Revitalizing Tumor Infiltrating Lymphocytes and IL-2 in Melanoma

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- I do not have any relevant financial relationships or disclosures

Learning Objectives

1. Discuss the mechanism of action of tumor infiltrating lymphocytes (TILs) and the role of aldesleukin (IL-2) in the treatment of melanoma.
2. Review the clinical evidence supporting the use of TILs and IL-2 in the melanoma treatment paradigm.
3. Identify challenges and considerations in implementing TIL-based therapy in clinical practice focusing on patient selection and adverse event management.
4. Describe supportive care strategies to optimize patient outcomes for TIL-based therapy.

Tumor Infiltrating Lymphocytes (TILs)

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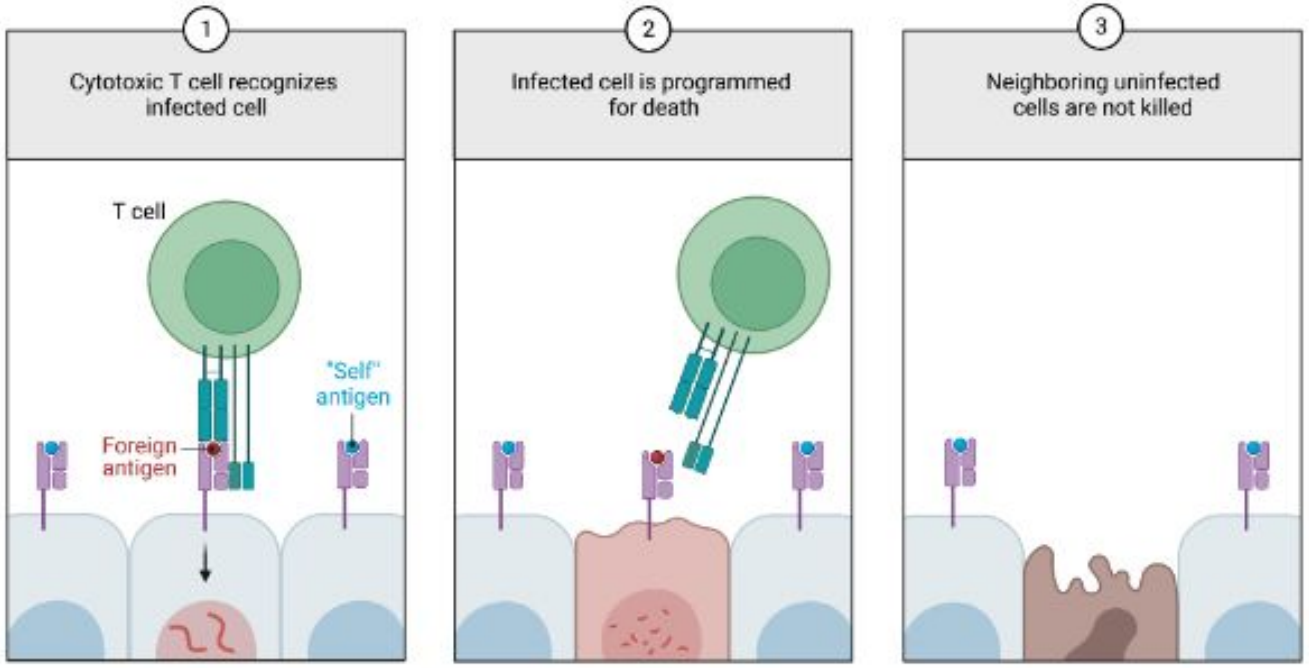
Immune Surveillance

- The immune system identifies and destroys foreign or abnormal cells
  - **Innate immunity (myeloid):** Nonspecific first line of defense
  - **Adaptive immunity (lymphoid):** Specific response that adapts to stimuli

- T-Cell Activation:



- Cytotoxic T-cells release perforins (cause leaky pores) and granzymes (break down proteins) to cause cell death
- Foreign antigen = tumor-specific antigen

Adapted from "Selective Antigen Recognition by Cytotoxic T Cells", by BioRender.com (2025). Retrieved from https://app.biorender.com/biorender-templates

Monjazeb AM, et al. *Front Oncol*. 2013; 3: 197.

History of Immunotherapy

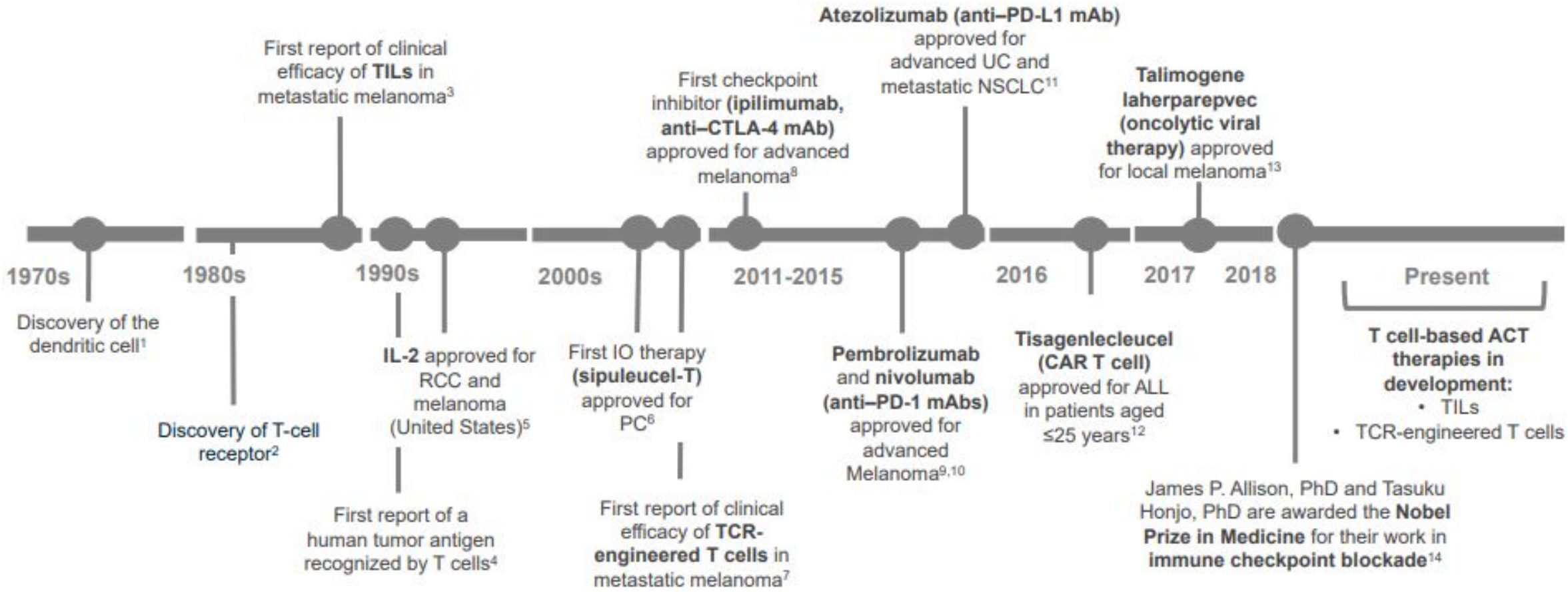
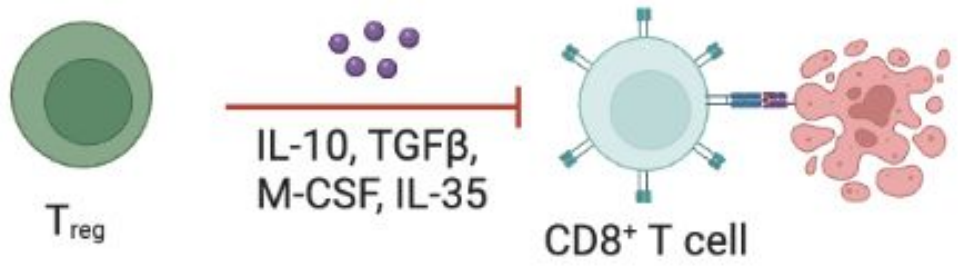


