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REVIEW ARTICLE

Tumor-Infiltrating Lymphocyte Therapy for the Treatment of Metastatic Melanoma

Katy K. Tsai[1] · **Krishna V. Komanduri**[1]

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Abstract
Tumor-infiltrating lymphocyte (TIL) therapy is a type of personalized immunotherapy that harnesses the antitumor activity of endogenous immune cells. While TIL therapy has been in clinical investigation since the 1980s and shown promising signs of clinical activity in immunogenic solid tumors such as melanoma, more recent improvements in product manufacturing and characterization have demonstrated consistent efficacy and the feasibility of administering TIL therapy on a larger scale. Lifileucel was granted accelerated approval by the US Food and Drug Administration in February 2024 for the treatment of advanced melanoma, marking the first regulatory approval of a TIL product, and also the first approval of cellular therapy for the treatment of any solid tumor. Despite this landmark event, questions remain surrounding optimal TIL timing, sequencing with other treatment modalities, and the optimal TIL repertoire and phenotype. Innovations in cellular engineering are expected to improve the antitumor efficacy and safety profile of TIL therapy, advance our understanding of how best to deliver TIL therapy, and provide hope for paradigm-shifting approaches in the treatment of advanced melanoma as well as for other solid tumors.

1 Introduction

On 16 February, 2024, the US Food and Drug Administration (FDA) granted accelerated approval to lifileucel, a tumor-infiltrating lymphocyte (TIL) therapy, for patients with advanced melanoma previously treated with immune checkpoint inhibitors and BRAF inhibitors, if a BRAF V600 mutant. This landmark event represented not only the first approved TIL therapy, but the first approval of cellular therapy for the treatment of any solid tumor. Here, we provide an overview of the current and anticipated future state of TIL therapies for melanoma and other solid tumors.

2 Cellular Therapy for Solid Tumors: A Conceptual Understanding

Adoptive cell therapy (ACT) with TIL is a personalized immunotherapeutic strategy that harnesses the antitumor activity of endogenous immune cells. Immune effector cells, including cytotoxic (CD8+) and helper (CD4+) T cells, home to the tumor microenvironment, recognizing and targeting tumor-associated antigens and mediating tumor cell lysis. Tumor-infiltrating lymphocytes are harvested by surgical resection of tumor tissue, followed by ex vivo expansion to billions of cells with coculture using the T-cell growth factor interleukin (IL)-2 [1]. This autologous TIL product is reinfused after a course of non-myeloablative lymphodepletion (NMA-LD) with cyclophosphamide and fludarabine, facilitating TIL expansion [2, 3]. After TIL administration, additional IL-2 is given to support in vivo expansion of the infused cells [4, 5].

While TIL therapy falls under the broader category of ACT, it is distinguished from other ACTs, such as engineered T-cell receptor (TCR) T-cell and chimeric antigen receptor T-cell (CAR-T) therapies based on its scope of tumor antigen recognition. The T-cell population in the TIL product recognizes a broad range of tumor antigens, perhaps contributing to increased anti-tumor efficacy; in contrast, engineered TCR T cells and CAR-T therapies target a single tumor antigen, which may account for their relatively more modest activity in solid tumors to date. Additionally, TIL therapy involves enhanced recognition of only tumor-specific antigens, thus lowering the likelihood of developing

✉ Krishna V. Komanduri
krishna.komanduri@ucsf.edu

[1] Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA

Key Points

Tumor-infiltrating lymphocyte (TIL) therapy with an unmodified TIL product from Iovance received accelerated approval in the USA in February 2024 for the treatment of advanced melanoma that is progressing after treatment with immune checkpoint inhibitor therapy and, for those with a BRAF mutation, a BRAF inhibitor.

Tumor-infiltrating lymphocyte therapy is resource intensive and comprises multiple steps, including tumor tissue procurement surgery, a 7-day course of non-myeloablative lymphodepleting chemotherapy, a TIL infusion, up to six doses of interleukin-2, followed by recovery and hospital discharge with a close outpatient follow-up. The successful and safe delivery of TIL therapy requires close multidisciplinary collaboration from inpatient and outpatient providers experienced in these agents, with support from an intensive care unit.

US Food and Drug Administration approval of the first unmodified TIL product for advanced melanoma has opened the door to accelerated innovations to improve the antitumor efficacy and safety profile of TIL therapy, which will provide the basis for paradigm-shifting approaches in the treatment of advanced melanoma and other solid tumors.

the "on-target, off-tumor" side effects frequently observed with CAR-T therapy in patients with solid tumors, which generally targeted shared tumor-associated antigens [6, 7].

Melanoma is considered to be an immunogenic tumor, characterized by a high, ultraviolet, radiation-induced mutational burden and therefore a high neoantigen burden [8, 9]. Dermatopathologist Wallace Clark first applied the term "tumor-infiltrating lymphocytes" to refer to cancer-reactive immune cells, and in 1969 reported that TIL were recruited in the radial growth phase of melanoma and induced regression in primary tumors [10]. The presence of TIL in primary melanomas was associated with increased survival and a decreased risk of metastasis [11–13], while the presence of TIL in metastatic lesions appeared to confer similar benefit [14]. These factors, and the limited systemic treatment options for advanced melanoma, led to the development of ACT with TIL for melanoma [15].

