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Expert consensus guidelines on management and best practices for tumor-infiltrating lymphocyte cell therapy

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ABSTRACT

Adoptive cell therapy with autologous, ex vivo-expanded, tumor-infiltrating lymphocytes (TILs) is being investigated for treatment of solid tumors and has shown robust responses in clinical trials. Based on the encouraging efficacy, tolerable safety profile, and advancements in a central manufacturing process, lifileucel is now the first US Food and Drug Administration (FDA)-approved TIL cell therapy product. To this end, treatment management and delivery practice guidance is needed to ensure successful integration of this modality into clinical care. This review includes clinical and toxicity management guidelines pertaining to the TIL cell therapy regimen prepared by the TIL Working Group, composed of internationally recognized hematologists and oncologists with expertise in TIL cell therapy, and relates to patient care and operational aspects. Expert consensus recommendations for patient management, including patient eligibility, screening tests, and clinical and toxicity management with TIL cell therapy, including tumor tissue procurement surgery, non-myeloablative lymphodepletion, TIL infusion, and IL-2 administration, are discussed in the context of potential standard of care TIL use. These recommendations provide practical guidelines for optimal clinical management during administration of the TIL cell therapy regimen, and recognition of subsequent management of toxicities. These guidelines are focused on multidisciplinary teams of physicians, nurses, and stakeholders involved in the care of these patients.

INTRODUCTION

Adoptive cell therapy (ACT) with tumor-infiltrating lymphocytes (TILs) for treatment of solid tumors involves the direct surgical resection and prosection of a patient's tumor tissue and ex vivo expansion of TIL, which reverses the dysfunctional state acquired in the tumor microenvironment by improving the phenotypic, functional, and tumor-reactive profile.^{1,2} Because TILs are obtained directly from a portion of the patient's tumor tissue, they are differentiated from chimeric antigen receptor (CAR) T-cell therapy by

their polyclonality and ability to recognize and target a multitude of patient-specific tumor neoantigens to mediate tumor cell lysis.³

The Surgery Branch at the National Cancer Institute (NCI) began the pioneering research efforts in TIL cell therapy in the 1980s. Studies in patients with metastatic melanoma treated with non-myeloablative lymphodepletion (NMA-LMD), TIL, and interleukin-2 (IL-2) confirmed clinical safety and demonstrated significant efficacy, with objective tumor regression in up to 55% of patients.^{4,5}

Since then, several studies from the NCI and other groups have aimed to optimize the regimen in patients with metastatic melanoma.⁶⁻¹⁰ Access to TIL has increased with the adoption of centralized manufacturing, increasing the number of sites available to offer this therapy. Current trials accrue multiple tumor types. Lifileucel, the first US Food and Drug Administration (FDA)-approved autologous, cryopreserved TIL cell therapy product, showed clinically meaningful activity (independent review committee-assessed objective response rate (ORR) of 31.4% and median duration of response not reached at a median follow-up of 36.5 months) in a phase 2 study in 153 patients with heavily pretreated advanced (unresectable or metastatic) melanoma after progression on immune checkpoint inhibitor (ICI) and targeted therapies (if *BRAF*-mutated).¹¹ A recent phase 3 study conducted in the Netherlands and Denmark showed significant improvement in progression-free survival (7.2 vs 3.1 months; HR 0.50 (95% CI 0.35 to 0.72); $p < 0.001$) and substantially higher ORR (49% vs 21%) with TIL cell therapy compared with ipilimumab in patients with unresectable or metastatic

melanoma who had received a maximum of 1 prior line of systemic therapy.¹² The robust efficacy of TIL cell therapy observed in pretreated patients suggests the potential benefit of this approach soon after the failure of first-line ICI therapy. Early data are promising in patients with advanced or metastatic non-small cell lung cancer^{13 14}; recurrent, metastatic, or persistent cervical carcinoma¹⁵; head and neck squamous cell carcinoma^{16 17}; and breast cancer.¹⁸

As the use of TIL therapy becomes more widespread, historic best practices on management of high-dose IL-2 need to be reconsidered in the setting of the TIL cell therapy regimen, where IL-2 is used to support T-cell activity rather than used as a therapeutic agent.

To this end, the TIL Working Group, composed of an internationally recognized multidisciplinary team including surgeons, hematopoietic stem cell transplant physicians, and solid tumor oncologists with expertise in TIL cell therapy, has been established in collaboration with industry experts to aid healthcare practitioners in better understanding and administering TIL cell therapy. The TIL Working Group members have treated numerous patients with TIL cell therapy in single-center and multi-center trials. Based on the evidence in the literature and members' experience with treating patients with TIL cell therapy, the group has developed expert consensus recommendations for patient management, including pretreatment assessments, IL-2 administration, and toxicities. Additionally, key operational considerations and future directions for the success of TIL cell therapy are addressed. These are general consensus recommendations for TIL therapy, but for any patient on clinical trials, protocols should be followed strictly. Individual protocols may differ from these consensus recommendations.

GENERAL OVERVIEW OF STEPS IN TIL CELL THERAPY

The overall course of TIL cell therapy and patient journey is illustrated in online supplemental figure 1. The process begins after discussion of treatment options and preliminary assessment for appropriateness of TIL by the primary oncologist. Potentially eligible patients who decide to pursue TIL cell therapy would be ideally referred to an authorized treatment center (ATC). TIL ATCs are centers that are certified to provide TIL cell therapy in the setting of a clinical trial or standard of care. To be deemed an ATC, the centers should have treatment teams that have experience treating patients with TIL cell therapy and/or care team providers who have undergone a training program in TIL treatment. Once a patient has been deemed a candidate for TIL cell therapy, the TIL Good Manufacturing Practice (GMP) manufacturer (both in commercial and academic settings) is notified and the required materials for tumor tissue procurement surgery are obtained. The surgeon identifies the optimal lesion(s) and surgical approach in coordination

with the treating physician. Currently, at least 1.5 cm (1.5–4 cm diameter) of tumor is required for TIL production. A tumor tissue procurement surgery is performed during which the surgical team resects and prosects (trims and fragments) the tumor tissue under sterile conditions, often in collaboration with pathology (taking utmost care that any material used for pathology review is kept separate from the tissue used for TIL manufacturing). The tumor tissue is transported for TIL manufacturing in sterile media containing hypothermosol, amphotericin B, and gentamicin, and prepared fresh on the day of tumor tissue procurement surgery and stored at 2°C–8°C until arrival of the courier for transport pick-up. In an academic/institutional setting, the TIL product is usually manufactured on site in a local certified GMP facility, whereas in a commercial setting, fresh tumor tissue is shipped to a centralized GMP facility to initiate TIL cell therapy manufacturing. Once manufactured, the cryopreserved TIL infusion product is shipped back to the ATC, where it is infused under the supervision of the treatment team.

Prior to TIL infusion, patients receive a non-myeloablative lymphodepleting regimen (generally comprising fludarabine and cyclophosphamide), typically lasting 5–7 days, which may be administered in the inpatient or outpatient setting at the discretion of the treatment team. For current TIL products, cryopreserved TIL (thawed prior to infusion) or freshly manufactured TIL are infused after completing non-myeloablative lymphodepletion, followed by administration of a short course of high-dose bolus IL-2 every 8–12 hours over a period of 2–5 days to support growth and activity of the infused TIL (detailed below). In studies investigating lifileucel TIL cell therapy, up to 6 doses of IL-2 were often administered,^{19 20} whereas other studies investigating TIL cell therapy have used up to 15 doses of IL-2.¹² Currently, hospital admission is required for supportive care and monitoring during TIL infusion and high-dose IL-2 administration. Patients are discharged when deemed appropriate by the inpatient care team, after sufficient hematological recovery and improvement from any IL-2 toxicity, approximately 14 days after TIL infusion (described in more detail below; online supplemental table 1). Some patients will require hydration and/or transfusion support of packed red blood cells and/or platelets after discharge, so close follow-up and coordination of care is needed. As with other cellular therapies, patients are encouraged to remain close to the treatment center (within 30–50 miles or <1 hour) with a designated caregiver for preplanned period (online supplemental figure 1). Thirty days after TIL infusion is a reasonable milestone, but the period of time may be shorter or longer depending on institutional guidelines and patient fitness, comorbidities, and toxicities that occur with treatment.

CLINICAL MANAGEMENT OF PATIENTS RECEIVING TIL CELL THERAPY: GUIDELINES AND BEST PRACTICES

Patient identification and selection

Considering the challenges of managing advanced disease and the multiple steps involved in TIL cell therapy, timely and efficient planning and operational execution are critical. Patient selection for TIL cell therapy is a collaborative effort by a multidisciplinary team including the surgeon, medical oncologist, and often a cellular therapy or hematopoietic stem cell transplant physician. Case discussion should be performed in the setting of a multidisciplinary tumor board.

