Developing innovative strategies of tumor-infiltrating lymphocyte therapy for tumor treatment

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Abstract. Cancer is the main cause of global mortality, and thus far, effective therapeutic strategies for cancer treatment are in high demand. Adoptive transfer of tumor-infiltrating lymphocytes (TILs) represents a promising avenue in immunotherapy for the management of malignancies. The clinical safety and efficacy of TIL-based therapy have been established through numerous rigorous clinical trials. However, the efficacy of TIL infusion in inducing an anti-tumor response is limited to a subset of clinical patients with cancer. Therefore, there is an urgent need to develop innovative strategies aimed at enhancing the effectiveness of TIL-based therapy. In the present review, the developmental history of TIL-based therapy was systematically summarized and analyzed, while also presenting a unique perspective on enhancing the multi-dimensional anti-tumor capabilities of TILs. The insight and conclusions presented in this review may contribute to improving the efficacy of TIL-based therapy and expediting its development.

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Abbreviations: CAR, chimeric antigen receptor; TIL, tumor-infiltrating lymphocyte; ZFNs, zinc finger nucleases; PD-1, programmed cell death 1; TCR, T-cell receptor; TRAIL, TNF-related apoptosis-inducing ligand; FasL, Fas ligand; CRR, complete response rate; iPSCs, induced pluripotent stem cells; aAPC, artificial antigen-presenting cells; OV, oncolytic virus

Key words: tumor-infiltrating lymphocyte, adoptive cellular immunotherapy, anti-tumor properties, gene editing

Contents

1. Introduction

Cancer is one of the leading causes of mortality, accounting for ~10 million annual deaths worldwide. According to the latest report, it was estimated that annually, there were ~4,064,000 new cases and ~2,413,500 cancer-associated deaths in China (1). In the US, there were ~1,958,310 new cancer cases and ~609,820 cancer-associated deaths in 2023 (2). In addition, cancer also negatively affects a country's economic growth; the estimated global economic cost of cancer between 2020 and 2050 is $25.2 trillion (3). Of note, the burden of cancer shows a continuously increasing trend worldwide, and as such, cancer prevention and control are becoming an ever more prominent subject. However, the efficacy of treatments with traditional therapeutic strategies is limited, and thus, there is an urgent need to develop novel approaches for cancer treatment.

Chimeric antigen receptor (CAR)-T-cell therapy is a revolutionary success in cancer-adoptive cellular immunotherapy. Adoptive cellular immunotherapy is a promising strategy for curing cancer completely. Numerous remarkable clinical responses have been observed in treating certain subsets of B-cell leukemia or lymphoma with CAR-T cells; however, several challenges limit their therapeutic efficacy in solid tumors (4). Tumor-infiltrating lymphocyte (TIL) therapy is a type of adoptive cellular immunotherapy in which TILs are harvested from tumor tissues. After amplification *in vitro*, TILs are infused back into a patient and exert a specific killing

effect on cancerous cells (Fig. 1). Since TILs are autogenous lymphocytes that are not genetically modified, they exhibit T-cell receptor (TCR) clone diversity, superior tumor-homing ability and low off-target toxicity, and thus, there are scarcely any adverse reactions. Therefore, TIL-based therapy offers unique advantages in treating solid tumors compared with other adoptive cellular therapies (5,6).

However, in the tumor microenvironment, given the antigen heterogeneity and tumor evolution, the application of TIL-based therapy is still limited. In the present review, the fundamental studies on TIL-based therapy and clinical trials on improving the therapeutic effect of TILs are summarized. Furthermore, the current limitations, challenges and opportunities of TIL-based therapy are also discussed. Finally, the future developmental directions of next-generation TIL-based therapy are highlighted.

2. Relevant techniques and significant findings in the developmental trajectory of TIL-based therapy

In 1980, Yron *et al* (7) reported that lymphoid cells in suspension derived from tumor tissues were able to expand and grow continuously in the presence of T-cell growth factor without tumor cell proliferation. These lymphoid cells exhibited significant cytotoxic activity towards syngeneic tumor cells and normal fibroblasts grown in culture but did not lyse normal lymphoid cells. After 6 years, they reported a novel approach by adopting immunotherapy towards cancerous cells using TILs. Their results showed that in combination with cyclophosphamide, treatment with TILs and IL-2 cured all mice bearing MC-38-induced colon adenocarcinoma of advanced hepatic metastases, and up to 50% of mice were cured of advanced pulmonary metastases (8). These data provide a rationale for the use of TILs in the treatment of patients with advanced cancer.

In 1988, Topalian *et al* (9) performed a pilot study to investigate the feasibility and practicality of administering IL-2-expanded TILs in patients with metastatic cancer; two partial responses to therapy were observed and one additional patient with breast cancer experienced a partial regression of disease. In five of six patients with melanoma, TILs demonstrated lytic activity specific to the autologous tumor target. No toxic effects were observed that were directly attributable to TIL infusions. This study represents an initial attempt to use TILs as a means of immunotherapy in patients with cancer. Similarly, in 1994, Rosenberg *et al* (10) reported that treatment with TILs and IL-2, with or without cyclophosphamide, resulted in objective responses (ORs) in approximately one-third of patients with metastatic melanoma, and the treatment was safely administered. These results demonstrate the practicality of TILs for the treatment of patients with melanoma.

