Cancer Immunology, Immunotherapy (2024) 73:232
https://doi.org/10.1007/s00262-024-03793-4

REVIEW

Recent clinical researches and technological development in TIL therapy

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Received: 10 November 2023 / Accepted: 29 July 2024 / Published online: 12 September 2024

Abstract

Tumor-infiltrating lymphocyte (TIL) therapy represents a groundbreaking advancement in the solid cancer treatment, offering new hope to patients and their families with high response rates and long overall survival. TIL therapy involves extracting immune cells from a patient's tumor tissue, expanding them ex vivo, and infusing them back into the patient to target and eliminate cancer cells. This revolutionary approach harnesses the power of the immune system to combat cancers, ushering in a new era of T cell-based therapies along with CAR-T and TCR-therapies. In this comprehensive review, we aim to elucidate the remarkable potential of TIL therapy by delving into recent advancements in basic and clinical researches. We highlight on the evolving landscape of TIL therapy as a prominent immunotherapeutic strategy, its multifaceted applications, and the promising outcomes. Additionally, we explore the future horizons of TIL therapy, next-generation TILs, and combination therapy, to overcome the limitations and improve clinical efficacy of TIL therapy.

Keywords Tumor-Infiltrating Lymphocyte (TIL) Therapy · Cancer Immunotherapy · Solid Tumors · Adoptive Cell Therapy · Clinical Research · Technological Development

Introduction

TIL therapy was originally developed by Rosenberg's group starting in 1986 [1]. The premise involves extracting immune cells, known as TILs, from a patient's tumor, cultivating and expanding these cells in a laboratory, and then reintroducing them into the patient's body to target and destroy cancer cells. TIL therapy recognizes the uniqueness of each patient's cancer by recognizing the cancer neoantigens, activating the T cell immune response to eradicate tumor cells (that is called "cytotoxicity"). This precision medicine approach leads to higher response rates and increased chances of remission, which is nothing short of revolution in the oncology field. It is especially remarkable considering that Dr. Rosenberg discovered TIL's cytotoxicity ~ 40 years ago without many of the molecular toolkits that are becoming available only in the past 15 years.

Over the past few decades, substantial research efforts have been dedicated to the development of technologies for expanding tumor-infiltrating lymphocytes (TIL) [1–5], and T cells in general, along with the formulation of clinical procedures [6, 7]. TIL therapy has progressed to a point where it is on the cusp of an approval as a biological drug—lifileucel by Iovance (https://www.iovance.com) is upon BLA review by the FDA [8]. There are crucial areas that demand further advancement, including the evaluation of its potency, enhancing clinical effectiveness in both "hot" and "cold" cancers, optimizing the manufacturing process, reducing the overall production cost, and improving the accessibilities of this modality.

To successfully integrate TIL therapy into clinical practice (that is the "standard of care"), it is essential to expand its therapeutic applications in solid tumor types with severe unmet medical needs, establish optimal clinical protocols of treatment procedures, streamline manufacturing processes, and conduct pertinent clinical trials. Furthermore, ongoing research into next-generation TIL therapy is already underway, and the accumulation of real-world data will serve to foster and substantiate further advancements in this field.

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In this review, we will provide a highlight of the fundamentals, development, and limitations of current TIL therapy. We will also discuss the recent development of the next-generation TIL therapy.

Current status of adoptive cell therapy (ACT)

The advantages of TIL over CAR-T and TCR-T in solid cancer treatments

Adoptive cell therapy (ACT) represents a dynamic frontier in cancer treatment, harnessing the potential of the immune system to target and kill tumor cells. This approach encompasses various strategies, including tumor-infiltrating lymphocytes (TIL), gene-modified T cells expressing novel T cell receptors (TCR), and chimeric antigen receptors (CAR). These therapies have demonstrated considerable promise in various tumor types, and multiple clinical trials are conducted worldwide to further optimize this treatment modality [9].

CAR-T therapy has shown remarkable efficacy and success in treating hematological malignancies, such as B-cell acute lymphoblastic leukemia (ALL), certain types of non-Hodgkin lymphoma, and leukemia. Multiple CAR-T products have received regulatory approval and are gaining tremendous success in clinical applications [10–15]. However, CAR-T therapy has several considerable limitations: (1) it is associated with significantly severe side effect, such as cytokine release syndrome (CRS), acute respiratory distress syndrome (ARDS), and neurological toxicities [16]; (2) CAR-T therapy is highly effective for blood cancers; however, its effectiveness in treating solid tumors remains poor (Table 1).

Conversely, TCR-T therapy shows potential in targeting a wide range of solid tumors. It can be tailored to specific tumor antigens, including those derived from intracellular proteins presented by MHC molecules, making it more suitable for targeting solid tumors. In fact, it has demonstrated clinical efficacy in treating solid tumors rather than blood cancers, as discussed in numerous research and review articles on TCR-T therapies [17–20]. The FDA has accepted

Table 1 Comparison of adoptive cell therapy

Cellular therapy	TIL therapy	TCR-T therapy	CAR-T therapy
Targets	Target multiple tumor antigens	Target tumor antigen displayed on the cancer cell (MHC/peptide)	Target tumor antigen on the cancer cell surface
Raw material	Resected tumor tissue	Leukapheresis	Leukapheresis
Indications	Data available in melanoma, cervical, head and neck, and lung cancers, and other solid cancers	Data available in melanoma, synovial sarcoma, NSCLC, colorectal, ovarian, metastatic breast, metastatic pancreatic cancers, and other solid cancers	Relapsed/refractory multiple myeloma, relapsed/refractory large B-cell lymphoma, relapsed/refractory mantle cell lymphoma, diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, follicular lymphoma
Manufacturing	3–4 weeks	2–3 weeks	2–3 weeks
Toxicity	No unexpected off tumor tissue effects found Adverse events are manageable	Potential on target, off-tissue effects	Potentially sever adverse event, such as cytokine release syndrome
FDA approval	Iovance's AMTAGVI™ (lifileucel) receives FDA accelerated approval for advanced melanoma	FDA has accepted for priority review its Biologics License Application (BLA) for afami-cel (formerly ADP-A2M4), for advanced synovial sarcoma. The application has a Prescription Drug User Fee Act (PDUFA) target action date of August 4, 2024	ABECMA (Idecabtagene Vicleucel), 2021 BREYANZI (Lisocabtagene Maraleucel), 2021 CARVYKTI (Ciltacabtagene Autoleucel), 2022 KYMRIAH (Tisagenlecleucel), 2017 TECARTUS (Brexucabtagene Autoleucel), 2020 YESCARTA (Axicabtagene Ciloleucel), 2017
Clinical application	Currently most effective for melanoma and potentially other solid tumors	Solid tumors	Most suitable for blood cancers
Advantage	Possible recognition of multiple tumor antigens	Sensitive recognition	High affinity MHC independent
Disadvantage	Possible non-tumor-reactive	MHC-rescticted Possible off-target effect Targeted antigen only	Currently effective for blood cancer only Possible severe adverse event Cell surface antigens only

the Biologics License Application (BLA) for a TCR-T cell therapy, specifically afami-cel, for the treatment of advanced synovial sarcoma, granting it priority review (https://www.adaptimmune.com). The limitations of TCR-T therapy include: (1) The current field lacks very specific antigen targets, (2) there are potentially fatal side effects in addition to CRS and neurological toxicities when off-target toxicity (that is, TCR recognizes antigens expressed in normal tissues) is induced, (3) HLA restriction—HLA types need to be matched in order for alpha–beta TCR to function, and (4) the manufacturing process can be complex (Table 1).

