

Tumor-Infiltrating Lymphocyte Cell Therapy Educational Module

What Is TIL Cell Therapy?

Table of Contents

Immunology Background

Evolution of Immunotherapy Approach for Cancer Treatment

TIL Cell Therapy Mechanism of Action and Treatment Process

TIL Cell Therapy Clinical Data Summary

Requirements for Delivery of TIL Cell Therapy

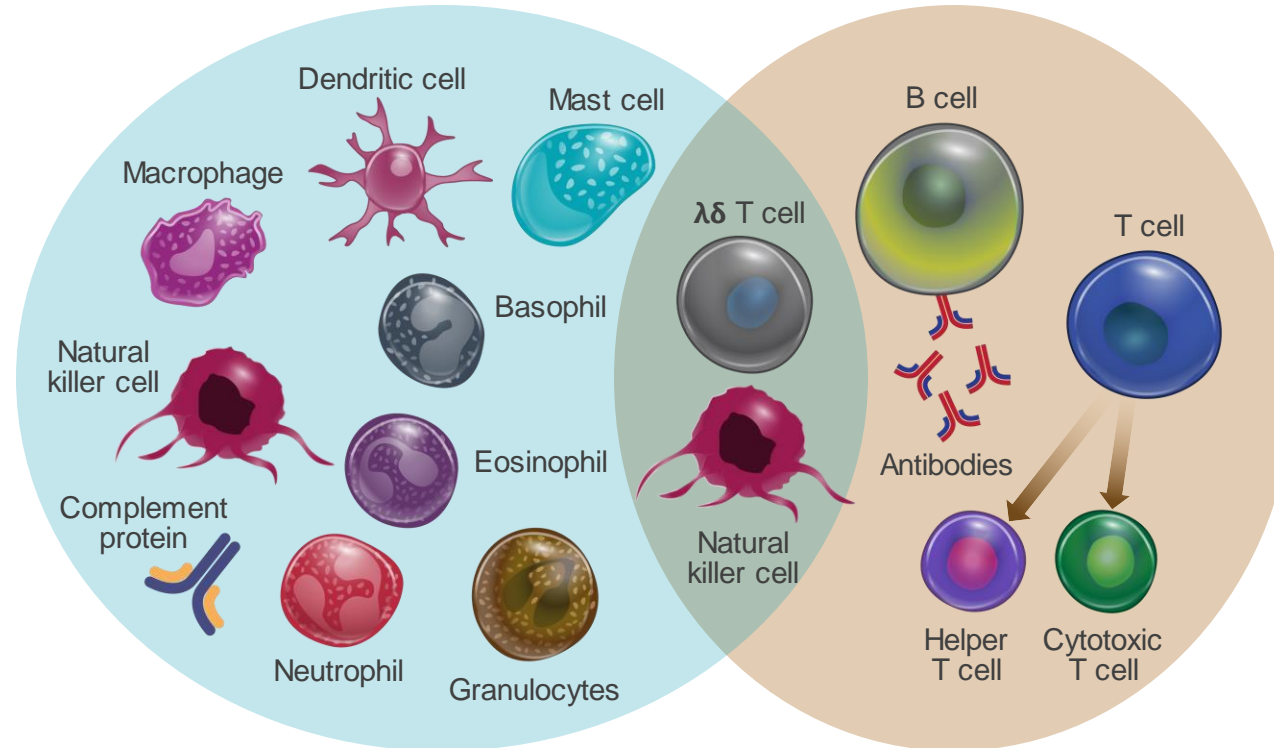
Summary

Backup

Immunology Background

The immune system identifies and destroys foreign or abnormal cells

Innate Immunity^{1,2}
Nonspecific first line of defense, activates adaptive response



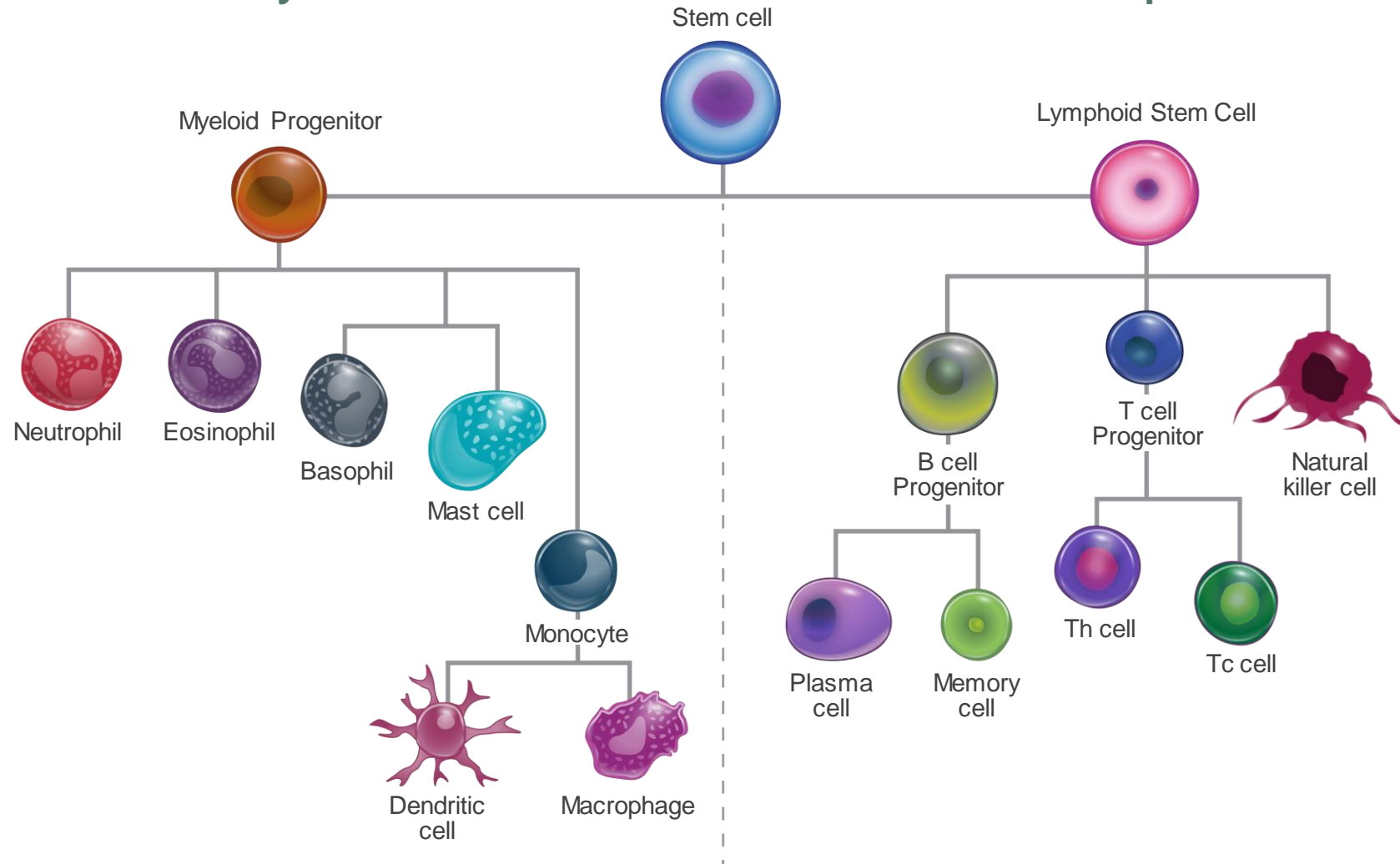
Adaptive Immunity^{1,2}
Specific response that adapts to diverse stimuli and has memory functions

Immune Surveillance^{1,2}
Identification and destruction of foreign or abnormal cells by innate and adaptive immunity

Individual cells in the immune system perform specialized roles in immune surveillance

Innate Immunity

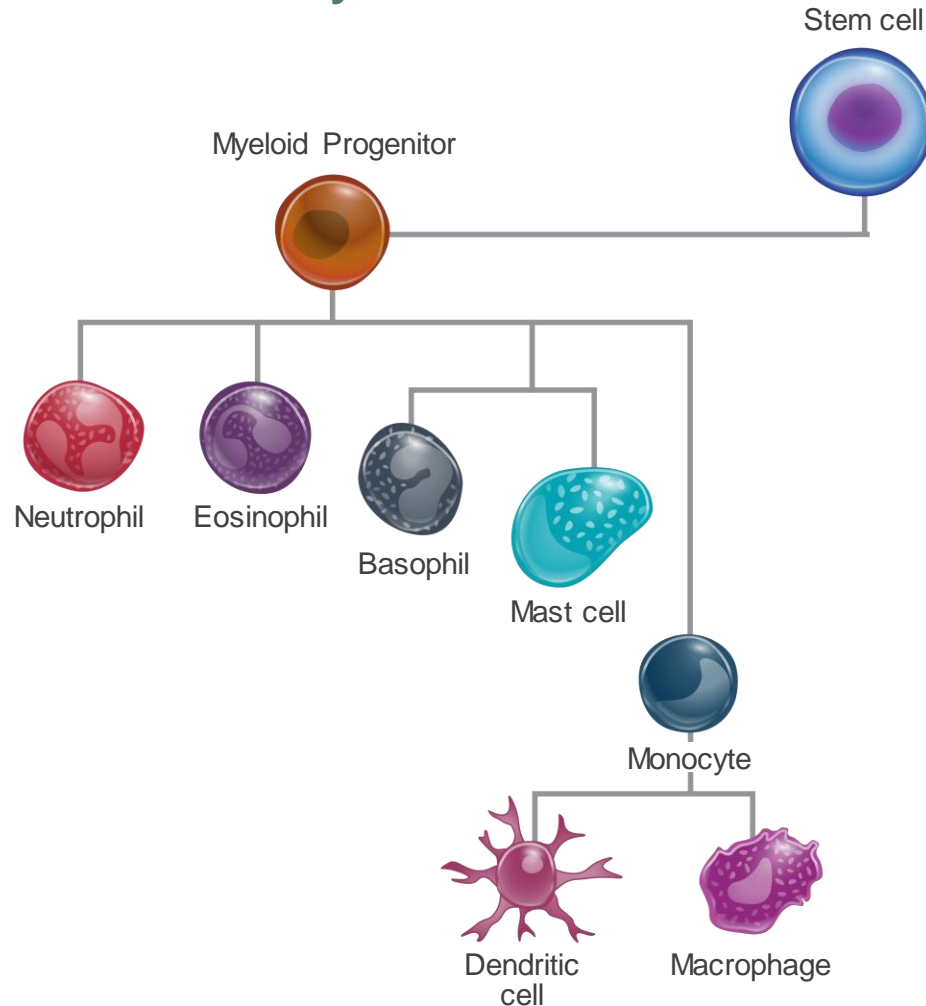
Adaptive Immunity



Tc, cytotoxic T cell; Th, helper T cell.
Janeway CA, et al. Immunobiology 5. The Immune System in Health and Disease. Fifth Edition. 2001.

Individual myeloid cells in the immune system perform specialized roles in immune surveillance

Innate Immunity



Cells in the innate immune system include

Neutrophils: Phagocytose and kill microorganisms

Eosinophils and basophils: Defense against parasitic infections

Mast cells: Involved in allergy and anaphylaxis

Dendritic cells: Antigen-presenting cells that activate T cells

Macrophages: Phagocytose and digest cellular debris

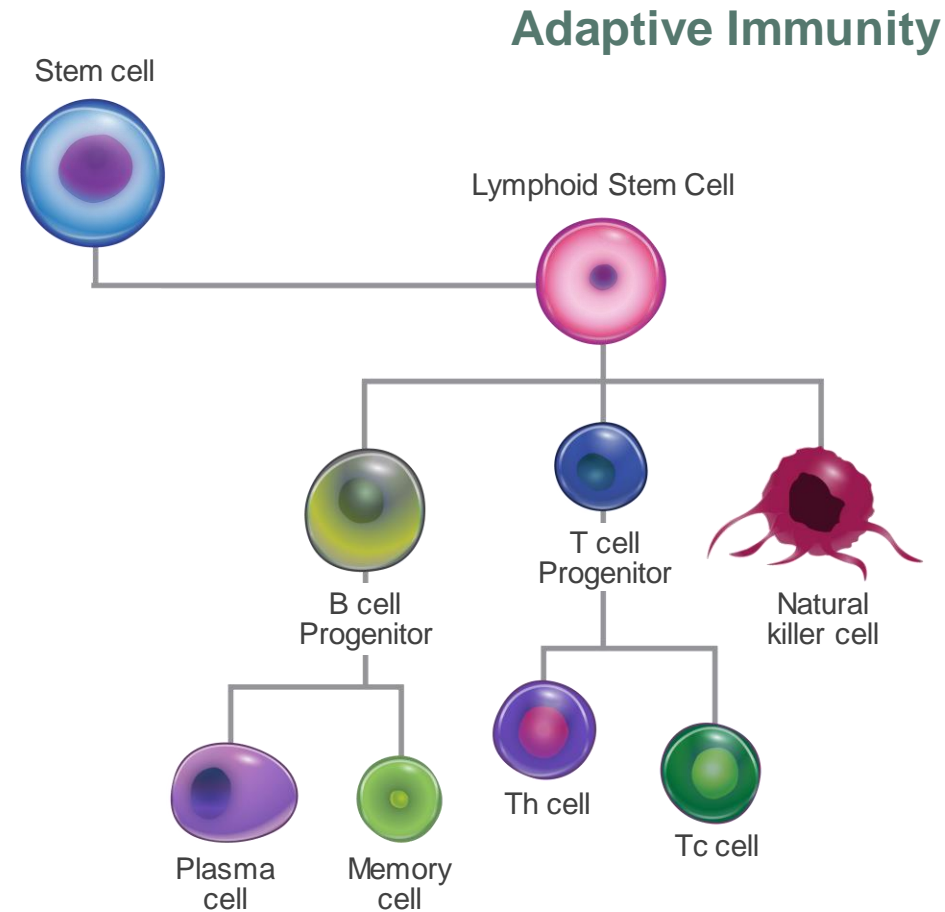
Individual lymphoid cells in the immune system perform specialized roles in immune surveillance

Lymphocytes in the adaptive immune system include

B cells: Produce antibodies for recognition of foreign/abnormal extracellular antigens

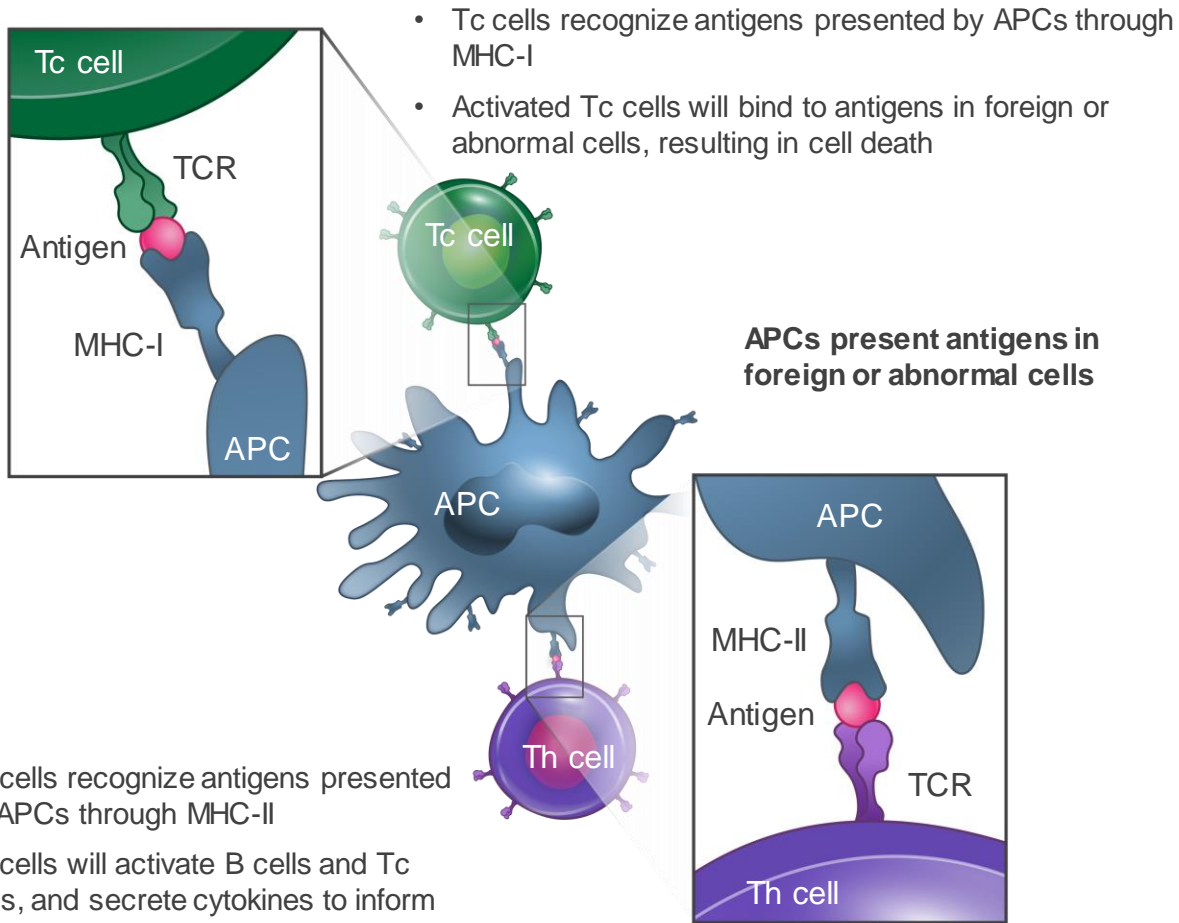
T cells: Direct recognition of foreign/abnormal intracellular antigens in APCs through TCRs