In 2008, Tran *et al* (11) developed an alternative 'young' TILs method; this approach used tumor tissue to rapidly expand TILs for administration without testing for tumor recognition. These younger TILs exhibited higher levels of antigen reactivity, longer telomeres and higher levels of CD27 and CD28. This strategy may facilitate the widespread application of TILs in tumor immunotherapy. In 2011, Itzhaki *et al* (12) described an efficient and reliable method for generating young-TILs for adoptive transfer therapy. Treatment with these young TILs resulted in an OR rate (ORR) of ~50% in patients with refractory melanoma (12). Thus, this approach may be adopted for the treatment of other types of malignancies.

In 2012, Jin *et al* (13) described a modified method for the initial culture and rapid expansion of TILs in gas-permeable flasks. TIL initiation and rapid expansion procedure in gas-permeable G-Rex flasks required fewer total vessels, less media, less incubator space and less labor than other approaches. In addition, TIL culture in G-Rex flasks facilitated the production of TILs for the treatment of patients. In the same year, Friedman *et al* (14) found that at least 20% of metastatic melanomas contained $CD4^+$ lymphocytes with specific tumor recognition by interferon-γ release assay. After lymphodepletion, a patient with widespread metastatic disease was administered TILs containing human leukocyte antigen (HLA) class II-restricted tumor activity with high-dose IL-2 therapy that mediated the regression of extensive metastatic disease in the liver and spleen. These results suggest a possible role for $CD4^+$ cells in the effectiveness of adoptive cell therapy.

In 2014, Tran *et al* (15) used a whole-exome sequencing-based method to demonstrate that TILs from a patient with metastatic cholangiocarcinoma contained $CD4^+$ T helper type 1 (Th1) cells recognizing a mutation in the erb-b2 receptor tyrosine kinase 2 interacting protein frequently expressed in cancer. Adoptive transfer mutation-specific polyfunctional $CD4^+$ Th1 cells may help a patient achieve prolonged stabilization of disease. Patients with cancer progression treated with $CD4^+$ Th1 cells have also been shown to exhibit tumor regression. These results provide novel evidence that $CD4^+$ T cells recognize and respond to mutated antigens, and this can be exploited to promote the regression of metastatic epithelial cancer.

In 2015, Zhang *et al* (16) genetically engineered TILs to secrete single-chain IL-12 selectively at the tumor site. The IL-12 encoding gene was driven by a nuclear factor of the activated T-cell promoter. In this first-in-man trial, administration of genetically engineered TILs mediated tumor responses in the absence of IL-2 administration using cell doses 10- to 100-fold lower than conventional TIL-based treatment. However, due to the toxicity of secreted IL-12, further improvements are necessary before this approach can be safely used in the therapy of patients with cancer. In addition, Beane *et al* (17) used zinc finger nucleases (ZFNs) directed against the gene encoding human programmed cell death 1 (PD-1) to genetically edit melanoma TILs, which resulted in a 76% reduction in PD-1 surface expression. Of note, the genetically edited TIL product showed improved *in vitro* effector function and a significantly increased polyfunctional cytokine profile compared to unmodified TILs. Furthermore, all donor cells displayed an effector memory phenotype and expanded on a large scale *in vitro*. However, the efficiency and safety of PD-1 gene-edited TILs for the treatment of metastatic melanoma requires further study.

The adoptive transfer of neoantigen-reactive TILs can result in tumor regression in patients with cancer. Thus, neoantigen-specific TCR identification is a key technology for improving the clinical efficacy of TILs. In 2017, Parkhurst *et al* (18) identified 27 TCRs from 6 patients that specifically recognized 14 neoantigens expressed by