TIL therapy can potentially be used to treat a wide range of cancer types as well as TCR-T therapy; it relies on extracting immune cells from patient's tumor. TILs are infiltrated into a tumor site. Therefore, these cells have already recognized the tumor as foreign antigen(s). By isolating and expanding, these TILs can be used to target and attack the cancer cells with precision. TILs are extracted from the patient's own tumor; hence, it is personalized for each patient. This minimizes the risk of rejection or graft-versus-host disease (GVHD) often associated with allogeneic therapies.

TIL therapy, while generally well tolerated and uneventful [21], does have associated risks and potential side effects. Administration may lead to transient dyspnea, chills, and fever immediately following infusion [22]. These adverse events (AEs) are not typically associated with elevated serum levels of circulating cytokines and should not be conflated with cytokine release syndrome, which is commonly observed with other cell therapies such as chimeric antigen receptor (CAR) T cell therapy [23].

Common AEs in TIL therapy include:

Lymphodepletion-related toxicity: Prior to TIL infusion, patients typically undergo a lymphodepleting regimen, which can lead to cytopenias and increased susceptibility to infections [22, 24, 25]. Management strategies include prophylactic antibiotics, antiviral medications, and antifungal agents, as well as supportive care measures such as growth factors for neutropenia.

IL-2-related reactions: Patients may experience fever, chills, or hypotension during or shortly after the TIL infusion due to IL-2 toxicity [24–28]. These reactions are usually manageable with supportive care and symptomatic treatment.

Other potential risks: As with any therapy involving immune modulation, there is always a risk of unforeseen adverse events, and close monitoring during and after treatment is essential.

By implementing these management strategies, we can mitigate the risks associated with TIL therapy and ensure a safer treatment experience for patients. We will include a detailed section on these safety aspects in our discussion to provide a more balanced and comprehensive overview.

TIL therapy has shown promise in achieving long-lasting responses, even in advanced or metastatic cancers [29–31]. Some patients have experienced complete and sustained remissions, suggesting that the treatment can provide lasting benefits [32–35]. TIL therapy is investigated for a wide range of solid tumors, including melanoma, cervical cancer, lung cancer, breast cancer, and more recently gastric intestinal cancers [36–39]. This makes it a potentially valuable treatment option for a range of cancer types. The FDA has approved a tumor-infiltrating lymphocyte (TIL) therapy called lifileucel (also known as Amtagvi) for the treatment of advanced melanoma, marking the first cellular therapy to treat solid tumors (https://www.iovance.com). Furthermore, TIL therapy can be used in combination with other immunotherapies, such as immune checkpoint inhibitors, to enhance its effectiveness. This combination approach may provide additive or synergistic benefits in treating cancer.

The limitations of TIL therapy include: (1) the current manufacturing process for TIL therapy is labor-intensive, time-consuming, and costly, and (2) tumor tissues are challenging to obtain, particularly in late-stage cancer patients (Table 1).

In summary, CAR-T cell therapy is most suitable for hematological malignancies, TCR-T cell therapy shows promise for solid tumors, and TIL therapy is currently most effective for melanoma and potentially other solid tumors. All offer promising approaches to cancer treatment as emerging T cell-based modalities, each with its own set of advantages and disadvantages. The selection of a specific immunotherapy approach should be carefully tailored to factors such as the type of cancer, the patient's medical condition, and the treatment's availability at the healthcare facility. Understanding the unique strengths and limitations of each therapy can guide their application in clinical practice to maximize patient outcomes.

TIL therapy up to date

The impact of prior anticancer treatments on TIL: evaluating effectiveness and characteristics

It is indeed important to consider the impact of prior treatments such as anticancer agents and irradiation on tumor-infiltrating lymphocytes (TILs) when harvesting them for therapy. Research indicates that the impact of chemotherapy and radiation on TILs may not significantly affect their effectiveness and characteristics [40–42]. TILs, which are extracted from a patient's tumor, expanded in a laboratory, and reinfused to fight cancer, have shown promising results even in patients who have undergone extensive prior treatments. Studies suggest that these pre-treatments do not greatly diminish the functional capacity of TILs, as the therapy can still induce substantial antitumor responses.

Clinical efficacy of TIL therapy in melanoma and resistance factors

In recent years, as TIL therapy has been developed to a certain level of maturity, a number of clinical trials are underway. Nearly 50 trials led by either academic groups (Table 2a) or biotech companies (Table 2b) are ongoing, targeting both immunologically "hot" solid tumors and more recently "cold" solid tumors. Melanoma has been studied in TIL therapy for 30 years and represents a highly immunogenic indication, with objective response rate (ORR) 36%-56%, progression-free survival (PFS) 3.7–7.5 months, overall survival (OS) 15.9–21.8 months in metastatic melanoma [24, 25, 34, 43–45]. Retrospective analysis of a single-center experience of non-selected autologous TIL study conducted by Pillai et al. and Instil Bio (https://instilbio.com) revealed that the ORR was 67%, complete response (CR) rate was 19%, and the disease control rate (DCR) was 86%, which was consistent with that observed in the prior PD-1 inhibitor subgroup (58%, 8%, and 75%, respectively) among 21 patients with advanced cutaneous melanoma. Median overall survival in all treated patients and the prior PD-1 inhibitor subgroup was 21.3 months. In total, 5 patients (24%) had durable ongoing responses (> 30 months post-TIL infusion) at data cutoff, and all patients who achieved CR remained alive and disease-free [46] (Supplementary Table 1).

The tumor microenvironment (TME) significantly influences the efficacy of immunotherapies, including TIL therapy. The TME comprises various components such as immune cells, cytokines, and stromal elements, which together create an environment that can either promote or inhibit antitumor responses. Immunosuppressive cells within the TME, including regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), play a crucial role in dampening the immune response against tumors [47]. These cells secrete immunosuppressive cytokines and metabolites that inhibit the activity of cytotoxic T lymphocytes (CTLs) and other antitumor immune cells. This suppression leads to several resistance mechanisms that contribute to treatment failure, such as antigen loss, T cell dysfunction or exhaustion, and impaired T cell migration to the tumor site [48–52].

Moreover, the TME can prevent effective T cell infiltration and recognition of tumor cells, further complicating immunotherapy outcomes. Strategies to counteract these challenges include targeting immunosuppressive pathways, enhancing T cell functionality, and improving TIL trafficking and persistence within the tumor [52, 53]. By addressing these aspects, the effectiveness of TIL therapy and other immunotherapies can be significantly improved, offering better clinical outcomes for patients with various solid tumors [51, 52].

Combining TIL with immune checkpoint inhibitors (ICI)

One strategy to enhance efficacy and durability of response is to combine TIL with ICI. Mullinax JE et al. conducted the combination study of TIL and Ipilimumab (cytotoxic T lymphocyte-associated antigen 4 blockade: CTLA-4 antibody). They reported 38.5% ORR, PFS 7.3 months (95% CI 6.1–29.9 months) in metastatic melanoma, and one of patients became a complete response at 52 months [54]. Furthermore, earlier line treatment with lifileucel (LN-144) and LN-145 plus pembrolizumab (programmed death 1: PD-1 antibody) combination led by Iovance demonstrated 67% ORR and CR rate of 25% in immune checkpoint inhibitors (ICI)-naïve advance melanoma patients [55, 56]. In addition to melanoma, in combination with pembrolizumab, it has also shown promise in advanced ICI-naive head and neck squamous cell carcinoma (HNSCC; $n = 18$, ORR = 38.9%) and advanced untreated cervical carcinoma ($n = 14$, ORR = 57.1%), respectively [55] (Supplementary Table 1).

TIL therapy application for other cancer types

TIL therapy has exciting potential to overcome tumor heterogeneity and induce deep and durable remissions in treatment-refractory cancers other than melanoma [24].