Pioneering studies by Steven Rosenberg and colleagues at the National Institute of Health Surgery Branch demonstrated the therapeutic value of ACT with cultured TIL in vivo [16]. Metastatic melanoma was among the first solid tumor types treated with TIL therapy [17, 18]. Promising results in the 1980s spurred further investigation of TIL therapy for melanoma in a number of subsequent phase I/II trials in subsequent decades. These trials demonstrated reproducible efficacy, with objective response rates (ORRs) of 33–72% and durable complete responses in about 20% of patients despite differences in lymphodepletion regimens, TIL manufacturing processes, TIL dose, and IL-2 administration [19–26]. Given available systemic therapies for melanoma, these trials primarily enrolled patients who had previously been treated with cytokine- or chemotherapy-based regimens. The parallel development and FDA approval of more effective drugs for advanced melanoma shifted the focus of TIL therapy to later lines of therapy.

3 Contextualizing TIL Therapy for Melanoma

Advances in melanoma therapy have included advancements in TIL manufacturing and administration, as well as the development of immune checkpoint inhibitors (ICI) and small-molecule inhibitors targeting the BRAF oncogene. In 2011, the FDA approved ipilimumab, an antibody targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4), for the treatment of unresectable/metastatic melanoma. BRAF inhibitors in combination with MEK inhibitors were approved in 2014, with work ongoing to improve the therapeutic profile of this combination such that three different combinations are presently FDA approved.

Subsequent approvals included pembrolizumab and nivolumab, antibodies against programmed cell death receptor-1 (PD-1) in 2014, and for the combination of ipilimumab plus nivolumab in 2015. Collectively, these agents have improved long-term survival with manageable toxicity profiles and ease of administration (ICI by peripheral intravenous catheter, BRAF and MEK inhibitors by mouth), and are thus frequently used treatments in the first-line treatment of melanoma, particularly ICI rather than BRAF/MEK inhibitors given that BRAF/MEK therapy requires the presence of a BRAF V600 driver mutation, which is not present in all melanomas [27], and also given results from a phase III trial showing improved survival outcomes in patients with BRAF V600 mutant melanoma when given combination ICI rather than BRAF/MEK inhibitors in the first-line setting [28].

These advances have translated to significant improvement in 5-year overall survival for patients with advanced melanoma, from about 10% with cytotoxic chemotherapy alone, to around 50% [29]. Recent data report a landmark 10-year overall survival of approximately 50% in patients with advanced melanoma with combination anti-PD-1/CTLA-4 therapy [30]. Even so, half of patients will still experience disease progression from either primary or secondary resistance to ICI and/or molecularly targeted therapies. Therefore, a significant unmet need for alternative treatment strategies remains.

Given the resource-intensive nature of TIL harvest, production, and administration, single-center studies at institutions capable of on-site cell therapy manufacturing have driven TIL development. However, in the modern era of checkpoint inhibitor therapies, new trials were required to establish the benefit of TIL therapy in patients previously treated with ICI (and BRAF inhibitors, if BRAF V600 positive). With the expansion of cell therapy technologies enabling off-site manufacturing facilities, key phase II and III trials were launched to re-evaluate TIL efficacy in the era of ICI.

4 Regulatory Approval of TIL Therapy for Melanoma

Modern TIL studies have incorporated improvements in manufacturing and product characterization. The C-144-01 trial was a multicenter, international, single-arm, multicohort phase II trial testing an unmodified TIL product, lifileucel, in 153 patients with advanced melanoma. Cohort 1 of the trial included patients receiving non-cryopreserved TIL generated using a manufacturing process different from that for lifileucel; patients in Cohort 2 and registrational Cohort 4 received cryopreserved lifileucel, and Cohort 3 included patients from Cohorts 1, 2, and 4 who were retreated with lifileucel [31]. Tumor-infiltrating lymphocytes were centrally manufactured (published 22-day manufacturing process, with a 33-day harvest-to-infusion time). Patients were heavily pretreated, with a median of three lines of prior therapy including PD-1/L1-containing regimens [31, 32].

In cohort 2 ($N=66$), nearly all patients had prior progression of disease despite prior anti-PD-1/L1 exposure; 17 patients harbored BRAF V600 mutant melanoma and 88% had progressed on BRAF-targeted agents; 34 patients had already previously received combination anti-PD-1/CTLA-4 therapy as first-line (23%) or subsequent-line (29%) therapy [32]. An ORR of 36% and disease control rate (DCR) of 80% were noted in the overall cohort, with the highest ORR of 41% and DCR of 81% observed in patients who had disease progression after initial anti-PD-1/L1 therapy ($n=42$).