At present, patients being considered for TIL cell therapy will have advanced disease that has progressed on prior lines of treatment including an antiprogrammed cell death protein 1 (anti-PD-1)-containing regimen, but they must be well enough to be able to wait for TIL manufacturing and scheduling of infusion, which may range from 22 to 60 days, and tolerate all components of the regimen, including the surgical procedure, non-myeloablative lymphodepletion (NMA-LD), cell infusion and IL-2. Patients must have at least one tumor lesion amenable to resection for TIL manufacturing and in clinical trials, an additional site of measurable disease has been required for response assessment. Efficacy in the setting of completely resected disease is unknown. Specific recommendations for surgical site selection have been described by Mullinax *et al.*²¹

Critical parameters to consider when determining patient eligibility include performance status, renal function, cardiac function, and pulmonary reserve. Some considerations regarding these are described below.

1. Performance status

- a. Tolerance of non-myeloablative lymphodepletion/IL-2 and organ reserve.
- b. In patients with rapidly progressive disease, the patient's condition immediately prior to starting non-myeloablative lymphodepletion will need to be anticipated.
- c. Bridging therapy between the time of surgery and the start of NMA-LD should be considered in patients with a high disease burden and in patients with rapidly progressive disease or organ impairment that may prevent the patient from receiving TIL cell therapy. The optimal bridging therapy is one that is patient-specific and disease-specific and does not have major associated adverse events (AEs), as patients need to be eligible for TIL cell therapy after bridging, with adequate hematopoietic, renal, and hepatic recovery; however, in practice, such bridging therapy oftentimes does not exist. Careful consideration should be given to the necessary time to recover and clear the selected bridging therapy so as to avoid delaying initiation of the TIL cell therapy regimen. If bridging therapy contains cardiotoxic agents, consider repeating echocardiography, and if it contains pulmonary toxic agents, pulmonary function test (PFT) should be repeated. Regimens

that require steroid administration should generally be avoided (discussed below).

2. Brain metastasis

- a. Patients with untreated brain metastasis should generally not be considered for TIL cell therapy outside of a clinical trial due to the risk of intracranial bleeding, as data regarding safety and degree of efficacy are evolving. All lesions highly suspicious for active brain metastases should be treated and demonstrate stability without neurological symptoms post-treatment when a patient is off steroids prior to consideration of TIL cell therapy (see the section on special populations).

3. Bowel metastases

- a. Patients with bowel metastases should be evaluated carefully for TIL therapy, as there is potentially an increased risk of bleeding and infection. However, given the lack of effective alternative therapies for these patients, risks and benefits should be weighed carefully and discussed with the patient.

4. Renal function: Includes multiple considerations

- a. Adequate renal function (glomerular filtration rate ≥ 40 mL/min) is imperative for tolerating the full regimen.
- b. IL-2
 - i. Renal toxicity secondary to IL-2: Includes both prerenal kidney injury related to third spacing of fluid and hypotension and intrinsic renal toxicity.
 - ii. Compromised renal function limits the ability to administer non-steroidal anti-inflammatory drugs (NSAIDs) which are an important premedication prior to IL-2 administration and other supportive agents with nephrotoxic risk.
 - iii. If there is evidence of any compromised renal function at baseline, consider avoiding/limiting NSAIDs to prevent worsening of renal function.
 - iv. IL-2 administration may have a cascade effect: IL-2 administration could lead to more injury leading to oliguria, which can worsen peripheral edema/effusions and other organ toxicity.
 - v. It is also important to note that IL-2 is cleared renally, so decrease in renal function will impair the ability to eliminate IL-2 and increase the duration of exposure to/toxicity of IL-2.
- c. Creatinine levels should be within normal limits and creatinine clearance (CrCl) should be >60 mL/min by the Cockcroft-Gault formula. Although allowed in clinical trials, the TIL cell therapy regimen should be used with caution in patients with CrCl of 40–60 mL/min and may need to be modified:
 - i. In patients with impaired renal function, fludarabine dose should be reduced (20 mg/m² in patients with CrCl 50–79 mL/min and 15 mg/m² in patients with CrCl 40–49 mL/min).

- ii. The threshold to discontinue IL-2 due to toxicity should be lowered.
 - iii. Nephrotoxic agents (including NSAID premedication for IL-2) should be avoided.
 - iv. Diuretics should be avoided for at least 24 hours after the last dose of IL-2.
 - d. We would not recommend this therapy in patients with CrCl<40 mL/min as it has not been studied in such patients and the risks may outweigh benefits.
5. Cardiac function
- a. Functional cardiac reserve is necessary, as the TIL cell therapy regimen, IL-2, associated fluid shifts, hypotension, and compensatory tachycardia place stress on the myocardium.
 - b. A left ventricular ejection fraction >45% on echocardiogram obtained during screening and New York Heart Association functional classification Class <1 are required.
 - c. Patients who have a history of ischemic heart disease, angina, or clinically significant atrial and/or ventricular arrhythmias must undergo a cardiac stress test.
 - d. A cardiologist should be consulted if there are any abnormalities and a risk/benefit discussion with the cardiologist is advised before pursuing TIL cell therapy.
6. Pulmonary reserve
- a. This is important given the pulmonary risks of the regimen, such as pleural effusions, volume overload, and pulmonary edema. In addition, patients often require supplemental oxygen during treatment.
 - b. Particular attention needs to be paid with certain malignancies, such as lung cancer, that are associated with smoking and associated comorbidities such as chronic obstructive pulmonary disease (COPD)/interstitial lung disease. Spirometry should be performed in these patients and diffusing lung capacity for carbon monoxide (DLCO) should be measured; patients with moderate to severe impairment, that is, DLCO<50% or <40%, respectively, may not be candidates for TIL cell therapy.
 - c. Patients should not require continuous oxygen supplementation prior to surgery.
 - d. Although not required for all patients, screening PFT is recommended for patients having any of the following:
 - i. History of cigarette smoking of ≥20 pack-years.
 - ii. Cessation of smoking within the past 2 years or still smoking.
 - iii. History of pneumonitis (including related to prior cancer treatment), COPD or asthma.
 - iv. Significant signs or symptoms of respiratory dysfunction, such as cough, wheezing, dyspnea, rales on auscultation, and abnormal chest X-ray.
 - v. History of pleural drainage within the past 3 months.
 - e. Postbronchodilator values: Forced expiratory volume in 1 s (FEV₁)/forced vital capacity>70% or FEV₁>50% of predicted normal is recommended.
 - i. If a patient is unable to perform reliable spirometry due to abnormal upper airway, a 6 min walk test may be used to assess pulmonary function. Patients who are unable to walk a distance of at least 80% predicted for age and sex or who demonstrate evidence of hypoxia at any point during the test (oxygen saturation<90%) should not receive TIL therapy due to the risk profile.
 - f. A pulmonologist should be consulted in patients with borderline pulmonary reserve.
 - g. Patients with FEV₁<1.5L may need chronic inhaled steroids and/or bronchodilators.
 - h. For patients with pleural effusions, consideration of drainage or placement of temporary in-dwelling catheter is reasonable.
- In addition to the considerations above, patients should have adequately recovered from any prior therapy toxicity (including from prior immunotherapy) and should not require immunosuppressive dose of systemic steroids (typically no more than 10 mg of daily prednisone or equivalent steroid for physiological replacement) or biological agents; we expect this recommendation may evolve as clinical experience increases. Additionally, patients should meet institutional hematological parameters for starting non-myeloablative lymphodepletion and should not have uncontrolled active infections. In accordance with TIL study protocols, patients should not receive live or attenuated vaccines within 28 days prior to beginning the NMA-LD or within 3 months after the last dose of IL-2 and until the patient's absolute neutrophil count (ANC) is ≥1000/mm³. In accordance with US Centers for Disease Control and Prevention (CDC) recommendations, patients vaccinated within a 14-day period before starting immunosuppressive therapy or while receiving immunosuppressive therapy should be considered unimmunized and should be revaccinated at least 3 months after therapy is discontinued provided that immune competence has been restored.²²
- Online supplemental table 2 details additional considerations for TIL cell therapy eligibility as well as screening assessments that must be conducted.