A pilot study was conducted in patients who had advanced epithelial ovarian carcinoma with intraperitoneal (IP) TIL. There were no measurable responses due to possible manufacturing difficulties; however, ascites regression tumor and CA-125 reduction were observed [57].

Karbach J et al. reported that complete and durable tumor remission was observed after three TIL infusions for a patient with metastatic hormone-refractory prostate cancer (mHRPC) expressed New York esophageal squamous cell carcinoma 1 (NY-ESO-1) treated with in vitro expanded tumor-infiltrating lymphocytes (TILs) in conjunction with IL-2 and ICI [58].

In Phase I study of adjuvant immunotherapy with autologous TILs in locally advanced cervical cancer, 9 of 12 patients (75.0%) attained a complete response, with a disease control duration of 9–22 months [59] (Supplementary Table 1).

Stevanović *et al.* reported a phase II study of TIL therapy for human papillomavirus (HPV)-associated epithelial cancers (NCT01585428) in 2019. ORR in cervical cancer was observed 28% and 18% for non-cervical cancer patients. Two of the responses in cervical cancer were complete and lasted 67 and 53 months after treatment. The magnitude of HPV reactivity of the infused TILs was associated with clinical response. HPV-associated cancers also harbor somatic gene mutations (mutated neoantigens) and epigenetically dysregulated genes (cancer germline antigens) that may be targeted by the TILs. [30].

Table 2a List of investigator initiated clinical trials actively recruiting patients

NCT number	Target indications	Intervention/treatment	Sponsor/collaborators	Country	Phases
NCT05141474	Epithelial tumors, solid tumor	Neoantigen-selected TILs	Vall d'Hebron Institute of Oncology|Banc de Sang i Teixits	Spain	Early Phase 1
NCT05724732	Advanced gynecologic tumors	Autologous TILs	RenJi Hospital	China	Early Phase 1
NCT05238818	Metastatic or recurrent gynecological tumors	TIL (GT202)	Obstetrics and Gynecology Hospital of Fudan University	Chia	Phase 1
NCT04812470	Metastatic uveal melanoma, metastatic cutaneous melanoma	TIL	Vastra Gotaland Region|Miltenyi Biomedicine GmbH	Sweden	Phase 1
NCT03991741	Metastatic melanoma, locally advanced refractory/recurrent melanoma, metastatic head and neck cancer, locally advanced refractory/recurrent head and neck cancer	TIL	University of California, San Diego	USA	Phase 1
NCT05869539	Advanced melanoma	TIL with ANV419 (IL-2Rb/gagonist)	University Hospital, Basel, Switzerland|Anaveon AG	Switzerland	Phase 1
NCT05470283	Metastatic melanoma	Membrane Bound IL15 (OBX-115) in combination with acetazolamide	M.D. Anderson Cancer Center	USA	Phase 1
NCT05768347	Urothelial carcinoma, non-invasive bladder urothelial carcinoma	TIL	H. Lee Moffitt Cancer Center and Research Institute|United States Department of Defense	USA	Phase 1
NCT04643574	Solid tumor	TIL enriched for tumor antigen specificity (NeoTIL)	Centre Hospitalier Universitaire Vaudois	Suisse	Phase 1
NCT01946373	Melanoma	TIL plus Dendritic cell vaccine	Karolinska University Hospital	Sweden	Phase 1
NCT06084299	Advanced liver cancer	TIL	Tongji Hospital	China	Phase 1
NCT05681780	Non-small cell lung cancer, stage IV non-small cell lung cancer, recurrent non-small cell lung cancer	TIL stimulated with CD40L plus nivolumab	H. Lee Moffitt Cancer Center and Research Institute	USA	Phase 1|Phase 2
NCT04611126	Metastatic ovarian cancer, metastatic fallopian tube cancer, peritoneal cancer	TIL in combination with nivolumab, relatlimab, and ipilimumab	Herlev Hospital	Denmark	Phase 1|Phase 2
NCT03801083	Locally advanced, recurrent, or metastatic biliary tract cancers	TIL	University of Pittsburgh	USA	Phase 2
NCT03467516	Metastatic uveal melanoma	TIL	University of Pittsburgh	USA	Phase 2
NCT03935893	Gastric cancer, colorectal cancer, pancreatic cancer, sarcoma, mesothelioma, neuroendocrine tumors, squamous cell cancer, merkel cell carcinoma, mismatch repair deficiency, microsatellite instability	TIL	University of Pittsburgh	USA	Phase 2
NCT01174121	Metastatic colorectal cancer, metastatic pancreatic cancer, metastatic ovarian cancer, metastatic breast carcinoma, metastatic endocrine tumors, neuroendocrine tumors	Young TIL plus pembrolizumab	National Cancer Institute (NCI)|National Institutes of Health Clinical Center (CC)	USA	Phase 2

Table 2a (continued)

NCT number	Target indications	Intervention/treatment	Sponsor/collaborators	Country	Phases
NCT02621021	Melanoma	Young TIL in combination pembrolizumab	National Cancer Institute (NCI)|National Institutes of Health Clinical Center (CC)	USA	Phase 2
NCT02133196	Advanced non-small cell lung cancer, squamous cell carcinoma, advanced NSCLC, adenosquamous carcinoma, adenocarcinoma	Young TIL	National Cancer Institute (NCI)|National Institutes of Health Clinical Center (CC)	USA	Phase 2

The phase I study of the combination nivolumab and TIL for the patients with advanced NSCLC was recently completed at H. Lee Moffitt Cancer Center (NCT03215810). Initial tumor regression occurred in 68.8% (11 of 16) of patients at first CT scan performed one month after TIL infusion, and the median best change was 35.5% (range + 20 to − 100). Two out of 16 patients achieved complete responses ongoing 1.5 years later. In exploratory analyses, T cells recognizing multiple types of cancer mutations were detected after TIL treatment and were enriched in responding patients. Neoantigen-reactive T cell clonotypes increased and persisted in the peripheral blood after treatment. [39].

Tran E *et al.* demonstrated that TILs from 9 out of 10 patients with metastatic gastrointestinal cancers contained CD4+ and/or CD8+ T cells that recognized one to three neo-epitopes derived from somatic mutations expressed by the patient's own tumor. Interestingly, in one patient with metastatic colon cancer a human leukocyte antigen (HLA) –C*08:02–restricted T cell receptor from CD8+ TILs targeted the $KRAS^{G12D}$ hotspot driver mutation. [60]

Colorectal, pancreatic, and ovarian cancers are traditionally known to be less responsive to immunotherapy [61]. Despite no objective responses being observed, 63% of patients exhibited stable disease [61] (Supplementary Table 1). Notably, one patient with pancreatic cancer experienced a reduction in tumor burden lasting over a year, with no new safety signals identified [61]. This suggests potential antitumor activity even in cancers typically resistant to such treatments.

In-depth immunological characterization paving the way of TIL therapy improvement

Tumor-reactive TIL selection (Fig. 1A)

TIL selection based on IFN-γ before rapid expansion procedure (REP)

In study by Rosenberg et al., tumor-reactive TILs which exhibited interferon-gamma (IFN-γ) in co-culture with autologous tumor cells or cancer cell lines in vitro were preselected for REP [29]. Adoptive cell transfer (ACT) using this selected TIL method showed ORR 49% (12% CR and 37% PR) in patients with metastatic melanoma. However, this method required extra time and materials for testing and TIL expansion [62].