In the registrational cohort 4 ($N=87$), the ORR was 29%. Cohorts 2 and 4 were pooled because of identical eligibility, lifileucel manufacturing process, treatment regimen, and central response assessment. All patients had received prior anti-PD-1/programmed cell death ligand 1 therapy; 81.7% of the patients had also received anti-CTLA-4 therapy, and 53.6% had received combination anti-PD-1/anti-CTLA-4 therapy; 25.5% of the patients had received BRAF/MEK inhibitors. More than half of the patients ($n=83$ [54.2%]) were considered primary refractory to prior anti-PD-1/programmed cell death ligand 1 therapy. When analyzed together (published full analysis set, $N=153$), demonstrated ORR was 31.4% (95% confidence interval [CI] 24.1–39.4), progression-free survival was 4.1 months, and median overall survival was 13.9 months (95% CI 10.6–17.8). Together, these phase II data demonstrated the efficacy and safety of lifileucel in prospectively enrolled patients with advanced melanoma in the post-ICI setting, forming the basis for FDA accelerated approval of lifileucel in February 2024.

It should be noted that the primary efficacy data to support the FDA Biologics License Applications process ultimately considered ORR and duration of response (DoR) data from a subset ($n=73$) of patients from Cohort 4. Cohort 2 was excluded from efficacy analysis based on the FDA requirement for a blinded independent central review for tumor response, and some patients were excluded from the FDA's analysis of Cohort 4 because of the receipt of less than the recommended dose threshold. The FDA-recommended lifileucel dose is 7.5×10^9 viable cells, based on expected main efficacy measures of ORR and DoR in adult patients with unresectable/metastatic melanoma previously treated with an anti-PD-1 therapy, and if BRAF V600 mutation positive, treatment with a BRAF inhibitor with or without a MEK inhibitor. The ORR in this population was 31.5% (95% CI 21.1–43.4), and median DoR was not reached (95% CI 4.1 to not reached) [33, 34].

Another landmark TIL study is the pivotal M14TIL trial, the first multicenter phase III trial launched with fresh autologous TIL (TM001) generated by participating institutions in the Netherlands and in Denmark, using harmonized TIL manufacturing without commercial involvement [35]. This study randomized patients with unresectable stage IIIC/IV melanoma in a 1:1 ratio to receive either ipilimumab monotherapy or TIL with lymphodepletion and IL-2. Of 168 subjects, 86% who had received prior anti-PD-1 therapy in the adjuvant or first-line metastatic setting, ORR with ipilimumab or TIL were 21% (95% CI 13–32) and 49% (95% CI 38–60), respectively. Median progression-free survival was 3.1 months versus 7.2 months ($P<0.001$) and median overall survival, 18.9 months versus 25.8 months. An application for marketing authorization by the European Medicines Agency is currently in development for TM001.

Together, these results demonstrate consistent efficacy and the feasibility of larger scale TIL therapy, providing hope for paradigm-shifting approaches in advanced melanoma in addition to other solid tumors. Trials are ongoing to evaluate the efficacy of lifileucel alone in non-small cell lung cancer (NCT04614103) and endometrial cancer (NCT06481592); and lifileucel in combination with anti-PD-1 for melanoma (NCT05727904); head and neck squamous cell carcinoma, non-small cell lung cancer (NCT03645928), and cervical cancer (NCT03108495).

4.1 TILs in Melanoma: Unmet Needs

Despite dramatic advances in melanoma therapy, critical areas of unmet need remain, including those related to the site of metastasis as well as the melanoma subtype. For example, patients with melanoma brain metastasis have a poorer overall prognosis, and lower objective response rates and survival compared with those without brain metastasis [36, 37]. The magnitude of benefit of TIL for the treatment of melanoma involving the central nervous system remains unclear. The risk of TIL-mediated neurologic adverse events (AEs) from infiltration into the central nervous system is a concern, as are the potential risks of chemotherapy and IL-2 administration in patients with active brain metastases (e.g., thrombocytopenia and bleeding risk) [38]. Encouraging intracranial responses have been reported, [39], and remain an active area of investigation (NCT05640193) [40].

Melanoma is a very heterogeneous disease, with pathogenesis and clinical behavior/clinical outcomes differing widely based on the subtype. Increasingly, it is recognized that the melanoma subtype/genomic profile may influence disease response to various modes of immunotherapy. For example, acral, mucosal, and uveal melanoma are known to be less responsive to ICI compared with cutaneous melanoma [41–43]. Because most TIL studies have focused on cutaneous melanoma, less is known about outcomes of TIL therapy in patients with these subtypes. Cohorts 2 and 4 on the C-144-01 trial enrolled mostly patients with cutaneous melanoma ($n = 83$ [54.2%]), with mucosal ($n = 12$ [7.8%]) or acral ($n = 10$ [6.5%]) melanoma comprising the minority of the enrolled population [31]. In a post-hoc analysis presented at the European Society for Medical Oncology in 2023, outcomes of the 12 patients with mucosal melanoma treated with lifileucel were clinically meaningful and durable (ORR 50%, median duration of response not reached) [44]. These data further support the potential benefit of TIL in this difficult-to-treat melanoma subtype. Given the small sample size, pooling these results with real-world data from lifileucel-treated patients, along with retrospective outcomes from previously treated TIL patients, would be highly informative.

