Supplement to:

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

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Supplementary Table 1. Discharge considerations

Parameters	Discharge criteria
ANC	>500 cells/mm^3 or trending to >500 cells/mm^3 in next 24 hours
Temperature	Afebrile for 24 hours after stopping IV antibiotics and fluconazole (~7–10 days post TIL infusion)
Pulmonary status	Return to baseline or near baseline prior to treatment after diuresis

ANC, absolute neutrophil count; IV, intravenous.

Supplementary Table 2. Patient selection considerations and pre-operative assessments

System	Eligible	Contraindications*	Assessments
General	• Unresectable or metastatic disease • Age ≥18 years • ECOG PS 0 or 1 • Estimated life expectancy ≥3 months	• Generally avoid in cases of rapidly progressive disease • History of hypersensitivity to any component or excipient of the regimen • Chronic steroid therapy >10 mg of prednisone (or equivalent) for any reason	• Height (only at screening), weight • Examination of extremities, head, eyes, ears, nose, and throat, dermatological, musculoskeletal, and psychiatric (mental status) • BMI and BSA • Temperature • CT scan of chest, abdomen, pelvis, and additional anatomic regions (eg, extremities, neck) per disease history and clinical symptoms • High-resolution CT with PO/IV contrast or contrast-enhanced MRI for assessing radiographic tumor response†
Cardio-vascular	• LVEF >45% or NYHA Class <1 • Patients ≥ 60 years of age with history of ischemic heart disease, angina, or clinically significant atrial and/or ventricular arrhythmias must have a cardiac stress test • Cardiology consult in patients with abnormal cardiovascular function		• Cardiovascular examination, pulse rate, blood pressure
Pulmo-nary	• FEV_1 >50% of predicted normal or FEV_1/FVC >70% of normal • Not requiring continuous O_2 supplementation at BL • Screening PFT for: – Cigarette smoking ≥20 pack-years – Ceased smoking within past 2 y or still smoking – Significant respiratory dysfunction – History of COPD or asthma – Pleural drainage within the last 3 mo	• Documented FEV_1 ≤60% • 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 6-minute walk test (SpO_2 <90%) • Patients with moderate to severe lung impairment (DLCO <50% and <40%, respectively)	• Respiratory system examination • Respiratory rate • Pulse oximetry during IL-2 administration
Central nervous system	• No brain metastases on MRI imaging • Patients with definitively treated brain metastases must be stable by MRI imaging for ≥14 days prior to beginning the non-myeloablative lymphodepletion preconditioning regimen	• Untreated brain metastases	• Neurological examination MRI of the brain if positive for central nervous system involvement at screening or baseline, or as clinically indicated
Gastro-intestinal	• SGPT and SGOT ≤3 times ULN (≤ 5 times ULN in patients with liver metastasis) • Total bilirubin ≤2 mg/dL (≤3 mg/dL for patients with Gilbert syndrome) • Patients with acute/chronic hepatitis infections may be enrolled if the viral load by PCR is undetectable with/without active treatment		• Gastrointestinal (abdomen, liver) examination • Serum alkaline phosphatase, SGPT, SGOT, total bilirubin, direct bilirubin, LDH, total protein
Renal	• Estimated creatinine clearance >60 mL/min using the Cockcroft-Gault formula • Estimated creatinine clearance ≥40-60 mL/min using the Cockcroft-Gault formula with dose reduction/avoidance of nephrotoxic agents	• Creatinine clearance <40 mL/min	• BUN, creatinine, sodium, potassium, chloride, total CO_2 or bicarbonate