Natural killer cells: Recognition of virus-infected cells and tumor cells

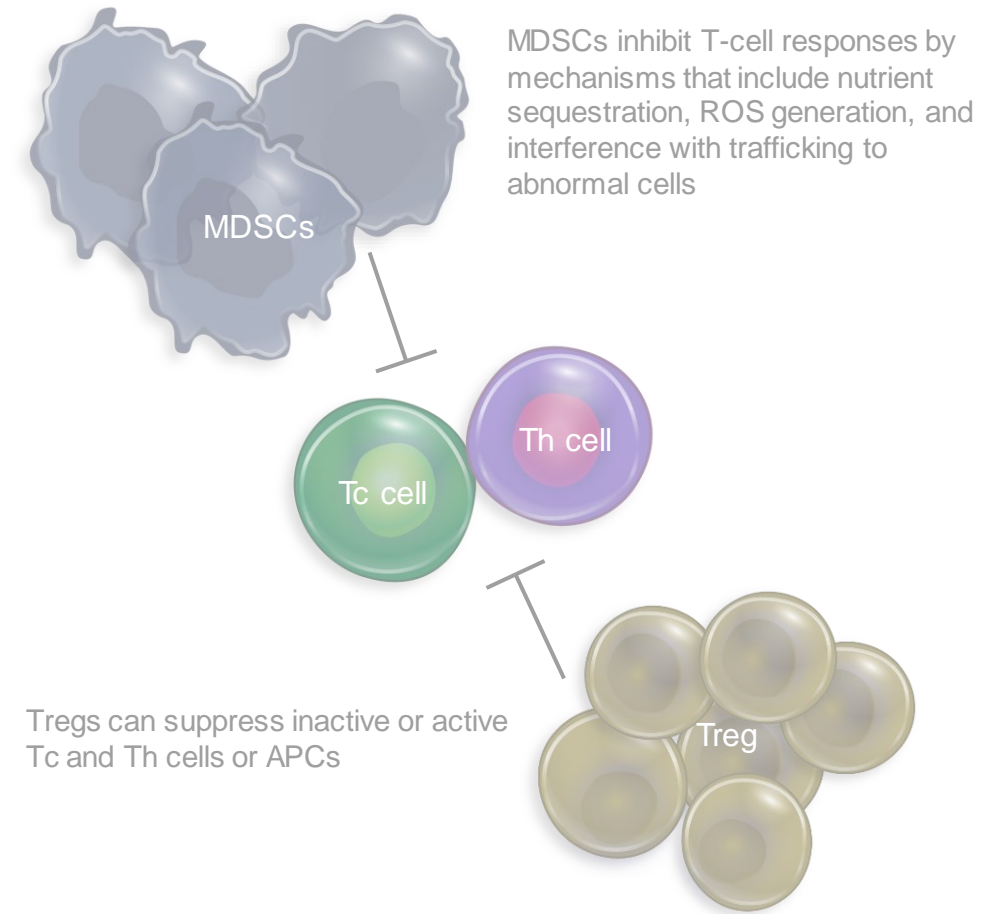


T-cell activation and regulation drives immune surveillance

T-cell activation



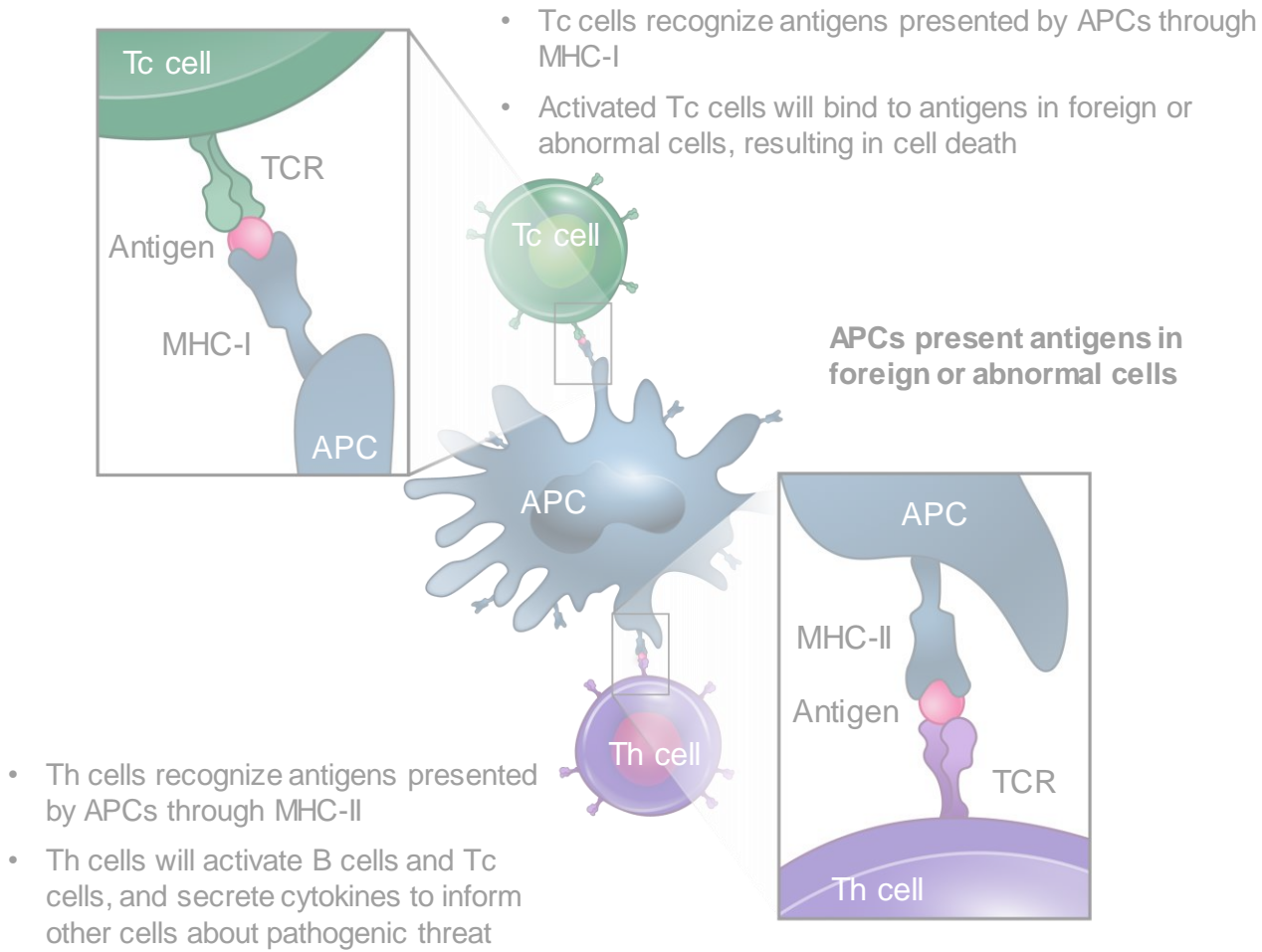
T-cell regulation



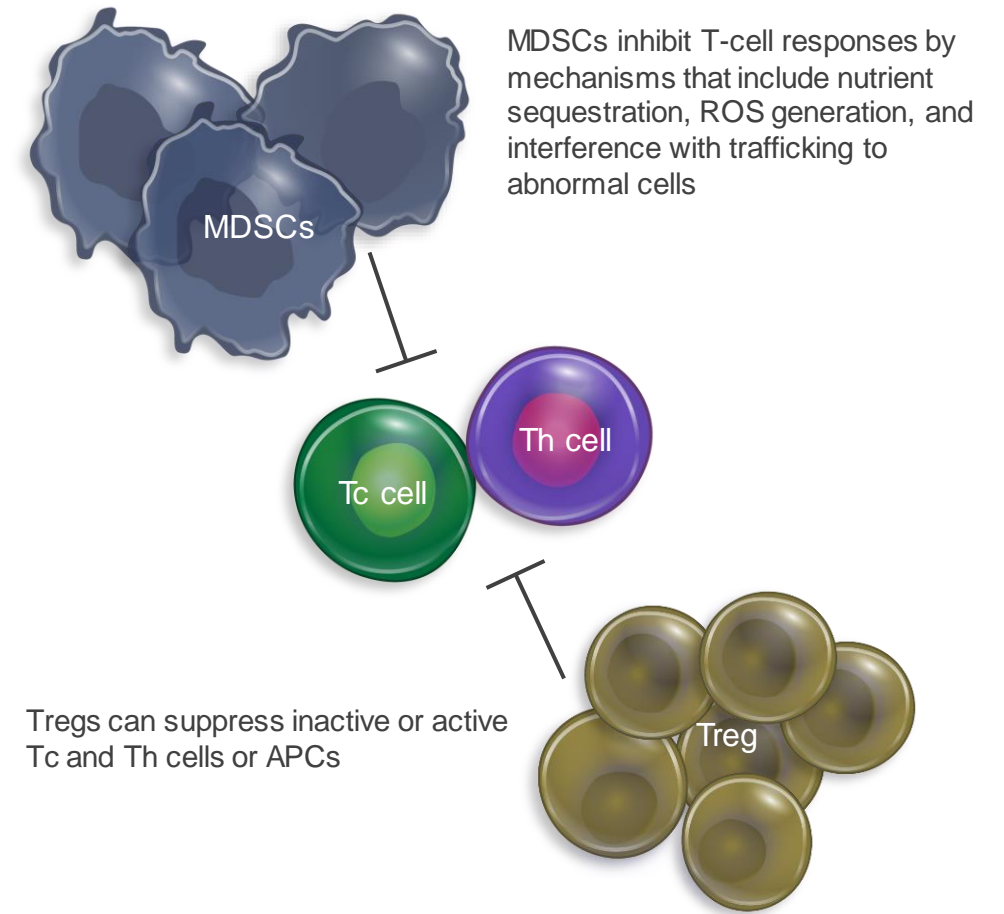
APC, antigen-presenting cell; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; ROS, reactive oxygen species; Tc, cytotoxic T cell; TCR, T-cell receptor; Th, helper T cell; Treg, regulatory T cell.
Monjazeb AM, et al. *Front Oncol.* 2013;3:197.

T-cell activation and regulation drives immune surveillance

T-cell activation



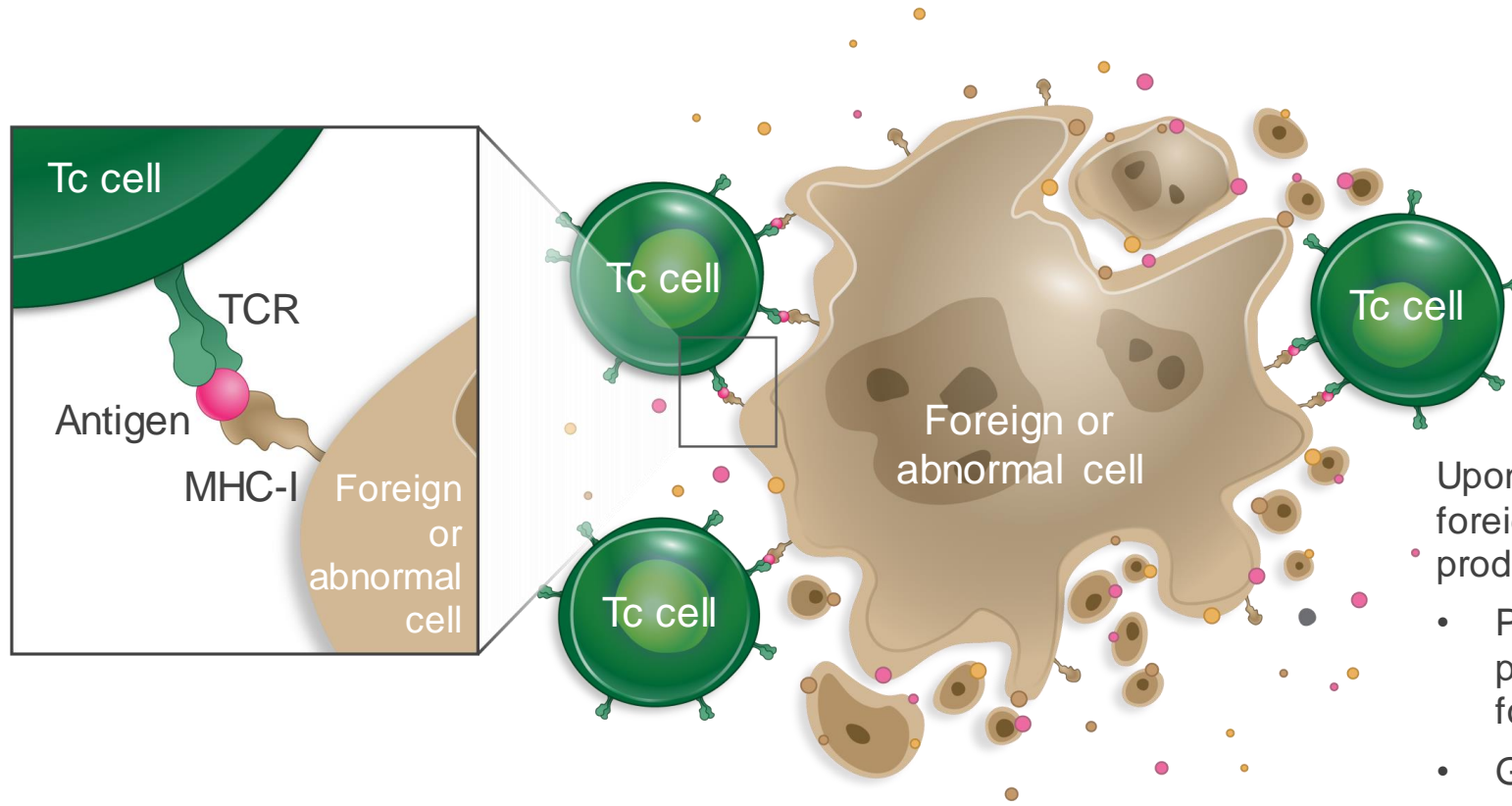
T-cell regulation



APC, antigen-presenting cell; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; ROS, reactive oxygen species; Tc, cytotoxic T cell; TCR, T-cell receptor; Th, helper T cell; Treg, regulatory T cell.
Monjazeb AM, et al. *Front Oncol.* 2013;3:197.

T cells bind to foreign or abnormal cells, including tumor cells, through antigen recognition

Tc cells bind to antigens on the surface of foreign or abnormal cells through TCR-MHC-I complexes

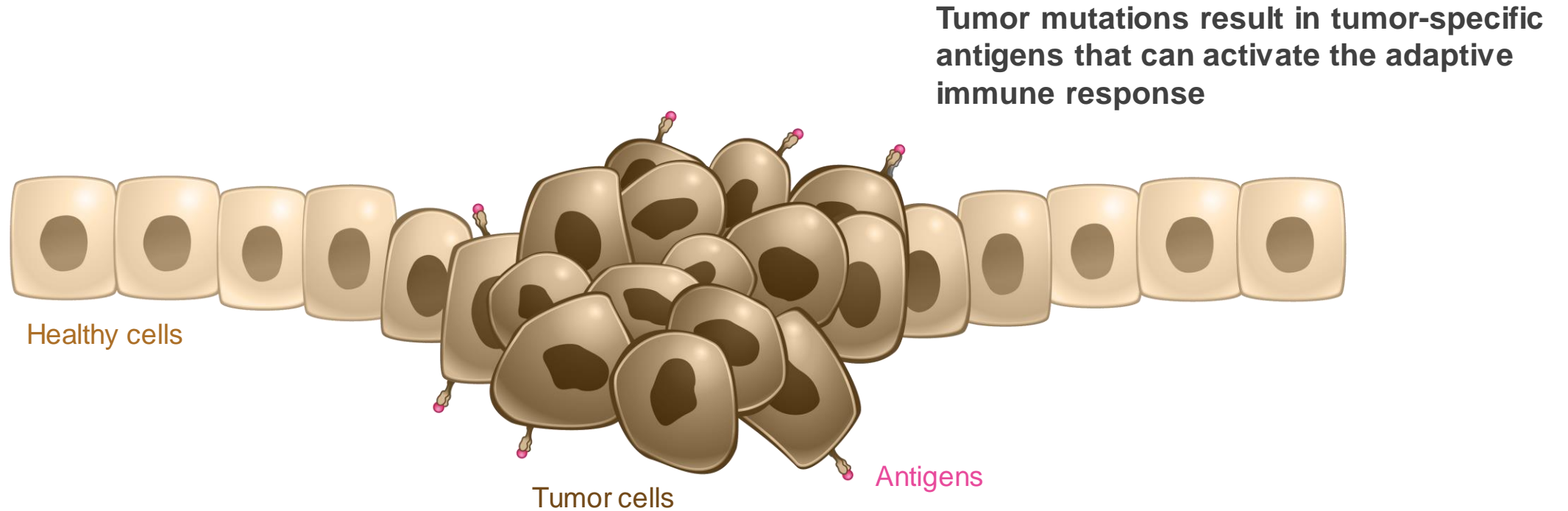


Upon Tc cell recognition of a foreign or abnormal cell, Tc cells produce perforins and granzymes.