Young TILs

Another key development is the so-called "young TILs" starting in 2008–2010. It reduces the initial expansion period

Table 2b List of company sponsored clinical trials actively recruiting patients

NCT number	Target indications	Intervention/Treatment	Sponsor/Collaborators	Country	Phases
NCT05729399	Solid tumor	GT201	Grit Biotechnology	China	Early Phase 1
NCT05333588	Glioblastoma multiforme, adult	Autologous TILs	Hebei Senlang Biotechnology Inc., Ltd	China	Early Phase 1
NCT05098184	Advanced melanoma	GC101 TIL	Shanghai Juncell Therapeutics|Shanghai 10th People's Hospital	China	Early Phase 1
NCT05142475	Breast cancer, treatment side effects, advanced breast cancer, effects of immunotherapy	Autologous TILs	Shanghai Juncell Therapeutics|Shanghai 10th People's Hospital	China	Early Phase 1
NCT05098197	Advanced hepatobiliary-pancreatic cancers	Autologous TILs	Shanghai Juncell Therapeutics|Shanghai 10th People's Hospital	China	Early Phase 1
NCT04960072	Refractory/relapsed gastrointestinal tumors	TIL	Shanghai Juncell Therapeutics|Shanghai 10th People's Hospital	China	Early Phase 1
NCT05087745	Advanced solid tumor	TIL	Shanghai Juncell Therapeutics|Sir Run Run Shaw Hospital	China	Early Phase 1
NCT05941936	Solid tumor	LM103	Suzhou BlueHorse Therapeutics Co., Ltd	China	Early Phase 1
NCT05902520	HNSCC, melanoma, gynecologic cancer, colorectal cancer, lung cancer, urogenital cancer	CD39+CD103+CD8+ Tumor-isolated T Cells (AGX148) Alone or Combined With siRNA Modulation of PD-1 (AGX148/PH-762)	AgonOx, Inc.|Phio Pharmaceuticals Inc.|Providence St Joseph Health	USA	Phase 1
NCT05475847	Advanced cervical cancer	C-TIL052A	Fudan University|Cellular Biomedicine Group Ltd	China	Phase 1
NCT05430360	Solid tumor	GT201	Grit Biotechnology	China	Phase 1
NCT05628883	Metastatic melanoma, unresectable melanoma, acral melanoma, mucosal melanoma, cutaneous melanoma, ocular melanoma, uveal melanoma, iris melanoma, conjunctival melanoma, non-cutaneous melanoma	TBio-4101 (an Autologous Selected and Expanded TIL)	H. Lee Moffitt Cancer Center and Research Institute|Turnstone Biologics, Corp	USA	Phase 1
NCT05176470	Locally advanced melanoma, pathologic stage IIIB cutaneous melanoma AJCC v8, pathologic stage IIIC cutaneous melanoma AJCC v8, pathologic stage IIID cutaneous melanoma AJCC v8, Stage IV melanoma	LN-144 (lifileucel) and pembrolizumab	Iovance Biotherapeutics, Inc.|Ohio State University Comprehensive Cancer Center	USA	Phase 1
NCT05607095	Uveal melanoma, melanoma, metastatic Uveal melanoma, metastatic melanoma	Lifileucel (LN-144)	Memorial Sloan Kettering Cancer Center|Iovance Biotherapeutics, Inc	USA	Phase 1
NCT05573035	Relapsed/refractory metastatic or locally advanced melanoma and selected solid tumor malignancies	Epigenetically reprogrammed TIL (LYL845)	Lyell Immunopharma, Inc	USA	Phase 1
NCT05417750	Advanced malignant solid tumors	GC101 TIL (dose escalation)	Shanghai Juncell Therapeutics	China	Phase 1
NCT05576077	Breast cancer, colorectal cancer, uveal melanoma, cutaneous melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma	TBio-4101 (an autologous selected and expanded TIL) and pembrolizumab	Turnstone biologics, corp	USA	Phase 1
NCT04217473	Metastatic melanoma	Adenovirus TILT-123	TILT Biotherapeutics Ltd	Finland	Phase 1

Table 2b (continued)

NCT number	Target indications	Intervention/Treatment	Sponsor/Collaborators	Country	Phases
NCT04842812	Solid Tumor (brain, breast, colorectal, liver, lung cancer)	TILs and CAR-TILs targeting HER2, Mesothelin, PSCA, MUC1, Lewis-Y, GPC3, AXL, EGFR, Claudin18.2/6, ROR1, GD1, or B7-H3	Second Affiliated Hospital of Guangzhou Medical University|Guangdong Zhaotai InVivo Biomedicine Co. Ltd	China	Phase 1
NCT05361174	Unresectable melanoma, metastatic melanoma, stage III non-small cell lung cancer, stage IV non-small cell lung cancer	PD-1 Knockout TIL (IOV-4001)	Iovance Biotherapeutics, Inc	USA	Phase 1|Phase 2
NCT04426669	Gastrointestinal epithelial cancer, gastrointestinal neoplasms, cancer of gastrointestinal tract, gastrointestinal cancer, colorectal cancer, pancreatic cancer, gall bladder cancer, colon cancer, esophageal cancer, stomach cancer	Genetically engineered, neoantigen-specific TIL (CISH inhibited using CRISPR)	Intima Bioscience, Inc.|Masonic Cancer Center, University of Minnesota	USA	Phase 1|Phase 2
NCT03108495	Cervical carcinoma	LN-145	Iovance Biotherapeutics, Inc	USA	Phase 2
NCT03645928	Metastatic melanoma, squamous cell carcinoma of the head and neck, non-small cell lung cancer	LN-144 (lifileucel)/LN-145 in combination with checkpoint inhibitors or TIL LN-144 (lifileucel)/LN-145/LN-145-S1	Iovance Biotherapeutics, Inc	USA	Phase 2
NCT04614103	Metastatic non-small cell lung cancer	LN-145	Iovance Biotherapeutics, Inc	USA	Phase 2
NCT05727904	Metastatic melanoma, unresectable melanoma, melanoma	LN-144 (lifileucel) plus pembrolizumab	Iovance Biotherapeutics, Inc	USA	Phase 3