Image available at: tilworkinggroup.com

Role of Immune Escape

- Tumors have developed mechanisms for escape from cytotoxic T-cells:
  - Loss of antigen
  - Reduction in major histocompatibility complex (MHC)
  - Increase in regulatory T-cells suppress cytotoxic and helper T-cells
  - Upregulation of immune checkpoint molecules

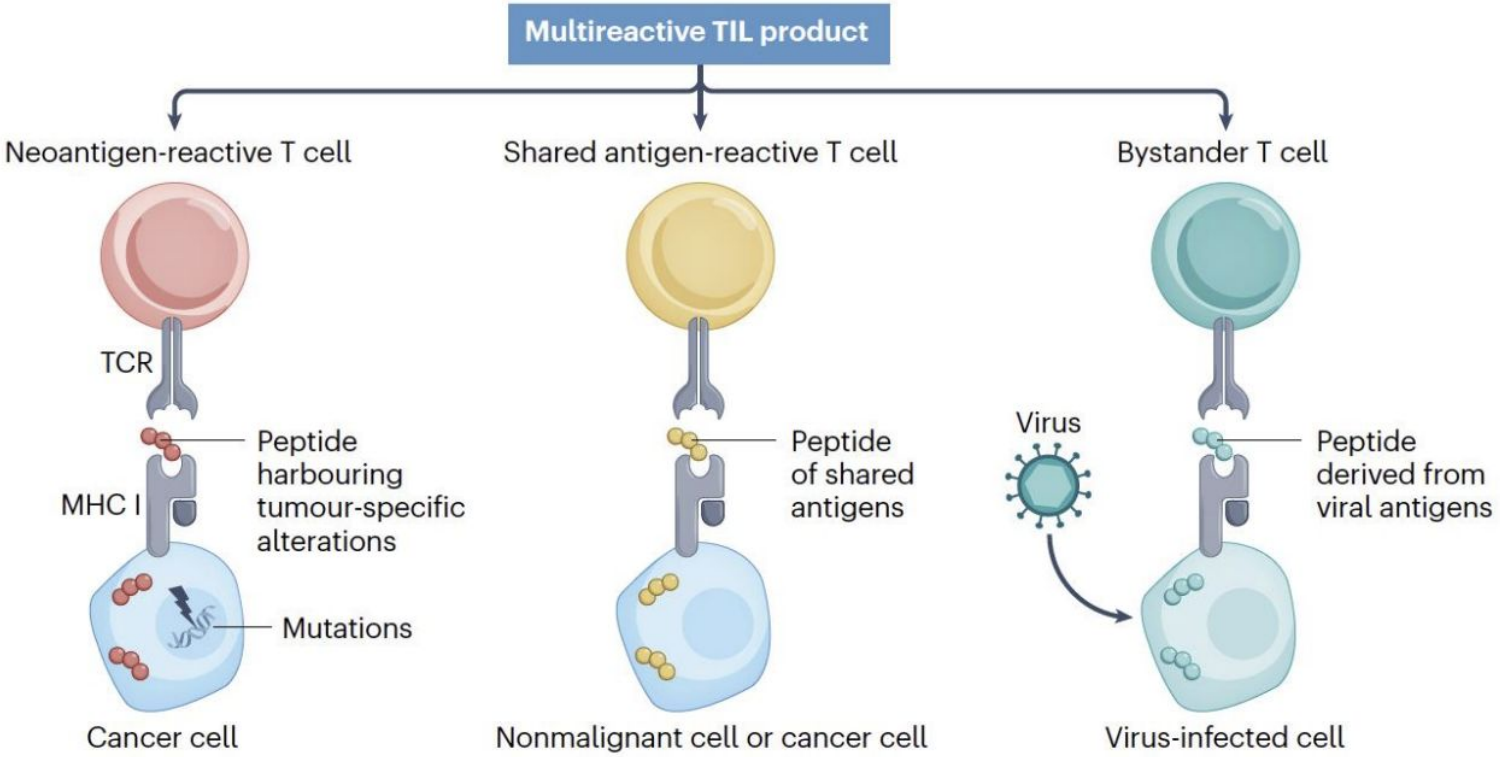


Adapted from "The Tumor Microenvironment: Overview of Cancer-Associated Changes", by BioRender.com (2025). Retrieved from https://app.biorender.com/biorender-templates

Monjazeb AM, et al. *Front Oncol*. 2013; 3: 197.

TILs

- Tumor infiltrating lymphocytes (TILs) = activated cytotoxic T-cells that have migrated to the tumor microenvironment



- Polyclonal population consisting of CD4+ and CD8+ T-cells
- Directed at multiple antigens including:
  - Neoantigen-reactive T-cells
  - Shared antigen-reactive T-cells
  - Bystander T-cells