- Perforins form pores in the plasma membrane of the foreign or abnormal cells
- Granzymes enter through the pores, break down proteins, and lyse the cell

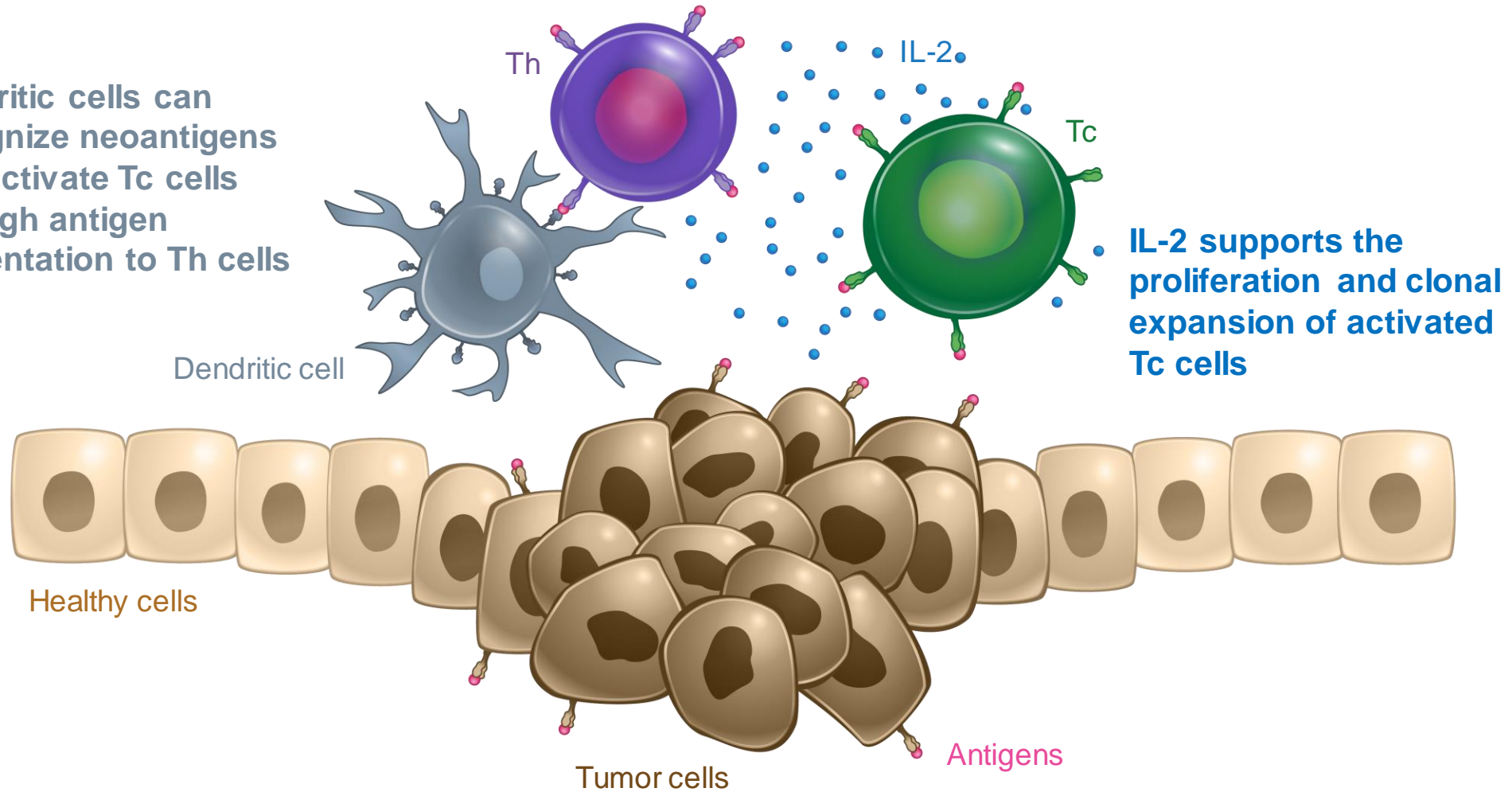
Mutations in tumor DNA cause changes in tumor protein sequences that can be recognized by the immune system as tumor-associated antigen

T cells can detect and eliminate tumor cells through neoantigen recognition

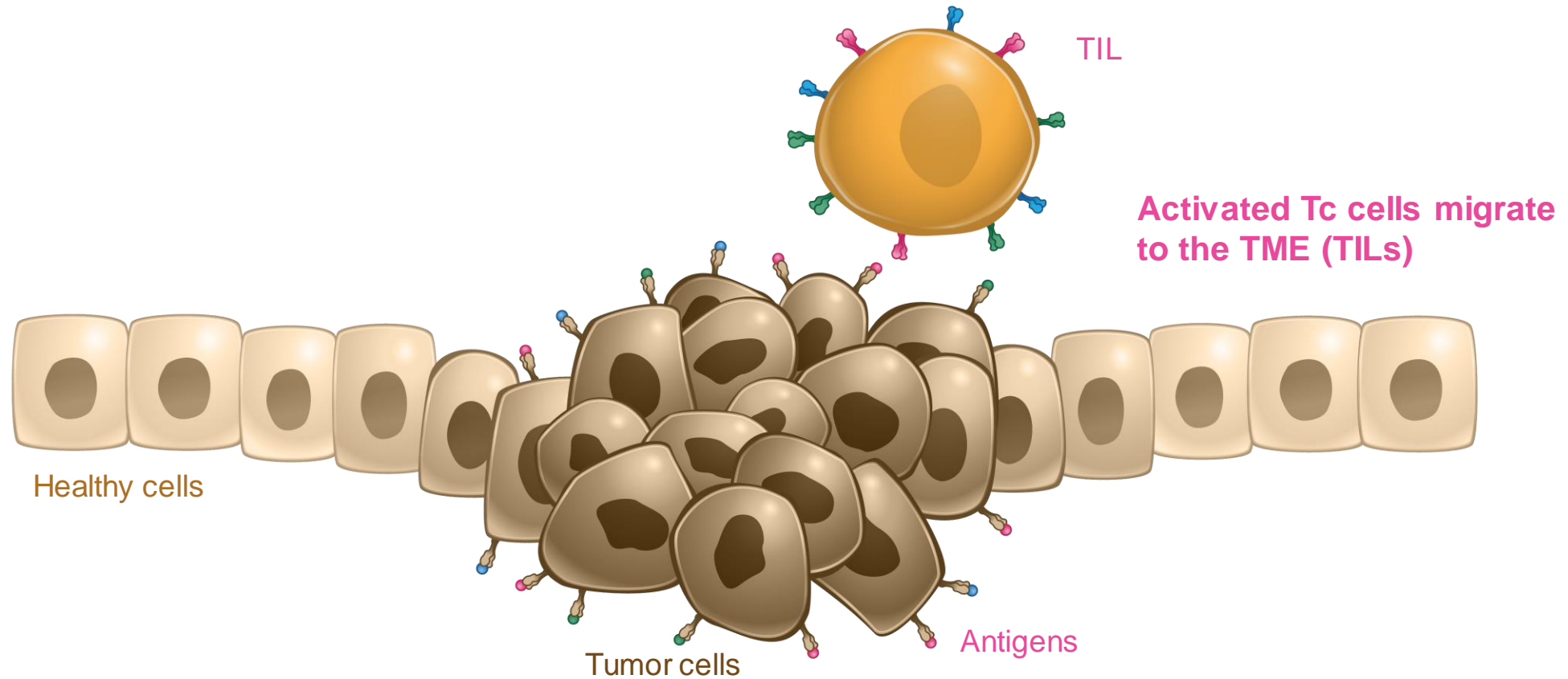


Dendritic cells present antigens to Th cells, which activate Tc cells

Dendritic cells can recognize neoantigens and activate Tc cells through antigen presentation to Th cells



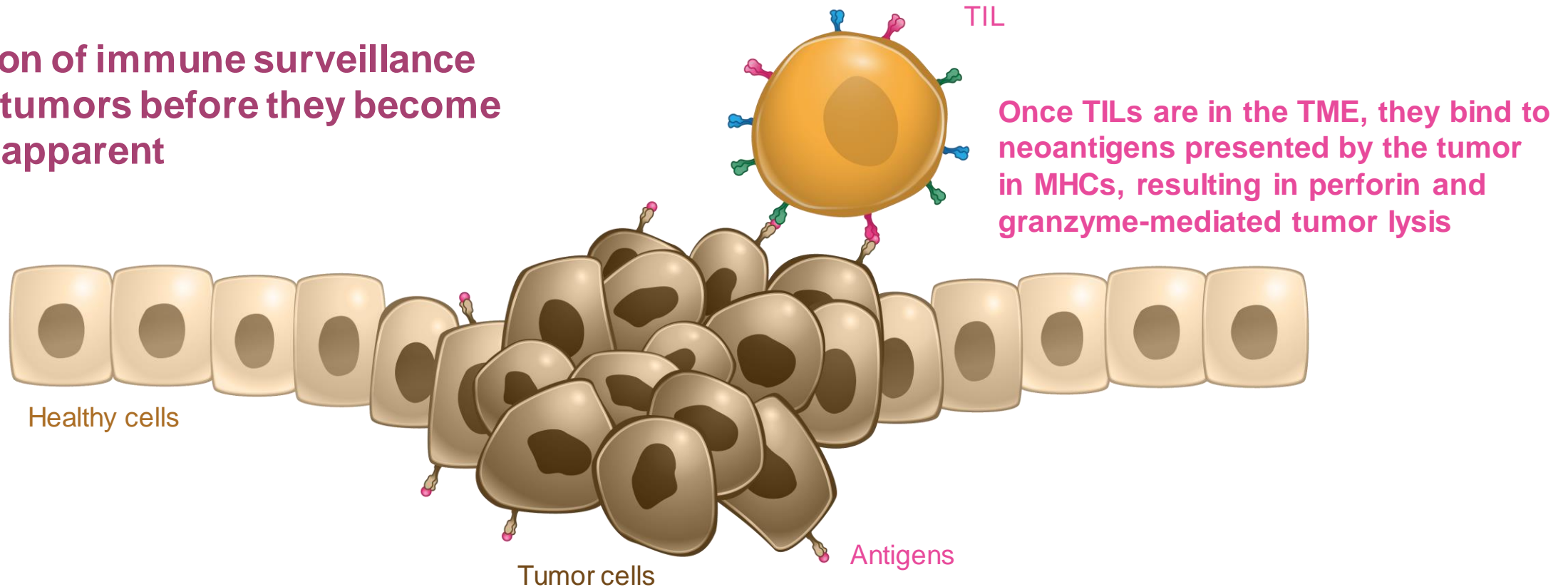
Activated Tc cells migrate to the tumor microenvironment



Tc, cytotoxic T cell; Th, helper T cell; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment.
Monjazebe AM, et al. *Front Oncol.* 2013;3:197.

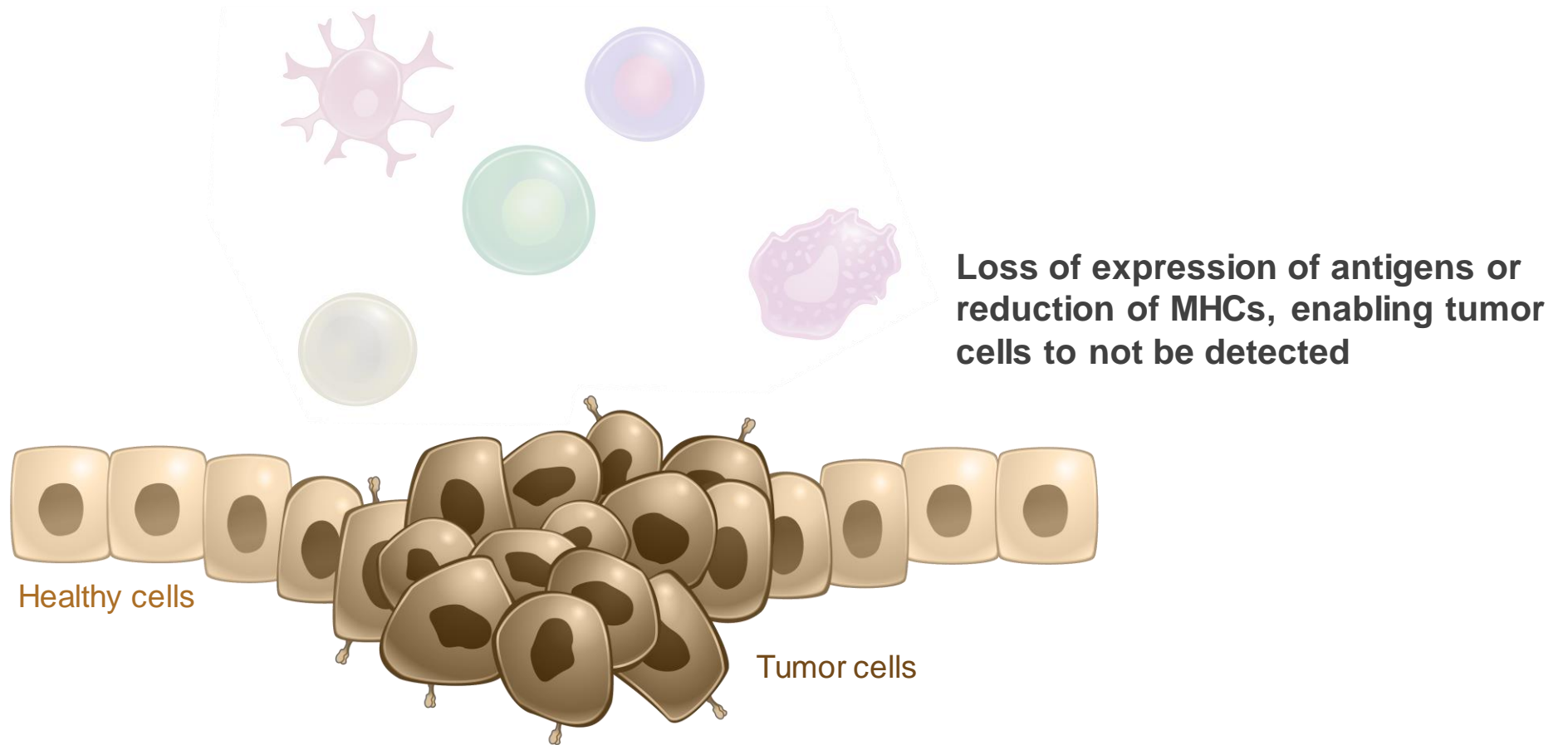
Activated Tc cells in the TME (TILs) lyse tumor cells after antigen recognition

Completion of immune surveillance destroys tumors before they become clinically apparent