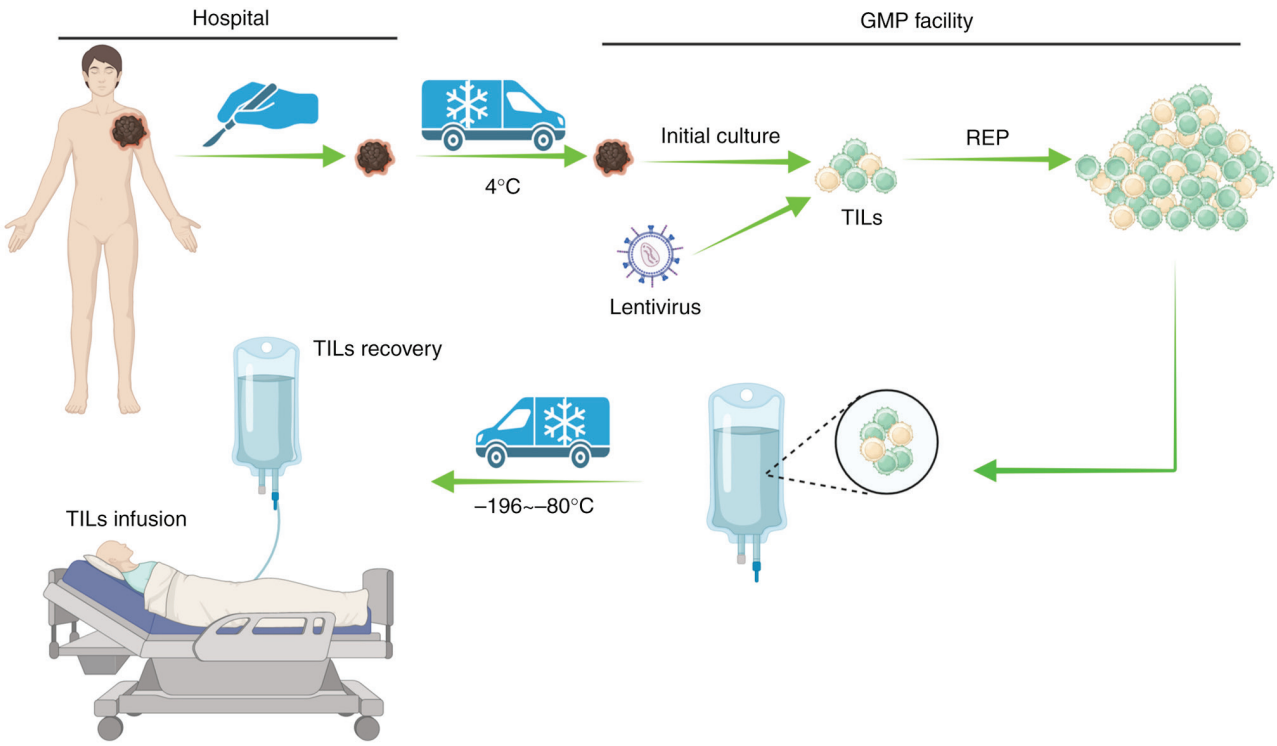

Figure 1. Workflow of TIL therapy. TIL, tumor-infiltrating lymphocyte; GMP, good manufacturing practice; REP, rapid expansion protocol.

autologous tumor cells. In 2018, Lu *et al* (19) developed a new TCR identification approach, which included the co-culture of TILs with tandem minigene-transfected or peptide-pulsed autologous antigen-presenting cells and identification of paired TCR sequences using single-cell RNA sequencing analysis. Transduced T cells with these TCRs specifically recognized the neoantigens. This strategy provides an efficient procedure to identify neoantigen-specific TCRs for use in future clinical applications for patients with cancer.

In 2019, Nguyen *et al* (20) used a modified TIL protocol with a lower dose of IL-2 in a single-institution phase II clinical trial in patients with unresectable, metastatic melanoma. Their results showed that this novel protocol of low-dose IL-2 following adoptive cell transfer of TILs was feasible and clinically effective (20).

In 2020, Krishna *et al* (21) identified a memory-progenitor CD39-negative stem-like phenotype ($CD39^{-}CD69^{-}$) by using high-dimensional analysis of human adoptive cell therapy products. These TILs were associated with complete cancer regression and TIL persistence. In addition, these TILs were capable of self-renewal, expansion, persistence and a superior antitumor response *in vivo*.

In 2021, Sinicrope *et al* (22) reported that lower circulating adiponectin levels were not only associated with an increase in TIL densities in colon cancer but also with an enhanced antitumor immune response.

In 2022, Chamberlain *et al* (23) used clustered regularly interspersed short palindromic repeats (CRISPR)-CRISPR associated 9 (Cas9) gene editing to knock out PD-1 in TILs; an 87.53% reduction in cell surface PD-1 expression was observed, and PD-1 knockout did not affect the final fold expansion of TILs. These results demonstrated that a non-viral, non-plasmid-based CRISPR-Cas9 gene editing method can be feasibly adopted into a TIL-based adoptive cell transfer therapy protocol to produce treatment products without any evident negative effects.

In 2023, Forsberg *et al* (24) produced CAR-TILs through transducing a lentiviral vector encoding an anti-HER2 CAR construct. CAR-TILs were able to eradicate melanoma in patient-derived xenograft mouse models, even in the absence of antigen presentation by HLA. Furthermore, the tolerable and anti-tumor activity of CAR-TILs was also observed in four companion dogs. These results demonstrated that CAR-TIL therapy is a promising approach for improving the tumor-targeting capacity of TILs.

Representative techniques and significant findings in the development of TIL-based therapies are summarized in Fig. 2.

3. Molecular and cellular mechanisms underlying the function of TILs

TCRs are heterodimers comprised of an α and β chain; the TCR repertoire is highly diverse and allows T cells to specifically recognize multiple types of major histocompatibility complex I-presenting tumor antigens. After interaction with tumor antigens, TCRs complex with CD3ϵ/γ/δ/ζ subunits to ensure signal transduction and drive the antigen-specific immune response to the cancer cell (25,26).

The TCR and CD3 complex transduces extracellular signals to intracellular effectors and thus has an irreplaceable role in the activation of TILs and their anti-tumor properties. The activated TILs secrete various cytokines to improve the killing effect on tumor cells. For example, activated TILs upregulate the expression of CD107a on the cell surface, which is associated with cytotoxity and degranulation. Furthermore, the cellular signals increase the release of IFN-γ, a key effector