Hemato-logic	• ANC ≥1000/mm^3 • Hemoglobin ≥8.0 g/dL • Platelets ≥100,000/mm^3	• Patients requiring transfusions or growth factor support to meet parameters should undergo investigation to assess etiology and reversible causes should be addressed prior to consideration of TIL therapy regimen.	• CBC with differentials, when available: WBC count with differentials (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), RBC count, hemoglobin, hematocrit, MCV, MCH, platelet count • INR and PT or INR and aPTT
Immune-related	• No active autoimmune or checkpoint inhibitor induced immune-related adverse events requiring >10 mg of prednisone (or prednisone equivalent)	• Active uveitis that requires treatment	• If history of uveitis, need eye exam to rule out active uveitis requiring treatment)
Infections		• Active systemic infections requiring systemic antibiotics	• Human immunodeficiency virus (HIV-1 and HIV-2) antibody titer • HbsAg, anti-HBc, HCV-Ab • Syphilis (RPR or VDRL) • HSV-1, and HSV-2 IgM serology or PCR assay • CMV antibody titer, including IgM or PCR assay • EBV panel, including IgM or PCR assay
Genito-urinary	• Patients of childbearing potential or their partners willing to take precautions to avoid pregnancy during treatment and for 12 months after receiving the treatment regimen	• Patients who are pregnant or breastfeeding	
Endocrine	• Patients with immunotherapy-related endocrinopathies stable for at least 6 weeks (eg, hypothyroidism), and controlled with hormonal replacement (non-corticosteroids) • Adrenal insufficiency requiring 10 mg of prednisone (or equivalent) or less		• Blood glucose • TSH and free T_4
Others		• Coagulation disorders • Major illnesses of the immune system • Seropositive for HIV-1 or -2 antibodies; HbsAg, anti-HBc, or HCV Ab; syphilis; CMV and EBV; HSV-1 and -2 • Primary immunodeficiency, such as SCID and AIDS • Received a live or attenuated vaccine within 28 days of beginning the non-myeloablative lymphodepleting preconditioning regimen	• Serum albumin, calcium total, magnesium total, phosphorus, total creatine kinase, and uric acid
Tumor resection criteria	• At least 1 resectable lesion (or aggregate of lesions resected) of a minimum 1.5 cm and less than 4 cm in diameter post-resection to generate TIL • Surgical tissue removal possible with minimal morbidity (defined as any procedure for which expected hospitalization is ≤3 days)	• ≥ Grade 2 hemorrhage within 14 days prior to enrollment	

*Some of these contraindications are under active investigation and may be modified pending trial results.

†If a patient has a known allergy to CT contrast material, alternate modality to be used. In cases where contrast is strictly contraindicated, a non-contrast scan will suffice.

ANC, absolute neutrophil count; aPTT, activated partial thromboplastin time; BL, baseline; BMI, body mass index; BUN, blood urea nitrogen; BSA, body surface area; CBC, complete blood count; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CT, computed tomography; DLCO, diffusing lung capacity for carbon monoxide; EBV, Epstein-Barr virus; ECOG PS, Eastern Cooperative Oncology Group performance status; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HBc, hepatitis B core antigen; HbsAg, hepatitis B surface antigen; HCV Ab, hepatitis C antibody; HSV, herpes simplex virus; ICI, immune checkpoint inhibitor;IL-2, interleukin-2; INR, international normalized ratio; IV, intravenous; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MRI, magnetic resonance imaging; NYHA, New York Heart Association; PCR, polymerase chain reaction; PFT, pulmonary function test; PO, per oral (by mouth); PT, prothrombin time; RBC, red blood cell; RECIST, Response Evaluation Criteria in Solid Tumors; RPR, rapid plasma reagin; SCID, severe combined immunodeficiency; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; SpO_2, oxygen saturation; T_4, thyroxine; TIL, tumor-infiltrating lymphocyte; TSH, thyroid-stimulating hormone; ULN, upper limit of normal; VDRL, Venereal Disease Research Laboratory; WBC, white blood cell.

Supplement Table 3. Pre-non-myeloablative lymphodepletion checklist

Verification of TIL production	• Verification of sufficient TIL production from the manufacturer prior to beginning non-myeloablative lymphodepletion
Physical examination	• Weight • ECOG PS evaluation • Gastrointestinal; cardiovascular; extremities; head; eyes, ears, nose, and throat; respiratory system; dermatological; musculoskeletal; neurological; and psychiatric examinations
Vital signs	• Pulse rate, pulse oximetry, respiratory rate, blood pressure, and temperature
Blood and urine tests	• Seronegative for HSV IgM or PCR assay • Hematology: CBC with differentials, WBC with differentials, RBC counts, 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, total creatine kinase, and uric acid • Serum pregnancy test for patients of childbearing potential
Antibiotic and antiviral prophylaxis	• Antibiotic prophylaxis: Levofloxacin/ciprofloxacin 500 mg orally daily or equivalent, until ANC >500mm^3 • Pneumocystis prophylaxis: Trimethoprim-sulfamethoxazole 1 single/double strength tablet orally three times per week (or alternative) started along with chemotherapy • Antiviral: Acyclovir 400 mg or valacyclovir 500 mg BID orally (or alternative) started along with chemotherapy
CVC/PICC line	• Dual or triple lumen large bore tunneled CVC/PICC line

ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; BID, twice daily; BUN, blood urea nitrogen; CBC, complete blood count; CVC, central venous catheter; ECOG PS, Eastern Cooperative Oncology Group performance status; HSV, herpes simplex virus; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; PCR, polymerase chain reaction; PICC, peripherally inserted central catheter; RBC, red blood cell; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; TIL, tumor-infiltrating lymphocyte; WBC, white blood cell.

Supplementary Table 4. "Cheat sheet" for management of TIL regimen and potential complications

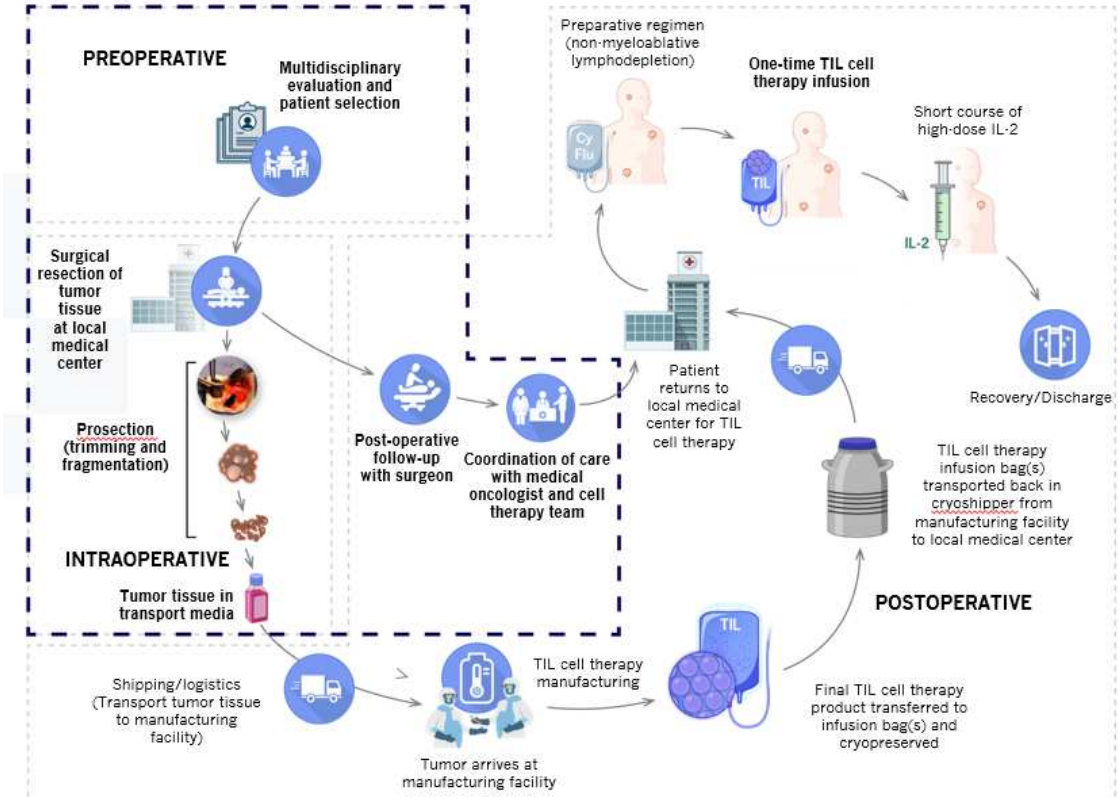
	IL-2 infusion		TIL Cell therapy infusion	Non-myeloablative lymphodepletion
THERAPY PREP	Supportive therapy prior to IL-2 administration: - **Acetaminophen** every 4–6 hours - **Indomethacin** OR other **NSAID** - **Pantoprazole** 40 mg PO/IV daily or **famotidine** 20mg PO/IV BID (or equivalent) - **Antiemetics** **Antihypertensive** medications should be discontinued 24 hours prior to IL-2 administration If a dose is held, can cautiously consider dosing at the next scheduled dose if the patient has recovered fully - If 2 consecutive doses are held, discontinue IL-2 permanently Once IL-2 is completed/discontinued, **NSAIDs**, **meperidine**, and other related medications should be stopped 12 hours later		Premedication: - Acetaminophen or equivalent - Diphenhydramine or another H1-histamine antagonist **Corticosteroids** should be used only to treat **immediate life-threatening conditions** - Steroids could diminish the efficacy of TIL cell therapy Cryopreserved TIL infusion products require thawing according to the manufacturer's specification prior to infusion TIL infusion should be initiated approximately 24 hours after completion of non-myeloablative lymphodepletion	Patients receive a preparative **non-myeloablative lymphodepletion regimen** that typically lasts for 5 - 7 days, which may be administered in the **inpatient** or **outpatient** setting at the discretion of the treating physician **Premedication: Palonosetron, ondansetron,** or **granisetron** or equivalent **CVC/PICC line** in place
MONITORING FOR AEs	**Recommended Monitoring:** - **Vitals** every 4 hours - **Pulse oximetry** every 8 hours - **Telemetry monitoring** - Strict **intakes** and **outputs** every 8 hours - **Neurologic** assessment every 8 h - H**ematologic panel**, **complete metabolic panel**, **blood chemistry** before each dose, **serum creatinine** - **Electrocardiogram** if persistent tachycardia - Daily weight monitoring	**Fevers/Chills/Rigors:** - These symptoms can become severe and refractory, leading to other issues such