A crucial need for therapeutic options exists for uveal melanoma [45]. Uveal melanoma commonly metastasizes to the liver, and melanoma-specific survival once metastatic disease develops is the poorest amongst melanoma subtypes, with few effective treatment options available. Aggressive ICI therapy with a combination anti-PD-1/anti-CTLA-4 blockade effects only up to 18% of the ORR [43]. Treatment with tebentafusp, a first-in-class TCR-bispecific fusion protein specific for glycoprotein 100 and CD3, has resulted in an overall survival improvement but is an option only for HLA-A*02:01-positive patients [46, 47], calling into question whether ACT-TIL might be an effective treatment option for this melanoma subtype. Despite its described immunoresistance, the harvest and ex vivo expansion of uveal melanoma TIL from both primary and metastatic sites has proven feasible [48, 49], with described clinical responses. In a phase II study of 20 patients with evaluable uveal melanoma, seven (35%) had objective responses, including one complete response at 21 months [30], with trials ongoing [50].

4.2 Lifileucel: Safety and Practical Considerations

While TIL approval has constituted a landmark in melanoma therapy, it is critical to understand the practical challenges posed by successful administration of TIL therapy. Efficient workflows and timely execution are essential given the high level of personnel/financial resourcing, logistic planning, and clinical expertise required to manage advanced disease and coordinate delivery of a multistep therapeutic product (Fig. 1).

5 Patient Selection

Patient selection for TIL therapy requires collaboration from a multidisciplinary team, including a surgical oncologist, medical oncologist, and a cell therapy and/or hematopoietic stem cell transplant physician. To be considered eligible for TIL therapy, patients will need to demonstrate evidence of advanced disease that has progressed on a previous anti-PD-1-containing regimen, but they must also be well enough to withstand all components of TIL therapy, including surgery, NMA-LD chemotherapy, a TIL infusion (including the wait associated with TIL manufacturing), and an IL-2 infusion. Patients must meet a number of parameters with regard to adequate performance status and organ function (e.g., cardiac, renal, pulmonary function), which is covered in detail in previously published expert consensus guidelines [50]. To ensure patient safety and appropriate selection, all relevant providers should review potentially eligible patients in the setting of a multidisciplinary tumor board.

5.1 Surgical Resection

Lifileucel manufacture requires the provision of ≥ 1.5 to ≤ 4.0 cm of tumor tissue in an aggregate diameter, which is subsequently prosected and shipped to a centralized good manufacturing practice facility to initiate lifileucel manufacturing. In the registrational C-144-01 trial, tumor-resection AEs were defined as AEs related to surgery that started after tumor resection and before the start of NMA-LD chemotherapy (or < 30 days of resection, if NMA-LD was not received). Tumors were resected from diverse sites