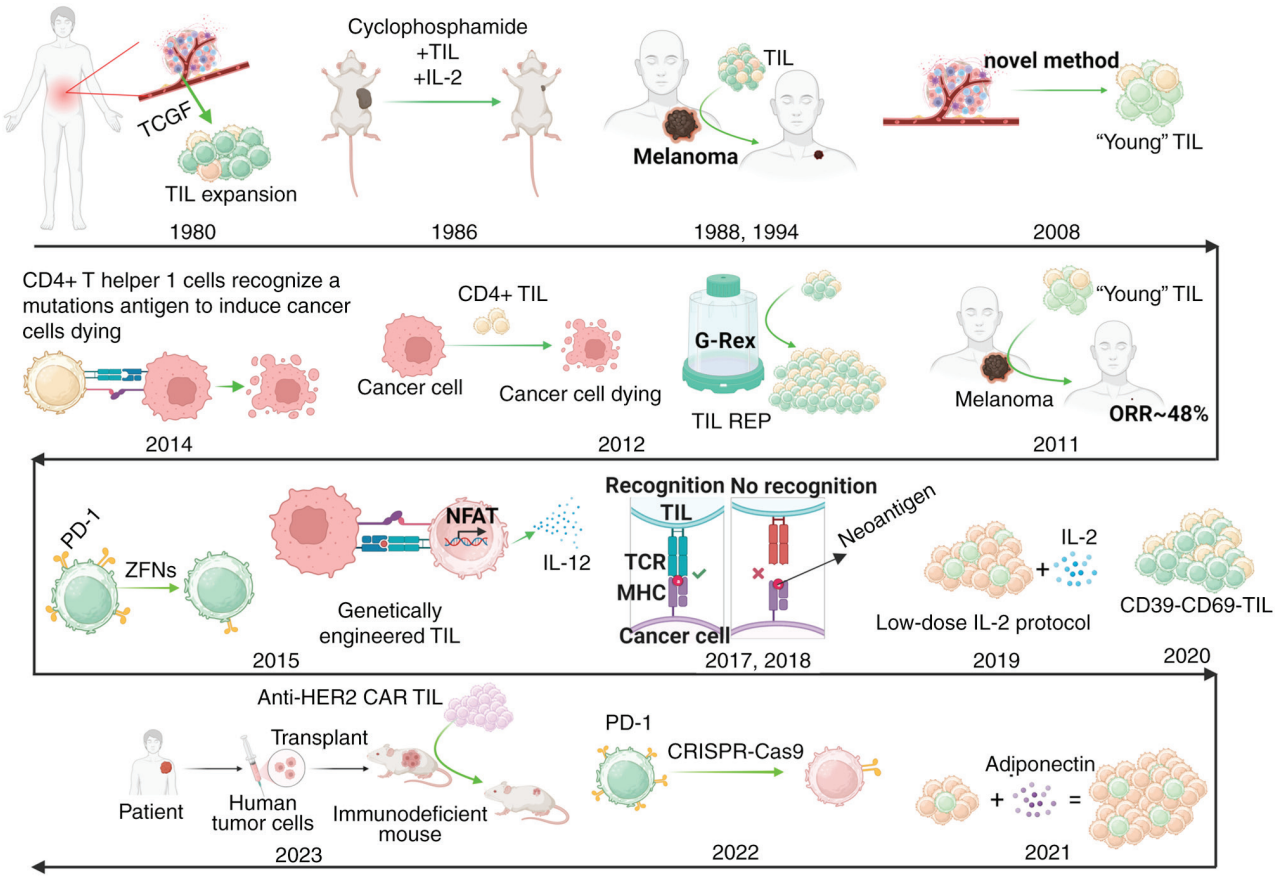

Figure 2. Representative techniques and findings in the development history of TILs. TILs, tumor-infiltrating lymphocytes; TCGF, T cell growth factor; REP, rapid expansion protocol; ZFNs, zinc finger nucleases; NFAT, nuclear factor of activated T cells; TCR, T-cell receptor; MHC, major histocompatibility complex; CRISPR-Cas9, clustered regularly interspersed short palindromic repeats-CRISPR associated 9; HER2, human epidermal growth factor receptor 2.

of TIL anti-tumor activity (27). Importantly, IFN-γ can induce apoptosis of both Fas-high and Fas-low cancer cells by upregulating apoptosis-related proteins, such as caspase-3, Bak and Fas (28). Activated TILs produce more TNF-α, perforin and granzyme, which further enhance the anti-tumor function of TILs (29-31). TNF-α initiates cancer-cell apoptosis through p53-dependent pathways by upregulating death receptor-4 and death receptor-5 (32). Interestingly, TNF-α can also improve the sensitivity of cells to Fas ligand (FasL)-induced cell death (33). Granzyme and perforin are released by TILs and have both been shown to exert anti-cancer properties in several types of cancer. Perforin and granzyme B prevent the formation of cancer cell foci and induce cancer-cell apoptosis (34,35). Mechanistically, granzyme B induces the accumulation of reactive oxygen species, which results in dysregulated mitochondrial function and cytochrome c release, and therefore, accelerated cancer cell apoptosis (36,37).

The interaction between TCRs and tumor antigens upregulates the expression of death ligands on the surface of TILs, including TNF-related apoptosis-inducing ligand (TRAIL) and FasL. TRAIL and FasL can recognize TRAIL receptor and Fas on cancer cells, respectively, and induce cancer-cell apoptosis (38-40). Binding of TRAIL to its receptor activates caspase family-dependent apoptosis signaling, such as caspase-8, -10 and -3. The activated caspase-8 leads to mitochondrial outer membrane permeabilization via the Bid-Bax/Bak pathway, which results in mitochondrial disorder and cytochrome C release, accelerating cancer-cell apoptosis (41). Furthermore, FasL binds Fas to induce cancer-cell apoptosis through the MAPK, NF-κB and caspase-8 signaling pathways (42).