Information is available at https://clinicaltrials.gov/

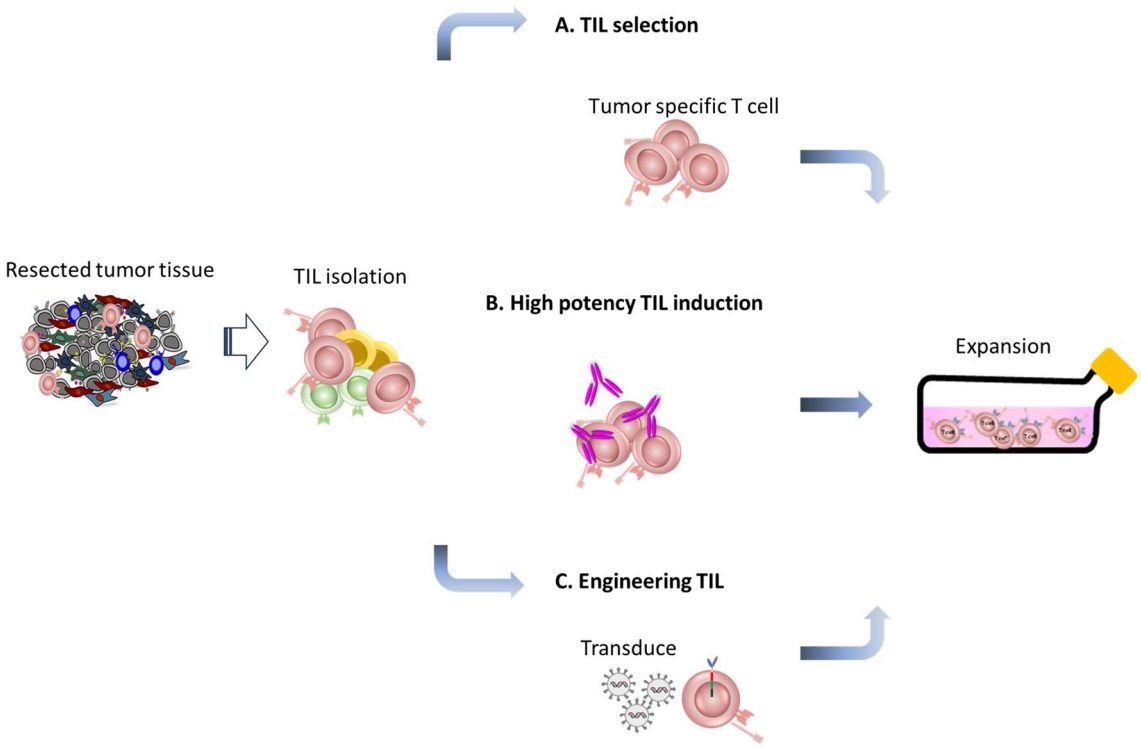

Fig. 1 Schematic overview of the next-generation TIL production. TILs are isolated from freshly resected tumor tissue and grown. Tumor-reactive cells are selected, based on expression markers, such as CD39+/CD103+, PD-1, CD39-/CD69-, and 4-1BB; B tumor tissue is cultured in the presence of antibodies, such as 4-1BB and OX40, C genetic modifications on TILs to enhance TIL functionality

by isolating TILs with enzymatic digestion of tumors and expands them instead of waiting for them to migrate out of tumor fragments in culture [63, 64]. The "young TIL" demonstrated that they have longer telomeres and higher levels of CD27 and CD28 compared to TIL expanded from fragments [65]. In one study using these "young TILs," 10 out of 20 patients with metastatic melanoma (50%) achieved an objective clinical response with 2 complete remissions and eight partial responses (with progression-free survival ranging from 3 to 18 months).

Neoantigen-reactive TILs

Technological advancement over the past decades enabled the development of neoantigen-reactive and high potent TIL, which is considered as the "next-generation TIL therapies" [66]. By mapping 55 neoantigen-specific TCR clonotypes (NeoTCRs) from 10 metastatic human tumors, including breast, melanoma, and colon, to their single-cell transcriptomes, Lowery et al. identified signatures of CD8+ and CD4+ neoantigen-reactive TILs. Neoantigen-specific TILs exhibited tumor-specific clonal expansion [67]. In phase I/II feasibility study with TIL therapy for metastatic melanoma, immune monitoring using exome sequencing demonstrated that neoantigen-specific T cells were detectable in TILs. In the two CR patients in whom neoantigen-specific T cell responses were analyzed, persistence of neoantigen-specific T cell was detectable for up to 3 years after TIL infusion [35]. Achilles Therapeutics (https://achillestx.com) developed VELOSTM manufacturing process to selectively expand clonal neoantigen-specific TILs (cNeT) by co-culturing TILs with autologous dendritic cells-loaded peptides corresponding to the patient's identified neoantigens.

The products are currently under clinical studies with patients with NSCLC (NCT04032847) and melanoma (NCT03997474) [68].

Phenotypic markers

Various technological developments in recent years enabled investigators to identify and sort tumor-reactive TILs based on phenotypic markers, such as programmed cell death 1 (PD-1), CD39-/CD69-, and CD39+/CD103+. These enrichment strategies would improve clinical efficacy, and further development for clinical manufacturing and clinical study are needed.

PD-1 PD-1 is highly expressed on tumor-reactive T cells; Inozume et al. showed that sorted and expanded CD8+PD-1+ T cells in tumor digests showed much

higher tumor-specific IFN-γ production compared with CD8+PD-1- T cells and PD-1 receptor can be a useful biomarker for enriching tumor-specific T cells from fresh melanomas [69]. Similarly, Fernandez-Poma SM et al., reported that PD-1+CD8 TILs maintain the tumor-specific TCRs repertoires and their ability to recognize tumor cells after in vitro expansion compared to PD-1- or bulk CD8 TILs. In tumor mice model, PD-1+CD8 TILs improved the survival compared to PD-1- CD8 TILs ($p < 0.05$), and the combination with PDL-1 blockade enhanced tumor regression ($p < 0.001$) compared to PDL-1 blockade alone, although the fold expansion of PD-1+CD8 TILs was 10 times lower than that of PD-1- cells, which suggested outgrowth of PD-1- cells was the limiting factor in the tumor specificity of cells derived from bulk CD8 TILs. The combined administration of anti-PDL-1 mAb with PD-1+TILs would be critical in the treatment of large established tumor [70]. PD-1-selected TIL therapy was withdrawn unfortunately due to funding issue (NCT04223648).

CD39-/CD69- Single-cell analysis of TILs from 7 CRs and 9 NRs by mass cytometry (CyTOF) revealed heterogeneous expression of 34 T cell surface markers (CD2, CD3, CD4, CD5, CD7, CD8a, CD9, CD11a, CD16, CD25, CD27, CD28, CD39, CD44, CD45, CD45RA, CD45RO, CD49D, CD57, CD69, CD95, IL7R, OX40, 41BB, CTLA4, KLRB1, CXCR3, CCR4, CCR5, CCR7, LAG3, ICOS, PD-1, and TIM3). Machine learning-based unsupervised clustering to define T cell clusters with activation/exhaustion states to classify patients according to their clinical outcome revealed CD69 and CD39 expression as two crucial features of most clinical relevance and associated with complete cancer regression. Manual visualization of TIL CyTOF profiles identified a cluster, which was fourfold more abundant in complete responder relative to non-responder (corrected $p = 0.0264$), corresponded to CD8+ T cells with abundant CD44, CD27, CD28, and low expression of TIM3, characterized in prior studies as memory-like and stem-like T cells. Higher numbers of CD8+CD39-CD69- cells in the infused TIL products were significantly associated with improved PFS ($p < 0.0001$, HR = 0.255, 95% CI 0.1257–0.5186) and melanoma-specific survival (MSS, $p < 0.0001$, HR = 0.217, 95% CI 0.101–0.463) in metastatic melanoma who enrolled and underwent TIL infusion. Furthermore, in tumor mice model, isolated CD39-CD69- CD8+ T cells let to substantial tumor regression and improved survival in a dose-dependent manner compared to CD39+CD69+ CD8+ T cells.

These findings suggest the strategies to isolate and expand stem-like neoantigen-specific T cells, or the engineering of T cells to have stem-like attributes, might provide opportunities for the development of more effective T cell-based immunotherapies [71] (CD39-CD69-).

CD103+/CD39+ Duhen et al. identified a unique population of co-expression of CD39 and CD103 in CD8 TILs, which have a tissue-resident memory T cells (T_{RM}) phenotype and express elevated levels of exhaustion markers. CD103+CD39+ double-positive (DP) TILs exhibited over 2 times greater tumor reactivity against autologous tumor compared to the double negative (DN) or single positive (SP) CD8 TILs. High frequency (above the mean frequency; 32.9%) of DP CD8 TILs is associated with better OS compared to low frequency (below 32.9%) of DP TILs in a cohort of HNSCC (3-year survival: 89% vs. 56%; $p = 0.0191$). In addition, sequencing of the CDR3 region of the TRB genes revealed that DP CD8 TILs were more oligoclonal compared to memory CD8 T cells in the peripheral blood and to DN and SP CD8 TILs. These indicate that using CD39 and CD103 to identify tumor-reactive CD8 TILs in solid tumors will lead to important mechanistic insights, and selection of those population possibly enhances clinical outcome of TIL therapy [72].