Surgery for tumor tissue procurement

Tumor tissue procurement surgery should ideally be planned within 2 weeks of patient identification to minimize the risk of significant disease progression prior to TIL administration. Choosing the optimal anatomic resection site is critical to reduce patient morbidity and potential contamination of the TIL infusion product and requires close communication and collaboration between surgeons and medical oncologists. Surgical considerations and best practices for tumor tissue procurement have been previously described by Mullinax *et al.*²¹

Surgical resection of a portion of the patient's tumor provides the starting material for TIL cell therapy and is carried out at the ATC. TIL infusion products have been successfully manufactured using diverse sites for tumor tissue procurement such as skin, lymph nodes, subcutaneous tissue, lung, liver, spleen, peritoneum, musculoskeletal sites, and breast.^{19 20} The current standards are that the operating surgeon resects approximately 1.5–4 cm diameter tumor tissue from a single lesion or an aggregate of smaller lesions, prosects (trims and fragments) the tumor tissue, taking care to remove necrotic and fatty tissue, and places it in sterile media for shipment to the manufacturing facility. Institutions should establish clear standard operating procedures for chain of custody and chain of identity from operating room to shipment and pathology review of the tumor sample if needed. The surgeon conducts postoperative follow-up, and the medical oncologist and/or cell therapy team typically coordinates the next steps in care.

Possible complications resulting from surgery for tumor tissue procurement depend on the surgical site and can include wound dehiscence, infections of the resected area, and anastomotic/staple line leaks, which could affect patient fitness for non-myeloablative lymphodepletion and thus TIL infusion.²³ An earlier study conducted at the Chaim Sheba Cancer Research Center in Israel reported 0% surgical mortality or major morbidity in patients receiving TIL cell therapy for metastatic melanoma; minor morbidity included only wound complications.²⁴ Grade 3/4 tumor tissue procurement AEs related to surgery were seen in only 3% of patients in the recent C-144-01 trial and included cellulitis and post-surgical site-related AEs (n=2 each) and nausea, abdominal pain, and hypoxia (n=1 each); no patient had surgery-related AEs that prevented TIL infusion.^{19 25} In the Netherlands Cancer Institute and National Center for Cancer Immune Therapy (NKI/CCIT) trial, AEs related to surgery were reported in 17% of patients, 63% of which were postsurgical wound infections requiring antibiotics (J. Haanen, MD, personal communication, September 27, 2023).

AEs related to the tumor tissue procurement surgery should be minimized and promptly managed by the surgical team. This starts with thoughtful selection of the tumor to be excised for TIL propagation. In general, a soft-tissue resection is favored over a visceral resection to minimize AEs. Regarding a required visceral resection, a minimally invasive approach is favored. It is preferable to avoid a bowel resection, but if required, small bowel resection is preferred over large bowel resection. In a patient with both lung and liver targets, a lung resection would generally be preferred. Prompt identification of surgical complications requires frequent communication with the patient. Early use of antibiotics in the setting of suspected postoperative infection is important. Deep abscesses should be drained percutaneously rather than via an open approach; a closed suction drain is placed when the abscess is substantial. Patients should be followed by the surgical team until resolution of significant tumor

tissue procurement-related AEs. AEs should be promptly communicated to the cellular therapy team so that any required scheduling adjustments may be undertaken. The patient who has experienced a significant tumor tissue procurement-related AE should ideally be approved in advance of lymphodepletion by the surgical team.

Non-myeloablative lymphodepletion

Non-myeloablative lymphodepletion prepares the tumor microenvironment by reducing the competition of the infused TIL for homeostatic cytokines (IL-7 and IL-15), and eliminating immunosuppressive cells, including regulatory T cells (Treg) cells and myeloid-derived suppressor cells, thus optimizing the milieu for activity of the infused TIL cells.^{26–29}

A dual or triple lumen large bore tunneled central venous catheter line 12–14.5 Fr or peripherally inserted central catheter (PICC) line is inserted at the time of hospital admission. This provides venous access for delivery of the non-myeloablative lymphodepletion regimen, infusion of TIL, IL-2 administration, and subsequent supportive care measures (antibiotics, transfusions, etc). The line may be kept in place until recovery.

The non-myeloablative lymphodepletion regimen conventionally used in TIL cell therapy clinical trials includes cyclophosphamide 60–120 mg/kg and fludarabine 75–125 mg/m². These doses are higher than those used in chimeric antigen receptor (CAR) T-cell therapy (cyclophosphamide 30 mg/kg and fludarabine 75 mg/m²) and generally lower than those in hematopoietic stem cell transplantation (cyclophosphamide 100–120 mg/kg and fludarabine 125–180 mg/m²).^{30 31} Our experience based on the C-144-01 trial for lifileucel and the NKI/CCIT trial currently supports the use of cyclophosphamide 60 mg/kg intravenous daily (×2 doses), followed by fludarabine 25 mg/m² intravenous daily (× 5 doses), although this may need to be adjusted for renal function and body mass index, as detailed in the renal toxicity section below.^{12 20} Exploration of lower dosing strategies is ongoing and may be integrated in the TIL regimen in the future.

The checklist in online supplemental table 3 can be used for planning prior to non-myeloablative lymphodepletion.

Management of non-myeloablative lymphodepletion toxicity Cytopenias

Cytopenias generally develop during and immediately after non-myeloablative lymphodepletion, with the lowest platelet counts occurring approximately 3–5 days after initiation of lymphodepletion, lowest lymphocyte counts on or around the day of TIL infusion (~7 days after initiation of lymphodepletion), and lowest neutrophil counts observed ~3–4 days after TIL infusion (~10–11 days after initiation of lymphodepletion). Platelet counts often recover by ~12–14 days, lymphocyte counts by ~4–7 days (which are mostly the infused TIL), and neutrophil counts by ~6–14 days after TIL infusion.^{6 32} In some patients,

cytopenias can persist for weeks and repeated packed red blood cell or platelet transfusions may be required even after hospital discharge.

Granulocyte colony-stimulating factor (G-CSF; filgrastim) or biosimilar to treat neutropenia can be safely initiated the day after TIL infusion and is strongly recommended to reduce the incidence of myelosuppression and infections and potentially shorten the duration of hospitalization. The recommended duration of G-CSF administration varies but should be continued until the ANC is at least $500/\text{mm}^3$. This is distinct from CAR T-cell therapy where G-CSF warrants cautious usage because of its association with cytokine release syndrome (CRS) severity.³³ TIL cell therapy is generally not associated with significant CRS. Patients typically receive filgrastim or biosimilar $5\mu\text{g}/\text{kg}/\text{day}$ subcutaneously daily starting from the day after TIL infusion until neutropenia is resolved per standard of care at the treating institution. Using daily complete blood counts as a guide, patients should receive platelets and packed red blood cells as needed. Hemoglobin levels should be maintained at $\geq 7.0\text{g}/\text{dL}$ and platelets $>30\,000/\text{mm}^3$ (unless patient is receiving anticoagulants) or per institutional standards for patients with comorbidities. Only irradiated blood products should be used for transfusion.

Antibiotic, antiviral and antifungal prophylaxis

Short-term and long-term antibiotic prophylaxis should be given to prevent opportunistic infections in the setting of drug-induced immunodeficiency. Antibacterial prophylaxis with levofloxacin or ciprofloxacin 500mg orally daily or equivalent should be started with the onset of neutropenia and continued until the ANC is $>500/\text{mm}^3$. To prevent pneumocystis infection, prophylaxis is commenced with chemotherapy, typically trimethoprim-sulfamethoxazole orally three times per week (or alternative). Antiviral prophylaxis should be commenced with chemotherapy, typically with acyclovir 400mg or valacyclovir 500mg orally (or alternative) two times per day.

Duration of antipneumocystis and antiviral prophylaxis may vary per standard of care at the treating institution. The TIL Working Group recommends continuing these for 6 months (at least 3 months) post-TIL infusion and/or until CD4 counts $>200\text{cells}/\text{mm}^3$; they can be stopped earlier if the absolute lymphocyte count (ALC) recovers to normal range. If ALC has not normalized by 3 months, then CD4 counts should be assessed, and prophylaxis continued if CD4 counts are $<200\text{cells}/\text{mm}^3$.

Fluconazole 400mg orally daily or another suitable fungal prophylaxis regimen as per standard of care at the treating institution should be started for antifungal prophylaxis on the day of TIL infusion and continued until ANC is $>1000/\text{mm}^3$.

Management of infections

In the setting of fever (temperatures $\geq 38.0^\circ\text{C}$), patients should be carefully screened for infections. It is important to note that while IL-2 often causes fever, broad-spectrum

antibiotics should be initiated for any neutropenic fever that occurs during the regimen. If fever is observed, urine and blood cultures should be performed; chest X-ray and sputum analysis are indicated in the event of pulmonary symptoms. During IL-2 administration, fever may be masked due to scheduled NSAIDs and acetaminophen. Therefore, in neutropenic patients exhibiting persistent hypotension or oliguria unresponsive to intravenous fluids, a high degree of suspicion for infection should be entertained and broad-spectrum antibiotics should also be considered. Fever during the time of bone marrow recovery is also common but should be a diagnosis of exclusion. Initiating or continuing administration of TIL or IL-2 to patients with neutropenic sepsis—or sepsis of any etiology—is not recommended.