Klobuch et al., Nat Rev Clin Oncol 2023

TIL Manufacturing

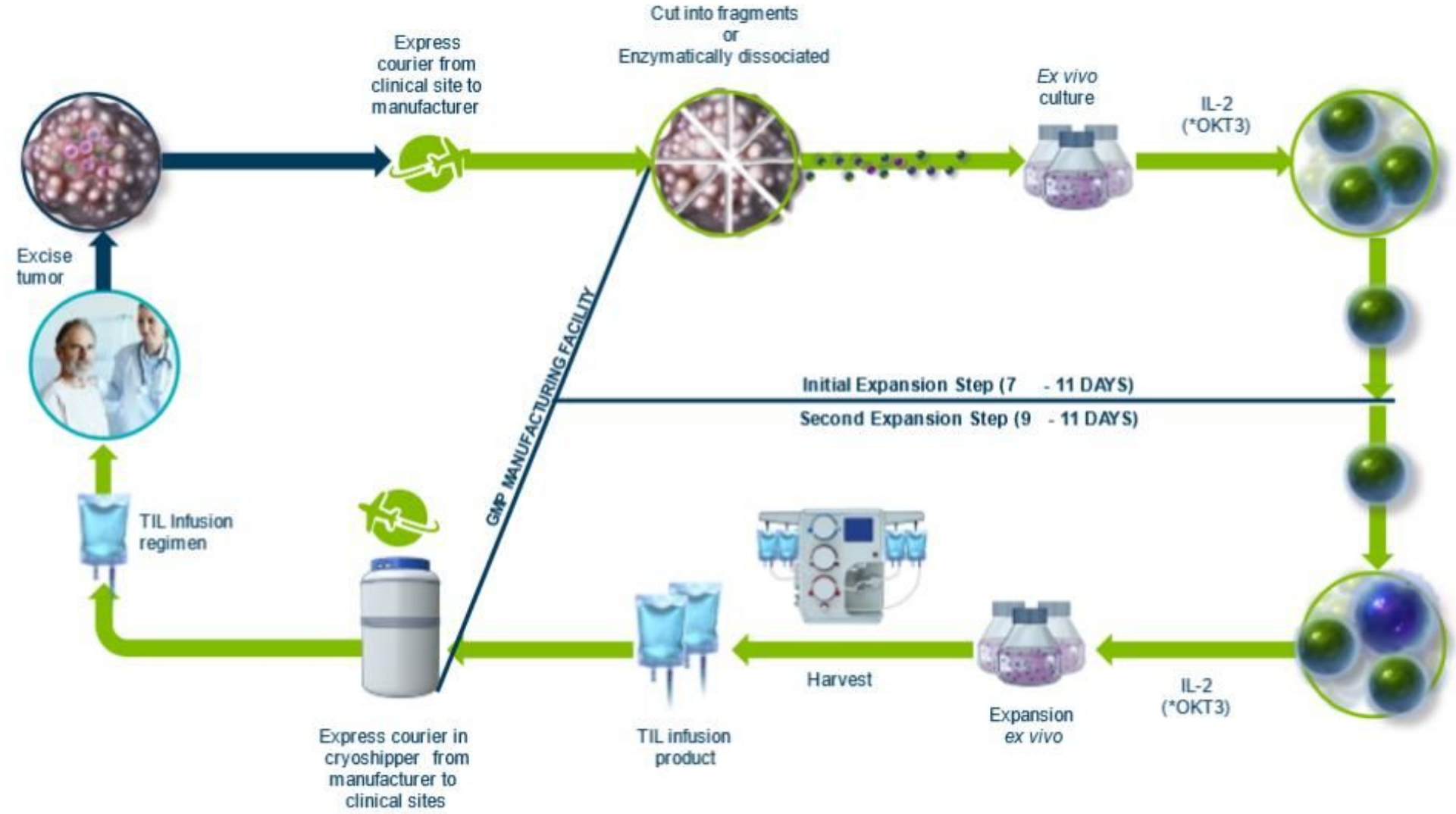
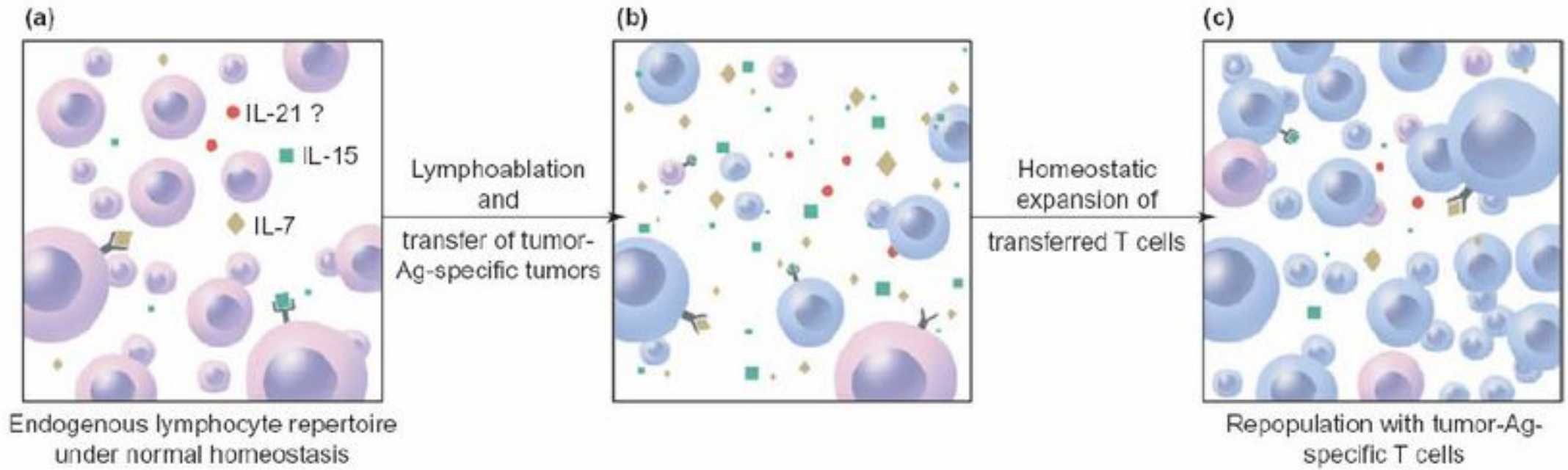


Image available at: https://www.sec.gov/Archives/edgar/data/1425205/000155837022001976/iova-20211231x10k.html

Role of Lymphodepletion

- Eliminates CD4+CD25+ regulatory T-cells
  - Enhances CD8+ T-cell activity and function
    -
- Removes endogenous lymphocytes to create a sink for IL-7, IL-15, and IL-21 to augment cytotoxic T-cell activity



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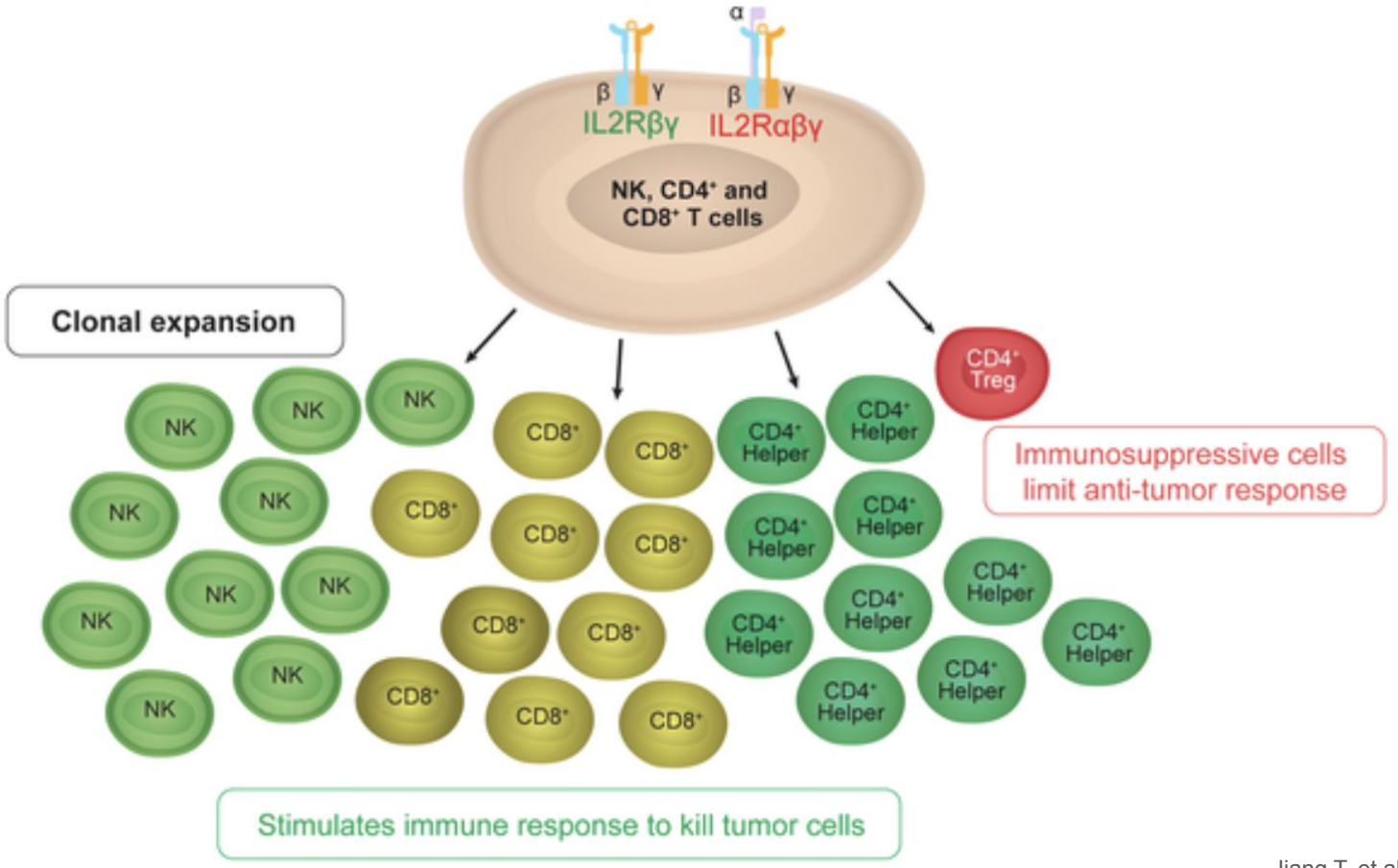
Gattinoni L, et al. *J Exp Med.* 2005; 202(7): 907-12.
Klebanoff C, et al. *Trends Immunol.* 2005; 26(2): 111-7.