CD137+(4-1BB) A member of the TNF-receptor (TNFR) superfamily, CD137 (4-1BB; TNFRS9) is known as inducible costimulatory receptor and expresses on activated T and natural killer (NK) cells [73]. CD137+ cells positively isolated by flow sorting or magnetic beads were enriched populations as functional and antigen-specific T cells [74, 75]. Seliktar-Ofir et al. successfully isolated CD137+ cells by magnetic bead separation, and these cells demonstrated significantly increased a cytotoxicity against autologous tumor cells compared to unselected cells (% cytotoxicity of CD137+ fraction 52.3 ± 10.6%; % cytotoxicity of unseparated 6.6 ± 6.6%; $p \leq 0.007$). The CD137 + fraction was examined for large-scale expansion. The CD137+ fraction reached a similar fold expansion as unseparated TIL ($p = 0.78$) or CD137− TIL ($p = 0.12$) on day 14. The CD137+ fraction of TIL recognized the mutated peptide increase by 3.2-fold compared to unseparated TIL. The direct implementation of the CD137 separation method may improve the clinical outcome of TIL therapy [76]. The phase II trial with 4-1BB selected TIL for metastatic melanoma patients was terminated unfortunately due to low accrual and change in research focus (NCT02111863).

High-potency TIL induction (Fig. 1B)

Efficient expansion of TILs is essential for manufacturing and successful clinical effects. Addition of agonistic 4-1BB antibody improved TIL expansion from primary bladder tumors regardless of pre-treatment with chemotherapy, and they exhibited tumor reactivity against autologous tumor cells [77, 78]. Notably, adding 4-1BB co-stimulation promoted the functional enhancement of CD8 TILs as well as enhancement of anti-PD-1-mediated reinvigoration of

exhausted CD8 TILs from both the primary tumor sites and the metastatic sites of patients with ovarian cancer [79]. Targeting 4-1BB together with anti-PD-1 blocking antibodies in TIL culture could be a promising strategy for improving the poor responses to immunotherapy in metastatic ovarian cancer.

OX 40 (CD134, TNFRSF4) is a potent costimulatory receptor that can potentiate T cell receptor signaling on the surface of T cells [80]. Administration of OX40 promotes proliferation and survival of T cells via the NF-kB pathway and has been shown to enhance CD8 infiltration and decreases immune suppression in mouse model [81]. Anti-OX40 agonistic antibody on the ex vivo expansion significantly increased CD8+ T cells and maintained TCR Vβ repertoire [82].

Engineering TIL (Fig. 1C)

There are several directions to engineering TIL using genetic engineering techniques to (1) enhance antitumor activity, (2) improve ex vivo expansion efficiency, (3) decrease TIL suppression and exhaustion, (4) reduce IL-2 toxicity.

Genetical engineering to enhance antitumor activity

CRISPR Recent studies have explored gene editing approaches, such as "clustered regularly interspaced short palindromic repeats-Cas (CRISPR-Cas9)," to enhance TILs' tumor-killing potential. By editing specific genes involved in T cell suppression and exhaustion, researchers try to create TILs with improved cytotoxicity and resistance to immunosuppression [83]. Fix SM et al. implemented CRISPR/Cas9-mediated knockout of transforming growth factor beta receptor 2 (TGFBR2), which is TGF-β-resistant TILs from ovarian cancer patients before undergoing a REP. TGFBR2-knockout TILs are protected from the immunosuppressive effects of TGF-β (Fig. 2A) on effector cytokine production, proliferation, and cytotoxicity, when compared to non-transfected control and Cas9 mock transfected TIL ($p < 0.001$). However, it is worth noting that these approaches of CRISPR modified TILs did not alter the ex vivo expansion efficiency, immunophenotype, nor the TCR clonal diversity of TIL, and may not improve the treatment efficacy, especially for "cold tumors" [84]. Similarly, Arthofer et al. reported that genetic editing of the cytokine-induced SH2 protein (CISH) enhanced T cell effector function. CISH is a novel intra-cellular immune checkpoint molecule and an important negative regulator of T cell signaling and antigen-specific effector function [85]. In a pre-clinical mice model, CISH knockout results in enhanced tumor regression when combined with PD-1 mAb blockade [86]. Intima Bioscience, Inc. (https://www.intimabioscience.com) is currently conducting a clinical trial initially at the University of Minnesota Masonic Cancer Center for patients with metastatic gastrointestinal epithelial cancer administering TIL which CISH has been inhibited using CRISPR (NCT04426669).

Transduce TIL using a retrovirus platform The chemokine receptor CXCR2 and its ligands are intimately involved in tumor regulation and growth, and its functional inhibition

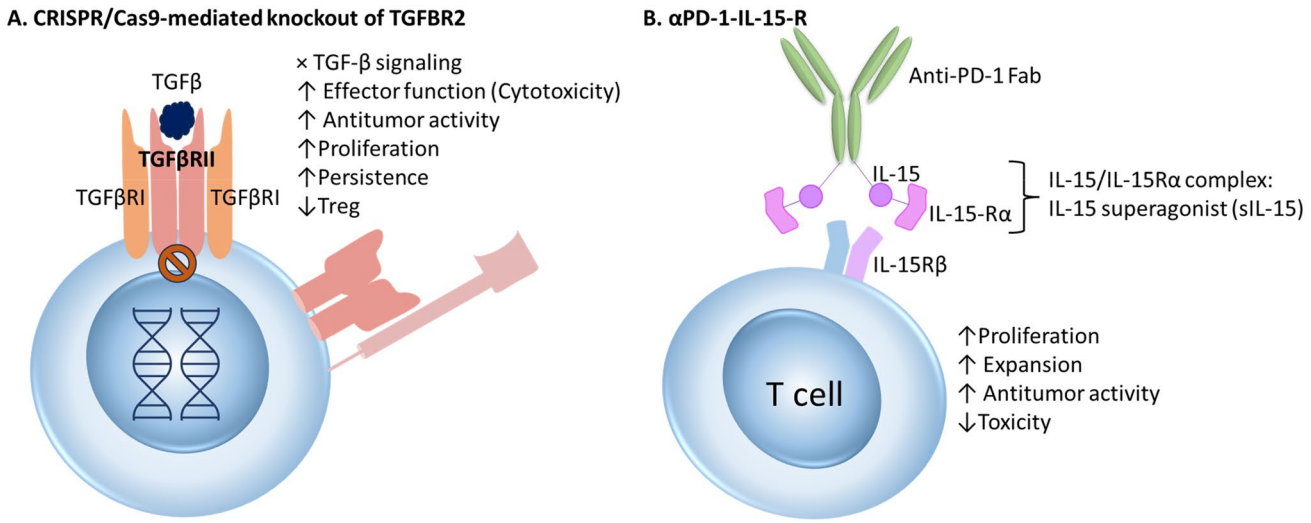

Fig. 2 Mechanistic overview of the next-generation TIL strategy. **A** CRISPR/Cas9-mediated knockout of TGFBR2: knockout of the TGF-β receptor II (TGFBR2) gene would render T cells resistant to TGF-β-mediated immunosuppression, consequently, increase effector function and persistence. **B** IL-15/IL-15Rα complex named IL-15 superagonist (sIL-15) greatly enhances IL-15 bioactivity. The Fc domain of αPD-1 antibody to conceal sIL-15 binding to the IL-15Rβ. Anti-PD-1 antibody not only anchored the concealed sIL-15 on PD-1+CD8+ T cells directly but also exposed sIL-15 activity to these cells. This would enable to decrease toxicity while maintaining efficacy

shows promising results in several cancer types [87]. By using CXCR2, it facilitated TIL migration into the tumor. This modification displayed an improved in vivo antitumor activity [88, 89]. Forget MA et al. optimized procedure to genetically modify TIL. The transduction levels of CXCR2 at infusion were ranging from 31.28 to 57.82% and expanded raged from 465- to 3096-fold expansion [4]. CXCR2 and nerve growth factor receptor (NGFR) transduced TIL for treating metastatic malignant melanoma had been conducted and completed at M.D. Anderson Cancer Center (NCT01740557) as of April 2023.