Renal toxicity

To monitor cyclophosphamide-induced urinary and renal toxicity, urinary sediment should be checked regularly for the presence of erythrocytes and other signs of toxicity. Hydration with forced diuresis (as clinically warranted) and frequent bladder emptying can reduce the frequency and severity of bladder toxicity. To reduce the risk of hemorrhagic cystitis, in addition to intravenous fluids, mesna and/or furosemide may be administered as per institutional standards.

Gastrointestinal toxicity

Both cyclophosphamide and fludarabine can cause nausea, vomiting, anorexia, abdominal pain or discomfort, diarrhea, stomatitis, and hemorrhage. Steroid use is prohibited for prevention of gastrointestinal symptoms to avoid possible adverse effects on the infused TIL. A 5-HT₃ antagonist such as palonosetron, granisetron, or ondansetron or equivalent as per institutional standard should be given to treat nausea and additional non-steroidal antiemetics can be used as needed.

TIL infusion

Cryopreserved TIL infusion products require thawing according to manufacturer's specification prior to infusion. TIL infusion is often initiated approximately 24 hours after completion of non-myeloablative lymphodepletion. However, a period of a few days may be indicated to ensure that adequate recovery from lymphodepletion. The following assessments should be performed on the day of and prior to TIL infusion:

1. Full physical examination, including weight, vital signs, gastrointestinal, cardiovascular, extremities, head, eyes, ears, nose, and throat, respiratory system, dermatologic, musculoskeletal, neurologic, and psychiatric examinations.
2. Blood tests, including hematology, chemistry, and inflammatory markers such as C reactive protein and ferritin.
3. Additional tests should be guided by findings from physical exam and blood tests.

Table 1 Pre-TIL infusion checklist*

Cryopreserved TIL infusion product	▶ TIL product to be thawed per manufacturer's specification.
Fluid balance	▶ The goal is to ensure that the patient is at baseline weight prior to infusion.
Physical examination	▶ Weight. ▶ Gastrointestinal; cardiovascular; extremities; head; eyes, ears, nose, and throat; respiratory system; dermatologic; musculoskeletal; neurologic; and psychiatric examinations. ▶ Baseline ophthalmology exam in patients with a history of uveitis from prior therapy to ensure complete resolution.
Vital signs	▶ Pulse rate, O ₂ saturation, respiratory rate, blood pressure, and temperature.
Blood tests	▶ Hematology: CBC with differentials (WBC with differentials, RBC, hemoglobin, hematocrit, MCV, MCH, platelet count). ▶ Blood chemistry: Sodium, potassium, chloride, total carbon dioxide or bicarbonate, creatinine, glucose, BUN, albumin, calcium total, magnesium total, phosphorus, alkaline phosphatase, ALT/SGPT, AST/SGOT, total bilirubin, direct bilirubin, LDH, total protein, and uric acid. ▶ Inflammatory markers: CRP and ferritin.
Line placement	▶ Central venous catheter/PICC line.
Premedication	▶ Acetaminophen or equivalent. ▶ Diphenhydramine or other H ₁ -histamine antagonist.
Medications available at bedside during infusion	▶ Continued acetaminophen (650 mg q4h) or equivalent. ▶ Indomethacin (50–75 mg q6h) or equivalent. ▶ Pantoprazole (40 mg) or famotidine (20 mg) or equivalent. ▶ Meperidine (25–50 mg) and/or hydromorphone. ▶ Epinephrine and diphenhydramine. ▶ Other medications as per institutional standards.
Other concurrent medications	▶ All other concurrent medications to be stopped during TIL infusion.

*Pre-non-myeloablative lymphodepletion checklist is provided in online supplemental table 3.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CBC, complete blood count; CRP, C reactive protein; IV, intravenous; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; NSAID, non-steroidal anti-inflammatory drug; PICC, peripherally inserted central catheter; q4h, every 4 hours; q6h, every 6 hours; RBC, red blood cell count; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; TIL, tumor-infiltrating lymphocyte; WBC, white blood cell count.

The safety profile of the TIL cell therapy regimen in solid tumors is well characterized, with toxicity primarily associated with non-myeloablative lymphodepletion regimen and IL-2. Occurrence of on-target or off-target cell-mediated toxicity is rare.^{4 7 9 13 15 20} Premedication includes acetaminophen and diphenhydramine or other H₁-histamine antagonist. Prophylactic use of systemic corticosteroids is not allowed under any circumstances as there is concern that steroids could diminish the efficacy of TIL cell therapy; such medications should be used only to treat immediate life-threatening conditions. We generally recommend against hydration with TIL infusion. Further, no other medications are given concurrently during TIL infusion. [Table 1](#) details a checklist that can be used for planning prior to TIL infusion.

Management of TIL infusion toxicity

Infusion-related reactions with TIL have been reported in <4% of patients; appropriate emergency medications (eg, epinephrine and diphenhydramine) should be available at bedside during infusion, and institutional emergency guidelines should be followed as needed, noting that steroids should only be administered in life-threatening conditions if other interventions have failed. Vital sign monitoring is recommended every 30 min during infusion then hourly (± 15 min) for 4 hours, and routinely (every 4–6 hours) thereafter, unless otherwise

clinically indicated, for up to approximately 24 hours post-TIL infusion. Avoiding significant changes in volume status is critical, and diuresis is recommended as tolerated to get back to near euvolemia (eg, as close as possible to admission weight) prior to IL-2 infusion to minimize volume-related complications of capillary leak syndrome.

Autoimmune-like toxicity resulting in uveitis and vitiligo, although rare, has also been reported in melanoma,^{12 29} but this has not been an issue in other tumor types. In melanoma, any patient with a history of uveitis from prior therapy should have a baseline ophthalmology exam to ensure no active signs of uveitis before beginning TIL therapy.

It is important to note that toxicities typically associated with other cellular therapies, such as high-grade CRS and immune-effector cell-associated neurotoxicity syndrome (ICANS), are generally not observed with TIL cell therapy. The general absence of these toxicities is likely attributed to the naturally occurring, patient-specific T-cell receptors expressed in the TIL product, which have already undergone immune selection for self-tolerance. By contrast, CARs and T-cell receptor (TCR) T cells are engineered with high avidity and/or costimulation in construct, which increases rapid proliferation but also is more apt to cause pathological inflammatory cascade.

Whereas CRS from CAR T-cell and TCR therapy is typically managed with tocilizumab and ICANS is typically managed with steroids, AEs with TIL cell therapy (eg, significant/recurrent fevers, hypoxia, neurological impairment) are more likely to be explained by alternative etiologies such as IL-2 or infection, and thus, are managed differently. Tocilizumab is not typically used in TIL cell therapy AE management. High-dose steroids (>10 mg prednisone equivalent) have been hypothesized to diminish TIL antitumor activity and thus are generally avoided.³⁴ Exceptions exist in cases of life-threatening emergency or fluid-refractory hypotension for patients with known adrenal insufficiency. For further guidance, please see the section on adrenal insufficiency below. Given the significant differences in management of TIL cell therapy toxicity versus CAR T/TCR therapy, all team members should receive extensive training to ensure toxicity is recognized and managed appropriately. Toxicity management pertaining to each component of TIL cell therapy is discussed within each section. We recommend a ‘cheat sheet’ overview of toxicity management be provided to all team members, particularly staff who may be covering overnight and may be less familiar with this treatment regimen (online supplemental table 4). Uveitis after TIL infusion usually responds to topical corticosteroid treatment.

IL-2 administration

The abbreviated course of high-dose IL-2 administered as part of the TIL cell therapy regimen functions as a supportive treatment to enhance T-cell activity and development after TIL infusion and differs from IL-2 monotherapy given with therapeutic intent, which is administered at high doses in repeat cycles. Patients who receive IL-2 as part of the TIL cell therapy regimen differ from patients who receive IL-2 monotherapy, as they have received non-myeloablative lymphodepletion and are, therefore, cytopenic. Although we attempt to preserve euvolemia prior to IL-2 administration, patients can also experience considerable fluid weight gain from the necessary hydration associated with the non-myeloablative lymphodepletion regimen before receiving IL-2. We recommend diuresis in hemodynamically stable patients to try to achieve pretreatment body weight prior to beginning IL-2. Occasionally, prior to initiating IL-2, patients may experience significant toxicity from the non-myeloablative lymphodepletion regimen that affects their ability to safely receive IL-2. In these cases, it may be necessary to forgo IL-2 treatment. In a small, as-yet-unpublished study in patients deemed ineligible for IL-2 because of age, organ function, or other comorbidities (ClinicalTrials.gov NCT01468818), the ORR was 29.4% (5/17), suggesting that TIL may be effective in the absence of IL-2. Notably, we do not recommend TIL cell therapy for patients who are deemed in advance not to be candidates for IL-2, but it may need to be omitted if toxicity from the non-myeloablative lymphodepletion regimen makes the patient unfit for IL-2.