Role of IL-2

- IL-2 helps with proliferation and expansion of activated cytotoxic T-cells (TILs) in vivo



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Jiang T, et al. *OncoImmunology*. 2016; 5(6): e1163462.
Charych D, et al. *PLoS ONE*. 2017; 12(7): e0179431.

Overall TIL Logistics

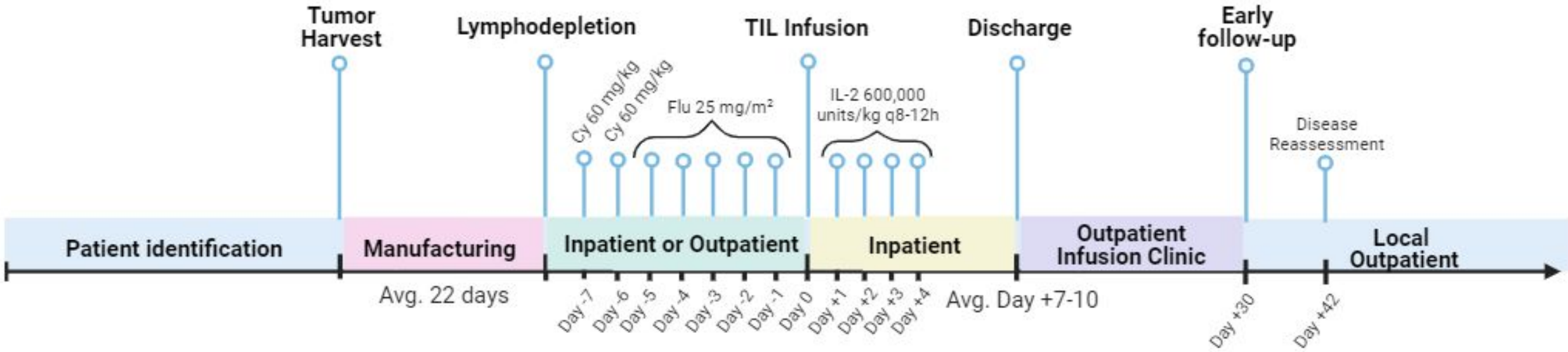


Image created by Biorender

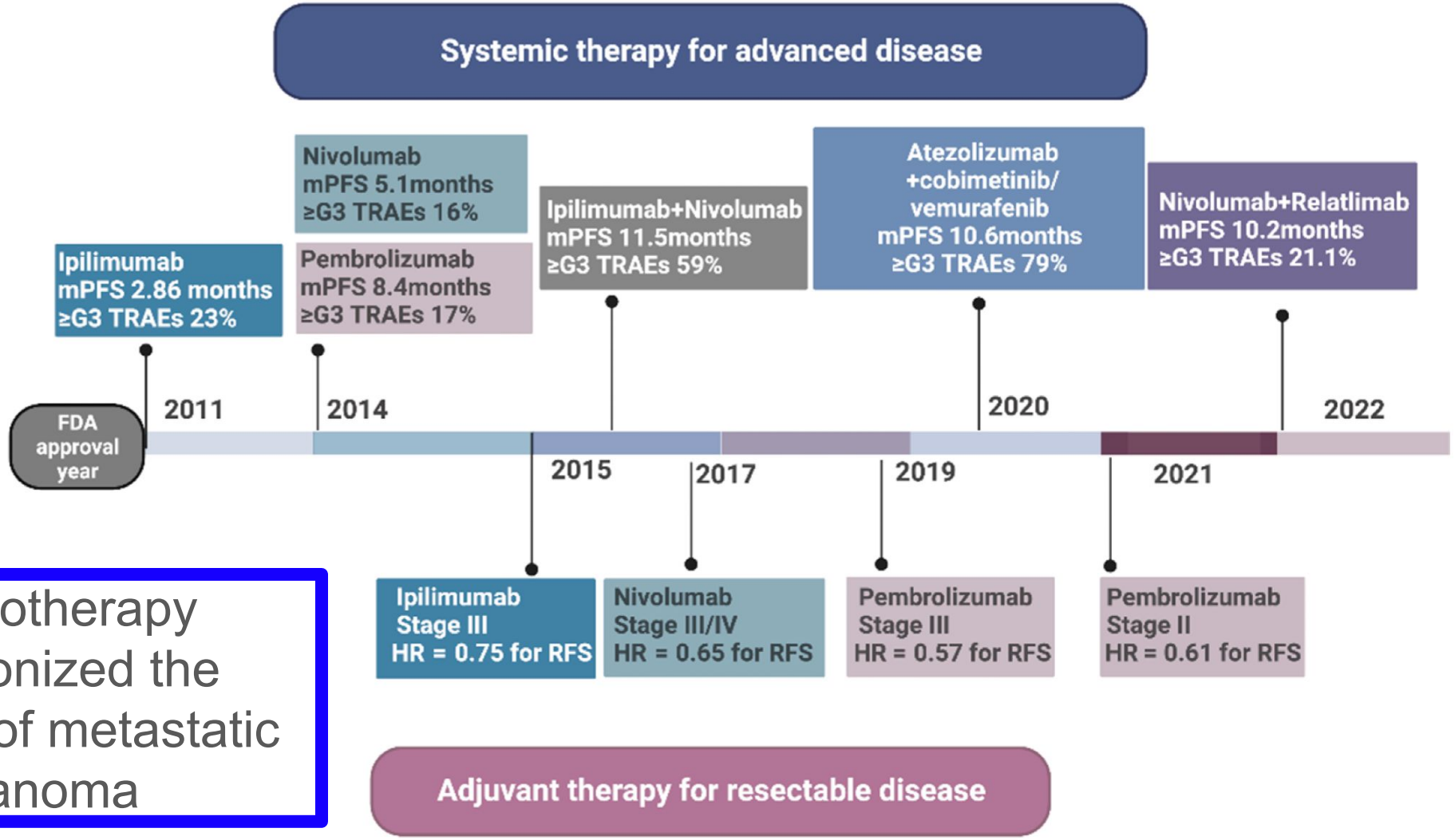
Landmark Studies for TILs in Melanoma

Melanoma: Background

- Estimated 104,960 new cases in 2025
  - 5th most common cancer in the United States
  - Incidence of cases rising by 1.2% each year
- 5-year relative survival for localized = 100%
- 5-year relative survival for metastatic = 35%
- Median age = 66 years
  - More common in non-Hispanic white males with fair complexion
  - Risk factors include prolonged or excessive exposure to artificial and natural sunlight, genetic mutations (BRAF V600E)
- Most common metastatic sites of disease:
  - Skin, lungs, liver, central nervous system

SEER database. Melanoma of the Skin. Accessed on May 12, 2025.
Sundararajan S, et al. *Stats Pearl Publishing LLC.* 2024. PMID: 29262232.
Image available at: https://miiskin.com/melanoma/symptoms-signs/

Melanoma: Treatment Landscape



Immunotherapy revolutionized the treatment of metastatic melanoma

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Knight A, et al. *Cancers*. 2023; 15(4): 1106.