Interleukin 12 (IL-12) enhances activity of CD4 and CD8 T cells, and infusion of IL-12 can mediate antitumor immunity in animal models [90]. However, systemic administration of IL-12 to cancer patients resulted in minimal efficacy and severe toxicity [91]. To improve TIL therapy, genetic engineering of TIL with a gene encoding IL12 was developed to deliver the potent cytokine selectively to the tumor site. In its first-in-man trial, 10 of 16 (63%) achieved an objective response; however, significant toxicities were seen such as liver dysfunction, high fevers (exceeding 40 °C), and sporadic life-threatening hemodynamic instability. Further scientific research and technology development are required before this approach can be safely used in the treatment of cancer patients [92].

Reprograming TIL

Another unique attempt is to reprogram exhausted TIL that possess T cell receptors (TCR) specific for tumor antigens into induced pluripotent stem cells (iPSC) to rejuvenate them for more potent ACT [93]. Islam et al. optimized the method to selectively reprogram tumor antigen-specific T cells from heterogeneous TIL populations by coculturing with autologous tumor cells and sorting the PD1+4-1BB+ CD8+ T cell population before reprogramming ACT [93]. Lyell (https://lyell.com) has developed "Epi-R reprogramming technology" to enrich CD8+T cells that express costimulatory markers (CD27, CD62L, and CD127) and stem-like T cells (CD39-CD69-), to enhance stemness (gene expression of CD28, TCF7, KLF3, SELL, BACH2, and LEF1), to reduce exhaustion associated genes (ENTPD1, TIGIT, CTLA4, LAYN, and LAG3), potential durable expansion, maintain polyclonality of TILs during production, and to enrich the TIL product with tumor-reactive T cells (LYL845) [94, 95]. The phase I clinical study using LYL845 for patients with relapsed and/or refractory metastatic or locally advanced melanoma, with expansion cohorts for patients with melanoma, NSCLC, and CRC is currently ongoing (NCT05573035), and initial data are expected in 2024.

Double trigger approach

A CD28/CD40-based chimeric CoStimulatory Antigen Receptor (CoStAR)-transduced TIL is distinctive therapeutic strategy that is led by Instil Bio. CoStAR-transduced TILs are expected to be elevated cytokine secretion, such as IFN-g, TNF-a and IL-2, enhanced tumor killing in vitro and in vivo, increased proliferative response, and reduced activation-induced cell death (AICD) [96]. Instil Bio is currently evaluating the product as ITIL-306 in their clinical trials with first patient.

Reduce IL-2 toxicity

Current TIL treatment regimens require high-dose IL-2 administration to support TIL survival in vivo, which limits their clinical applications due to IL-2-related toxicity. Obsidian Therapeutics (https://obsidiantx.com) is engineering TIL with membrane-bound IL-15 (mbIL15) to eliminate the dependence of TIL on exogenous IL-2, and they successfully expanded mbIL15-engineered TIL from both CRC and sarcoma ex vivo. A Phase I clinical trial of OBX-115 (engineered TIL product armed with mbIL15), a cytokine that is designed to remove the need for concomitant IL2 therapy, is currently evaluated in patients with metastatic melanoma (NCT05470283).

Another approach is an engineered an anti–PD-1 fusion with IL-15-IL-15Rα, whose activity was geographically concealed by immunoglobulin Fc region with an engineered linker (αPD-1-IL-15-R) to bypass systemic NK cells. Systematic administration of αPD-1-IL-15-R elicited extraordinary antitumor efficacy with undetectable toxicity. Mechanistically, cis-delivery of αPD-1-IL-15-R vastly expands tumor-specific CD8+T cells for tumor eradication. Additionally, αPD-1-IL-15-R upregulated PD-1 and IL-15Rβ on T cells to create a **feedforward activation loop**, thus rejuvenating TILs resulted in reducing tumor burden, improving survival, and also suppressing tumor metastasis compared to control ($p < 0.0001$). Collectively, renavigating IL-15 to tumor-specific PD-1+CD8+T cells, αPD-1-IL-15-R elicits effective systemic antitumor immunity [97] (Fig. 2B).

Recruiting and accumulating intravenously administered TIL within solid tumors

The challenge of recruiting and accumulating intravenously administered cells within solid tumors, such as in TIL therapy, is a critical issue in current adoptive cell therapies. This process is vital for enhancing the effectiveness of these therapies. Several strategies are explored to address this challenge. One approach involves enhancing the homing capabilities of TILs to the tumor site. Research indicates that tumor blood vessels play a crucial role in this process. High

endothelial venules (HEVs), which are specialized blood vessels found in some tumors, are particularly effective at recruiting lymphocytes from the bloodstream into cancerous tissues [98–102]. Targeting these HEVs or engineering TILs to better recognize and home to these structures could improve cell accumulation within tumors.

Another strategy is the use of combination therapies [103]. For instance, combining TIL therapy with immune checkpoint inhibitors like anti-PD-L1 has shown promise. This combination can enhance the recruitment and persistence of TILs in the TME by overcoming local immunosuppression and promoting T cell infiltration into the tumor [104, 105]. Moreover, optimizing the pre-conditioning regimens for patients before TIL infusion can sensitize tumor vasculature, making it more receptive to T cell infiltration [106, 107]. This involves using treatments that induce a more favorable TME for T cell homing and survival. However, determining the predictive value of PD-L1 expression in the context of combination therapy presents significant challenges. These limitations include therapy-induced changes in the immune microenvironment and the inherent heterogeneity of PD-L1 expression within tumors [108, 109]. Therapy can alter the local immune landscape, potentially affecting PD-L1 levels and their interpretability. Additionally, the variability of PD-L1 expression within different tumor regions complicates its use as a consistent predictive biomarker.

Overall, addressing the recruitment and accumulation of intravenously administered cells within tumors is multifaceted, involving improvements in cell engineering, combination therapies, and patient pre-conditioning. Further research and clinical trials are crucial to refine these strategies and enhance the efficacy of adoptive cell therapies against solid tumors.

In-depth understanding of TIL clonality through novel technologies

Single-cell sequencing on TIL to define active pharmaceutical ingredient

Tumor-reactive T cells have been demonstrated to bear a unique gene signature profile that can be captured by a single-cell sequencing analysis. Lowery et al. identified gene expression signatures of CD8+ and CD4+ neoantigen-reactive TILs, which can be considered as the "active pharmaceutical ingredient" in conventional pharmaceutical considerations. Specifically, this study [67] utilized single-cell RNA and T cell receptor sequencing to establish a training dataset, comprising 45,676 tumor-infiltrating lymphocytes (TILs) from 10 metastatic human tumors, and experimentally determined 55 neoantigen-specific T cell receptor clonotypes (NeoTCRs) as true positive. Gene expression signatures were subsequently identified for CD8+ and CD4+ neoantigen-reactive TILs, encompassing 243 and 40 genes, respectively. To assess the robustness of these gene signatures, an independent test set was employed, revealing experimental confirmation for 37 out of 73 predicted T cell receptor clonotypes (positive predictive value = 50.7%). It is worthy to note that only a specific subset of NeoTCRs, characterized by reduced differentiation and a more stem-like phenotype, demonstrated associations with both checkpoint inhibitor responses and adoptive cell therapy [71, 110–112].