Antihypertensive medications should be discontinued 24 hours prior to IL-2 administration, and vital signs and urine output should be checked 2 hours prior to the first IL-2 dose so that abnormalities can be addressed. We recommend checking serum creatinine prior to beginning IL-2 and then two times per day during administration to monitor any changes in renal function. Avoiding intravenous contrast and other nephrotoxins is advisable to minimize renal insult.

Supportive therapy prior to IL-2 administration includes acetaminophen every 4–6 hours or equivalent, indomethacin every 6 hours or other NSAID equivalent (recommended but should not be used if baseline renal function is poor and should be discontinued with any sign of decreasing urine output, rising creatinine, and when platelets are $<50\,000 \times 10^9/L$), pantoprazole 40 mg PO/intravenous daily or famotidine 20 mg PO/intravenous two times per day (or equivalent), meperidine 25–50 mg intravenous every 4 hours as needed and/or hydromorphone for rigors, antiemetics, and other medications as per institutional protocols. Maintenance fluids are typically not needed for IL-2 and fluids should be administered cautiously during active IL-2 treatment. Urine output should be assessed prior to each dose; the goal should be to maintain a urine output of at least 0.5 mL/kg/hour. We recommend holding IL-2 if the urine output falls below 4 mL/kg over a period of 8 hours.

The first IL-2 administration in the TIL cell therapy regimen should begin approximately 3–24 hours after the completion of TIL infusion at a dose of 600 000 IU/kg intravenous every 8–12 hours typically up to a maximum of 6 doses. To facilitate optimal staffing, some centers initiate IL-2 dosing the morning after TIL infusion, rather than overnight. It may be helpful to schedule IL-2 infusions around standard vital times; however, administration should not be scheduled immediately preceding nursing shift changes.

It is important to note that no clear correlation has been observed between the total number of IL-2 doses administered and efficacy of TIL cell therapy when the IL-2 was discontinued for toxicity.³⁵ IL-2 can be held or discontinued at the discretion of the treating clinician at any time. Based on our collective clinical experience, we would be extremely cautious with rechallenging a patient with high dose IL-2 who has required a previous dose hold. If one dose is held, dosing can resume at the next scheduled dose if the patient has sufficiently recovered but should be done so with significant caution. If two consecutive doses are held, IL-2 should be permanently discontinued. As such, we strongly encourage holding or discontinuing IL-2 in the setting of toxicity that does not rapidly resolve with supportive medications. After IL-2 completion or discontinuation, IL-2-related medications including NSAIDs and meperidine should be stopped 12 hours later. Many patients will require supplemental oxygen during IL-2 administration due to capillary leak syndrome and fluid shifts resulting in pulmonary edema. The use of diuretics during the IL-2 period is a subject of

debate. Some clinicians use intravenous diuresis with furosemide as needed in between IL-2 doses, with frequent electrolyte monitoring and replacement. If diuresis causes acute renal failure, IL-2 should be stopped.

Management of IL-2 toxicity

AEs resulting from IL-2 are typically transient due to its short half-life (distribution and elimination half-life of ~13 and ~85 min, respectively³⁶). Renal filtration is the major route of clearance with a clearance rate of approximately 120 mL/min³⁶ and effects peak several hours after exposure. AEs can be challenging to manage if not identified and treated early and appropriately. General IL-2 toxicity management guidelines are summarized in table 2.

Table 3 includes relative and absolute criteria based on IL-2 toxicity signs and symptoms to help determine when to skip IL-2 doses.

IL-2 toxicity should be assessed prior to each dose of IL-2 to determine if dosing is appropriate. If ≥ 3 relative criteria for IL-2 toxicity are present, corrective measures should be initiated and IL-2 dose should be skipped; if signs of toxicity are not reversible, IL-2 should be discontinued (table 4). Doses are skipped or omitted, not reduced or delayed (missed doses cannot be made up and the dose of IL-2 should not be adjusted).

Common AEs related to IL-2 that can be difficult to manage include rigors, fever, hypotension, shortness of breath, pulmonary edema, oliguria, and neurotoxicity. Patients should be counseled on the likelihood of these AEs. To help mitigate these issues, AEs should be carefully monitored and managed early. Ensuring euvolemia, closely monitoring vital signs and cognitive function, and holding or discontinuing IL-2 if toxicity does not rapidly resolve with supportive measures are ways to mitigate IL-2-related AEs.

Recommended monitoring includes the following:

1. Vitals every 4 hours (every 2 hours if receiving pressors; pressors are not required, except in occasional circumstances because persistent hypotension after IL-2 is an indication to hold or discontinue IL-2).
2. Pulse oximetry every 4 hours (every 2 hours if on pressors); if saturation is $< 92\%$, oxygen is started, and chest X-ray performed.
3. Telemetry monitoring during therapy (from time of TIL infusion to at least 24 hours after last IL-2 dose).
4. Strict intake and output every 8 hours.
5. Daily weight monitoring.
6. Neurologic assessment every 8 hours.
7. Hematologic panel (complete blood count with differential count and platelets), complete metabolic panel, blood chemistry before each dose, serum creatinine.
8. ECG if persistent tachycardia for > 2 hours.

Fevers, chills, and rigors

As noted previously, fevers, chills, and rigors are common side effects of IL-2, and all patients should be counseled to expect this. Chills and rigors typically occur within

1–2 hours of each dose, and fever occurs 1–2 hours after chills and rigors. Chills and rigors alone are not indications to hold or discontinue IL-2. These symptoms can become severe and refractory and lead to other issues such as respiratory deterioration if not identified quickly and managed appropriately. Prophylactic acetaminophen and NSAIDs should reduce severity and escalation of these symptoms. Management should include parenteral opioids, such as meperidine or hydromorphone per institutional standards. All fevers should trigger neutropenic fever protocols including empiric antibiotics. For guidance with respect to patients with adrenal insufficiency, please refer to appropriate section on TIL cell therapy considerations in special populations.

Pulmonary toxicity

To assess pulmonary side effects, physical examination and auscultation should be performed to check for rales in lung bases; chest X-ray should be obtained to assess for pleural effusions or pulmonary edema. Oxygen saturation should be maintained at $\geq 92\%$. We typically initiate oxygen therapy supportively if oxygen saturation is $< 95\%$. If the patient has an oxygen requirement, we recommend diuresis (if blood pressure can be maintained). Persistent oxygen requirement ($< 92\%$ on room air) that has not resolved prior to the time the next dose is due is an indication to hold IL-2. After discontinuation of IL-2, it is common for fluid shifts to cause pulmonary edema and pleural effusions may cause oxygen requirement requiring intravenous diuresis for days.

Hypotension

Hypotension is less common with the abbreviated course of high-dose IL-2 used for TIL than with therapeutic IL-2; about 8%–10% developed grade 3 or higher hypotension in clinical studies.^{12 19} Blood pressure target is based on baseline blood pressure and is assessed prior to each dose. For patients with blood pressure not meeting target, administer small (250–500 mL) normal saline (NS) or lactated Ringer's (LR) bolus over 30–60 min. Repeat blood pressure 30 min after intravenous bolus, and if not meeting target, repeat another 250 mL intravenous bolus. If hypotension persists despite intravenous fluid boluses, IL-2 may be discontinued. Some centers with significant historical experience with high-dose IL-2 and/or TIL use pressors such as dopamine 2 $\mu\text{g}/\text{kg}/\text{min}$ or phenylephrine 0.1 $\mu\text{g}/\text{kg}/\text{min}$ (may be titrated up to obtain target blood pressure); when phenylephrine can be weaned to 0.5 $\mu\text{g}/\text{kg}/\text{min}$ or less, it is safe to proceed with additional IL-2 dosing. However, use of pressors is not mandatory as it has been seen that the number of administered IL-2 doses is not associated with clinical outcomes when IL-2 is discontinued due to toxicity and abbreviated IL-2 dosing with discontinuation driven by clinical tolerance is feasible.³⁵