as **respiratory depression** **Pulmonary:** - **Pleural effusions** and **pulmonary edema** **Blood Pressure/ Hypotension**: - Assess prior to each dose **Renal Toxicity**: - Monitor **sCr** BID and **urine output** prior to each dose **Neurologic**: - Neurotoxicity is typically temporary - Progressive development of personality changes, hostility, confusion, disorientation, and hallucinations may require treatment with anti-psychotic drugs	**Vital Sign Monitoring**: - Every **30 min during infusion** then **hourly (± 15 minutes) for 4 hours**, and **routinely (every 4–6 hours)** thereafter for up to ~24 hours post-TIL infusion (unless otherwise clinically indicated) - Toxicities during the treatment period are typically due to non-myeloablative lymphodepletion and systemic IL-2 infusion - TIL cell therapy is associated with a **low rate** of on- and off-target cell-mediated toxicity - **CRS** and **ICANS** are not commonly observed with TIL cell therapy	**Cytopenias:** - Develop **during** and **immediately after** non-myeloablative lymphodepletion - **Platelet** counts recover by 12–14 days - **Lymphocyte** counts recover by 4–7 days - **Neutrophil** counts recover by 6–14 days **Opportunistic Infections**: - Short- and long-term **antibiotic**, **antiviral**, and **anti-fungal** prophylaxis should be initiated **Fever**: - **Temperatures ≥38.0°C** should be screened for infection **Cyclophosphamide-induced urinary and renal toxicity**: - Urinary sediment should be checked regularly for **erythrocytes** or other signs of toxicity **GI toxicity**: - **Steroid use** is **prohibited** for GI symptoms - Nausea, vomiting, diarrhea, abdominal pain, and stomatitis are common adverse events
MANAGEME	**Fevers/Chills/Rigors**: - **Acetaminophen** 650 mg by mouth every 4 hours - **Indomethacin** 50–75 mg every 6 hours or equivalent - **Meperidine** 25mg with option to repeat another dose within 30 mins as needed for rigors (25–50mg IV q4h PRN,) - **Hydromorphone** 0.5 mg IV every 15 min as needed for rigors, may repeat × 3 total doses[a] **Blood Pressure**:		**Infusion-related Reactions:** - Appropriate emergency medications (e.g., **epinephrine** and **diphenhydramine**) should be available at bedside during infusion - **Steroids** should only be administered **in life-threatening conditions** **Uveitis (melanoma)**:	**Neutropenia:** - **Filgrastim** or biosimilar **5 µg/kg/day** subcutaneously daily (starting from Day 1) until neutropenia is resolved **Thrombocytopenia & Anemia:** - Hemoglobin levels should be maintained **>7.0 g/dL** - Platelet levels should be maintained **>30,000/mm³** - Administer **platelets** and **packed red blood cells** as needed