Tumors can modify the tumor microenvironment to suppress the activity of TILs

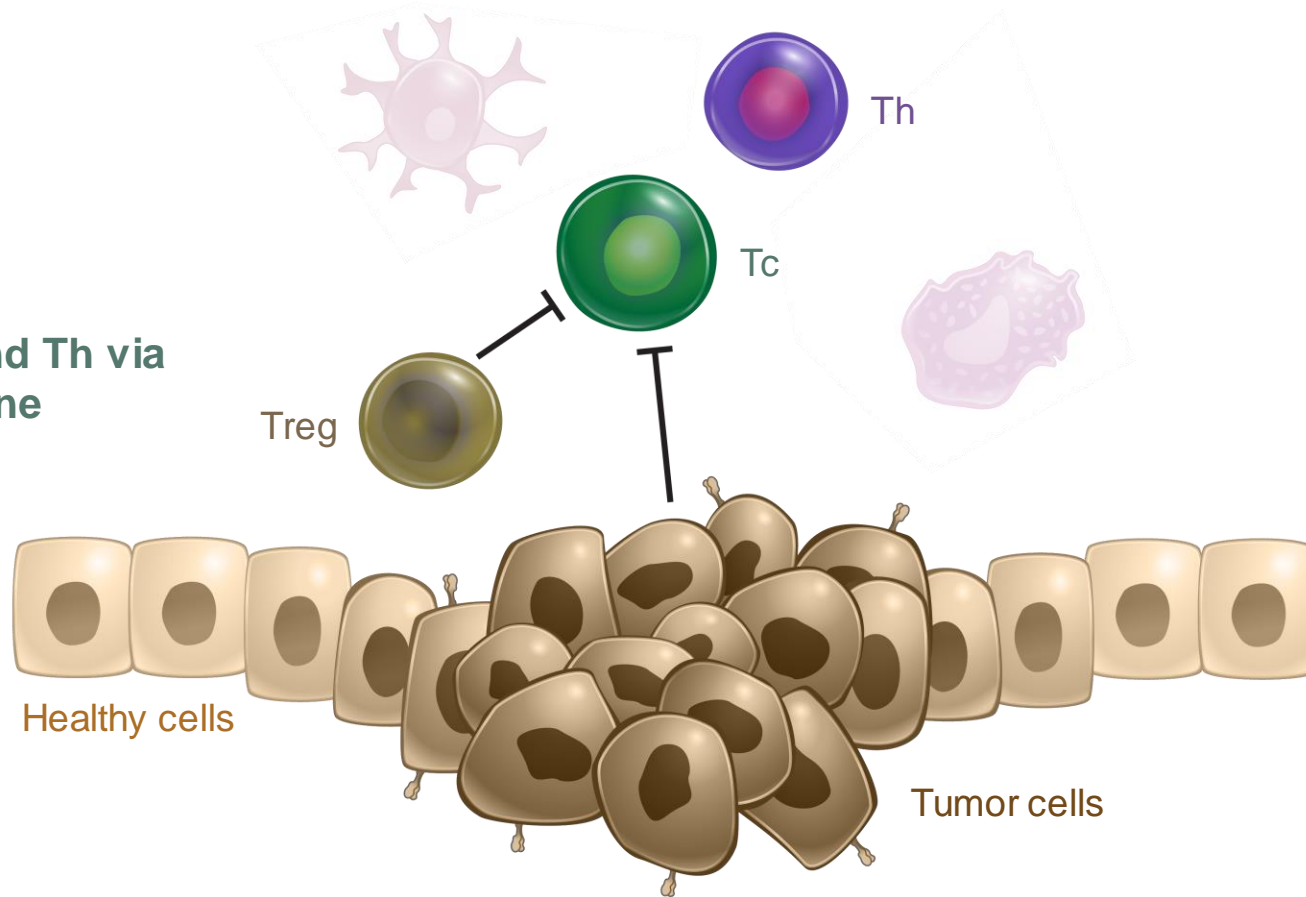
Tumors engage immunosuppressive mechanisms that lead to dysfunctional or fewer TILs in the tumor microenvironment



Tumors can modify the tumor microenvironment to suppress the activity of TILs

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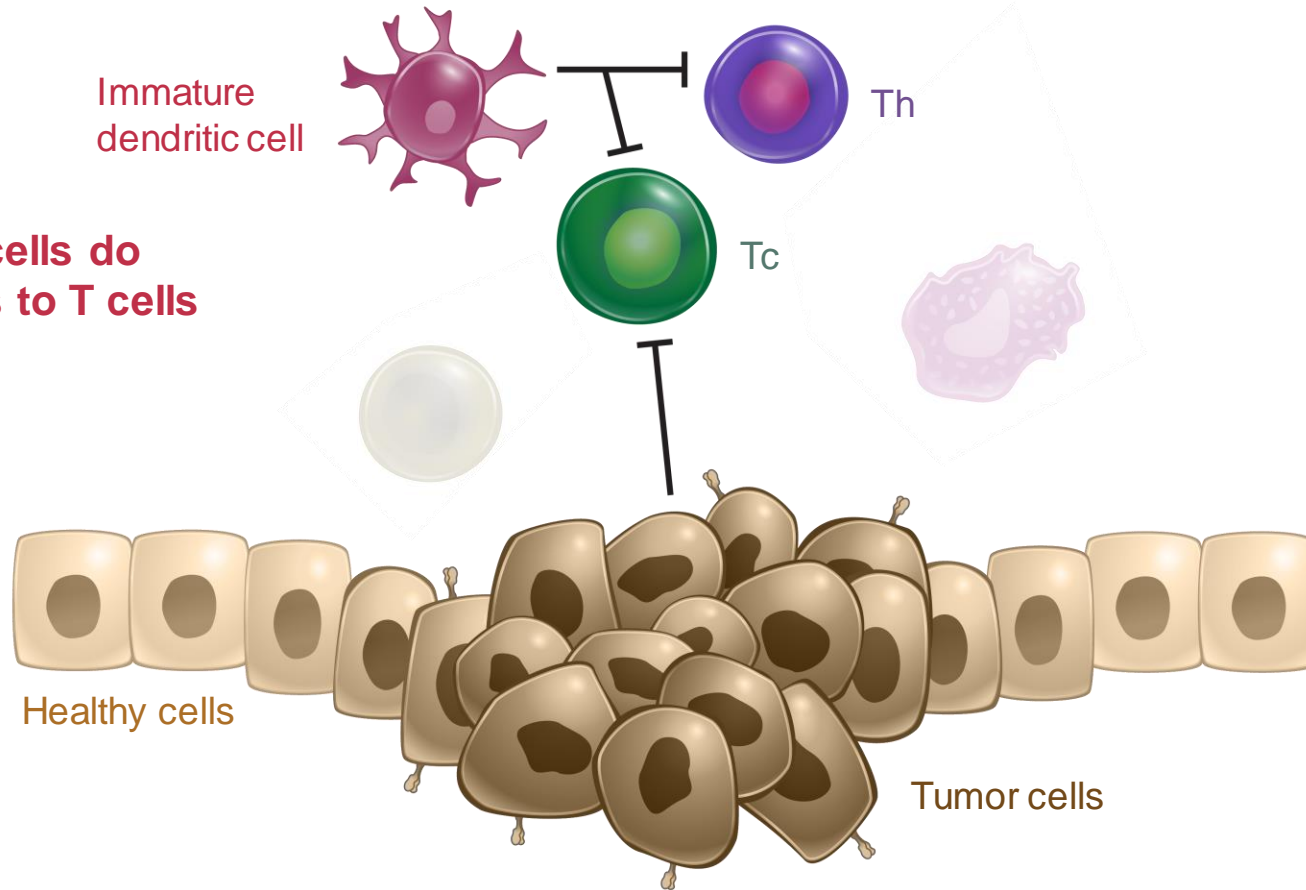
Suppression of Tc and Th via Treg, reducing immune surveillance



Tumors can modify the tumor microenvironment to suppress the activity of TILs

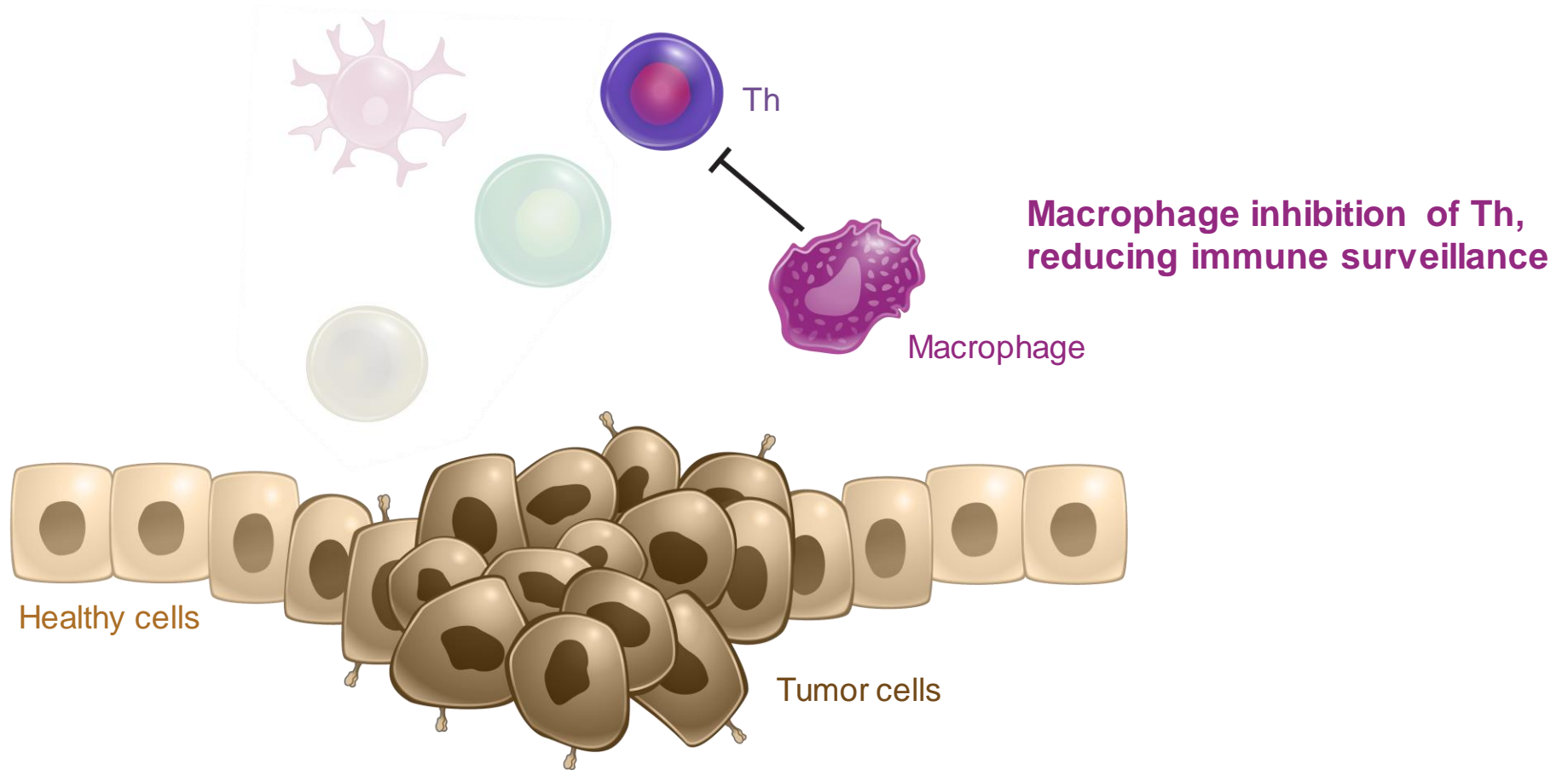
Tumors engage immunosuppressive mechanisms that lead to dysfunctional or fewer TILs in the tumor microenvironment

Immature dendritic cells do not present antigens to T cells



Tumors can modify the tumor microenvironment to suppress the activity of TILs

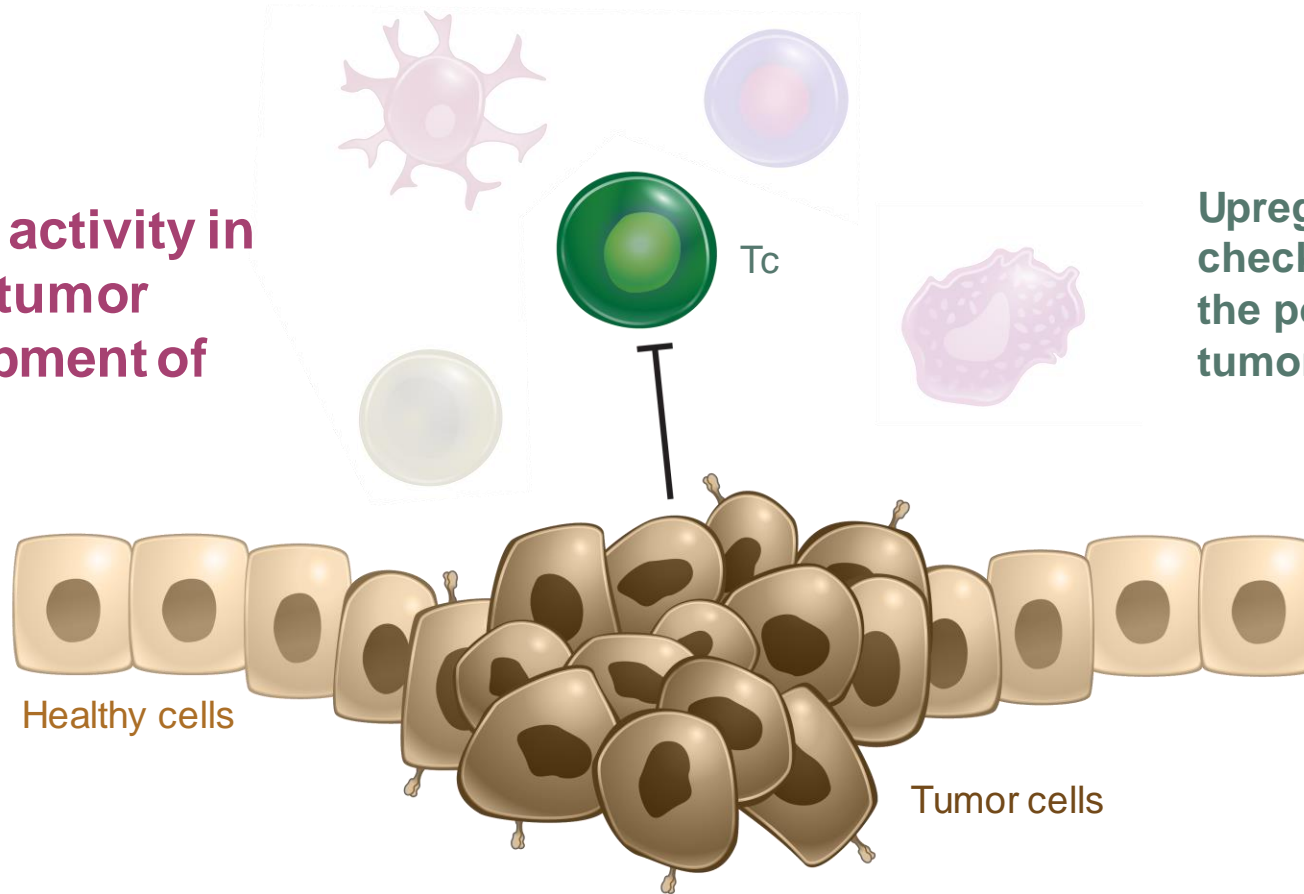
Tumors engage immunosuppressive mechanisms that lead to dysfunctional or fewer TILs in the tumor microenvironment



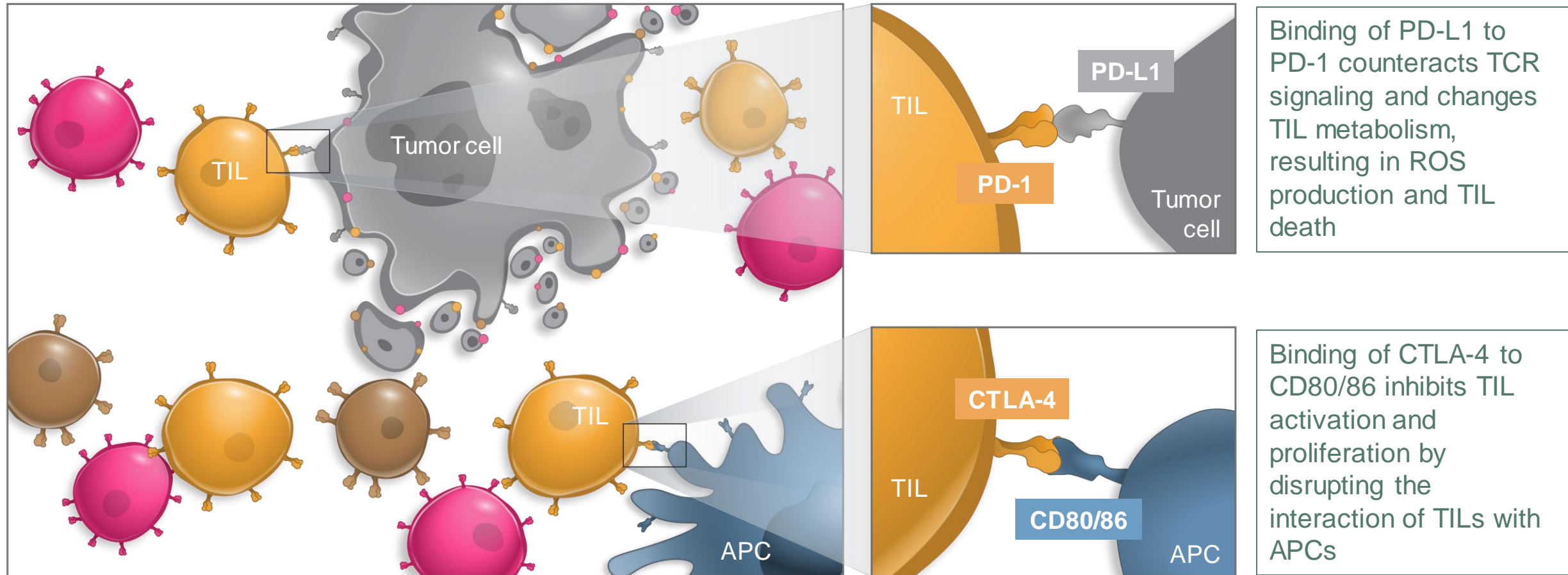
Tumors can modify the tumor microenvironment to suppress the activity of TILs

Tumors engage immunosuppressive mechanisms that lead to dysfunctional or fewer TILs in the tumor microenvironment

Suppression of TIL activity in the TME facilitates tumor growth and development of cancer



Engagement of checkpoint pathways aid in tumor evasion of TILs



APC, antigen-presenting cell; CD, cluster of differentiation; CTLA-4; cytotoxic T lymphocyte antigen-4; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; ROS, reactive oxygen species; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.
RaskovH, et al. *Br J Cancer*. 2021;124:359.