Melanoma: Prognosis

- Prior to immunotherapy, median survival = 6-9 months
- Combination immunotherapy = approximately 6 years

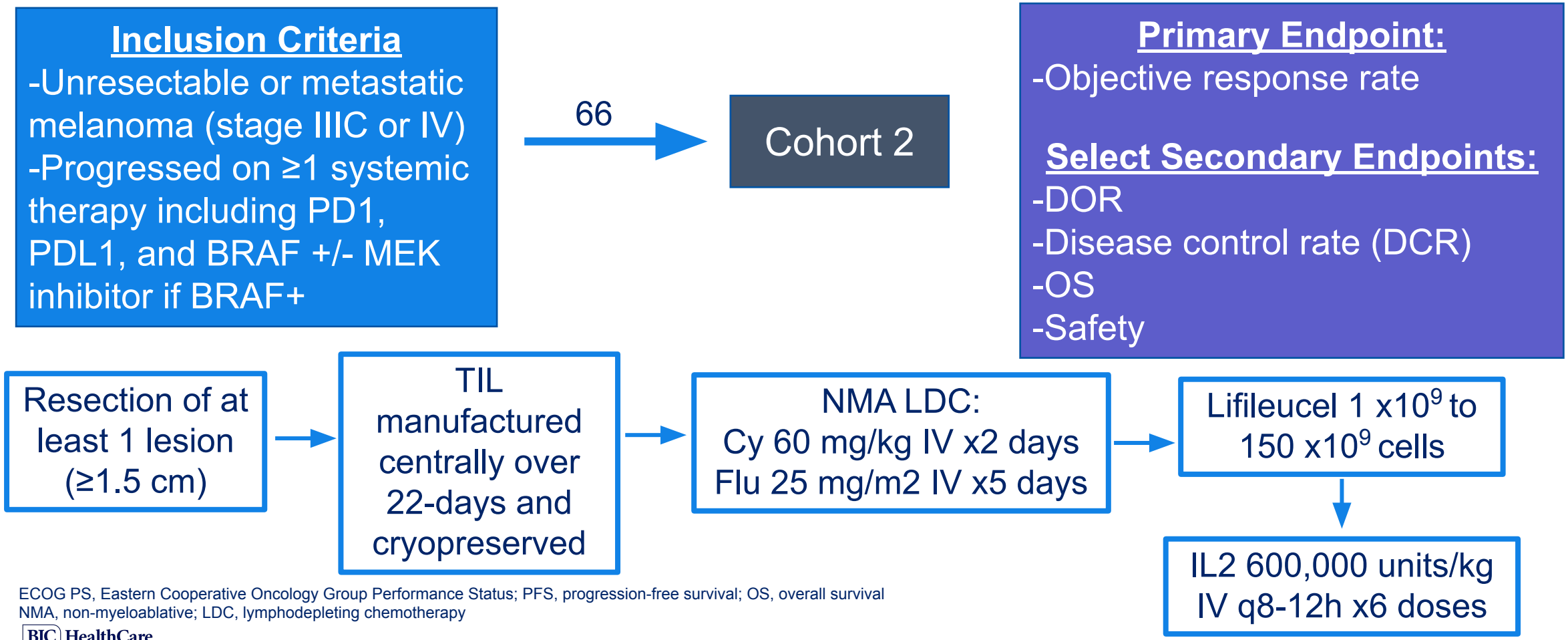
- 40-65% of patients have minimal or no response to immunotherapy
- Of immunotherapy responders, 30-40% develop resistance after 3 years
- 36% of patients discontinued immunotherapy due to toxicity
- 15-20% of BRAF+ patients do not respond to targeted therapy

- Limited chemotherapy response = 4-10%
- Median overall survival = 7 months

Knight A, et al. *Cancer (Basel)*. 2023; 15(4): 1106.
Gide T, et al. *Clin Cancer Res*. 2018; 24(6): 1260-1270.
Sarnaik A, et al. *J Clin Oncol*. 2021; 39: 2656-2666.

Phase II Study: Sarnaik, et al.

Design: Prospective, phase II, multicenter, open-label, single-arm



ECOG PS, Eastern Cooperative Oncology Group Performance Status; PFS, progression-free survival; OS, overall survival
NMA, non-myeloablative; LDC, lymphodepleting chemotherapy

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Sarnaik A, et al. *J Clin Oncol.* 2021; 39: 2656-2666.

Demographics: Sarnaik, et al.

Demographic	Cohort 2
Median age	55 yrs (20-79)
Stage IV	86%
Mean number of prior therapies	3.3
PD1 or PDL1	100%
CTLA4	80%
PD1 + CTLA4	52%
BRAF +/- MEK	88%
Primary refractory to prior PD1 or PDL1	64%
Progression to prior PD1 or PDL1	99%
Progression to prior CTLA4	77%
Mutated BRAF V600	26%

Treatment	Cohort 2
Median number of TIL cells	27.3 x10^9
Median number of IL-2 doses	5.5 (1-6)

- High tumor burden at time of treatment
- 42% of patients with baseline liver ± brain metastases

Sarnaik A, et al. *J Clin Oncol.* 2021; 39: 2656-2666.

Efficacy: Sarnaik, et al.

- Median follow-up = 18.7 months
- Median overall survival = 17.4 months
- Responses observed across all sub-groups
- 81% of patients had a reduction in tumor burden
- Time to best response = 1.4 months
- 25% of responding patients progressed

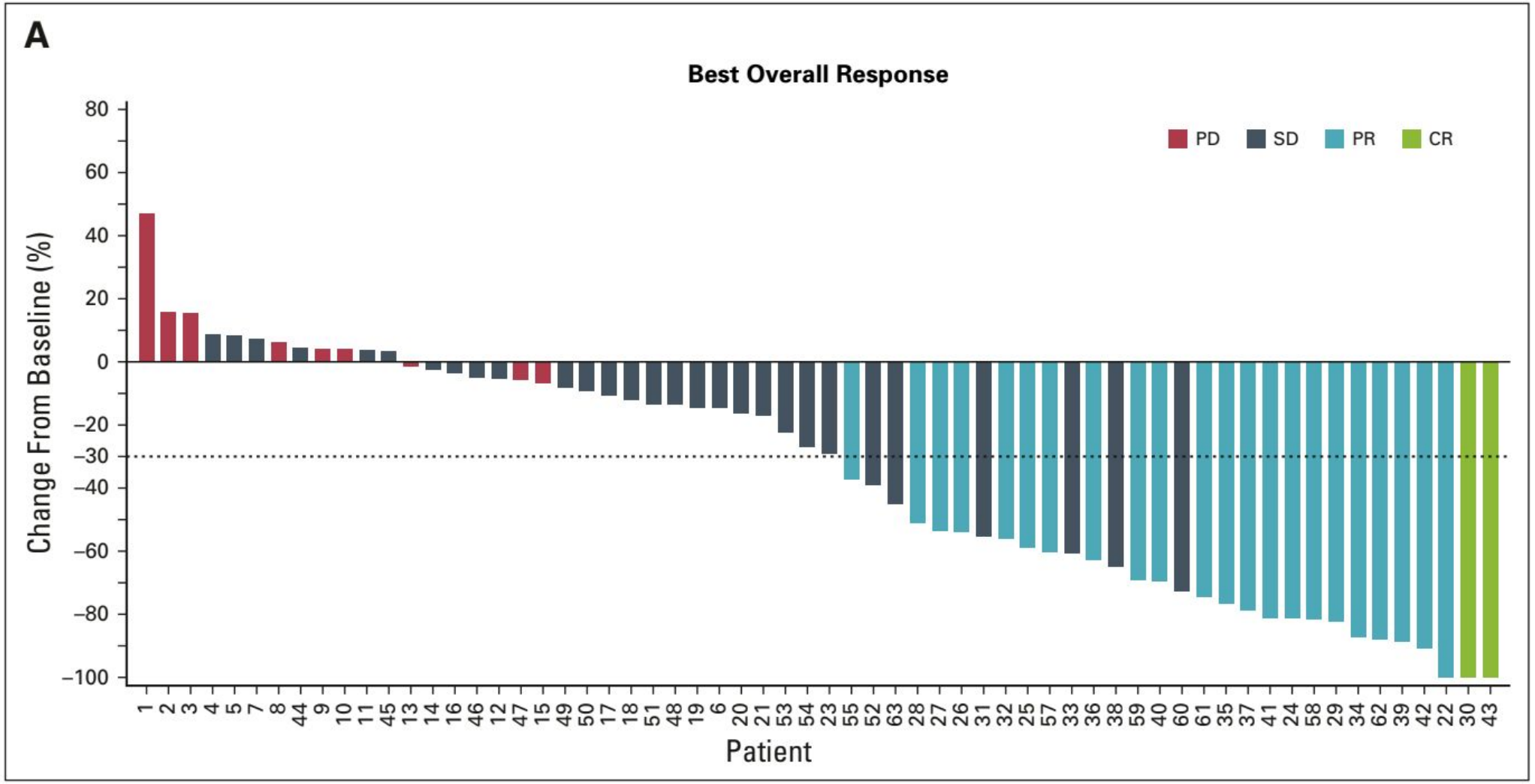
- 4-year long-term data:
  - Median follow-up = 48.1 months
  - Median overall survival = 13.9 months
  - Overall response rate = 31.4%

Key Endpoints	Cohort 2 n = 66
Overall response rate	24 (36%)
Disease control rate	53 (80%)
Best overall response rate	
Complete response	2 (3%)
Partial response	22 (33%)
Stable disease	29 (44%)
Progressive disease	9 (14%)
Not evaluable	4 (6%)

Length of response in responders (months)	% of patients
≥12	54.2%
≥24	39.6%
≥36	33.3%
≥48	20.8%

Sarnaik A, et al. *J Clin Oncol.* 2021; 39: 2656-2666.
Medina T, et al. *J Immunother Cancer.* 2023; 11(Suppl 1): A1-A1731.