Taken together, TILs show powerful anti-tumor properties via multiple mechanisms (Fig. 3).

4. Novel strategies for augmenting the anti-tumor efficacy of TILs

The anti-tumor properties of TILs have innate advantages, such as high safety, TCR diversity and superior tumor-homing ability, amongst other benefits (5,6). However, there remain several challenges regarding their clinical use. Thus, innovative therapeutic strategies are required to improve the anti-tumor properties of TILs.

Current challenges in the utilization of TILs for cancer therapy. In the present review, 10 clinical trials were randomly selected and the clinical complete response rate (CRR) was analyzed. A total of 542 patients with different types of cancer were treated with TIL therapy and the CRR ranged from 2.4-75% with an average CRR of 14.15% (Table I) (43-52). As shown in Fig. 4, there were differences in CRRs among the different clinical trials. These clinical data indicated that the effectiveness and stability of TIL-based therapy requires further improvement.

Table I. Previous clinical data of tumor-infiltrating lymphocytes in cancer treatment (total patients, n=542).

No.	First author, year	Cancer type	Classification	Patients, n	CRR, %	(Refs.)
1	Goedegebuure, 1995	Melanoma	Metastatic melanoma	16	19	(43)
2	Goff, 2016	Melanoma	Metastatic melanoma	101	24	(44)
3	Huang, 2022	Cervical cancer	Locally advanced cervical cancer	12	75	(45)
4	Chesney, 2022	Melanoma	Advanced melanoma after progression on immune checkpoint inhibitors	153	5.2	(46)
5	Zacharakis, 2022	Breast cancer	Metastatic breast cancer, phase II	42	2.4	(47)
6	Stevanović, 2019	Cervical cancer	HPV-associated cervical cancer	18	11.11	(48)
7	Rosenberg, 2011	Melanoma	Metastatic melanoma	93	22	(49)
8	Queirolo, 1999	Melanoma	Metastatic melanoma	19	10.5	(50)
9	Dudley, 2010	Melanoma	Metastatic melanoma	33	6	(51)
10	Figlin, 1997	Renal cell carcinoma	Metastatic renal cell carcinoma	55	9.1	(52)

The average CRR was 14.15%. HPV, human papillomavirus; CRR, complete response rate.

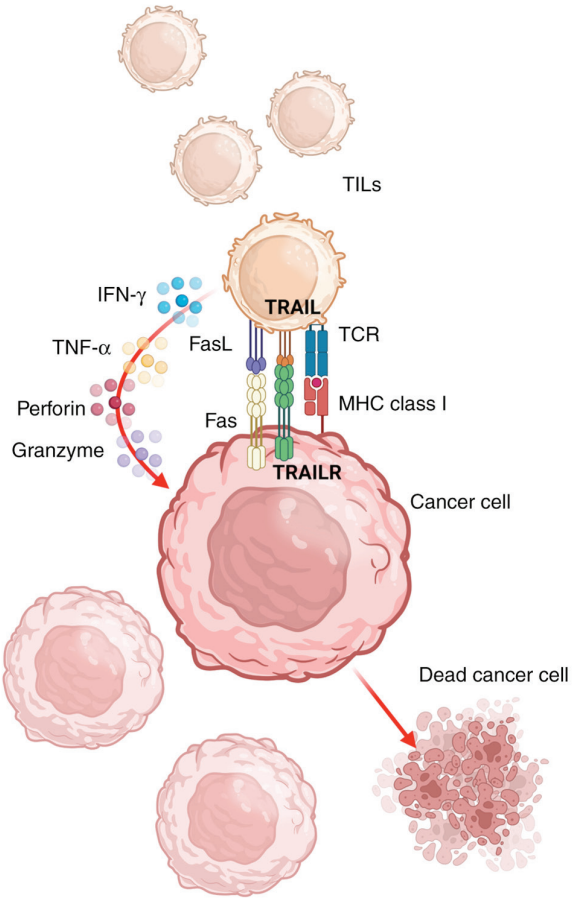

Figure 3. Mechanisms of TILs killing tumors. TILs, tumor-infiltrating lymphocytes; TCR, T-cell receptor; MHC, major histocompatibility complex; TRAIL, TNF-related apoptosis-inducing ligand; TRAILR, TRAIL receptor; FasL, Fas ligand; Fas, Fas cell surface death receptor; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α.

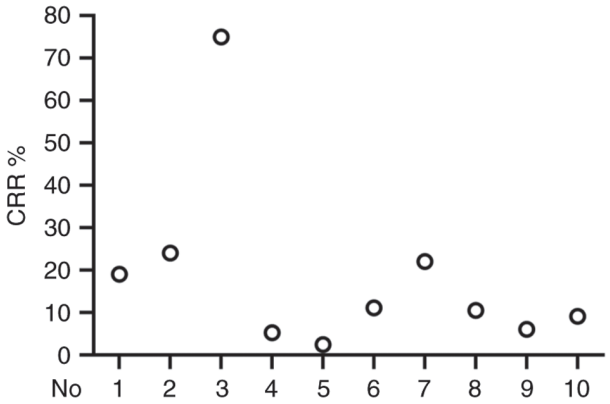

Figure 4. CRR of tumor-infiltrating lymphocyte therapy. CRR, complete response rate. No., study number according to Table I.

