. Moreover, the vaccination combination (DC and TILs) led to tumor regression and provided protection against tumor re-challenges. Poschke et al (2014) demonstrated the feasibility and safety of DC/TILs combination in a clinical trial with patients suffering from advanced metastatic melanoma [36]. Furthermore, many preclinical studies have proved that this combination approach resulted in a significant tumor regression [37, 38]. During the TILs treatment, a high concentration of IL-2 is needed. The addition of DC vaccination may reduce the need for lymphocyte depleting chemo conditioning and *in vivo* IL-2 support for the T cells. Kandalaft et al (2013) showed dendritic cell vaccination with bevacizumab and metronomic cyclophosphamide, followed by autologous adoptive T-cell therapy was practicable and well tolerated [39].

Aquilar-Cazares et al (2014) studied the density of intratumoral DCs and their impact on the density of TILs in 82 patients with non-small cell lung carcinoma (NSCLC) [40]. A 2.5-fold boost

Cancer type	Therapy	Number of patients	Infusion	Clinical outcomes	Reference
Melanoma	TIL	90	~4.1×10^{10}	TIL proved to be clinically effective in mediating tumor regression.	[10]
	TIL	44	~39.4×10^{9}	15 OR; 9 SD and 7 PD	[43]
	TIL	226	Variable	Success rate is higher in women and younger male patients.	[11]
	DC and TIL	8	DC: 0–72×10^{6} TIL: 1.1–20.6×10^{8}	Tumor regression and complete response for 50 months were observed.	[36]
	TIL	20	$36.5–57\times10^{9}$	10 OR; 4 SD; 6 PD	[16]
	TIL	99	Data not shown	95% PD	[11]
Colorectal	DC and TIL	49	Data not shown	Enhanced reactivity of CD8 tumor infiltrating lymphocytes (TILs) in patients with systemic tumor antigen specific immunity.	[19]
Brain Metastases melanoma	TIL	26	$10.8–104\times10^{9}$	High objective response rates were observed at both intracranial and extra cranial sites.	[18]
Ovary	DC and TIL	6	DC: $5–10\times10^{6}$ TIL: 5×10^{9}	2 PR 2 SD	[39]

Table 1: Data on the outcome of clinical trials using tumor infiltrating lymphocytes and dendritic cell vaccines.

TILs: Tumor infiltrating lymphocyte therapy; DC: Dendritic cell therapy; CR: Complete response; PR: Progressive Disease; SD: Stable disease; OR: Objective Response; PR: Partial Response

in TILs cell count was found in specimens with high infiltrating DCs densities compared to those with low DCs densities. The study showed that quality of life and survival of NSCLC patients improved with the combined treatment of tumor-DCs-TILs [41]. Fukunaga et al (2004) demonstrated that $CD8^+$ TILs together with $CD4^+$ TILs and dendritic cells improved the prognosis of patients with pancreatic adenocarcinoma [42]. The results showed that overall survival rate was significantly higher in $CD4^+/8^+$ patients (13 cases) compared to those in other groups $CD4^+/8^-$, $CD4^-/8^+$); $CD4^-/8^-$ (67 cases).

Conclusion

It is evident that the field of adoptive cellular therapy is growing fast. Several types of immunotherapies have shown great clinical impact. Cellular cancer therapies like dendritic cell therapy, activated T cell therapy and TILs are becoming more popular choices of cancer treatment due to the generation of clinically effective antitumor response and relatively low side effects. Based on the clinical trials data, TILs have shown good effectiveness in treating melanoma and other multiple groups of malignancies such as breast and brain metastasis. There have also been some clinical trials that failed to produce good results with TILs. The negative outcomes may be attributed to the advanced tumor stages of the patients. Despite considerable progress over the last decade, it is very difficult to draw a comprehensive conclusion on immunotherapeutic strategies in treating cancers. Treatment with combination of DC and TILs in patients with early stage cancer may provide us more insights in this field. Cancer immunotherapy needs further in-depth investigations on combination approaches that can improve long term efficacies and reduce the cost to a more affordable level.

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