Summary

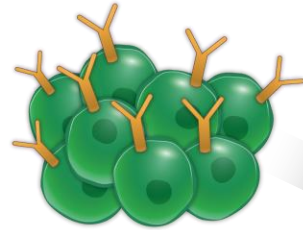
- Foreign or abnormal cells are eliminated through immune surveillance using the innate and adaptive immune systems
- T cells recognize antigens on the surface of foreign or abnormal cells, including tumors, and are activated and regulated by other cells in the immune system
 - T cells in the tumor microenvironment that bind to TSAs are called TILs
- Tumor cells can suppress the activity of TILs by inhibiting immune cells in the TME or upregulating of immune checkpoint molecules

Evolution of Immunotherapy Approach for Cancer Treatment

The immune system can be harnessed for the treatment of cancer

Adoptive cell transfer immunotherapy

TCR-engineered T cells
Peripheral blood T cells with modified TCR to recognize TAA



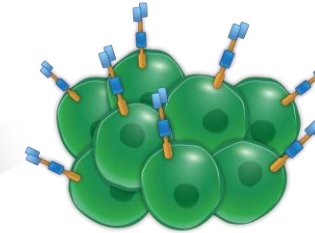
TIL

Autologous TILs expanded ex vivo and reintroduced in patient for tumor elimination



CAR T cells

Peripheral blood T cells expressing CAR to recognize TAA



Other immunotherapy

Cancer vaccines

Stimulate immune system against tumor



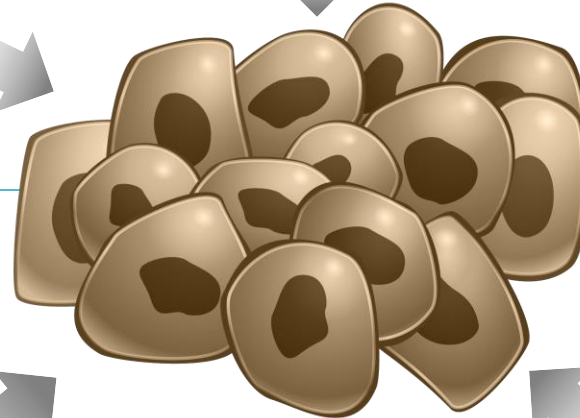
Cytokines

Stimulate proliferation of active T cells



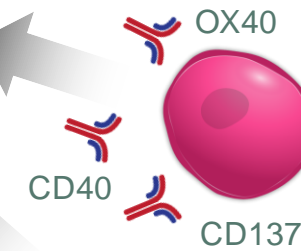
Oncolytic viral therapy

Infection of cancer cells with genetically engineered viruses that lead to cancer cell lysis



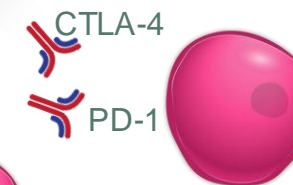
Immune agonists

Targeting costimulatory receptors boosts antitumor immunity



Checkpoint inhibitors

Inhibition of PD-1, PD-L1, or CTLA-4 with monoclonal antibodies that enable T-cell mediated tumor regression



Bispecific antibodies

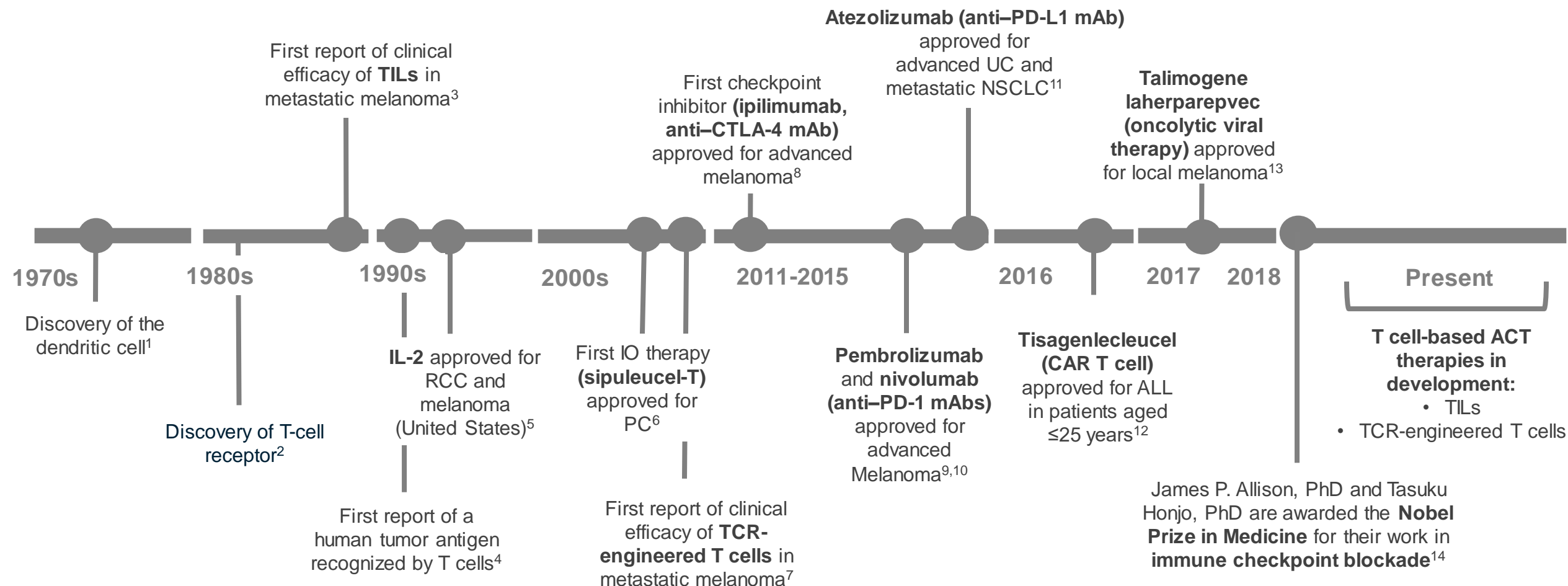
Direct engagement of T cells and tumor cells



CAR, chimeric antigen receptor; CD, cluster of differentiation; CTLA-4, cytotoxic T lymphocyte antigen-4; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TAA, tumor-associated antigens; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

Rahma OE. Overview of Immunotherapy for Cancer. Clinical Care Options. 2016.

Select milestones in cancer immunotherapy

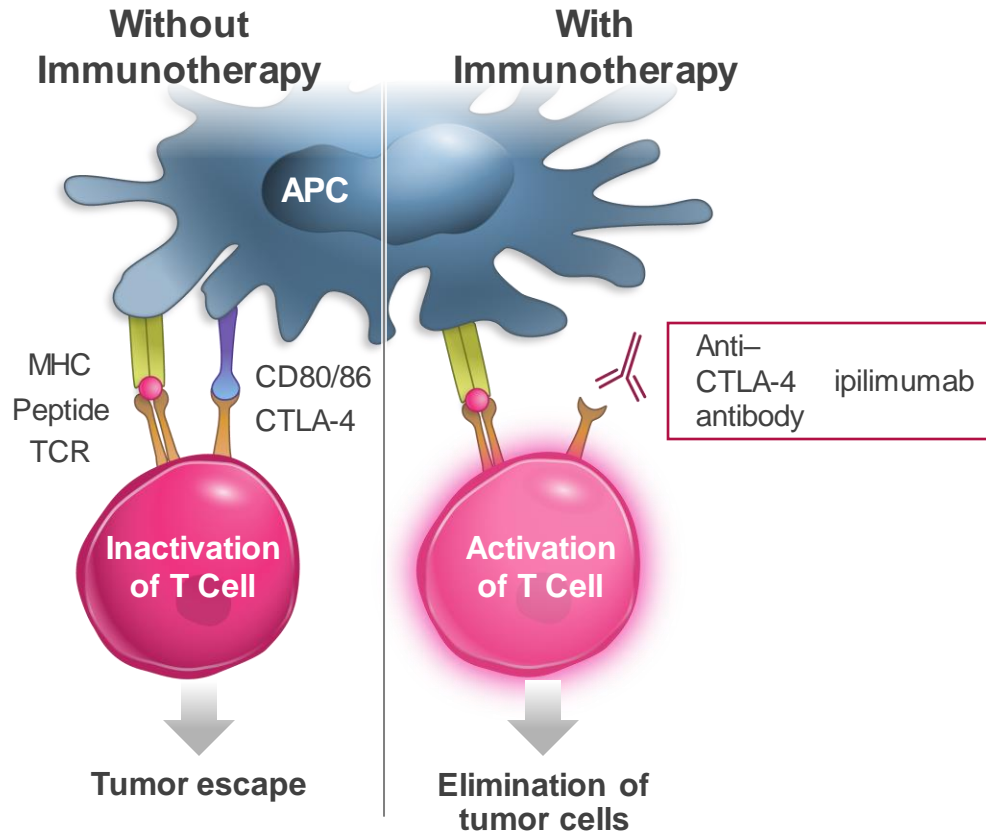


ACT, adoptive cell transfer; ALL, acute lymphoblastic leukemia; CAR, chimeric antigen receptor; CTLA4, cytotoxic T lymphocyte antigen-4; IL, interleukin; IO, immuno-oncology; mAb, monoclonal antibody; NSCLC, non-small-cell lung cancer; PC, prostate cancer; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; RCC, renal cell carcinoma; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; UC, urothelial carcinoma.

References in Notes section.

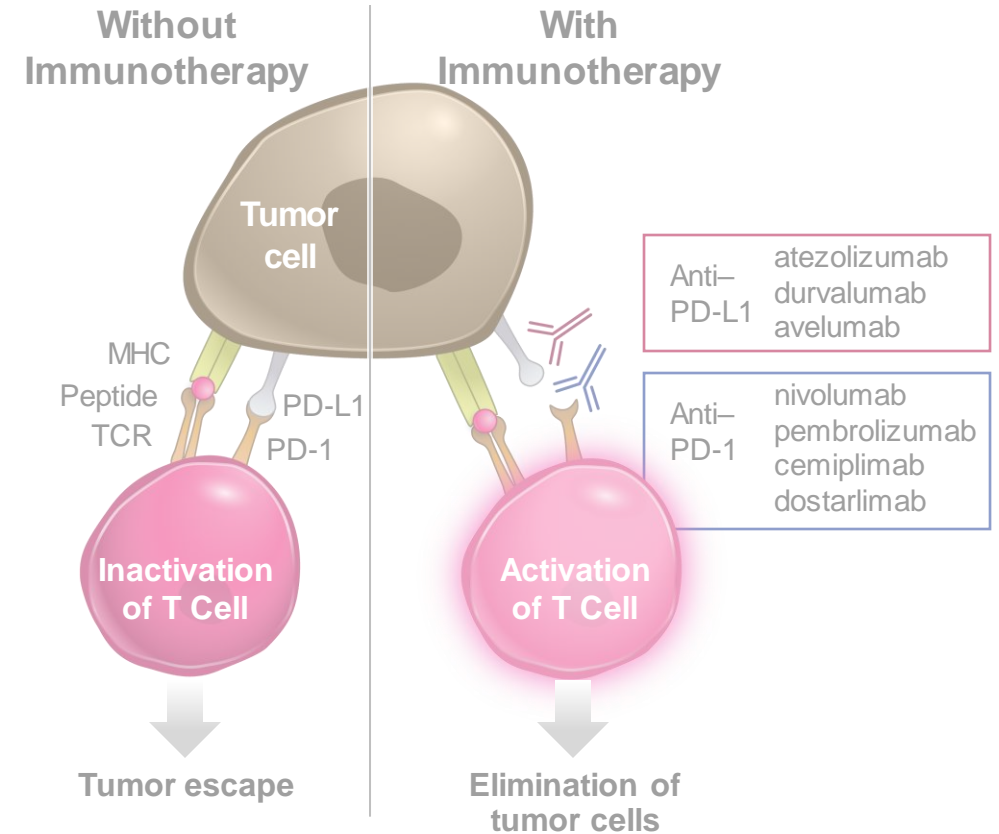
Immunotherapy with checkpoint inhibitors activates T cells within the tumor microenvironment

CTLA-4 inhibition



Inhibition of the CTLA-4/CD80(86) axis between APCs and T cells with anti-CTLA-4 antibodies leads to T-cell activation and tumor cell elimination

PD-1/PD-L1 inhibition

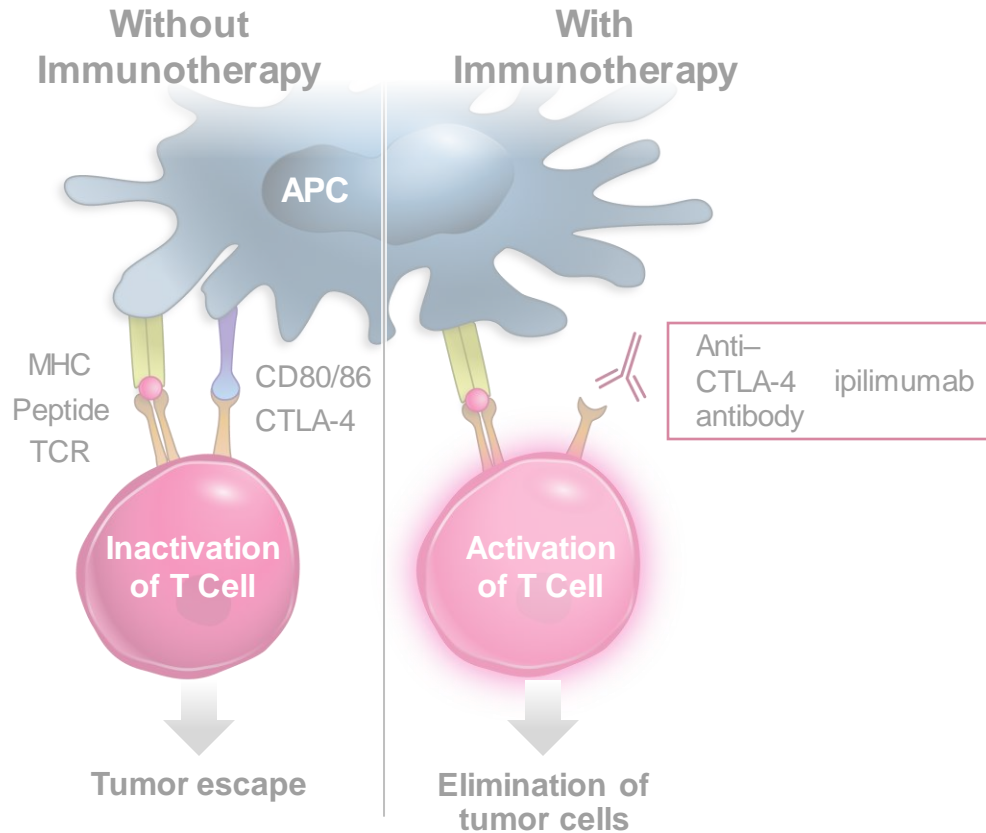


Inhibition of the PD-1/PD-L1(2) axis between tumor cells and T cells with anti-PD-(L)1 antibodies leads to T-cell activation and tumor cell elimination

APC, antigen-presenting cell; CD, cluster of differentiation; CTLA-4, cytotoxic T lymphocyte antigen-4; MHC, major histocompatibility complex; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; TCR, T-cell receptor.
Soularue E, et al. *Gut*. 2018;67:2056.