Mechanistic analysis revealed that the quality of TILs, expansion ability *in vivo*, exhaustion, immunosuppression and function disorder may be the leading causes of unstable clinical outcomes. Therefore, there is an urgent need to develop innovative therapeutic strategies to improve TIL-based anti-tumor therapies by addressing these issues.

Enrichment of functional TILs. Functional disorder of TILs in the tumor microenvironment results in the uncontrollable proliferation of malignant cells. Therefore, the enrichment of functional TILs and infusion of these TILs into patients is the 'first step' for preventing the growth of cancer cells.

Minimizing the TIL culture time and enriching more 'younger' TILs is essential to reducing the time it takes to treat a patient. Dudley *et al* (51) reported that 58% of patients treated with $CD8^+$ enriched young TILs and nonmyeloablative lymphodepletion resulted in an OR and a CRR of 9%. These data revealed that young TILs may be regarded as a novel source for clinical application.

Tumor-reactive stem-like TILs are capable of steady expansion, longer persistence, self-renewal and superior antitumor properties (21). CD39 and CD69 double negative

memory-progenitor stem-like TILs are strongly associated with TIL persistence *in vivo* and complete cancer regression (21). These stem-like TILs have a critical role in preventing cancer cell immune escape (53). In addition, induced pluripotent stem cells (iPSCs) are capable of self-renewal, maintaining their stemness, and have elongated telomeres. TIL-derived iPSCs are able to produce less-differentiated and tumor-specific T cells, which are a high-quality source of autologous TILs for cancer immunotherapy (54). Islam *et al* (55) selected $CD8^+$ $PD\text{-}1^+$ $CD137^+$ TILs and reprogrammed them into iPSCs, and the tumor-reactive TCRs of TIL-iPSCs were consistent with starting TILs. Furthermore, TIL-iPSCs were found to possess rare tumor antigen-specific TCRs, which were not found in cultured TILs. This approach may thus be used to enrich TILs that contain tumor antigen-specific TCRs.

Furthermore, Bhadurihauck *et al* (56) directly delivered SOX2, Oct-4 and NANOG proteins into the nucleus of tumor-infiltrating cytotoxic T-lymphocytes by utilizing a nuclear protein delivery system, which improved the proliferation and survival of these TICTLs. Teo *et al* (57) applied a simulated infective protocol to transform activated TILs into a $CD45RA^+$ central memory T-lymphocytes phenotype, which exhibited elongated telomeres, improved persistence and the powerful clearance ability of autologous acute myeloid leukemia blast cells. These approaches provide a promising novel avenue for tumor immunotherapy.

The anti-tumor properties of TILs can also be enhanced by optimizing the cell culture conditions. An overabundance of extracellular potassium suppresses T-cell effector functions by limiting nutrient uptake while improving the production of $CD8^+$ T-cells with enhanced *in vivo* persistence, stemness and tumor eradication ability (58). Acid accumulation induces methionine uptake and metabolism disorder by down-regulating the expression of SLC7A5, which alters H3K27me3 methylation on the promoter regions of genes related to T-cell stemness, and further increases their persistence and maintenance of a stem-like memory phenotype, and improves the anti-tumor properties of T cells *in vivo* (59). These mechanistic results may be used to develop a novel strategy for improving the treatment outcomes of T cell-based cancer immunotherapy.

Artificial antigen-presenting cells (aAPC) can be used to rapidly expand the clinical scale of TILs. TILs undergo rapid expansion under CD64/CD137 aAPC stimulation, and these aAPC-expanded TILs show a low CD4/CD8 ratio, fewer forkhead box (FOX)$P3^+$ cells and a larger population of tumor antigen-specific cells (60). CD86/CD137L/membrane-bound IL-15 aAPC can significantly increase the ratio of $CD8^+$T cell populations with effector-memory phenotype (61).

Collectively, these strategies summarized above can be used to enrich and expand functional TILs and further improve the persistence, stemness and survivability of said TILs.

Improved antitumor efficacy of TILs through gene editing. Gene editing is an effective approach to improving survival, resistance to immunosuppression and the tumor eradication ability of modified TILs (Fig. 5).

To increase the survival of TILs, Hsu *et al* (62) transduced IL-15 and herpes simplex virus-thymidine kinase (HSV-TK) genes into TILs and the transduced TILs secreted IL-15,

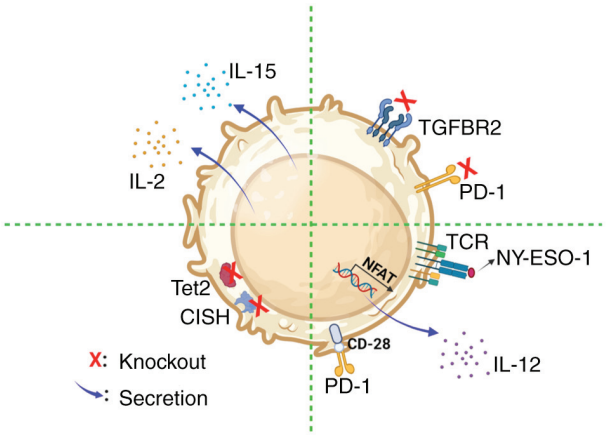

Figure 5. Schematic diagram of genetically modified tumor-infiltrating lymphocytes. TGFBR2, transforming growth factor β receptor 2; TCR, T-cell receptor; NY-ESO-1, New York-esophageal cancer 1; NFAT, nuclear factor of activated T cells; CISH, cytokine-induced SH2 protein; Tet2, ten-eleven translocation-2.