Efficacy: Sarnaik, et al.



Sarnaik A, et al. *J Clin Oncol.* 2021; 39: 2656-2666.

Toxicity: Sarnaik, et al.

- Transient adverse effects (AE) and primarily due to LDC and/or IL-2
- No new lifileucel AE reported after 6 months & at 4-year long-term follow-up

- Not associated with recrudescence of immune-related AEs
- Two patients died on treatment:
  - Intra-abdominal hemorrhage at tumor site
  - Acute respiratory failure

	Cohort 2 (N = 66)		
Preferred Term	**Any Grade**	**Grade 3 or 4**	**Grade 5**
No. of patients reporting at least one TEAE, No. (%)	66 (100)	64 (97)	2 (3)[a]
Thrombocytopenia	59 (89)	54 (82)	0
Chills	53 (80)	4 (6)	0
Anemia	45 (68)	37 (56)	0
Pyrexia	39 (59)	11 (17)	0
Neutropenia[b]	37 (56)	26 (39)	0
Febrile neutropenia	36 (55)	36 (55)	0

Sarnaik A, et al. *J Clin Oncol.* 2021; 39: 2656-2666.
Medina T, et al. *J Immunother Cancer.* 2023; 11(Suppl 1): A1-A1731.

Phase III Study: Rohaan, et al.

Design: Prospective, phase III, multicenter, open-label

Inclusion Criteria
-Age 18-75 yrs
-Unresectable or metastatic melanoma (stage IIIC or IV)
-PS 0-1
-Serum LDH ≤2 xULN
-Progressed on ≥1 systemic therapy including CTLA4

84 → TILs

84 → Ipi 3 mg/kg IV q3wk x4

Primary Endpoint:
-PFS

Select Secondary Endpoints:
-ORR
-OS
-HRQOL
-Safety

Resection of tumor → TIL manufactured at treatment center → NMA LDC: Cy 60 mg/kg IV x2 days Flu 25 mg/m2 IV x5 days → Lifileucel 5 x10^9 to 2 x10^{11} cells → IL2 600,000 units/kg IV q8h x15 doses

ECOG PS, Eastern Cooperative Oncology Group Performance Status; PFS, progression-free survival; OS, overall survival. Ipi, ipilimumab; NMA, non-myeloablative; LDC, lymphodepleting chemotherapy; HRQOL, health-related quality of life

Rohaan M, et al. *NEJM*. 2022; 387(23): 2113-25.

Demographics: Rohaan, et al.

- Patients who received TIL infusion, stayed in hospital for median of 17 days

Demographic	Total cohort (n = 168)
Median age	59 yrs (26-77)
Stage IV	98%
Previous systemic therapy	89%
Adjuvant PD1	24%
First-line PD1	62%
Primary refractory to prior PD1	64%
Mutated BRAF V600	43%
LDH 1-2x upper limit of normal	18%

Treatment in TIL cohort (n = 80)	
Median number of TIL cells	40.9 $x10^9$
Median number of IL2 doses	4 (0-10)
Treatment in Ipi cohort (n = 84)	
Median number of ipi doses	3 (1-4)

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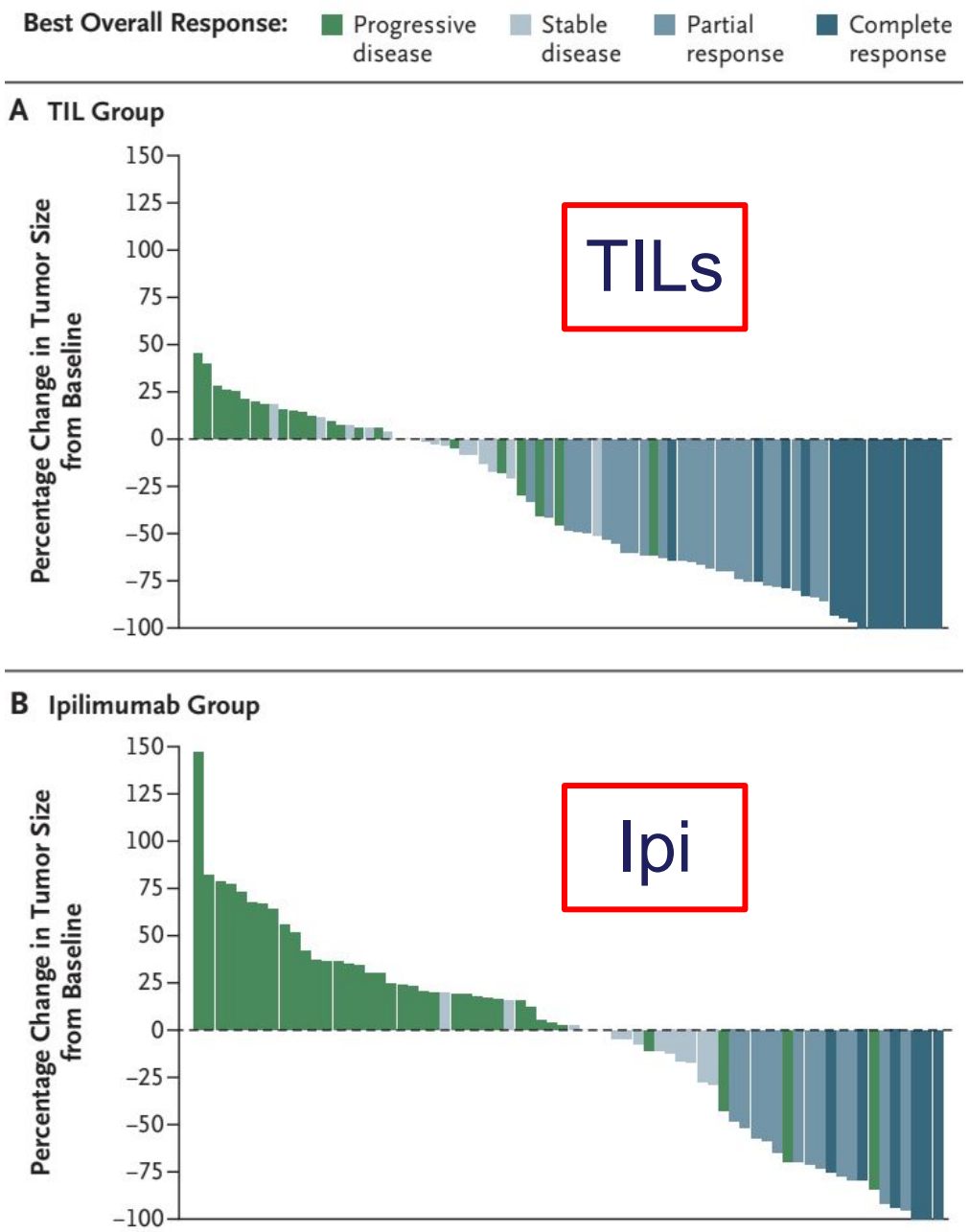
Rohaan M, et al. *NEJM*. 2022; 387(23): 2113-25.