NOTES	- If not meeting target, administer **NS** or **LR** 250–500 mL IV bolus over 30–60 min (repeat 250mL IV bolus if needed) **Urine Output:** - Target urine output at least 0.5 mL/kg/h - If not meeting target, administer **NS** or **LR** 500 mL IV bolus over 30 minutes (repeat 500 mL IV bolus if <50-80 mL/h) - If urine output is persistently low, stop IL2 and/or select centers consider **dopamine** at 2 µg/kg/min **Pulmonary**: - Initiate oxygen therapy if O_2 <95% and hold/discontinue IL-2 if O_2 remains below 92% at time for next dose of IL-2 - IV diuretics as needed **Cardiovascular**: - Sinus tachycardia >130 beats/min sustained for 1 h during IL2 - Assess telemetry/EKG for arrhythmias - Replace electrolytes as needed **Gastrointestinal**: - **Nausea/vomiting**: **ondansetron** 8 mg IV every 8 h 30 min prior to each dose - **Prochlorperazine** 10 g IV every 6 hours as needed, or **lorazepam** 0.5 mg IV every 6 hours as needed - **Loperamide** 2 mg every 2 hours as needed for diarrhea - **Diphenoxylate/atropine** 2 tablets by mouth every 6 hours as needed for diarrhea refractory to **loperamide** - **GI Prophylaxis**: **Pantoprazole** 40 mg by mouth/IV daily or **famotidine** 20 mg PO/IV BID or equivalent - Steroid use is prohibited for prevention of gastrointestinal symptoms **Dermatologic**: - **Diphenhydramine** 25 mg PO every 6 h or **hydroxyzine** 10 mg PO every 6 h for itching **Endocrine**: - Hypothyroidism may need to be supplemented with **levothyroxine** **Hematologic**: - **Anemia** – transfusion if Hgb ≤7 g/dL - **Thrombocytopenia** – transfusion if platelets <10,000 mm^3 **Infections:** - **Treat with appropriate antibiotic coverage as per institutional standards** **Edema/Capillary leak:** - IV diuretics as needed	- **Topical corticosteroid** treatment (e.g., **betamethasone**, **dexamethasone**, **prednisolone**, etc.) - **To Prevent Capillary Leak from IL2:** - **Diuresis** is recommended, as needed and tolerated, to bring a patient back to near **euvolemia**	**Antibiotic Prophylaxis**: - **Levofloxacin or ciprofloxacin** 500 mg orally daily or equivalent can be considered until **ANC is >500/mm^3** - **TMP/SMX** 1 single/DS tablet orally 3x/week to prevent **pneumocystis** infection **Antiviral Prophylaxis**: - **Acyclovir 400 mg or valacyclovir 500 mg** orally BID or equivalent administered with chemotherapy **Anti-fungal Prophylaxis**: - **Fluconazole 400 mg** orally daily on day of TIL infusion and continue until **ANC >1000 mm^3** **Fever:** - Broad-spectrum antibiotics for any **neutropenic fever**, **persistent hypotension**, or **oliguria** **Cyclophosphamide-induced urinary and renal toxicity:** - **Hydration with forced diuresis** - **Mesna** administered with cyclophosphamide depending upon dose of cyclophosphamide as per institutional standards to reduce risk of **hemorrhagic cystitis**

[a]Either meperidine or hydromorphone is given initially depending on institutional protocol, and if refractory, then the other is administered.

AE, adverse event; ANC, absolute neutrophil count; BID, twice daily; CRS, cytokine release syndrome; CVC, central venous catheter; GI, gastrointestinal; Hgb, hemoglobin; ICANS, immune-effector cell-associated neurotoxicity syndrome; IL-2; interleukin 2; IV, intravenous; LR, lactated Ringer's; NS, normal saline; NSAID, nonsteroidal anti-inflammatory drug; PICC, peripherally inserted central catheter; PO, per oral (by mouth); PRN, pro re nata (take as needed), sCr, serum creatinine; TMP/SMX, trimethoprim/sulfamethoxazole.

Supplementary Figure 1. Steps in TIL cell therapy and patient journey



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IL-2, interleukin 2; TIL, tumor-infiltrating lymphocyte.

Supplementary Figure 2. Operational considerations for TIL cell therapy

IL-2, interleukin 2; TIL, tumor-infiltrating lymphocyte.