which significantly prolonged their survival *in vitro* following withdrawal of IL-2; meanwhile, TILs expressing HSV-TK were readily eliminated by low concentrations of ganciclovir. Of particular importance, the transduced TILs maintained the ability to specifically recognize and eradicate melanomas, even without IL-2. In addition, transduced TILs with IL-2 exhibited prolonged TILs without IL-2 supplementation while preserving their tumor-targeting specificity (63). These reports show that genetic modifications could reduce the dependence of exogenous IL-2 and improve the survival of TILs.

For blocking immunosuppressive signals, Beane *et al* (17) efficiently reduced the surface expression of PD-1 in TILs using ZFNs; the effector function of these TILs was improved and the secretion of polyfunctional cytokines was also significantly increased. Importantly, these TILs showed an effector memory phenotype and rapidly expanded *in vitro*. Furthermore, using CRISPR-Cas9 to knockout and reduce PD-1 expression in TILs did not affect TIL expansion (23). In addition, Fix *et al* (64) used CRISPR/Cas9 to mediate transforming growth factor β (TGF-β) receptor 2 knockout, which resulted in TILs that were resistant to TGF-β signaling-induced immunosuppression, while the expansion efficiency, phenotype and TCR diversity of TILs were not affected. These results demonstrated that these methods could be applied to produce TILs that were resistant to immunosuppressive signals; these TILs may have significant value in clinical practice.

For improving the anti-tumor properties of TILs, Robbins *et al* (65) transduced T cells with a TCR, which was able to directly act against New York-esophageal cancer 1 (NY-ESO-1). Adoptive transfer of TCR-TILs to patients with $NY\text{-}ESO\text{-}1^+$ tumors showed a 45.5% ORR and 18.2% complete regression in patients with melanoma. Liu *et al* (66) constructed a CAR-TIL with a switch receptor, which contained an optimized PD-1 extracellular domain, and CD28 transmembrane and intracellular domains. These CAR-TILs were able to easily infiltrate into tumors, significantly decreased the tumor volume and were resistant to hypofunction induced by the tumor. Lee *et al* (67) revealed that ten-eleven translocation-2 (Tet2) deficiency contributed to the accessibility of chromatin

and the binding of transcription factors, which enhanced the activation of $CD8^+$ TILs. In addition, Tet2 inactivation increased the effector-like cell population and improved the anti-tumor effects of TILs. Furthermore, TILs genetically engineered to produce single-chain IL-12 using an nuclear factor of activated T-cells promoter led to the transduction of TILs to selectively secrete IL-12 at the tumor site to mediate the antitumor effects in the absence of IL-2 (16). Deletion of cytokine-induced SH2 protein using CRISPR/Cas9 improved TIL activation against tumor neoantigens (68). Similarly, utilization of CRISPR/Cas9 technology to inactivate SOCS1 resulted in augmentation of cytokine signal responsiveness and a notable enhancement in the efficacy of antitumor responses in murine models (69). These reports revealed that genetic editing offers a novel approach to improve the tumor eradication ability of TILs.

Taken together, these studies reveal that genetic modification can further improve the anti-tumor properties of TILs, and modification of TILs provides additional opportunities for designing effective therapeutic strategies to manage solid tumors.

Combination therapy. Combination therapy is regarded as an effective strategy for improving the effectiveness of adoptive TIL-based therapy.

Combination of TILs with oncolytic viruses (OVs). OVs are a type of naturally occurring genetically modifiable virus, which can specifically kill tumor cells without affecting normal cells. In recent years, OV treatment has shown promise as a therapeutic approach for tumor patients. Studies have indicated that combinations with OVs can improve the therapeutic efficiency of other approaches. For instance, intratumor injection of IL-2-armed OV resulted in the recruitment and accumulation of TILs, which have higher tumor specificity and contain fewer exhausted T cells and regulatory T cells. These TILs lead to tumor regression and prolong survival of mice with MC38 tumors (70). Mechanistically, OVs significantly enhance the tumor killing ability of TILs by inducing granzyme B secretion and increasing the population of cytotoxic cells (71).

Furthermore, administration of *ex vivo* tumor cultures with OVs [adenovirus (Ad)5/3-E2F promoter-24 bp deletion (E2F-D24)-hTNF-α-internal ribosome entry site (IRES)-hIL-2] induced the activation of $CD4^+$ and $CD8^+$ TILs and promoted the secretion of IFN-γ, C-X-C motif chemokine ligand 10, TNF-α and IL-2, which mediate anti-tumor responses (72). In addition, the combination of OVs with TILs in hamsters with pancreatic tumors resulted in a CRR of 100%. Interestingly, further study revealed that OVs could efficiently bind to TILs, and these TILs could carry and deliver OVs to tumors. As feedback, OVs further enhanced TIL cytotoxicity (73).