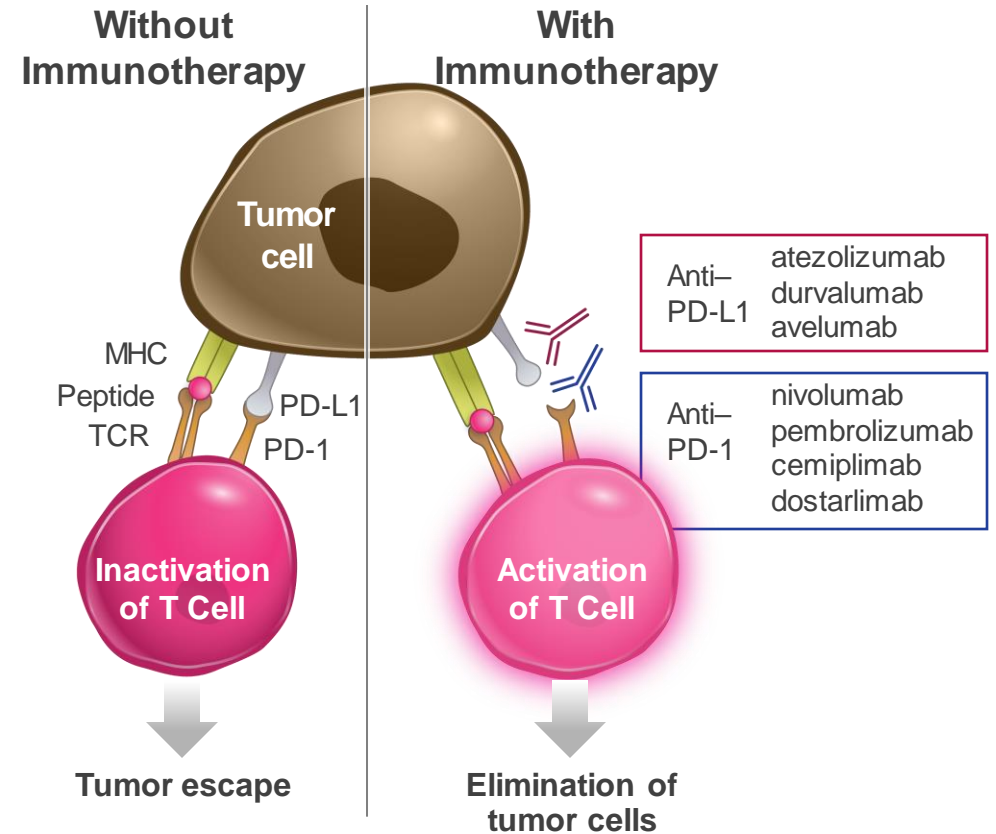
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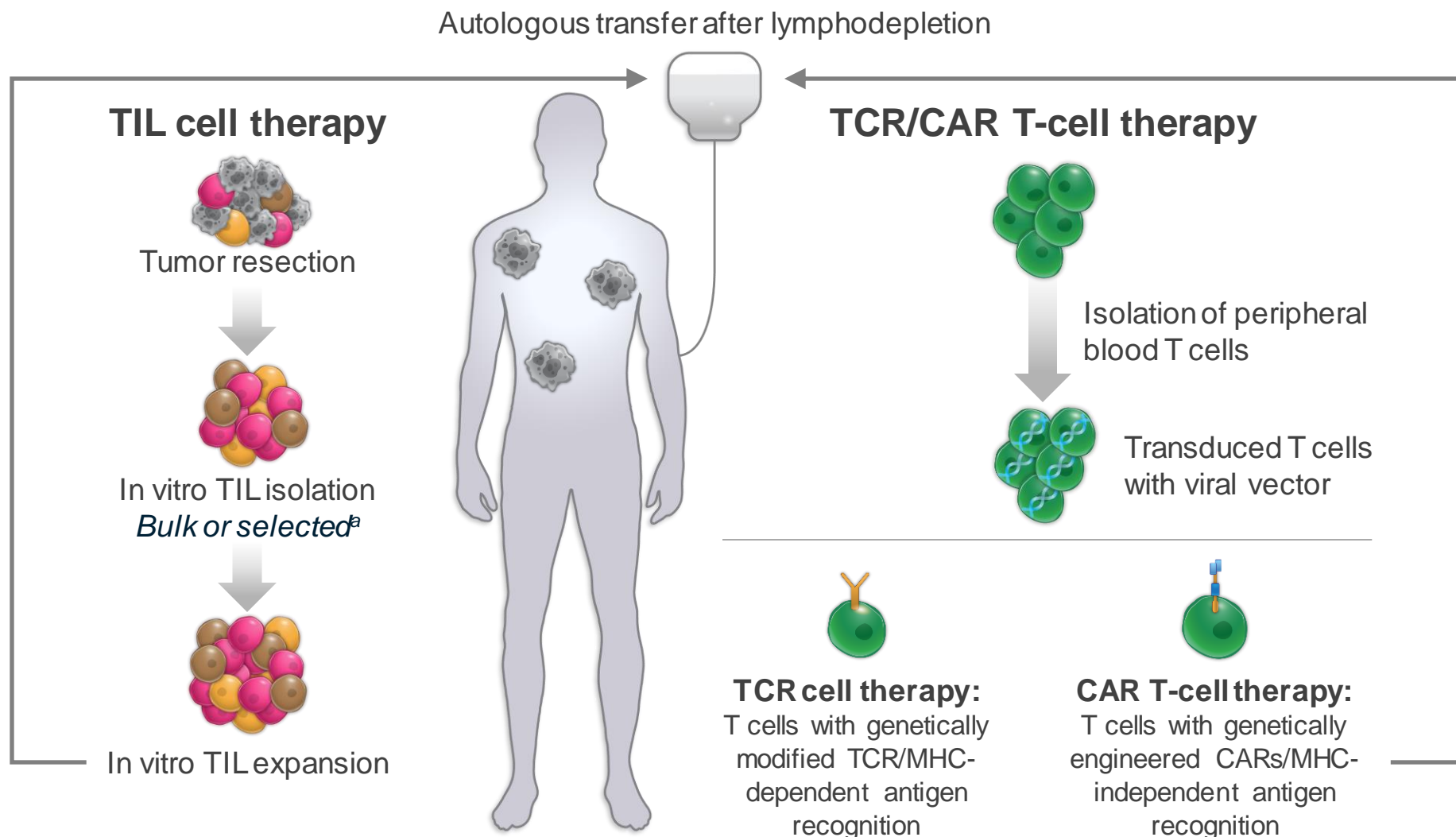
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Soularue E, et al. *Gut*. 2018;67:2056.

Adoptive cell transfer immunotherapy introduces autologous or genetically-modified T cells into the tumor microenvironment



CAR, chimeric antigen receptor; MHC, major histocompatibility complex; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

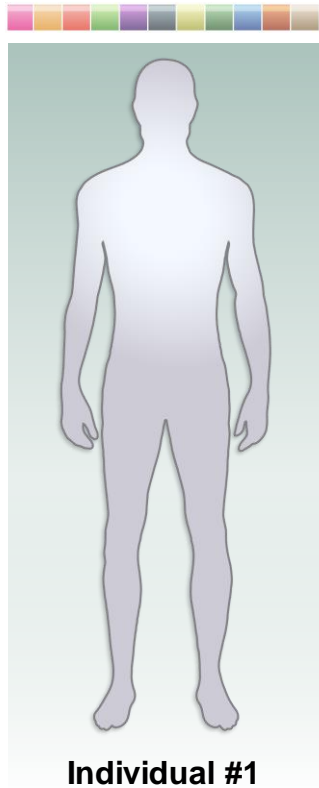
^a Bulk TIL refers to non-selected TILs; selected TILs refers to TILs selected against specific antigens.

Rohaani MW, et al. *Virchows Arch.* 2019;474:449.

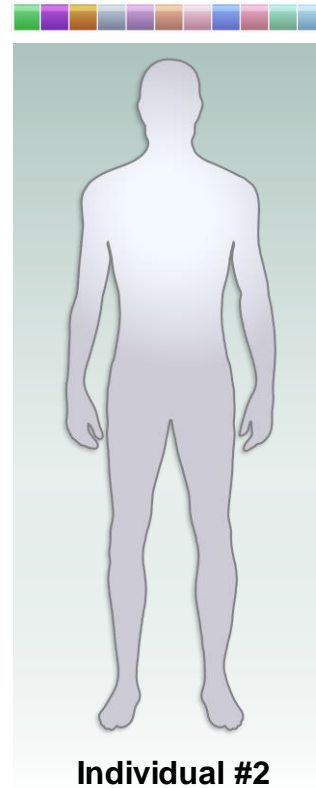
Rationale for TIL cell therapy in solid tumors

Solid tumors present a wide array of TSAs, yet fewer than 1% of TSAs are shared across patients with certain solid tumors¹

Individual #1 TSA profile



Individual #2 TSA profile



<1% Overlap
in TSAs



TILs are polyclonal and recognize a multitude of an individual's TSAs

TIL cell therapy has the potential to overcome challenges that make CAR T-cell and TCR therapy impractical in solid tumors, including^{2,3}

1. Delivery of CAR T cells or TCR-modified T cells into TME
2. Immunosuppression of CAR T cells or TCR-modified T cells in TME
3. Lack of heterogeneous TSA expression in all tumor cells
4. Incidence of CRS and other autoimmune AEs with CAR T cells and TCR-modified T cells

AE, adverse event; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; TSA, tumor-specific antigen.

1. Tran E, et al. *Nat Immunol*. 2017;18:255. 2. Ternyls D. CAR T Cells Push Forward in Solid Tumors Following Positive Results in Hematology. <https://www.targetedonc.com/view/car-t-cells-push-forward-in-solid-tumors-following-positive-results-in-hematology>. Accessed March 17, 2021. 3. Fardis M, et al. *Cell & Gene Therapy Insights*. 2020;6:855.

Differences in active and adoptive cell transfer immunotherapies

	Adoptive cell transfer immunotherapy ¹			Active immunotherapy ²
	TIL	CAR	TCR	Checkpoint inhibitors
Target	TSA	Non-MHC cell surface proteins	MHC-peptide complex	CTLA-4, PD-1, PD-L1
Specificity	Polyclonal	Monoclonal	Monoclonal	Monoclonal
Production	Isolation from tumor and expansion ex vivo	Isolation from peripheral blood, transduction, and expansion ex vivo	Isolation from peripheral blood, transduction, and expansion ex vivo	mAb production in vitro
Preparative/supportive regimens	Yes/Yes	Yes/No	Yes/Varies	No/No
Main toxicity	Lymphodepletion- and IL-2-related	Lymphodepletion-related, CRS, neurological	Lymphodepletion-related, CRS	Immune-related
Approved indications	In progress	ALL, BCL, DLBCL, FL, MCL ³⁻⁷	In progress	Melanoma, NSCLC, SCLC, UC, RCC, HCC, CSCC, HNSCC, MCC, HL, CC, BC ⁸⁻¹⁴

ALL, acute lymphoblastic leukemia; BC, breast cancer; BCL, B-cell lymphoma; CAR, chimeric antigen receptor; CC, colorectal cancer; CRS, cytokine-release syndrome; CSCC, cutaneous squamous cell carcinoma; CTLA-4, cytotoxic T lymphocyte antigen-4; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HCC, hepatocellular carcinoma; HL, Hodgkin lymphoma; HNSCC, head and neck squamous cell carcinoma; IL, interleukin; mAb, monoclonal antibody; MCC, Merkel cell carcinoma; MCL, mantle cell lymphoma; MHC, major histocompatibility complex; NSCLC, non-small cell lung cancer; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; RCC, renal cell carcinoma; SCLC, small cell lung cancer; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; TSA, tumor specific antigen; UC, urothelial carcinoma.

References in Notes section.

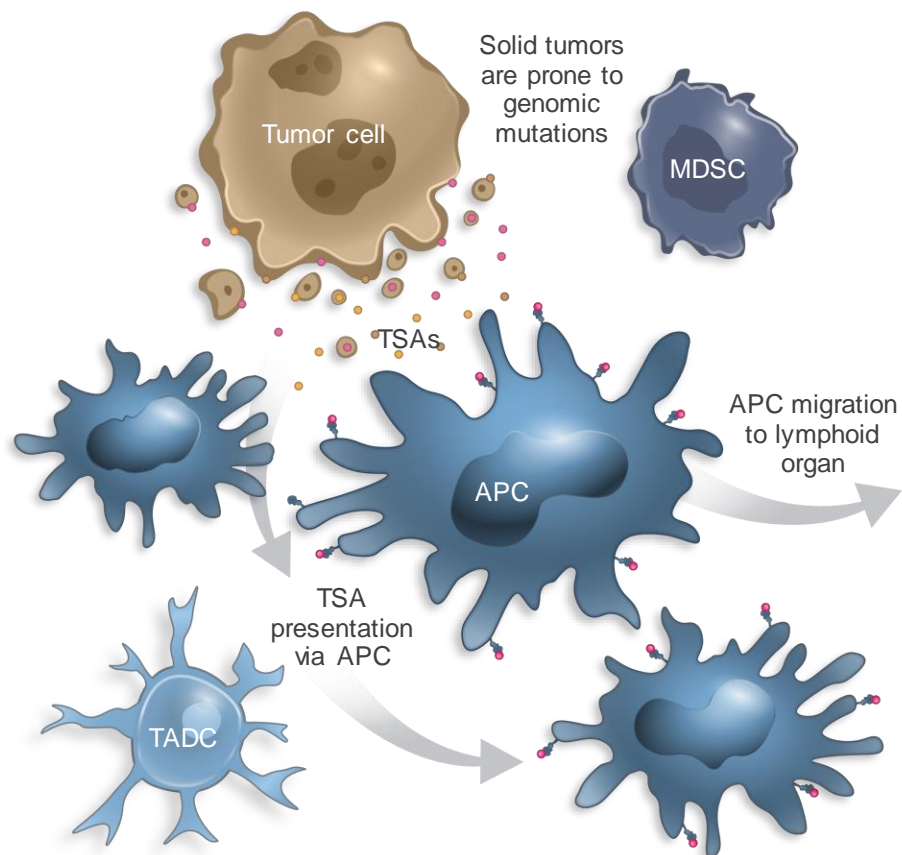
Summary

- The immune system can be harnessed for the treatment of cancer through adoptive cell transfer and other immunotherapies
 - Immunotherapy with checkpoint inhibitors activates T cells within the tumor microenvironment
 - Adoptive cell transfer immunotherapy introduces autologous or genetically-modified T cells into the tumor microenvironment
- There are three types of adoptive cell transfer immunotherapy
 - In TIL cell therapy, TILs are isolated from the tumor microenvironment, grown in vitro, and transferred to the same patient after lymphodepletion
 - In TCR/CAR T-cell therapy, peripheral blood T cells are isolated, transduced with TCR or CAR, grown in vitro, and transferred to the same patient after lymphodepletion

TIL Cell Therapy Mechanism of Action and Treatment Process

TILs bind to a myriad of TSAs and cause tumor cell death

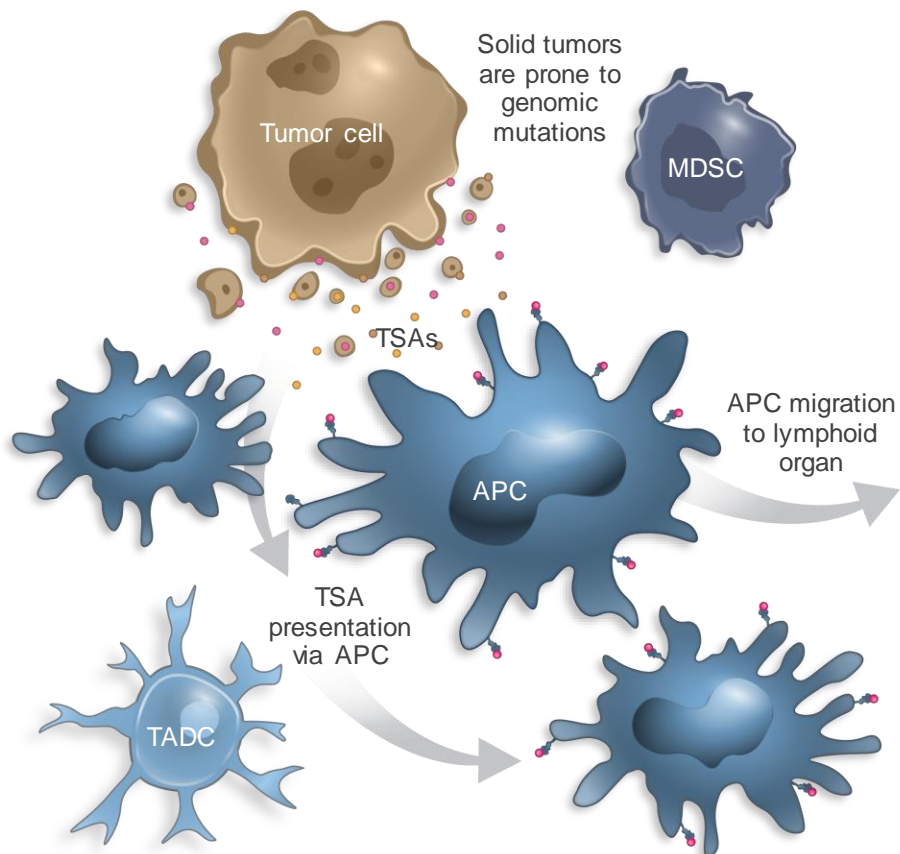
TSA antigen presentation via APC and APC migration from TME to lymphoid organ



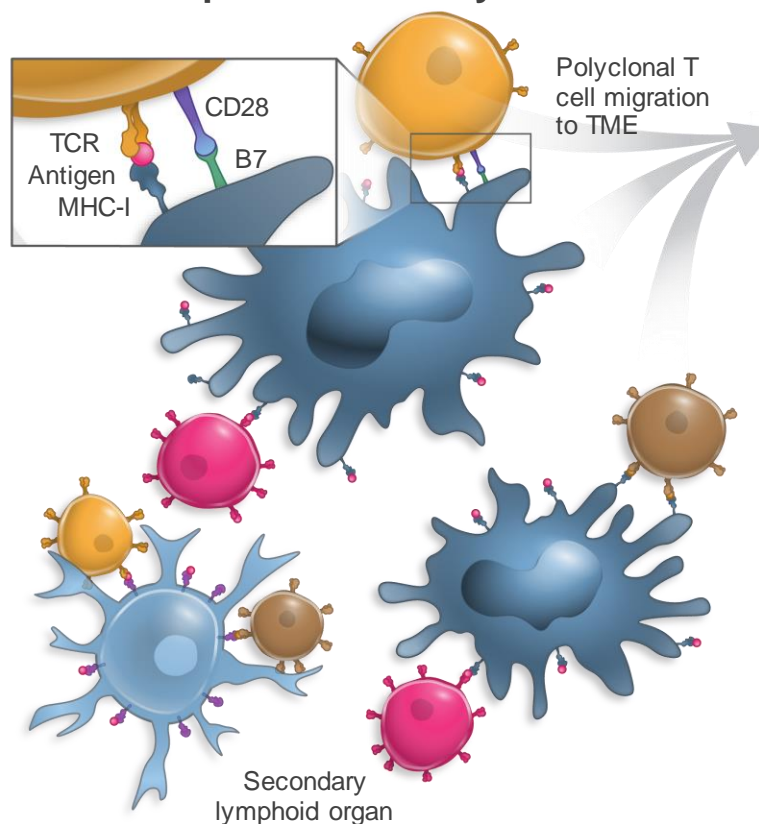
APC, antigen-presenting cell; TADC, tumor-associated dendritic cell; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; TSA, tumor-specific antigen
RaskovH, et al. *Br J Cancer*. 2021;124:359.