Ongoing challenges transforming laboratory therapy into industrial product

Robust CMC

Routinely manufacturing of TIL presents several challenges if it becomes a therapeutic option. As a pharmaceutic product, the first priority in manufacturing is robustness. TIL shares many challenges with other autologous therapies.

1. Variability in patient samples: each patient is unique with different clinical manifestation, and the intrinsic characteristics of tumor tissue as the starting material for TIL manufacturing can vary significantly in terms of quality and quantity.
2. Scaling up the manufacturing of TILs from small batch to a larger clinically relevant scale can be complex.
3. Quality control on materials, such as human AB serum and feeder cells, is critical for TIL expansion and activation. Commercially available human AB serum varies significantly based on source and lot [113, 114].
4. Maintaining the TILs in optimal culture conditions, including appropriate temperature, oxygen levels, and nutrient supply, is critical for TIL expansion and function.
5. Contamination with bacteria, fungi, or other microorganisms can compromise the quality and safety of product. Robust procedures for aseptic processing, ideally closed system, and quality control are essential.
6. Managing the supply chain for reagents, culture media, and equipment is vital to ensure consistent manufacturing since variations in these materials can affect the final product.
7. Robust analytical methods are needed to characterize the final product, including assessments of cell viability, phenotype, potency, and purity.

Overall, achieving robust CMC for TIL therapy manufacturing involves addressing these challenges to

ensure that final product is safe, effective, and consistently reproducible for patient treatment.

Health economy considerations

Recent years, as the pharmaceutical development of cell and gene therapies is becoming more exciting, a number of therapies have been introduced into the clinical applications and an exceptionally substantial number of candidates are in the pipelines. We all need to be mindful with their soaring prices and affordability of patients and even the affluent society like the USA.

The excessive cost of immunotherapy treatments is an additional and significant challenge. For example, immune checkpoint inhibitor therapies typically range in cost from $103,400 to $168,948, with an average cost of $148,431 [115, 116]. Newer treatments like ACT can be even more expensive than traditional methods.

While health insurance can reduce much of the financial burden for patients and their families, out-of-pocket costs continue to rise. This substantial expense can limit access for many patients and imposes a considerable financial burden on healthcare systems.

Scaling up TIL therapy production to treat a larger number of patients efficiently may help reduce costs per patient. Economies of scale can make the therapy more cost effective over time. Evaluating the effectiveness of TIL therapy compared to other cancer treatments, such as chemotherapy, radiation therapy, or immunotherapies like CAR-T cell therapy, is essential. This involves considering factors like response rates, survival outcomes, and quality of life.

Cost and manufacturing challenges

TIL therapy's manufacturing process is indeed complex and expensive, involving the isolation, expansion, and reinfusion of TILs into patients. The costs stem from the need for specialized facilities, extensive labor, and stringent quality control measures. Additionally, the personalized nature of the therapy means that each batch is patient specific, further driving up costs.

Strategies for improving scalability:

1. Process automation: Automating various stages of TIL production, such as cell isolation and expansion, can significantly reduce labor costs and increase efficiency [117]. Advances in bioreactor technologies and automated culture systems hold promise in this area [2, 118, 119].
2. Standardization and optimization: Developing standardized protocols for TIL expansion can improve consistency and reduce variability. Optimizing culture conditions to maximize TIL yield and potency can also enhance the overall efficiency of the process [2].
3. Partnerships and infrastructure investments: Collaborations between academic institutions, industry, and regulatory bodies can foster innovation and share the burden of high initial costs. Investing in dedicated TIL manufacturing facilities and shared infrastructure can also help achieve economies of scale.
4. Regulatory and reimbursement: Clear regulatory guidelines and supportive reimbursement frameworks are crucial for the widespread adoption of TIL therapy. Engaging with regulatory agencies early in the development process can facilitate smoother approval and market access.

TIL therapy can be expensive due to its autologous nature, which limits economies of scale when producing it lot by lot. The cost includes the entire process, from biopsy and cell isolation to cell expansion and infusion, as well as post-treatment monitoring and care. By addressing these cost and scalability challenges through innovative strategies and collaborative efforts, and by assessing the long-term cost-effectiveness of TIL therapy, we can pave the way for broader accessibility and adoption, ultimately benefiting more patients in need.

More importantly, continued research and clinical trials are necessary to refine TIL therapy protocols, improve its effectiveness, and reduce costs. Moore's law might be realized in cell and gene therapy space like many other technological achievements over the past 5–6 decades. Health economies may need to allocate resources to support such research efforts. Identifying right patients who are most likely to benefit from TIL therapy is important to ensure that resources are directed toward those who are most likely to derive clinical benefits.

Ethical and regulatory aspects

Ethical and regulatory issues are critical components that must be thoroughly addressed in the development and implementation of TIL therapy.

1. Patient selection criteria: The criteria for selecting patients for TIL therapy can raise ethical concerns, especially regarding fairness and equity. It is essential to ensure that these criteria are transparent, evidence-based, and designed to maximize benefits while minimizing potential biases [120]. Additionally, it is important to address how these criteria might exclude certain populations and to explore ways to make the therapy accessible to a broader range of patients.
2. Access disparities: Access to TIL therapy may vary significantly based on geographic, socioeconomic, and

institutional factors. Addressing these disparities is vital to ensure that all patients who could benefit from this treatment have the opportunity to receive it, regardless of their background or location.

3. Regulatory hurdles: Navigating the regulatory landscape for new therapies can be complex and time-consuming. Ensuring that TIL therapy complies with all necessary regulations while maintaining a focus on patient safety and treatment efficacy is a challenging but essential task. Streamlining these processes without compromising ethical standards could facilitate faster and broader access to TIL therapy.
4. Ethical landscape: Ethical considerations must be central to the development of TIL therapy. This includes ensuring informed consent, balancing risks and benefits, and safeguarding patient autonomy. Continuous ethical review and active stakeholder engagement are essential to address emerging ethical dilemmas and to ensure that patient welfare remains the highest priority.

Incorporating these considerations into the discussion can provide a more comprehensive understanding of the ethical landscape and future development of TIL therapy.

Conclusion

Undoubtedly, TIL therapy, like any groundbreaking medical advancement, presents its share of challenges. The complexity of the treatment process, human immune system, substantial costs involved, and the potential for adverse effects necessitate ongoing scrutiny and refinement. However, the medical community's commitment to overcoming these hurdles is inspiring, instilling confidence that TIL therapy will continue to evolve into a cornerstone of modern cancer care.

In conclusion, TIL therapy stands as the path toward more effective, personalized, and compassionate cancer treatments. Its potential to revolutionize the lives of patients and families cannot be overstated. As someone who values progress in medicine, I wholeheartedly commend the scientists, clinicians, and researchers who have brought TIL therapy to the forefront of oncology. This therapy is a testament to the indomitable spirit of human ingenuity and compassion in the face of one of the most challenging adversaries—cancer.

Recent research in TIL therapy has brought promising advancements that address key challenges in the field. Through enhancements in TIL selection, overcoming the suppressive TME, and the strategic used of genetic engineering, researchers are significantly enhancing the therapeutic efficacy and durability of TIL therapy. While TIL therapy still faces hurdles on the path to clinical implementation, the rapid progress in this field holds great promise for the future of cancer immunotherapy. Continued research efforts and collaborations between scientists, clinicians, and biotech companies are absolutely imperative to refine TIL therapy and expedite its translation into clinical practice, ultimately benefiting cancer patients worldwide.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00262-024-03793-4.

Acknowledgements None.

Author contributions SM developed the concept, coordinated author activities, revised the article, and provided final approval of the version to be submitted. LC, HL, HY, and FY contributed the revised the article.

Declarations

Conflict of interest The authors declare no competing interests.

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