Table 2 IL-2 toxicity management guidelines for TIL cell therapy

Toxicity	Management
Fevers/chills/rigors	<p>Fever above 100.5°C</p> <ul style="list-style-type: none"> ▶ Acetaminophen 650 mg PO q4h scheduled. ▶ Indomethacin 50–75 mg q6h or equivalent (stop if sCr>2mg/dL, decreased UOP, or platelets <50 000 x10⁹/L). ▶ Meperidine 25 mg with option to repeat another dose within 30 mins as needed for rigors (25–50 mg IV q4h PRN). ▶ Hydromorphone 0.5 mg IV every 15 min as needed for rigors, may repeat×3 total doses.* ▶ Preparation should be made beforehand, so intervention is possible in a timely fashion. ▶ Appropriate infectious workup and antibiotics as warranted.
Blood pressure	<p>Target blood pressure set on admission and assessed prior to each dose—assess ~2 hours prior to dose</p> <ul style="list-style-type: none"> ▶ If not meeting target, administer NS or LR 250–500 mL IV bolus over 30–60 min. ▶ Repeat blood pressure 30 min post IV bolus, if not meeting target, then may repeat another 250 mL IV bolus. If hypotension persists despite IV fluid bolus, we recommend IL-2 discontinuation; select centers with IL-2 experience use dopamine 2 µg/kg/min or initiate phenylephrine 0.1 µg/kg/min (may be titrated up to obtain target blood pressure); when phenylephrine can be weaned to 0.5 µg/kg/min or less, these centers reassess if it is safe to proceed with additional IL-2 dosing. In general, we recommend discontinuation of IL-2 in the setting of fluid-refractory hypotension.
Urine output	<p>To assess renal function, monitor serum creatinine prior to beginning IL-2 and then two times per day during administration urine output of at least 0.5 mL/kg/hour—assess two times per day, including about 2 hours prior to dose</p> <ul style="list-style-type: none"> ▶ If not meeting target, administer NS or LR 500 mL IV bolus over 30 min. ▶ Check urine output 1 hour post IV bolus, if <50–80 mL/hour, then may repeat another 500 mL IV bolus. Persistent low urine output despite IV fluid boluses, urine output <4 mL/kg over 8 hours, or serum creatinine 2.5–2.9 mg/dL are indications to hold IL-2. If persistent low creatinine clearance, we generally recommend discontinuation of IL-2. If creatinine clearance is persistently low, select centers with IL-2 experience initiate dopamine at renal perfusion doses of 2 µg/kg/min. If dopamine is initiated, urine output of 50 cc/hour must be established while off dopamine before additional IL-2 doses may be considered. NSAIDs and nephrotoxic agents should be withheld in the setting of renal injury.
Pulmonary	<ul style="list-style-type: none"> ▶ Physical exam with auscultation: check for rales in lung bases. ▶ Chest X-ray should be obtained to assess for pleural effusions or pulmonary edema. ▶ O₂ saturation should be maintained above 92%; may initiate oxygen therapy if O₂<95%. IL-2 doses should be permanently discontinued when patients require supplemental O₂ (<92% on room air) at timing of next dose (see table 3). If blood pressure can be maintained, diuresis can be tried to alleviate O₂ requirement.
Cardiovascular	<p>Sinus tachycardia >130 beats per min sustained for 1 hour</p> <ul style="list-style-type: none"> ▶ Assess fluid status and may administer NS or LR 500 mL IV fluid bolus. ▶ Assess telemetry/EKG for arrhythmias; continuously monitor and manage any new arrhythmias. ▶ Replete electrolytes. If arrhythmia or sustained tachycardia despite correction of reversible factors (hypotension, fever, dopamine), then may need to hold dose or stop IL-2 therapy
Gastrointestinal	<ul style="list-style-type: none"> ▶ Nausea/vomiting: scheduled ondansetron 8 mg IV q8h 30 min prior to each dose, prochlorperazine 10 mg IV q6h PRN, or lorazepam 0.5 mg IV q6h PRN. ▶ Diarrhea: PRN loperamide 2 mg every 2 hours as needed after ruling out gastrointestinal infection; diphenoxylate/atropine two tablets PO q6h PRN for diarrhea refractory to loperamide. ▶ Gastrointestinal prophylaxis: pantoprazole 40 mg PO/IV daily or famotidine 20 mg PO/IV two times per day (steroid use is prohibited for prevention of gastrointestinal symptoms). ▶ Transient cholestasis is reversible after discontinuation of IL-2 therapy.
Neurologic	<ul style="list-style-type: none"> ▶ IL-2 therapy should be withheld until the course of neurotoxicity can be established. ▶ Usually temporary. ▶ Anti-psychotic drugs may be required if there is progressive development of personality changes, hostility, confusion, disorientation, and hallucinations.
Dermatologic	<ul style="list-style-type: none"> ▶ Macular erythema, pruritus, desquamation. ▶ Itching: Diphenhydramine 25 mg PO q6h PRN or hydroxyzine 10 mg PO q6h PRN. ▶ Aveeno or Lubriderm (or equivalent) lotion TID. ▶ If rash is persistent/severe despite above measures, consider dermatology consult and use of topical steroid agents.
Endocrine	<ul style="list-style-type: none"> ▶ Hypothyroidism may need supplementation with levothyroxine if it persists after completion of therapy.
Infectious	<ul style="list-style-type: none"> ▶ 10%–30% incidence of staphylococcus bacterial infections. ▶ Prophylaxis as per institutional guidelines. ▶ Appropriate infectious workup and antibiotic coverage.
Edema/capillary leak	<ul style="list-style-type: none"> ▶ Closely monitor daily weights and rate of weight increase. ▶ Intravenous diuretics may be needed and are commonly administered >24 hours after completion of IL-2.

*Either meperidine or hydromorphone is given initially depending on institutional protocol, and if refractory to one, then the other is administered.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CTCAE, Common Terminology Criteria for Adverse Events; IL-2, interleukin-2; IL-2, interleukin-2; IV, intravenous; LFT, liver function test; LR, lactated Ringer's; NS, normal saline; PO, per orally (by mouth); PO, per oral (by mouth); PRN, pro re nata (take as needed); q4h, every 4 hours; q6h, every 6 hours; sCr, serum creatinine; sCr, serum creatinine; ULN, upper limit of normal; UOP, urinary output.

Table 3 Absolute and relative criteria by organ systems to skip or discontinue IL-2

System	Relative criteria	Absolute criteria
Cardiac	<ul style="list-style-type: none"> ▶ Sinus tachycardia (120–130 beats per min) 	<ul style="list-style-type: none"> ▶ Sustained (>1 hour) sinus tachycardia after correcting hypotension, fever, and tachycardia and stopping dopamine ▶ Development of clinically significant arrhythmia or cardiac event ▶ Hypotension refractory to fluid replacement
Gastrointestinal	<ul style="list-style-type: none"> ▶ Diarrhea 1000 mL/shift 	<ul style="list-style-type: none"> ▶ Diarrhea 1000 mL/shift×2 ▶ Grade 3 CTCAE hepatic impairment criteria: AST/ALT>5–20×ULN if baseline was normal; >5–20×baseline if baseline was abnormal; bilirubin >3–10×ULN if baseline was normal; >3–10×baseline if baseline was abnormal
Hemorrhagic		<ul style="list-style-type: none"> ▶ Frank blood in the sputum, emesis, or stool
Musculoskeletal	<ul style="list-style-type: none"> ▶ Extremity tightness 	<ul style="list-style-type: none"> ▶ Extremity paresthesias
Neurologic	<ul style="list-style-type: none"> ▶ Vivid dreams ▶ Emotional lability 	<ul style="list-style-type: none"> ▶ Hallucination, disorientation, or mental status changes not rapidly reversible
Pulmonary	<ul style="list-style-type: none"> ▶ New resting shortness of breath ▶ Rales 1/3 up chest 	<ul style="list-style-type: none"> ▶ New requirement of supplemental O₂ by nasal cannula for saturation ≥92% ▶ Endotracheal intubation ▶ Moist rales halfway up chest ▶ Pleural effusion requiring tap or chest tube
Renal	<ul style="list-style-type: none"> ▶ sCr increase by 50% from baseline or absolute sCr of 2.5–2.9 mg/dL ▶ CO₂<18 mEq/L 	<ul style="list-style-type: none"> ▶ Urine output <4 mL/kg over 8 hours ▶ sCr≥3 mg/dL ▶ sCr increase by 100% from baseline or absolute sCr≥3 mg/dL ▶ Persistent acidosis despite replacement

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTCAE, Common Terminology Criteria for Adverse Events; PRN, pro re nata (take as needed); sCr, serum creatinine; ULN, upper limit of normal.