Kudling *et al* (74) locally injected OVs (Ad5/3-E2F-D24-hIL-7) into a hamster model with tumors and observed a significant reduction in tumor growth and increased TIL infiltration. Mechanistic analysis revealed that oncolytic adenoviruses promoted the secretion of pro-inflammatory cytokines, and enhanced the activation and migration of cytotoxic T cells. In a clinical study, 18 stage IIIc/IV patients with melanoma were treated with TILs in combination with an adenovirus that expressed IFN-γ. This combination was tolerated by patients and the overall ORR was 38.5%, with a disease control rate of 46% (75).

Ye *et al* (76) developed a novel method to modify tumor cells with a HSV 1-based OV encoding TNF superfamily member 4 (OX40L) and IL-12 and transformed them into aAPCs. These infected tumor cells exhibited the features of APCs but with the improved activation and killing ability of TILs *in vitro*. Importantly, the combination administration of OV-OX40L/IL-12 and TILs led to complete tumor regression. In addition, activated T cells induced antitumor immunological memory and reprogrammed macrophages to an anti-tumor phenotype.

Collectively, these reports reveal that OVs not only improve the anti-tumor ability of TILs but also regulate the tumor microenvironment. This showcases the promising synergistic effect of TILs in combination with OVs in tumor therapy and demonstrates the feasibility of the clinical application of this novel therapeutic strategy.

TILs in combination with other drugs. Numerous drugs have been widely used in tumor treatment to significantly inhibit tumor growth by regulating multiple pathways. Recent studies discovered that several drugs can enhance the tumor killing ability of TILs.

Treatment with Ibrutinib plus Rapamycin restores TIL functions by blocking IL-2-inducible kinase and mTOR pathways. In addition, this combination therapy facilitated CD45RA re-expression in TILs and downregulated the expression of exhaustion markers (77). Ipilimumab plus TILs for metastatic melanoma treatment was well tolerated, the ORR was 38.5%, four of five patients maintained OR after 1 year and one achieved CR at 52 months (78). In addition, bispecific antibodies efficiently improve the killing effects of TILs on $HER\text{-}2^+$ ovarian cells (79). These approaches remodel the phenotype and enhance the therapeutic effects of TILs.

Combination administration with fibroblast activation protein (FAP)-4-1BBL and TCR activator notably improves TIL proliferation, activation and cytotoxicity. Mechanistically, FAP-4-1BBL induced secretion of IL-13 from TILs, which mediates tumor cell apoptosis dependent on IL-13α 1/2 receptors and the STAT6 pathway (31). Furthermore, the fusion protein PD-1Ab-IL21 promotes the expansion of memory stem $CD8^+$ T cells, which are tumor-specific and trigger anti-tumor immune responses (80). In addition, Pentoxifylline (PTXF) improved the ratio of cytotoxic TILs, increased IFN-γ levels, upregulated the expression of t-bet, suppressed the recruitment of regulatory TILs, decreased TGF-β levels and downregulated the expression of FOXP3. These changes induced by PTXF contributed to the anti-tumor responses of TILs (81). These combination strategies highlight the potential of TILs in combination with other therapeutics and/or adjuvants to improve the anti-tumor properties of TILs, and thus deserve further evaluation in clinical trials.

5. Conclusions and future perspectives

Successful cancer therapy remains a formidable challenge worldwide. Currently, traditional therapeutic strategies fall short in achieving a cure for cancer, resulting in substantial economic losses.

TIL therapy is an advanced immunotherapy approach for the treatment of cancer, which involves the collection and expansion of autologous TILs followed by their reinfusion into patients to specifically target and eliminate tumor cells. Currently, TIL therapy has demonstrated significant efficacy in treating malignant tumors, such as melanoma and colorectal cancer (44,45). Adoptive TIL therapy represents a promising strategy for cancer treatment, as TILs exhibit remarkable diversity in their TCR repertoire, possess tumor-homing capabilities and exert potent cytotoxic effects. Multiple clinical trials have consistently demonstrated the safety and efficacy of this approach (5,6).

However, TIL therapy also encounters several challenges and issues, including the acquisition of an adequate quantity and quality of immune cells, optimization of cell preparation processes, as well as addressing concerns related to immune evasion and drug resistance. These key issues necessitate urgent resolution. Furthermore, from a clinical perspective, immune toxicity resulting from the targeting of normal tissues is a significant concern due to the potential for immune cells to inadvertently harm healthy tissue during their attack on tumor cells. From a production standpoint, the protracted manufacturing cycle, exorbitant costs and challenging pricing structure associated with cell therapy impede patient accessibility and present commercial obstacles for this emerging treatment. Besides that, the infusion of TILs is ineffective in a subset of patients with cancer due to tumor heterogeneity and the intricate nature of the tumor microenvironment (Fig. 4).

Therefore, further enhancement of the anti-tumor properties of TILs in multiple dimensions is imperative, including the enrichment of functional TILs, gene editing techniques, and combination therapies. Gene editing represents one of the most efficacious strategies for modifying TIL characteristics and augmenting their tumor-killing potential, thereby paving the way for future clinical applications and commercialization of TILs. TIL infusion is a highly promising strategy for cancer treatment. Addressing clinical unmet needs and developing innovative TIL-based therapies will accelerate the growth of the TIL industry in tumor treatment.

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Availability of data and material

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Authors' contributions

Project administration: ZY, JS, AX and NL. Writing-original draft preparation: ZY, JS, YF, YZ and AX. Writing-review and editing: ZY, AX and NL. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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