TILs bind to a myriad of TSAs and cause tumor cell death

TSA antigen presentation via APC and APC migration from TME to lymphoid organ

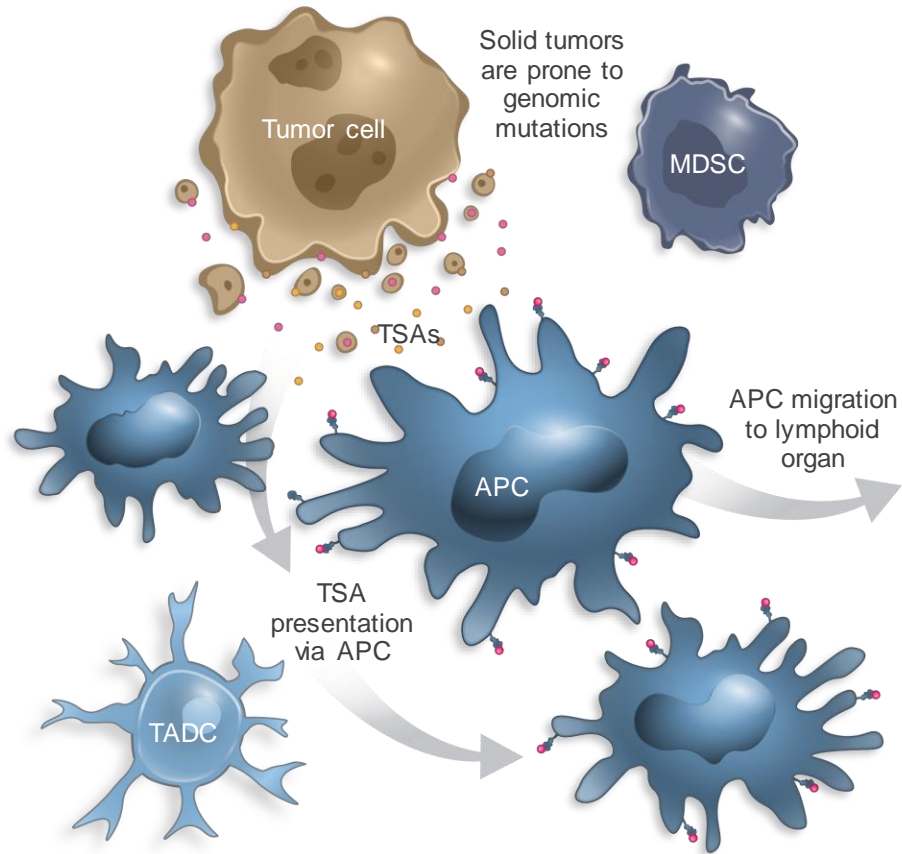


APCs prime naïve T cells to respond to an array of TSAs

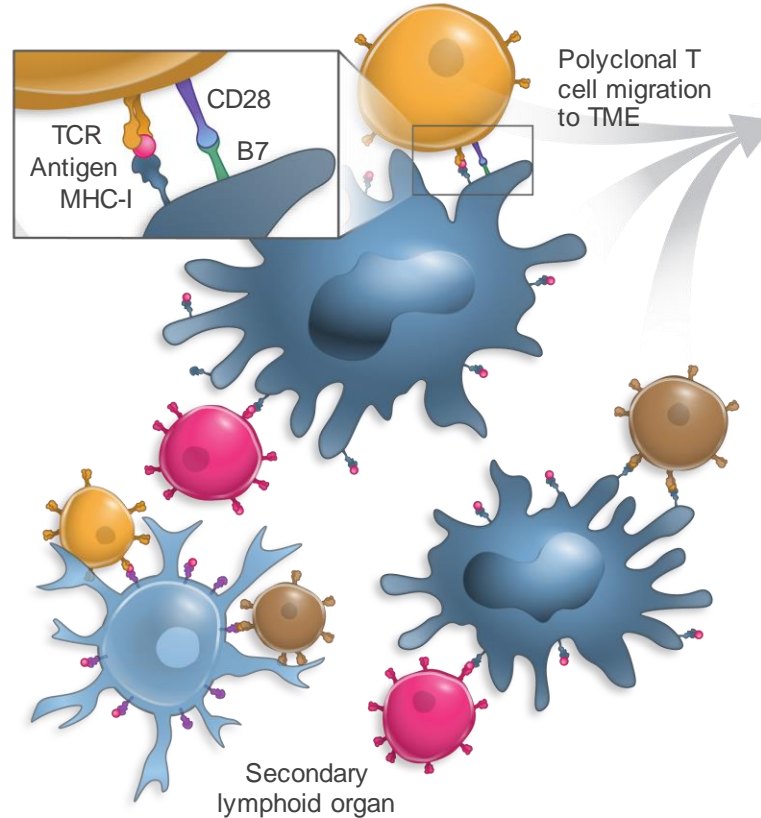


TILs bind to a myriad of TSAs and cause tumor cell death

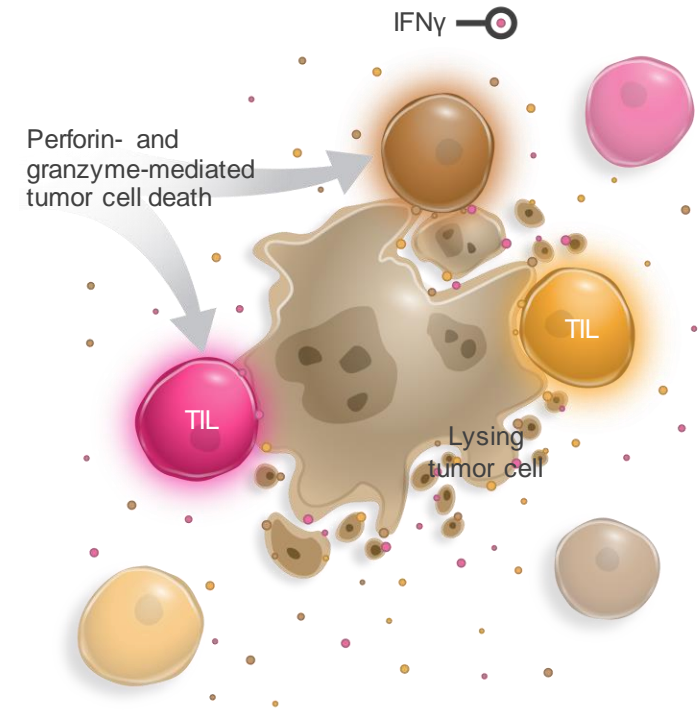
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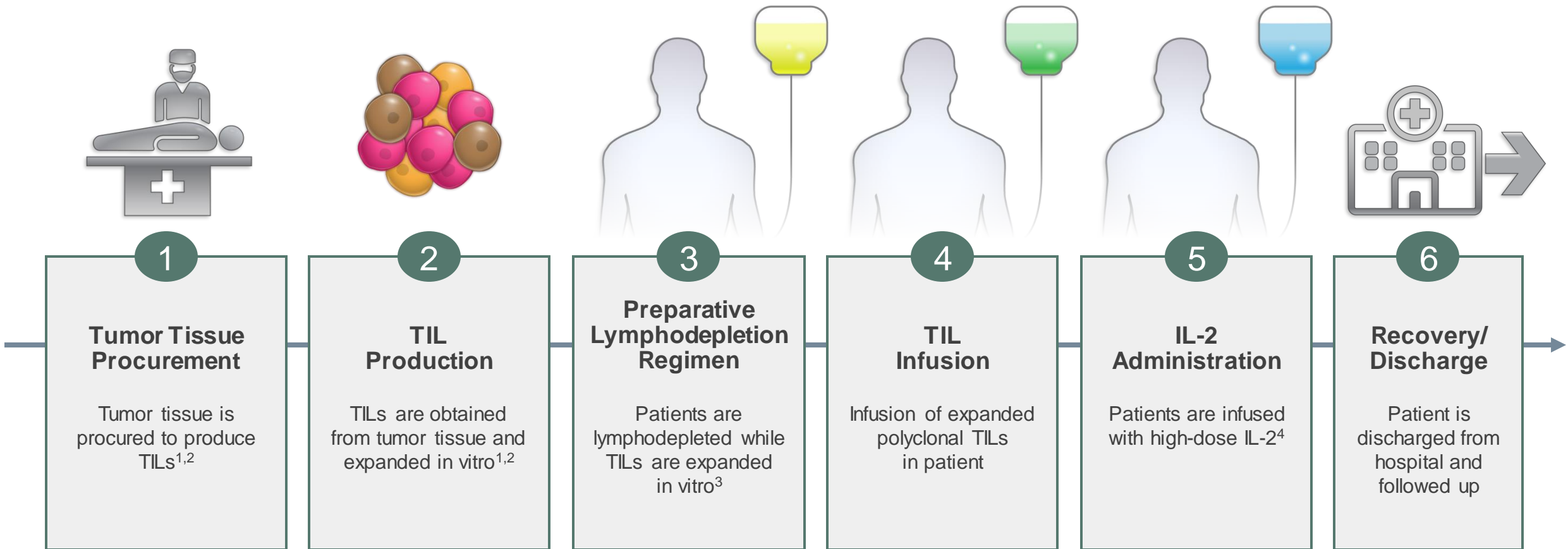


TILs enter TME, bind to TSAs, and cause tumor cell death



APC, antigen-presenting cell; IF, interferon; MHC, major histocompatibility complex; TADC, tumor-associated dendritic cell; TIL, tumor-infiltrating lymphocyte; TME, tumor microenvironment; TSA, tumor-specific antigen
RaskovH, et al. *Br J Cancer*. 2021;124:359.

TIL cell therapy stages



IL, interleukin; TIL, tumor-infiltrating lymphocyte.

1. Itzhaki O, et al. *J Immunother*. 2011;34:212. 2. Dudley ME, et al. *J Immunother*. 2003;26:332. 3. Dudley ME, et al. *J Clin Oncol*. 2008;26:5233. 4. Atkins MB, et al. *J Clin Oncol*. 1999;17:2105.

Tumor tissue is procured to produce TILs



1

Tumor Tissue Procurement

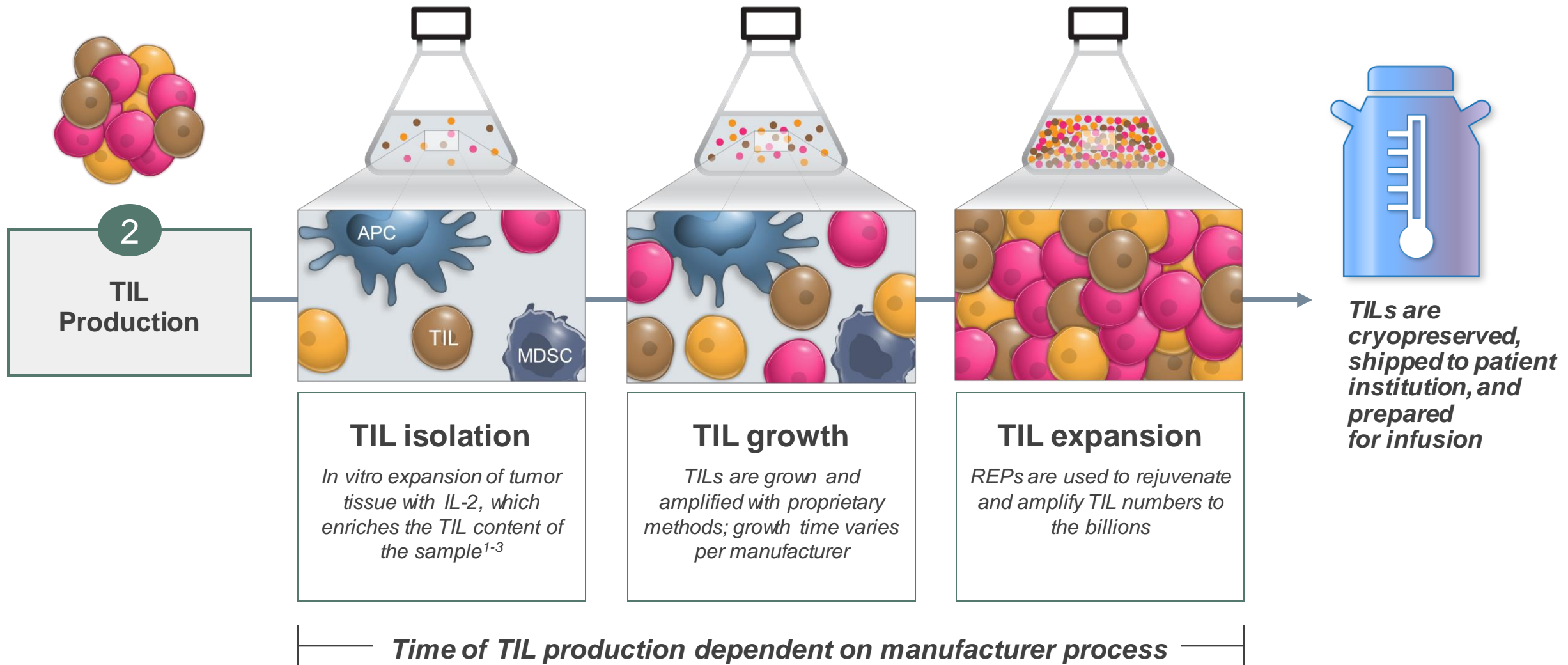
Objective

- To procure viable solid tumor tissue to ensure successful production of TILs

Considerations

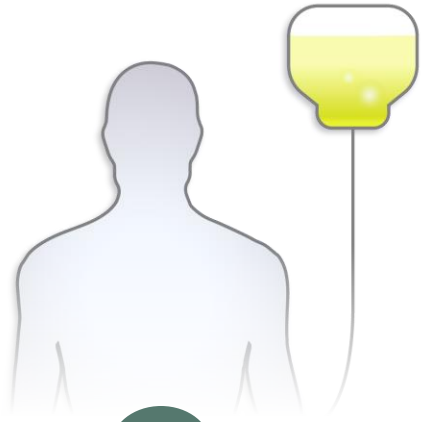
- Sufficient tumor sample of appropriate quality for TIL production
- Aseptic technique to limit sample contamination

TILs are produced in vitro with procured tumor tissue



APC, antigen-presenting cell; IL-2, interleukin-2; MDSC, myeloid-derived suppressor cell; REP, rapid expansion protocol; TIL, tumor-infiltrating lymphocyte.
1. Rohaan MW, et al. *Virchows Arch.* 2019;474:449. 2. Itzhaki O, et al. *J Immunother.* 2011;34:212. 3. Dudley ME, et al. *J Immunother.* 2003;26:332.

Patients are lymphodepleted before TIL administration



3

Preparative Lymphodepletion Regimen

Objective

Reduction of MDSC and TADC activity that limits the antitumor effect of TILs and eliminates T cells from host to decrease “cytokine sink”

Rationale

- Lymphodepletion has enhanced the antitumor effects of TILs in murine models, with a direct relationship between the extent of lymphodepletion and the magnitude of the antitumor effect of transferred TILs^{1,2}
 - Several mechanisms explain the enhanced antitumor effect of TILs with lymphodepletion, including the elimination of Tregs and cellular “sinks” for IL-7 and IL-15^{3,4}

Gy, gray; IL, interleukin; MDSC, myeloid-derived suppressor cell; NMA, non-myeloablative; TADC, tumor-associated dendritic cell; TBI, total-body irradiation; TIL, tumor-infiltrating lymphocyte; Treg, regulatory T cell.

1. Muranski P, et al. *Nat Clin Pract Oncol*. 2006;3:668. 2. Wrzesinski C, et al. *J Clin Invest*. 2007;117:492. 3. Antony PA, et al. *J Immunol*. 2005;174:2591. 4. Gattinoni L, et al. *J Exp Med*. 2005;202:907. 5. Dudley ME, et al. *J Clin Oncol*. 2008;26:5233.