Renal toxicity

To assess renal toxicity, monitor serum creatinine two times per day, urine output prior to each dose; urine output should be at least 0.5 mL/kg/hour. If urinary output targets are not met, NS or LR 500 mL intravenous bolus over 30 min can be administered. Urine output should be checked 1 hour after intravenous bolus; if it is <50–80 cc/hour, then another 500 mL intravenous bolus may be repeated. Persistent low urine output despite intravenous fluid boluses, urine output <4 mL/kg over 8 hours, or serum creatinine 2.5–2.9 mg/dL are indications to hold IL-2. Creatinine level (measured every 24 hours) should also be closely monitored and taken into consideration when deciding whether to hold IL-2; IL-2 administration is held if levels increase by 100%. For example, for patients with low muscle mass and low

baseline creatinine, an elevation from 0.5 mg/dL to 1 mg/dL may signal significant renal dysfunction and warrant at least holding a dose and we generally recommend discontinuation of IL-2. If CrCl is persistently low, select centers with previous IL-2 experience initiate dopamine at renal perfusion doses of 2 µg/kg/min. If dopamine is initiated, urine output of 50 cc/hour must be established while off dopamine before additional IL-2 doses may be considered. NSAIDs and nephrotoxic agents should be withheld in the setting of renal injury.

Neurological toxicity

Neurotoxicity due to IL-2 is not ICANS and should not be treated with steroids. ICANS has not been reported with TIL cell therapy. IL-2 therapy should be withheld until its course can be established, and we typically recommend

Table 4 When to skip or discontinue IL-2 based on absolute or relative criteria*

Observation category	Action
<3 relative criteria	Initiate corrective measure±skip IL-2
≥3 relative criteria	Initiate corrective measures, skip IL-2; Stop IL-2 if not reversible
Any absolute criteria	Initiate corrective measures, skip IL-2; Stop IL-2 if not reversible

*Absolute and relative criteria are detailed in Table 3.

discontinuing IL-2 permanently if there is evidence of IL-2-induced neurotoxicity. Neurotoxicity is typically temporary, but in the setting of renal injury, IL-2 may take longer to clear, and neurotoxicity may persist for longer duration. Progressive development of personality changes, hostility, confusion, disorientation, and hallucinations may require treatment with antipsychotic drugs. This can be exacerbated by hospital-induced delirium and psychoactive medications, and delirium should not be mistaken for ICANS.

General safety of the TIL cell therapy regimen

Most AEs with TIL cell therapy are transient in nature, though events can be severe and life-threatening, especially if not managed appropriately. Much of the toxicity is observed during the first 14 days of therapy when the patient is expected to be in the hospital, and the appearance of new grade 3 or 4 toxicity after that period is unusual. In the C-144-01 trial, all patients experienced cytopenias consistent with non-myeloablative lymphodepletion, and the most common grade 3/4 non-hematological treatment-emergent AEs were chills (75.0%), fever (51.9%), and febrile neutropenia (41.7%).^{11 19} In the phase 3 NKI/CCIT trial that compared TIL cell therapy with ipilimumab as first-line or second-line treatment in advanced melanoma, all patients in the TIL group had grade 3/4 neutropenia; the most common grade 3/4 AEs were febrile neutropenia (74%), hypophosphatemia (60%), and fever (45%).¹²

Discharge considerations

Patients may be discharged once ANC is >500 cells/ mm^3 or trending to >500 cells/ mm^3 in next 24 hours and patient is afebrile for 24 hours after stopping intravenous antibiotics and fluconazole (~7–10 days post TIL infusion). Platelet counts should be $>20\,000/\text{mm}^3$ independent of transfusion. G-CSF can be safely initiated the day after TIL infusion to reduce the incidence of myelosuppression and infections and shorten hospitalization duration. If oxygen was required during treatment, the patient should be diuresed and maintained as an inpatient until return to near baseline pulmonary status. Antibiotics are administered per institutional standards and if the patient is to maintain intravenous access, line care should be established. Patients must be able to safely perform activities of daily living and it should be feasible to manage any ongoing toxicity as an outpatient. Some patients will require hydration and/or transfusion support after discharge, so close follow-up and coordination of care are needed. It is recommended that patients remain in close proximity to the treatment center (30–50 miles or <1 hour) for 30 days after infusion, especially if they experience extensive cytopenias, as risk of post-IL-2 infection is higher compared with when high-dose IL-2 is administered in the immunocompetent population. Additionally, patients should be provided with all the necessary contact information for their care team, so they

are aware of who needs to be contacted in case of any complications/emergencies.

TIL cell therapy considerations in special populations

Adrenal insufficiency

Adrenal insufficiency or hypophysitis, an endocrine immune-related AE seen with anti-PD-1/cytotoxic T lymphocyte 4 (CTLA-4) therapy and anti-lymphocyte activation gene 3 (LAG3) treatment, may develop in about 0.5%–6% of patients treated with ICI monotherapy and 9%–11% treated with combination therapy.^{37 38} Patients with adrenal insufficiency have been and can be safely treated with non-myeloablative lymphodepletion, TIL, and IL-2. In some cases, patients may develop hypotension and require 2–3 times the usual maintenance fluids during lymphodepletion and/or IL-2 administration. Fluids can often be infused during lymphodepletion and tapered prior to TIL/IL-2 infusion. Endocrinology consult is recommended at admission. For patients with known adrenal insufficiency and/or prior hypophysitis, physiological replacement steroids (<10 mg prednisone equivalent) should be continued at home dosing throughout the regimen. In our collective experience, many patients with adrenal insufficiency will not require stress-dose steroids and hence, we do not recommend administering them prophylactically. However, we have a low threshold to administer stress-dose steroids if hypotension develops in patients with known adrenal insufficiency. In the setting of adrenal insufficiency and fluid-resistant hypotension, stress-dose steroids should be administered in consultation with an endocrinologist per institutional standards.

Brain metastases

Very limited data are available regarding the safety of TIL cell therapy in patients with untreated or actively growing brain metastases. The NCI group published a retrospective report on TIL cell therapy in patients with melanoma brain metastases that demonstrated preliminary efficacy of this approach,³⁹ but with no published toxicity rates. The only significant toxicity that was reported was a subarachnoid hemorrhage in one patient who was thrombocytopenic from the non-myeloablative lymphodepletion regimen. A clinical trial to evaluate the feasibility and safety of TIL treatment in patients with active brain metastases is ongoing (NCT05640193).

MRI of the brain should be obtained prior to starting the non-myeloablative lymphodepletion regimen. Based on current data, we recommend that any untreated brain metastasis be treated with surgery or radiation therapy prior to beginning the TIL cell therapy process outside of a clinical trial. Patients with definitively treated brain metastases must be stable for ≥ 14 days prior to beginning the non-myeloablative lymphodepletion regimen. It is recommended to exercise caution with hemorrhagic

brain metastases. Patients who require steroids for symptomatic brain metastases should not be considered for TIL cell therapy. Because only patients with treated brain metastases are referred/recommended for TIL cell therapy outside of a clinical trial, antiseizure prophylaxis is not required unless the patient has had a seizure in the past. Consultation with the neurosurgery team is recommended if complications arise.

OPERATIONALIZING TIL CELL THERAPY

The multistep TIL cell therapy process requires close coordination among multiple specialties and departments; thus, careful planning of surgery, admission, discharge, and follow-up supports a positive patient experience. Some of the aspects to consider when establishing a TIL cell therapy service line are detailed below (online supplemental figure 2).

Streamlining patient selection and support

Identification of patients who may be candidates for TIL cell therapy may be a challenge, and the treatment window in which patients may be eligible could be narrow. Therefore, streamlining the referral pathway to ATCs is critical. Patient engagement materials and education through patient advocacy can help enhance awareness of this therapeutic option and improve understanding of the TIL cell therapy process and treatment expectations. Further, increased awareness among physicians will aid oncologists in considering how best to integrate this treatment modality in practice and ensure timely referral. Housing and supportive care considerations for patients and caregivers should be addressed to enable patient proximity (recommended distance is <1 hour) to the ATC for 30 days after TIL infusion. Social work evaluation is important to address social and caregiver ramifications and help to minimize financial encumbrance.