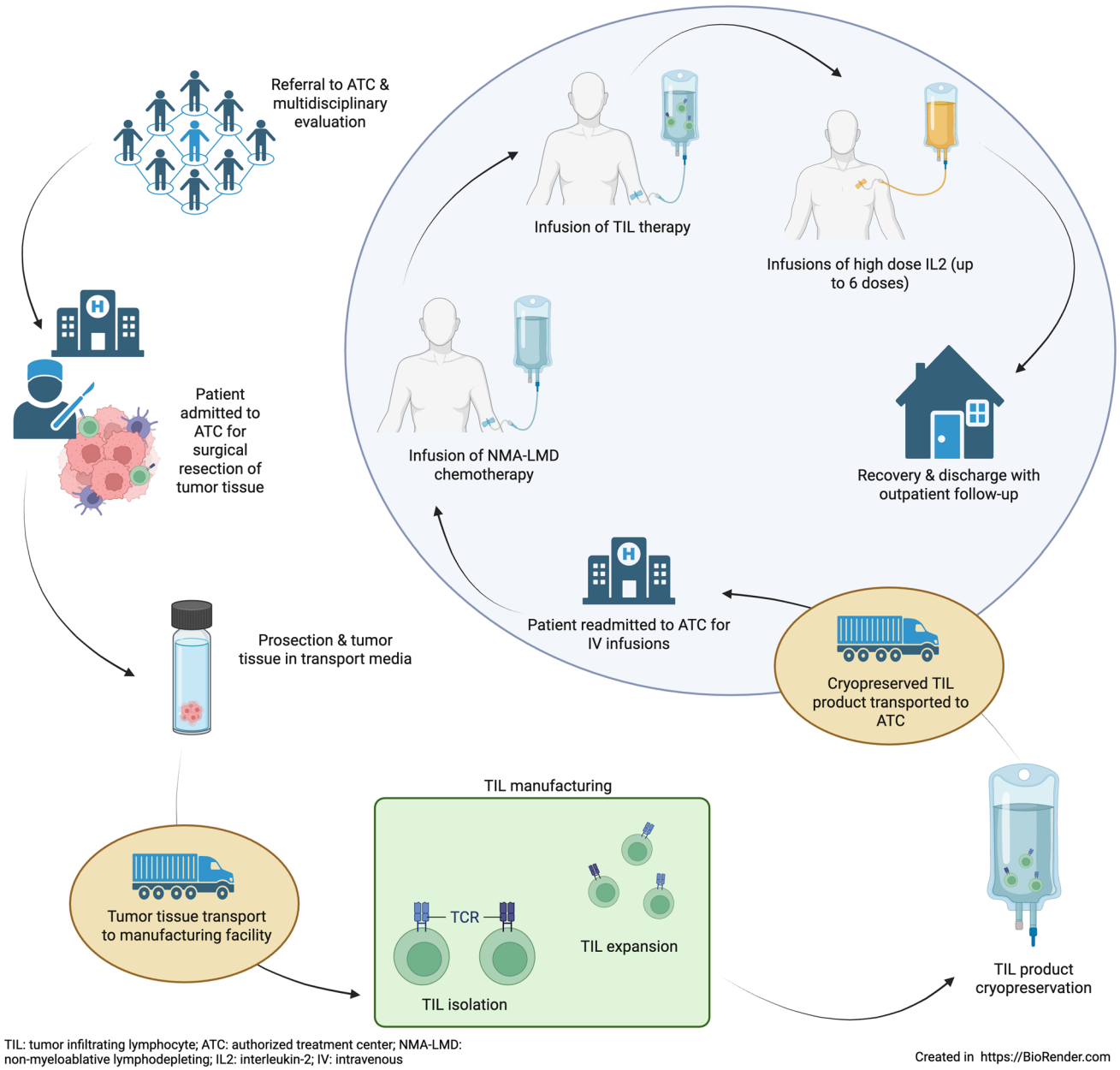

Fig. 1 An overview of the treatment journey for the patient receiving tumor infiltrating lymphocyte (TIL) therapy. Steps in the treatment journey include referral and evaluation, surgical resection of the tumor, transport of the tumor and isolation and expansion of TIL in the manufacturing facility, and transportation of the TIL product back to the treating center. The TIL product is infused following administration of lymphodepleting chemotherapy to the patient and followed by infusions of high dose interleukin-2 (IL2) in the inpatient setting

with overall low surgical morbidity: tumor-resection AEs of any grade were noted in 60 (31.7%) patients, with grade 3/4 tumor-resection AEs seen in six (3.2%) patients [31].

Surgical TIL harvest may not be possible in some patients because of the anatomic positioning of tumors in a high-risk site, or the presence of only low-volume disease. The logistics of surgical scheduling can add several weeks of delays, while surgery itself may induce risks of surgical morbidity, prolonged recovery time, and increased healthcare utilization costs. Innovations in cellular expansion could improve the TIL yield and reactivity, allowing for the harvest of a lower volume of tumor tissue by alternative and less invasive methods (e.g., core needle biopsy) and might decrease the fraction of patients who become TIL ineligible because of disease progression during the wait for surgery and TIL product manufacturing.

5.2 NMA-LD Administration

The traditional TIL therapy regimen consists of the administration of NMA-LD chemotherapy, followed by a one-time TIL infusion, followed by systemic high-dose IL-2. Nonmyeloablative lymphodepletion can be administered as an inpatient or outpatient at the discretion of the treatment team, although at present hospital admission is required for monitoring and supportive care during TIL and IL-2 infusion. The most common toxicities arising during TIL therapy are attributed to NMA-LD and/or IL-2, and administration of this regimen thus requires support from experienced and trained staff and providers as well as access to intensive care unit support.

The standard NMA-LD regimen established by Rosenberg and colleagues (and used for lifileucel) consists of a 7-day regimen: cyclophosphamide 60 mg/kg intravenously (IV) daily for 2 days, followed by fludarabine 25 mg/m^2 IV daily for 5 days. This optimizes the tumor microenvironment for activity of infused TIL by eliminating immunosuppressive cells (regulatory T cells, myeloid-derived suppressor cells) and reducing competition for homeostatic cytokines (IL-7, IL-15) [2, 3].

Expected treatment-emergent AEs (TEAEs) from NMA-LD include grade 3/4 cytopenia in most patients. Indeed, on the C-144-01 lifileucel study, all patients in the safety analysis set ($N=156$) experienced one or more TEAE (any grade). Grade 3/4 TEAEs occurred in $\geq 30\%$ of patients, including thrombocytopenia (76.9%), anemia (50.0%), and febrile neutropenia (41.7%). Patients must be monitored not only for direct toxicities (e.g., cyclophosphamide-induced hemorrhagic cystitis) and gastrointestinal toxicity (nausea, vomiting, diarrhea, stomatitis), but complications arising from pancytopenia and immunosuppression (bleeding, infection) [51].

The expected toxicities of NMA-LD complicate administration to patients with comorbidities, limiting candidacy for TIL therapy. As current TIL NMA-LD regimen doses are higher than those used in CAR-T therapy although lower than those used in hematopoietic stem cell transplantation [52], the optimal dose is not clear, with lower dose NMA-LD regimens under exploration. One small study analyzed data from a trial comparing the standard NMA-LD regimen with two-fold lower dosing of cyclophosphamide (30 mg/kg for 2 days) in TIL recipients with metastatic melanoma [53]. While a substantially lower toxicity incidence and shortened mean duration of inpatient stay were seen, the sample size was small ($n=8$); further prospective studies are required to optimize the NMA-LD dose and schedule. If a reduced dose of cyclophosphamide-fludarabine could result in similar TIL expansion and post-infusion persistence with reduced toxicity, this would likely facilitate wider adoption of TIL therapy, potentially on an outpatient basis, with a reduction in resource utilization costs.