Efficacy: Rohaan, et al.

- Median follow-up = 33 months

Efficacy Endpoints	TIL	Ipi
Median PFS	7.2 mo.	3.1 mo.
PFS at 6 months	52.7%	21.4%
Objective response rate	49%	21%
Complete response	20%	7%
Median overall survival	25.8 mo	18.9 mo
Overall survival at 2 years	54.3%	44.1%
Clinical benefit	68%	39%

- TIL patients had lower symptoms burden of fatigue, pain, and insomnia, but more symptoms of nausea and vomiting



Rohaan M, et al. *NEJM*. 2022; 387(23): 2113-25.

Toxicity: Rohaan, et al.

- More grade ≥ 3 AE occurred in TIL > Ipi
- Treatment-related serious AE Ipi (27%) > TIL (15%)
- ICU level care required in 8 TIL patients

Related to TIL ± IL-2	
Capillary leak syndrome	30%
Autoimmune sequelae	
Skin hypopigmentation	11%
Uveitis	8%
Hearing impairment	4%
Median neutrophil nadir	7 days

AE (Any grade)	TIL	Ipi
Neutrophil decrease	100%	0
Febrile neutropenia	74%	0
Diarrhea	45%	45%
LFTs	32%	22-27%
Rash	46%	34%
Fever	92%	12%
Chills	64%	0
Sinus tachycardia	50%	0
Hypotension	41%	0
SCr increase	36%	0
Pulmonary edema	32%	0
Hypoxia	24%	0

Rohaan M, et al. *NEJM*. 2022; 387(23): 2113-25.

Clinical Considerations

Patient Identification

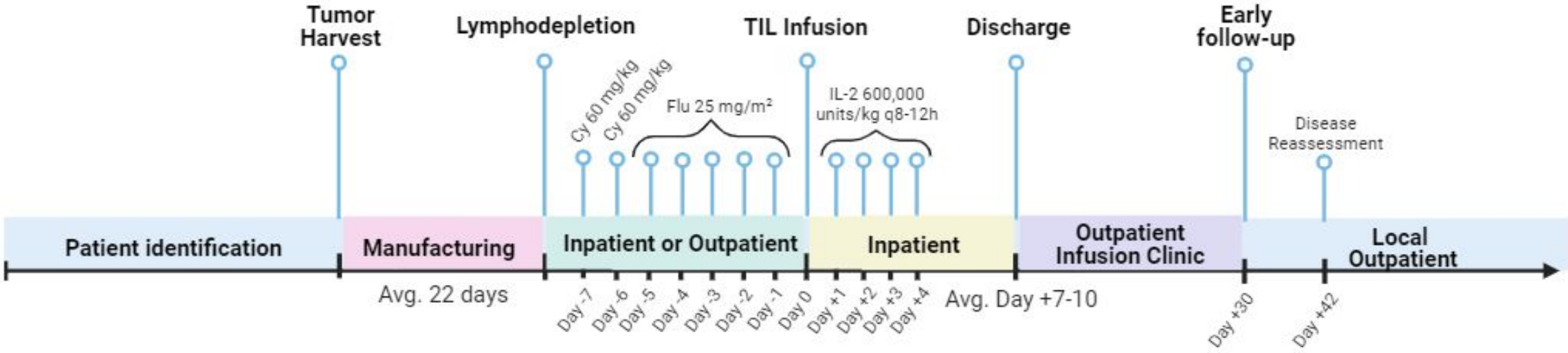
- Performance status
  - Can tolerate non-myeloablative lymphodepletion and IL-2 toxicity
- Pace of disease progression
  - Approximately 22 day manufacturing window
  - Bridging therapy if high burden of disease
- Site of disease
  - Brain metastases: Increased risk of bleeding. Site of metastases should be treated and considered stable prior to TIL therapy
  - Bowel metastases: Benefit should outweigh associated bleeding risk
- Recommended organ function reserve
  - Renal: GFR > 40 mL/min
  - Cardiac: LVEF > 45%
  - Pulmonary: FEV1 > 50%

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

TIL Timeline

- Tumor harvest: Should occur within 2 weeks of patient identification
- TIL manufacturing: Average time is 22 days per manufacturer
- Lymphodepletion: 5-7 days
- IL-2: 3-4 days
- Cytopenia: Recovery typically 7 days
- Short-term follow-up: 30-42 days

11-12 weeks



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Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.
Image created using Biorender

Lymphodepleting Chemotherapy

- Considered “non-myeloablative”
- Central line preferred, but chemotherapy compatible with peripheral access
- Chemotherapy:
  - Fludarabine 25 mg/m2 IV (30 minutes) x5 doses
  - Cyclophosphamide 60 mg/kg IV (60 minutes) x2 doses
- Antiemetics:
  - 5HT3 antagonist + NK1 antagonist ± olanzapine + breakthrough
  - No dexamethasone due to corticosteroid effect
- Cytopenias:
  - HSV Viral: Acyclovir or valacyclovir
  - PJP pneumonia: sulfamethoxazole-trimethoprim, atovaquone, pentamidine
  - Bacterial: Gram positive and gram negative coverage when ANC <500
  - Fungal: Fluconazole or micafungin when ANC <1000
  - Growth factor support starts day +1 and continues until ANC >1000

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

TIL Toxicity

- Infusion-related reactions are rare (reported in <4% of patients)
  - Manage per institutional standard with diphenhydramine, acetaminophen, famotidine, etc.
  - Use of corticosteroids should be reserved for life-threatening emergencies only
  - Monitor vital signs every 30 minutes during infusion, then hourly x4 hours, then per standard routine check
- Autoimmune sequelae (uveitis & vitiligo) following TIL infusion are rare
- Cytokine release syndrome (CRS) and neurotoxicity as seen with CAR T-cell therapy do NOT occur. Do NOT use tocilizumab or corticosteroids for management of side effects post-TIL infusion

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

TIL Supportive Care

- Recommend the following pre-medications to be administered 30-60 minutes prior to TIL infusion:
  - H1 histamine antagonist (i.e. diphenhydramine 25-50 mg PO or IV)
  - Acetaminophen 650 mg PO
- Order hypersensitivity or emergency medications to manage infusion-related reactions per institutional standard. Reserve hydrocortisone for life-threatening infusion-related reaction
- Administration through a central line
- May consider pre- and post- IV hydration due to DMSO preservative in cryopreserved TIL bags

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

IL-2 Pharmacology

- Dosed 600,000 units/kg IV (15 minute infusion) every 8-12 hours for total of 6 doses
  - Number of doses has not been correlated with efficacy
  - Typically started ~24 hours after TIL infusion
  - Most institutions prefer 12 hour dosing interval
- Biologic effects typically occur several hours after IL-2 dose
  - Chills and rigors can occur ~1-2 hours after infusion
  - Fevers can occur 2-4 hours after infusion
- Primarily cleared through the kidneys
  - Acute kidney injury can prolong IL-2 toxicity
  - Half-life is approximately 90 minutes

Aldesleukin [package insert]. San Diego, CA: Prometheus Laboratories, LLC; 2012.