Institutional capacity and infrastructure

One of the barriers to implementing TIL cell therapy is the capacity of the healthcare system to meet the demand in terms of resources. A state-of-the-art infrastructure is essential for all the steps in the treatment process, including scheduling logistics, patient referrals, surgery, TIL production, and infusion. Well-defined roles for the multidisciplinary teams, streamlined processes and optimal workflows, and clear communication among the TIL cell therapy team members are important for smooth treatment delivery. Institutions considering becoming ATCs will need to determine the number of patients who would be eligible for TIL cell therapy and their capacity based on hospital resources (eg, staffing, beds). Successful implementation will require staff training and education to provide adequate expertise; implementing infrastructure requirements, including appropriate staffing, appropriate storage and thawing conditions, inpatient beds in isolation rooms, monitored bed availability, and the availability of subspecialty consultants

to manage any complications of treatment. It will also be important to consider reimbursement strategies and overall cost-effectiveness based on the expected number of treated patients.⁴⁰ These challenges have been faced during implementation of CAR-T cell therapy and some critical success factors identified for successful implementation were collaboration among payers, manufacturers, and providers to streamline eligibility requirements and develop equitable reimbursement; better communication among providers and facility administrators within and across sites to evolve and optimize processes; and a commitment by manufacturers to generate robust and compelling health economic and outcomes research in support of these products.^{40 41} These lessons learnt from implementation of CAR-T cell therapy can be carried through to setting up processes for operationalizing TIL cell therapy.

Surgery and tumor resection

A consensus on preferred tumor resection sites and best practices to acquire the desired tumor tissue based on tumor type should be established. Different surgical specialists may be required for tumor resection according to anatomic location, so standardized workflows are critical to ensure that the process is reproducible and new team members can be easily integrated. To mitigate the risk of tumor tissue contamination, any equipment or instrument that comes into contact with the tissue during and after resection needs to be sterile, including instruments used for prosection and transfer media for transport. Once the tumor tissue is resected and prosected by the surgeon, additional logistics should be considered, as streamlining and standardizing workflow is critical to successful tissue procurement and TIL manufacturing. The portion of the tumor tissue designated for TIL manufacturing should be placed directly in the sterile medium and sent to the cell manufacturing facility via prearranged courier. Transport media must be prepared fresh on the day of tumor procurement using aseptic techniques in the operating room.

Shipping logistics

Aseptic handling is required not only through tumor tissue procurement and placement into transport media, but through the packaging process as well. The two supply chains involved in TIL cell therapy, that is, transport of the tumor tissue from the ATC to the manufacturer and of the TIL infusion product from the manufacturer back to the ATC are complex and require careful handling, chain of custody, and accurate record management. Precise scheduling is crucial, as it can impact manufacturing and patient infusion. After resection, the tumor specimen container should be maintained at 2°C–8°C in a refrigerator until arrival of the prearranged courier for pick-up. Once manufactured, the final TIL infusion product requires cryopreservation during transportation from the manufacturer back to the ATC. Couriers will need to have contingency measures in place for unexpected delays

such as transportation holdups and will need to ensure that cryogenic temperatures are maintained precisely during transport. The TIL infusion product should be kept frozen throughout any handling prior to preparation for thawing and infusion. A strong collaboration among manufacturers, couriers, the receiving laboratory, and clinical teams is essential to manage logistics efficiently.

Nursing support

A nurse navigator who functions as a TIL cell therapy coordinator should be involved in the patient's treatment journey from the initial discussion with the patient about the therapy, treatment process, and effects of the treatment. Clear guidelines are needed to determine which nursing staff will service TIL cell therapy (solid tumor staff or hematology staff who may already have experience and training for CAR T-cell therapies) and who will perform coordination of care during and after the regimen is administered. Other operational considerations include education programs and training for staff and creating educational sheets and seminars for staff in relation to dosing, safety mitigation strategies, emergencies, and care escalation.

Pharmacy support

Pharmacy provides clinical pharmacist support during treatment. A clinical pharmacist's responsibilities throughout this process may include patient evaluation; order set creation for non-myeloablative lymphodepletion; TIL product preparation and infusion; order set creation for IL-2 administration and management of expected symptoms; comprehensive patient, caregiver, and staff education; transitions of care; and pharmacovigilance and monitoring. Receipt, storage, and thawing of the TIL product can also be performed by appropriately trained pharmacies; in some facilities, this may be performed in a cell therapy laboratory.

Cell therapy laboratory

In some centers, cell therapy laboratories may be responsible for TIL manufacturing. In addition, these laboratories support the processing and storage of tumor tissue; process development; lot release testing and quality control; and preparation for infusion including thawing and delivery to the bedside. Laboratory staff have extensive GMP-compliant manufacturing, quality management, and regulatory experience to ensure compliance.

Manufacturing

The individualized nature of TIL cell therapy involving generation of autologous products is a complex process that includes tumor tissue procurement surgery, prosecution, transportation to a manufacturing facility, TIL product manufacturing (expansion and reinvigoration), and transportation of the finished product back to the ATC where TIL cell therapy is administered to the patient. To overcome the traditional lengthy, cumbersome manufacturing processes that employed open-culture systems, improvements to manufacturing have

focused on decreasing production time by enhancing TIL expansion capacity *in vitro*, minimizing the number of manufacturing steps performed in an open system, and incorporating a more closed system to minimize contamination, as well as exploring techniques to identify tumor-specific TIL in the original tumor sample to maximize expansion of this subset of lymphocytes.⁴² Production failures, contamination, long manufacturing turnaround times, and difficulties with transportation of the product are some of the challenges that must be considered. With manufacturing advancements, manufacturing success rates of 90%–98.8% have been reported.^{7 12 14 19 43 44} Automation, standardization of processes, and environmental control are required to reduce contamination and ensure optimal TIL yield in a timely manner. The potency of TIL drug product is assayed using a matrix approach that provides a comprehensive picture of the potency and identity by the selection of key complementary functional and phenotypic cell attributes. Functional attributes include the quantitation of IFN- γ secretion in response to T-cell stimulation by antibody coated beads and in coculture with a target cell line. Development of elements of the matrix was informed by the generally understood mechanism of action of TIL, extensive experience with the product and its characterization methods, evaluation of potential Critical Quality Attributes, and years of manufacturing and clinical experience for use in metastatic melanoma.

Data/electronic medical record management

The FDA currently requires manufacturers of CAR T-cell therapies to monitor patients for safety for up to 15 years.⁴⁵ Unmodified TIL cell therapy does not require the same monitoring since it is not genetically engineered. In case of similar monitoring requirements for future genetically modified TIL cell therapies, ATCs must consider data collection for long-term follow-up including defining the entities responsible for creating data platforms, ensuring accuracy of data collection, logistical challenges of long-term tracking, and funding requirements. Ordering tools and documentation for TIL cell therapy-specific assessments will need to be established. Syncing electronic medical records for patient medical history and TIL cell therapy information across multiple teams throughout the patient journey will be crucial right from patient selection to management of AEs.

FUTURE DIRECTIONS IN TIL CELL THERAPY AND PRODUCT MANUFACTURING

While the most extensive experience with TIL cell therapy is in the setting of non-*uveal* melanoma, this treatment modality is also being investigated in other solid tumor types such as non-small cell lung cancer,^{13 14} cervical cancer,¹⁵ head and neck squamous cell carcinoma,^{16 17} breast cancer,¹⁸ *uveal* melanoma,⁴⁶ and colon cancer.⁴⁷ Distinct comorbidities and organ compromise with other tumors warrant consideration during TIL cell therapy and should include, at the very least,

a consultation from a physician who has expertise with that tumor type.

In the case of lung cancer, the associated distinct pulmonary and cardiac comorbidities, as well as older age and smoking, must be considered for surgery and treatment. Minimally invasive surgical techniques such as video-assisted thoracoscopic surgery wedge resection can be used for lung tumor tissue procurement. Holding or discontinuing IL-2 may need to be considered earlier due to low pulmonary/cardiac reserve. We continue to refine the use of TIL cell therapy in relation to melanoma and other solid tumors.

TIL cell therapy manufacturing protocols have advanced in recent years to increase TIL yield and quality, making TIL cell therapy a potentially more viable treatment option for larger numbers of patients with different tumor types. Strategies to further enhance clinical and safety outcomes across various indications are being investigated, including optimizing the dosing of non-myeloablative lymphodepletion, TIL modification strategies such as neoantigen selection and gene editing, and novel IL-2 analogs. Novel areas for continued innovation in TIL cell therapy are emerging regularly.

CONCLUSIONS

While the advancement of TIL cell therapy and related outcomes provides a promising outlook for patients in need of new options, unique challenges related to patient care and management will need to be addressed leveraging the knowledge and experience of teams such as the TIL Working Group. Educating physicians on administration of the regimen and managing toxicities is crucial to ensure that patients derive optimum benefit from the therapy. The best practices detailed here are intended to provide a framework for the complex issues involved in the management of patients receiving TIL cell therapy and may be of use to oncologists, intensivists, nurses, and other stakeholders involved in managing these patients. As with any novel therapy, these guidelines will be revisited as the field evolves.

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