5.3 Lifileucel Administration

Infusion of the cryopreserved TIL product is generally tolerated well. The median infused TIL dose was 21.1×10^9 cells on C-144-01 (range, 1.2×10^9–99.5×10^9 cells), and serious anaphylactic or infusion-related reactions were rare. Anaphylactic and infusion-related reactions reported by investigators on C-144-01 as related specifically to lifileucel were described in six (3.8%; grade 1/2) and two (1.3%; grade 3/4) patients, respectively.

Six deaths occurred within 30 days after a lifileucel infusion, two of which were attributed to progression of disease, and four of which were attributed to AEs. Of the four TEAE deaths, three were assessed by the investigators as not related to lifileucel but to NMA-LD and/or IL-2 (pneumonia, arrhythmia, acute respiratory failure). One was noted as possibly related to TIL/related to all components of the regimen (intra-abdominal hemorrhage) [31].

5.4 IL-2 Administration

After a TIL infusion, high-dose IL-2 is infused as cytokine support. The lifileucel regimen recommends bolus IL-2 at 600,000 IU/kg IV be given every 8–12 hours, for up to six doses. The median number of IL-2 doses administered on C-144-01 was 6.0 (range, 0–6) [31].

Administration of high-dose IL-2 is associated with a number of challenging dose- and schedule-dependent side effects potentially affecting a wide range of organ systems, including capillary leak syndrome, which can result in hypotension and reduced organ perfusion, fevers/chills/rigors, pulmonary edema, cardiac arrythmias, and various other gastrointestinal, dermatologic, and neurologic issues. Interleukin-2 thus requires administration by experienced trained providers in a hospital setting with an intensive care unit level of care available [54].

Given that the IL-2 administered post-TIL infusion is intended to be T-cell supportive rather than directly therapeutic, IL-2 is typically given to physiologic tolerance, and can be held or discontinued at any time by the treating provider if there are toxicity concerns [51]. In fact, in a post-hoc analysis of C-144-01, no clear correlation was observed between the total number of IL-2 doses given and the efficacy of TIL cell therapy when IL-2 was discontinued for toxicity. The ORR to lifileucel was 38%, 31%, and 31% in patients who received one to two, three to four, and five to six doses of IL-2, respectively ($p=0.87$) [55].

The optimal dose of IL-2 remains to be established. The resource-intensive nature of IL-2 administration and the

challenging toxicity profile, particularly in lymphodepleted patients, presents an opportunity to explore alternative dosing regimens of post-TIL IL-2, as well as to consider alternative cytokine support and novel delivery mechanisms.

6 Strategies for Improvement: Next-Generation TILs

The need for standard NMA-LD and IL-2 regimens has been associated with toxicities of TIL, including lifileucel. A number of trials with next-generation TILs are ongoing to identify rational methods of improving the safety profile and the antitumor efficacy of TIL in melanoma and in other solid tumors (Table 1). As T cells require three signals to activate and expand (TCR engagement with an antigen, a costimulatory receptor signal, and a cytokine signal [56]), strategies that improve intra- and/or extracellular signaling are needed to optimize T-cell activation [7, 57].

6.1 Optimizing Extracellular Cytokine-Induced T-Cell Activation

Enhancing cytokine-induced T-cell activation can be accomplished by exploring novel methods of cytokine inclusion with TIL products, as well as by exploring local delivery of alternative cytokines besides IL-2. Interleukin-15 is a cytokine that activates and expands CD8+ and natural killer cells and maintains TIL function [2]. As it does not expand CD4+ T cells and regulatory T cells, it is an attractive alternative to IL-2 [58]. OBX-115 (Obsidian Therapeutics) is a membrane-bound IL-15 (mbIL015)-engineered autologous TIL. Because of inducible and regulatable expression of mbIL-15 using a carbonic anhydrase-2 drug-responsive domain, expression is inducible using oral acetazolamide administration. In the presence of acetazolamide, OBX-115 TIL express mbIL-15, while mbIL-15 is degraded by the proteosome in its absence. Acetazolamide is well tolerated and can be re-dosed to reactivate and re-expand persistent OBX-115 TILs in circulation. In addition, given the membrane tethering to reduce systemic exposure and ability to regulate cell-surface IL-15 expression, the need for IL-2 is eliminated; the first-in-human study of this agent is under investigation with options for a standard or lower dose regimen of cyclophosphamide/fludarabine. Data presented for the first ten infused patients showed promising efficacy (ORR 44.4%, including two complete responses) in ICI-refractory melanoma, and an improved safety profile as compared with IL-2-containing TIL regimens: no dose limiting toxicities (DLTs) occurred at any dose level, and there were no grade 4/5 and limited grade 3 non-hematologic TEAEs [59].

GT201 (GRIT Biotechnology) is an engineered TIL also incorporating membrane-bound, constitutively active IL-15. Seven patients with tumors including non-small cell lung cancer, melanoma, cervical cancer, and ovarian cancer were treated in a phase I trial with standard NMA-LD and IL-2. Encouraging responses were observed, with 42.9% of patients achieving partial response, and two patients with stable disease. The safety profile was promising, with no grade ≥ 3 AEs attributed to GT201, and all grade ≥ 3 AEs attributed to NMA-LD and/or IL-2 either recovered or grade ≤ 2 within 14 days [60], although the sample size is limited.