IL-2 Toxicity

- Capillary leak syndrome = compromises vascular endothelium causing third spacing of fluids
  - Intravascular depletion impacts most organs including brain, heart, liver, lungs, kidneys, and circulatory system
  - Typically presents with flu-like symptoms, hypotension, hypoxia, weight gain, edema, and decreased urine output
- Impairs neutrophil trafficking
  - Staphylococcus infections reported in 10-30% of patients in earlier studies
- Adrenal insufficiency
  - Patients with immunotherapy-related adrenal insufficiency may require stress-dose steroids during acute toxicity period
- Rash & musculoskeletal changes
- Gastrointestinal
  - Moderate emetic risk
- Cytopenias

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

IL-2 Supportive Care

- Start supportive care medications 60 minutes prior to the first dose of IL-2
- Stop the medication 12 hours after the last dose of IL-2
- Supportive medications may include:
  - Acetaminophen 650 mg PO every 4 hours
    - Consider reducing if LFT abnormalities are present
  - Indomethacin 50 mg PO every 6 hours OR naproxen 375 mg PO every 12 hours
    - Reduce or discontinue if renal impairment or PLT <50,000
  - Famotidine 20 mg PO/IV every 12 hours
  - Ondansetron 8 mg PO/IV every 8 hours
  - Meperidine 25-50 mg IV every 4 hours as needed for rigors or chills
- Avoid IV fluid due to third spacing of fluids

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

IL-2 Management by System

Toxicity	Management
Fever, chills, and/or rigors	Continue scheduled acetaminophen and NSAID - Administer meperidine 25 mg with option to repeat another dose within 30 minutes for rigors - If refractory to meperidine, consider hydromorphone 0.5 mg IV every 15 minutes as needed for rigors (maximum = 3 total doses) - Infectious disease work-up and management of febrile neutropenia. Continue prophylactic antimicrobials.
Blood pressure	Target BP is set on admission and assessed prior to each dose. Assess 2 hours prior to dose - If not meeting target- give 500 mL IV bolus - Fluid boluses can be given until O2 requirement develops though would consider escalation of care at >2L above maintenance requirements in 24-hour period - If hypotensive despite IVF, initiate pressors.
Urine Output	SCr monitored twice daily Urine output >0.5 mL/kg/hr- assess twice daily including 2 hours prior to dose - If not meeting goal, then give IV bolus and check urine output 1 hour post IV bolus and repeat IV bolus if still not at goal
Pulmonary	O2 saturation should be maintained ≥92%, initiate O2 if <95%. -If O2 requirement at time of IL2 dose, then criteria to hold -If BP will tolerate, can diurese to relieve O2 requirement

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

IL-2 Management by System (continued)

Toxicity	Management
Gastrointestinal	Nausea - Continue scheduled antiemetics > administer breakthrough prochlorperazine or lorazepam as needed Diarrhea - Loperamide and/or diphenoxylate/atropine as needed Hemorrhage - Continue famotidine as scheduled for prophylaxis
Neurologic	Withhold IL2, usually temporary and reversible with IL2 clearance - Manage with antipsychotics if clinically warranted
Endocrine	Hypothyroid and adrenal insufficiency - May require additional supplementation for pre-existing conditions
Pulmonary	O2 saturation should be maintained ≥92%, initiate O2 if <95%. - If O2 requirement at time of IL2 dose, then criteria to hold - If BP will tolerate, can diurese to relieve O2 requirement
Capillary Leak	Monitor daily standing weights and urine output prior to each dose of IL2 - May consider use of diuresis ± IV bolus if not meeting UOP goal

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

Considerations for IL-2 Dose Interruptions

System	Relative Criteria	Absolute
Cardiac	Sinus tachycardia >120 BPM	-Sustained sinus tachycardia (>1 hr) after correction hypotension and fever -Elevated troponin -Arrhythmia -Hypotension refractory to IVF
Gastrointestinal	Diarrhea >1000 mL/shift	-Diarrhea >1000 mL/shift x2 -AST/ALT ≥grade 3 per CTCAE
Hemorrhagic	Guaiac and sputum positive	Frank blood in sputum, emesis, or stool
Musculoskeletal	Extremity tightness	Extremity paresthesia
Neurologic	Vivid dreams, emotional lability	-Hallucinations, disorientation, or mental status changes not reversible -Persistent crying
Pulmonary	Resting SOB Rales 1/3 up chest	-New O2 requirement at time of next IL2 dose -Moist rales ½ up chest -Pleural effusion requiring drainage
Renal	-SCr Increase by 50% from baseline or absolute SCr 2.5-2.9 -CO2 <18	-Urine <4 mL/kg over 8 hours -SCr increase by 100% from baseline or absolute SCr ≥3 -Persistent acidosis despite replacement

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

Criteria to Hold or Discontinue IL-2 Doses

- Allow dosing delays up to 2 hours if patient improves within parameters, but do not delay the next dose
- Administer IL-2 doses at least 8 hours apart (ideally 12 hrs)
- If dose is held for more than 24 hrs -> stop IL-2
- If 2 consecutive doses are held -> permanently discontinue IL-2

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

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

Discharge Considerations

- May vary from institution based on infrastructure & experience
- Absolute neutrophil count >500
  - In the absence of GCSF
  - Antibacterial and antifungal prophylaxis should be discontinued
  - Treated or absence of infection
- Platelet count >20,000
  - Independent of platelet transfusions
- Resolution of hypoxia
  - Patient should return to baseline pulmonary status (on room air)
- Central line removal
- Maintain activities of daily living
- Should stay within 1 hour for 30 days post-TIL infusion

Betof Warner A, et al. *J Immunother Cancer*. 2024; 12: e008735.

Key Takeaways

- Tumor infiltrating lymphocytes are surgically removed, mass produced, and reinfused back into the patient to provide autologous tumor-specific immune cells.

- TILs provide an effective and novel treatment strategy in patients with metastatic melanoma who have failed conventional immunotherapy and/or targeted therapy.

- The toxicity and logistical considerations for TIL therapy can be a barrier to treatment access and requires a multidisciplinary team to safely and efficiently mobilize this therapy in clinical practice.

Patient Case Example

A 53 year old male with metastatic melanoma (BRAF V600E negative) who recently progressed on nivolumab presents to clinic. He recently heard on the news that lifileucel was FDA approved and he asks if he is a candidate for this novel treatment.

PMH: Diabetes (on metformin at home, well controlled), OSA (uses CPAP at home), hypertension (on lisinopril, well controlled)

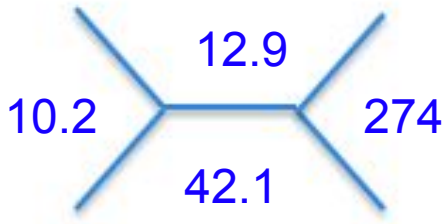
Exam: Well appearing caucasian male with a performance status of 0.

Patient Case Example

Pertinent findings on exam & imaging:

- Patient noted to have progression of disease in liver and lymph node metastases

Pertinent labs:



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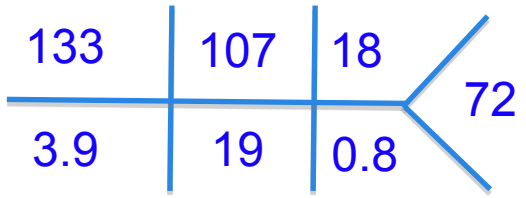

Patient Case Question #1

Is this patient a candidate for TIL therapy?

1. Yes, all metastatic melanoma patients are candidate for TIL therapy after failing PD1 or targeted therapy
2. Yes, the patient meets the FDA approved indication and can safely tolerate TIL therapy based on his clinical presentation
3. No, he needs to fail ipilimumab prior to being a candidate for TIL therapy
4. No, he will have more clinical benefit with use of chemotherapy over TIL therapy

Patient Case Question #2

The patient is eligible and proceeds with TIL therapy. He has received 2 IL-2 doses thus far. Prior to the scheduled 3rd dose of IL-2, he becomes anuric (UOP <4 mL/kg), hypotensive (79/48), and requires 2L NC for hypoxia. What is the best response to this clinical situation?

1. Administer furosemide 20 mg IV to improve urine output
2. Administer meperidine 25 mg IV for capillary leak syndrome
3. Administer IL-2 as scheduled since efficacy of TIL therapy is based on completion of all 6 planned doses
4. Hold IL2 and administer an IV bolus

Questions?

Thank you for your attention!

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Thank You!

Questions?