C-TIL051 (AbelZeta Pharma), a non-engineered TIL therapy manufactured with a proprietary process to maximize yield, and in an ongoing phase I trial for patients with non-small cell lung cancer, is being given in combination with pembrolizumab as well as NKTR-255 (IL-15 receptor agonist). This strategy might allow lower cytokine dosing and prolonged IL-15 activity, although clinical safety and efficacy data are not yet available (NCT05676749).

Interleukin-7 supports T-cell proliferation and survival [2], and is another alternative to IL-2 currently under clinical investigation. GC203 (Juncell Therapeutics) is a TIL therapy engineered to constitutively express membrane-bound IL-7 (mbIL-7). Twenty patients with recurrent advanced ovarian cancer were enrolled to this phase I trial, and received a 3-day lymphodepletion regimen consisting of hydroxychloroquine 600 mg on day 1, and cyclophosphamide 20 mg/kg/day on days 1 through 3, followed by a GC203 TIL infusion, and no IL-2 support [61]. The ORR of 33.3% included two (11.1%) patients with a complete response and four (22.2%) patients with a partial response. The most common TEAEs were elevated C-reactive protein, fever, and fatigue, with only one patient experiencing severe neutropenia, suggesting the potential for membrane tethered IL-7 to be a safer alternative to high-dose bolus IL-2.

Interleukin-12 is a proinflammatory cytokine that stimulates the differentiation of CD4+ T cells into Th1 cells, thereby promoting an antigen-specific immune response, and increasing the production of interferon-γ through the increased proliferation and activation of cytotoxic effector cells [62]. Systemic administration of IL-12 can cause severe side effects, including fatigue, vomiting, diarrhea, headaches, and other flu-like symptoms, constraining its therapeutic potential [63]. Efforts to engineer TILs to secrete IL-12 with antigen stimulation showed potential efficacy, but with unacceptable side effects, limiting its development [64]. Two agents in preclinical development show promise. IOV-5001 (Iovance Biotherapeutics) is an NFAT-inducible membrane-bound IL2 (mbIL-12)-engineered TIL that has demonstrated minimal IL-12 shedding and the potential for improved safety [65]. Another agent is an NFAT-inducible and cytoDRiVE-regulatable mbIL-12-engineered TIL (Obsidian Therapeutics), engineered to restrict spatial and

Table 1 Selected list of actively accruing trials investigating TILs for solid tumors

ClinicalTrials.gov ID	Phase	Sponsor	TIL product	Cancer Type
NCT05430373	I	Grit Biotechnology	GT101; unmodified TIL	Any solid tumor
NCT05729399	I	Grit Biotechnology	GT201; membrane-bound IL-15-engineered TIL	Any solid tumor
NCT06145802	I	Grit Biotechnology	GT-316; dual knockout CRISPR/Cas9 engineered TIL	Gynecologic cancers
NCT05676749	I	AbelZeta Pharma	C-TIL051; non-engineered TIL	NSCLC
NCT05468307	I	Juncell Therapeutics	GC203; membrane-bound IL-7-engineered TIL	Gynecologic cancers
NCT06060613	I/II	Obsidian Therapeutics	OBX-115; membrane-bound IL-15-engineered TIL	Melanoma, NSCLC
NCT05361174	I/II	Iovance	IOV-4001; transcription activator-like effector nucleases (TALEN)-mediated PDCD-1 gene inactivation engineered TIL	Melanoma, NSCLC
NCT06237881, NCT06598371	I/II	KSQ Therapeutics	KSQ-001EX; SOCS1/Regnase-1 dual-knockout CRISPR/Cas9 engineered TIL	NCT06237881: Melanoma, NSCLC, HNSCC; NCT06598371: Melanoma, NSCLC, HNSCC, CRC, pancreatic cancer, cervical cancer
NCT04426669, NCT05566223	I/II	Intima Bioscience	CISH-inactivated TIL; cytokine-inducible SH2 domain-containing protein (CISH) knockout CRISPR/Cas9 engineered TIL	NCT04426669: CRC, pancreatic cancer, gallbladder cancer, esophageal cancer, gastric cancer; NCT05566223: NSCLC
NCT03991741	I	University of California, San Diego	Autologous TIL	Melanoma, HNSCC
NCT04614103	II	Iovance	Lifileucel (LN144/145)[a]	Melanoma, NSCLC, HNSCC
NCT04111510	II	Iovance	Lifileucel (LN1244/145)	Triple-negative breast cancer
NCT03645928	II	Iovance	Lifileucel (LN144/145)[a] ± checkpoint inhibitors	Melanoma, NSCLC, HNSCC
NCT03935893	II	University of Pittsburgh	Unmodified TIL	Gastric cancer, esophageal cancer, CRC, pancreatic cancer, sarcoma, mesothelioma, neuroendocrine carcinoma, squamous cell carcinoma, MCC, mismatch repair deficient and/or microsatellite unstable cancers
NCT03801083	II	University of Pittsburgh	Unmodified TIL	Biliary tract cancers

CRC colorectal cancer, *HNSCC* head and neck squamous cell carcinoma, *IL* interleukin, *MCC* Merkel cell carcinoma, *NSCLC* non-small cell lung cancer, *TIL* tumor-infiltrating lymphocyte

[a]LN-144 and LN-145 are TIL expansion products differentiated only by indication: LN-144 (lifileucel) is the product for melanoma and LN-145 is used in all other solid tumor indications

temporal IL-12 regulation via inducible elements [65]. Clinical safety data are forthcoming for these engineered TILs.

6.2 Optimizing Extracellular T-Cell Activation by Improving TCR-Mediated Antigen Recognition

The adaptive immune response to cancer is predicated on TCR recognition of tumor neoantigen clonal heterogeneity. While unmodified TILs such as lifileucel and ITIL-168 (an alternative unmodified TIL product by Instil Bio; previously in clinical development but now discontinued) have shown that an unrestricted TCR repertoire can counteract tumor heterogeneity with clinical benefits [31, 32, 66], enriched TIL recognizing tumor neoantigens might yield more effective TILs [67, 68]. Clinical studies of predefined neoantigen-specific TILs are underway to create TIL and TCR therapy products with a higher fraction of tumor-specific TCRs [69–71].

Conventional non-engineered TILs reflect T-cell heterogeneity that may not generate ideal responses, such as a variable proportion of CD4+ and CD8+ T cells, a low proportion of "stem-like" memory progenitor CD8+CD39-69- and other memory T cell subsets, and a high proportion of inhibitory marker expression (e.g., PD-1, LAG3, TIM3, TIGIT) [72, 73]. Surface phenotypic restriction of narrower tumor-reactive T cells for inclusion in TIL products [74, 75] is being explored. Engineered feeder cell lines (e.g., K562) can constitutively express co-stimulatory molecules, including CD137L (4-1BBL), CD86, IL-15, and IL-21, and can expand T cells to scalable numbers while still preserving clonal diversity [76], while maintaining a CD8+ tumor-reactive proliferative phenotype.

Innovations in cell culture might improve TILs, i.e., media/reagents that improve stemness or antigen-reactive TCR capture could produce a more effective product [77]. LYL845 (Lyell) used proprietary expansion protocols for their non-engineered TIL, which were epigenetically reprogrammed to generate effector T cells with putatively reduced exhaustion and improved stemness/persistence/antitumor activity. LYL845 was in phase I clinical development for melanoma, non-small cell lung cancer, and CRC 17 [78], but was discontinued by Lyell in October 2024.

6.3 Optimizing Intracellular and Extracellular Enhancements of T-Cell Activation and Effector Function

Synthetic co-stimulation of T-cell signaling may mitigate known mechanisms of immune escape and TIL resistance. One strategy to include a synthetic costimulatory antigen receptor (CoStAR) molecule with dual CD28 and CD40 domains on healthy donor T cells targeting the tumor-associated antigen CEA led to increased T-cell activation and long-term proliferation even in the absence of IL-2 [79, 80] and could warrant clinical investigation.

6.4 Intracellular Optimization of T-Cell Fitness

As T-cell exhaustion occurs via activation-induced inhibitory signaling, interrupting these pathways using gene knockout technology could resensitize the immune system to T-cell-mediated antitumor activity. One approach is to knock out the PD-1 protein *PDCD-1* in TILs [81, 82] to prevent binding to programmed cell death ligand 1 on tumor cells, thus simulating the therapeutic effects of a PD-1 blockade and enabling tumor detection and elimination. IOV-4001 (Iovance) uses transcription activator-like effector nucleases (TALEN)-mediated PDCD-1 gene inactivation to knockout PD-1 protein expression on TIL, and reports increased reactivity compared with non-engineered TIL [83]; a first-in-human trial is ongoing (NCT05361174) [84].

KSQ-001EX (KSQ Therapeutics) TILs have been engineered using CRISPR/Cas9 to knock out the suppressor of the cytokine signaling 1 (*SOCS1*) gene; *SOCS*1 downregulates T-cell signaling using cytokines including interferon-γ [85], and in its absence cytokines are able to activate T-cell-mediated antitumor activity. KSQ-004EX dual-knockout TIL additionally inactivates Regnase-1, a regulatory ribonuclease that destabilizes IL-6 and IL-12 and thus reduces immune responses. SOCS1 and Regnase-1 were identified in a CRISPR screening process to be the most potent antitumor knockout combination [86]. This agent is in clinical investigation, with results still unpublished (NCT06237881, NCT06598371).

A third knock-out approach has been proposed for TIL, involving the cytokine-inducible SH2 domain-containing protein (CISH), which is associated with downregulation of intracellular T-cell effector signaling pathways [87]. A neoantigen-specific CRISPR/Cas9 CISH knock-out TIL (Intima Bioscience) is in phase I/II development (NCT04426669, NCT05566223).

7 Conclusions

Recent landmark studies have demonstrated consistent reproducible clinical activity of TIL therapy in advanced melanoma, leading to the regulatory approval of lifileucel in the USA.

Although challenges exist for these relatively complex therapies, TILs are now a standard therapy for relapsed melanoma. Accelerated innovations in cell engineering will further improve the safety and efficacy of TIL therapies. While many questions remain surrounding optimal TIL timing, sequencing, combinations with other agents, and the optimal

TIL repertoire and phenotype, several next-generation products are in clinical development, and poised to advance our understanding of how to optimize TIL therapy for patients with melanoma and other cancers.

Declarations

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Consent to Participate Not applicable.

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