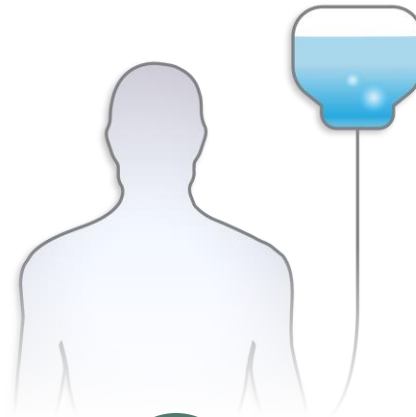
After lymphodepletion, patients are infused with TILs, followed with IL-2 infusion



4

TIL Infusion

- One-time infusion of autologous TILs
- Polyclonal TILs that recognize TSAs that are patient-specific



5

IL-2 Administration

- Short course of high-dose IL-2
- Supports T-cell activity



6

Recovery/Discharge

- Recovery/reconstitution of patient blood cell counts before leaving hospital

Summary

- Tumor cell death mediated by TILs involve
 - Presentation of multiple TSAs by APCs
 - APC priming on naïve T cells with multiple TSAs
 - T cells primed with multiple TSAs enter the TME (TILs), bind to different TSAs, and cause tumor cell death by releasing perforins and granzymes
- TIL cell therapy involves
 - Tumor tissue procurement to produce TILs
 - TIL production in vitro
 - Preparative lymphodepletion
 - Infusion of TILs
 - IL-2 administration
 - Recovery from treatment and discharge from hospital

TIL Cell Therapy Clinical Data Summary

Select completed TIL cell therapy clinical trials in patients with melanoma (1 of 2)

Year	Sponsor	N	Number of prior therapies ^a	Lymphodepletion regimen	TIL type ^b	IL-2 doses	ORR, %	DCR, %	DoR, mo
1988 ¹	NCI	20	ND	Cy	Bulk	3-14	55	ND	2 to <13
1994 ²	NCI	86	2-3	± Cy	Bulk	≤15	34	ND	2 to 53+
2002 ³	NCI	13	ND	Cy + Flu	Selected	5-12	46	ND	2 to 24+
2005 ⁴	NCI	35	2-3	Cy + Flu	Bulk	4-15	51	ND	2 to 30+
2010 ⁵	NCI	17	ND	Cy + Flu ± TBI	Bulk/ Selected	4-10	41	ND	4 to 44
2012 ⁶	MD Anderson Cancer Center	31	≥2	Cy + Flu	Bulk	2	58	97	ND
2012 ⁷	H. Lee Moffitt Cancer Center	19	2-4	Cy + Flu	Selected	≤15	26	47	ND

Cy, cyclophosphamide; DCR, disease control rate; DoR, duration of response; Flu, fludarabine; IL, interleukin; NCI, National Cancer Institute; ND, not disclosed; ORR, overall response rate; TBI, total-body irradiation; TIL, tumor-infiltrating lymphocyte.

^aPatients in these studies did not receive checkpoint inhibitors as prior treatment. ^bBulk TIL refers to non-selected TILs; selected TILs refers to TILs selected against specific antigens.

References in Notes section.

Completed TIL cell therapy clinical trials in patients with melanoma (2 of 2)

Year	Sponsor	N	Number of prior therapies ^a	Lymphodepletion regimen	TIL type ^b	IL-2 doses	ORR, %	DCR, %	DoR, mo
2012 ¹	Copenhagen University Hospital	11	ND	Cy + Flu	Bulk	ND	18	36	ND
2013 ²	Sheba Medical Center	80	ND	Cy + Flu	Bulk/young	≤15	40	81	ND
2016 ³	NCI	101	ND	Cy + Flu ± TBI	Bulk	5 to >8	53	ND	14+ to 53+
2019 ⁴	Iovance Biotherapeutics	66 ^c	3	Cy + Flu	Bulk	≤6	38	80	NR
2019 ⁵	University Health Network/Princess Margaret Cancer Centre	12	0-3	Cy + Flu	Bulk	9-10	25	58	ND
2020 ⁶	Netherlands Cancer Institute	13	2-3	Cy + Flu	Bulk/young	1-9	38	46	2 to 108+

Cy, cyclophosphamide; DCR, disease control rate; DoR, duration of response; Flu, fludarabine; IL, interleukin; ND, not disclosed; NR, not reached; ORR, overall response rate; TIL, tumor-infiltrating lymphocyte.

^aPatients in studies highlighted in light green did not receive prior immune checkpoint inhibitors; patients in studies highlighted in dark green received prior immune checkpoint inhibitors. ^bBulk TIL refers to non-selected TILs; young TILs were cultured for ≤2 weeks. ^cCohort 2 only.

References in Notes section.

TILs in patients with metastatic melanoma: study design and endpoints

First clinical study of TIL infusion with prior lymphodepletion and subsequent IL-2 treatment

N=20

Patient characteristics

- Aged ≥ 18 years with metastatic melanoma
- Prior systemic therapy (n=14)

**Surgical resection
of tumor**

Bulk TIL production^a
4-8 weeks

Treatment

Treatment

Cyclophosphamide
(25 mg/kg)

36 h

Bulk TIL infusion
($\leq 2 \times 10^{11}$ cells, 30-60 min)
 ≤ 7 infusions, not cryopreserved

*After first
TIL infusion*

IL-2 infusion
(100,000 IU/kg, every 8 hours)
until DLT

Endpoints

ORR, DOR, safety

DOR, duration of response; DLT, dose-limiting toxicity; IL, interleukin; ORR, overall response rate; TIL, tumor-infiltrating lymphocyte.

^a Bulk TIL refers to non-selected TILs.

Rosenberg SA, et al. *N Engl J Med*. 1988;319:1676.

Efficacy and safety of TILs in patients with metastatic melanoma

First clinical study of TIL infusion with prior lymphodepletion and subsequent IL-2 treatment

Efficacy

Response, n (%)	Patients (N=20)
ORR	11 (55)
CR	1 (5)
PR	10 (50)
NR	9 (45)

- IL-2 doses ranged from 3 to 14
- Responses were observed in patients who were IL-2 treatment-naïve and who had progressed after IL-2 treatment
- DOR ranged from 2 to >13 months

Safety

n (%)	Patients (N=20)
Hyperbilirubinemia	20 (100)
Thrombocytopenia	17 (85)
Anemia	16 (80)
Hypotension	13 (65)
Nausea/vomiting	11 (55)
Chills	10 (50)
Elevated creatinine	10 (50)
Oliguria	10 (50)
Diarrhea	9 (45)
Neutropenia	4 (20)

CR, complete response; DOR, duration of response; IL, interleukin; NR, no response; ORR, overall response rate; PR, partial response; TIL, tumor-infiltrating lymphocyte.
Rosenberg SA, et al. *N Engl J Med*. 1988;319:1676.

TILs in patients with metastatic melanoma: study design and endpoints

Clinical study of TIL infusion with/without prior lymphodepletion and subsequent IL-2 treatment

N=86

Patient characteristics

- Aged ≥ 11 years with metastatic melanoma
- ECOG PS ≤ 3
- Prior systemic therapy (n=83))

**Surgical
resection
of tumor**

**Bulk TIL
production^a
2-12 weeks**

Treatment

Treatment

**No
cyclophosphamide**

**Cyclophosphamide
(25 mg/kg)**

36 h

Bulk TIL infusion
($\sim 1.5 \times 10^{11}$ cells, 30-60 min)
 ≤ 4 infusions, not cryopreserved

After first
TIL infusion

IL-2 infusion
(720,000 IU/kg, every 8 hours)
 ≤ 15 doses

Endpoints

ORR, DOR, safety

DOR, duration of response; ECOG PS, Eastern Cooperative Oncology Group performance status; IL, interleukin; ORR, overall response rate; TIL, tumor-infiltrating lymphocyte.

^a Bulk TIL refers to non-selected TILs.

Rosenberg SA, et al. *J Natl Cancer Inst.* 1994;86:1159.

Efficacy and safety of TILs in patients with metastatic melanoma

Clinical study of TIL infusion with/without prior lymphodepletion and subsequent IL-2 treatment

Efficacy

Response, n (%)	No CPM (n=29)	CPM (n=57)	All (N=86)
ORR	9 (31)	20 (35)	29 (34)
CR	4 (14)	1 (2)	5 (6)
PR	5 (17)	19 (33)	24 (28)

- Lymphodepletion with cyclophosphamide did not result in significant improvement in response or changes to DOR (2 to 53+ months)

Safety

Gr 3-4 events, n	No CPM	CPM	All
TEAEs	51	94	145
Chills	5	35	40
Pruritus	3	3	6
Mucositis	1	1	2
Nausea	12	50	62
Diarrhea	4	29	33
Malaise	7	6	13
Oliguria ^a	10	22	32
Anuria ^b	2	4	6

CPM, cyclophosphamide; CR, complete response; DOR, duration of response; Gr, grade; IL, interleukin; ORR, overall response rate; PR, partial response; TEAE, treatment-emergent adverse event; TIL, tumor-infiltrating lymphocyte.

^a Oliguria defined as <80 mL/8h. ^b Anuria defined as <240 mL/24h.

Rosenberg SA, et al. *J Natl Cancer Inst.* 1994;86:1159.

TILs in patients with metastatic melanoma: study design and endpoints

Clinical study of TIL infusion with prior lymphodepletion (with/without TBI) and subsequent IL-2 treatment

N=101

Inclusion criteria:

- Aged ≥ 18 years with metastatic melanoma
- ECOG PS < 2

**Surgical
resection
of tumor**

**Bulk TIL
production**

Treatment

Treatment

**Cy (60 mg/kg/d) + Flu
(25 mg/m²/d)**

**Cy (60 mg/kg/d) + Flu
(25 mg/m²/d) +
1200 Gy TBI**

5 d

**Bulk TIL infusion
not cryopreserved**

**IL-2 infusion
(720,000 IU/kg,
every 8 hours)
until DLT**

Endpoints

ORR, OS, safety

Cy, cyclophosphamide; DLT, dose-limiting toxicity; ECOG PS, Eastern Cooperative Oncology Group performance status; Flu, fludarabine; Gy, gray; IL, interleukin; ORR, overall response rate; OS, overall survival; TBI, total body irradiation; TIL, tumor-infiltrating lymphocyte.
Goff SL, et al. *J Clin Oncol*. 2016;34:2389.

— NMA

— 1,200 TBI

Id safety of TILs in patients with metastatic melanoma

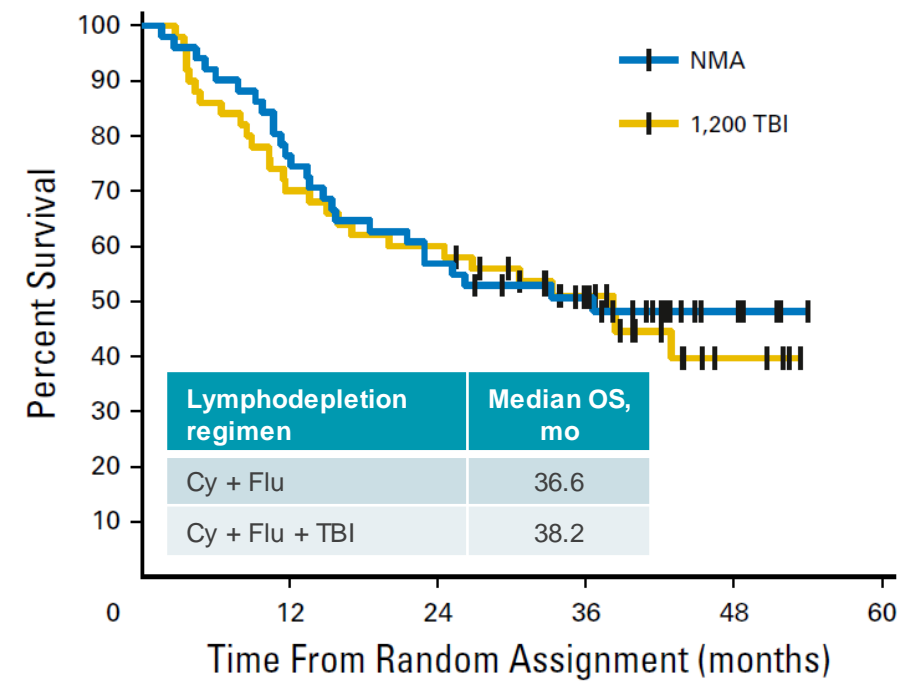
clinical study of TIL infusion with prior lymphodepletion (with/without TBI) and subsequent IL-2 treatment

ORR
Median follow-up: 40.9 months

Response, n (%)	Cy + Flu (n=51)	Cy + Flu + TBI (n=50)	Total (N=101)
ORR	23 (45)	31 (62)	54 (54)
CR	12 (23)	12 (24)	24 (24)
PR	11 (22)	19 (38)	30 (30)

- Mean IL-2 doses received: 8
- Results were similar using chemotherapy preparative regimens with or without addition of TBI
- The toxicities of treatment were largely a result of nonmyeloablative chemotherapy and administration of IL-2

OS
Median follow-up: 40.9 months



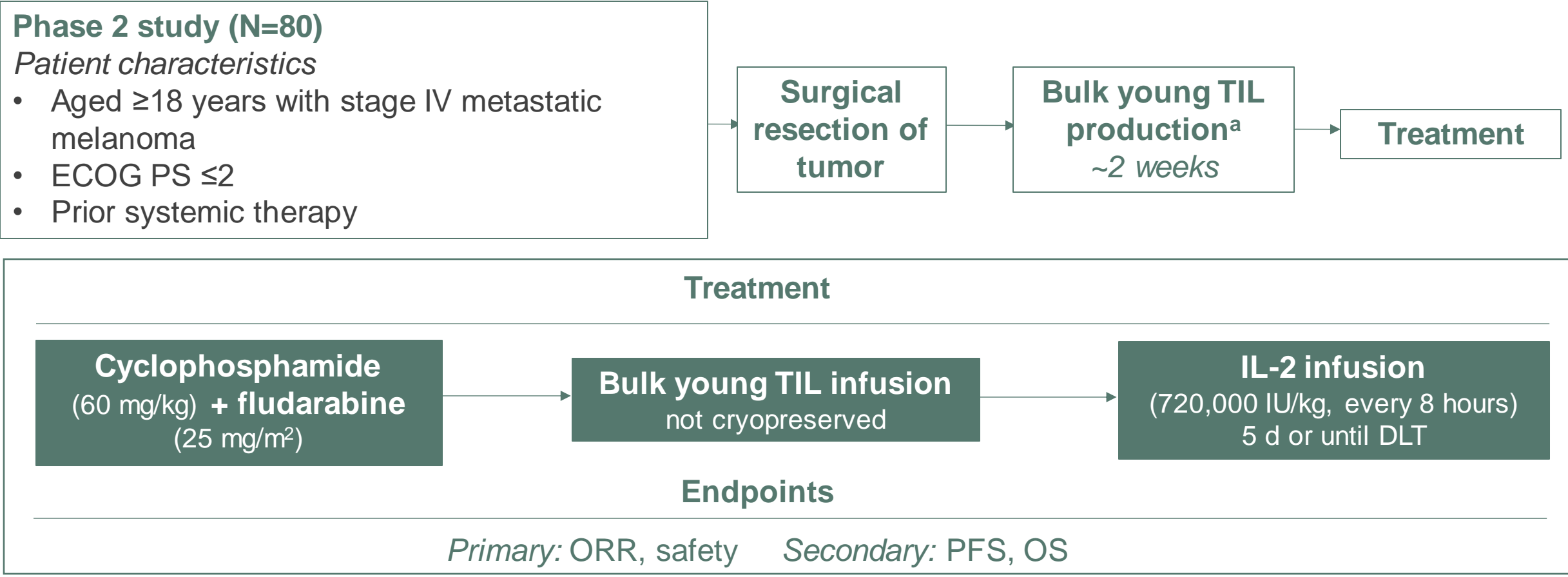
No. at risk						
NMA	51	39	30	21	6	0
1,200 TBI	50	35	30	18	4	0

CR, complete response; Cy, cyclophosphamide; Flu, fludarabine IL, interleukin; ORR, overall response rate; OS, overall survival; PR, partial response; TBI, total body irradiation; TIL, tumor-infiltrating lymphocyte.
Goff SL, et al. *J Clin Oncol.* 2016;34:2389.



TILs in patients with metastatic melanoma (NCT00287131): study design and endpoints

Clinical study of young TIL infusion with prior lymphodepletion (cyclophosphamide + fludarabine) and subsequent IL-2 treatment^{1,2}



DLT, dose-limiting toxicity; ECOG PS, Eastern Cooperative Oncology Group performance status; IL, interleukin; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; TIL, tumor-infiltrating lymphocyte.
^a TILs cultured for two weeks and not selected against antigens.
1. Besser MJ, et al. *Clin Cancer Res.* 2013;19:4792. 2. Clinicaltrials.gov. <https://clinicaltrials.gov/ct2/show/NCT00287131>. Accessed March 17, 2021.



Efficacy of TILs in patients with metastatic melanoma (NCT00287131)

Clinical study of TIL infusion with prior lymphodepletion (cyclophosphamide + fludarabine) and subsequent IL-2 treatment

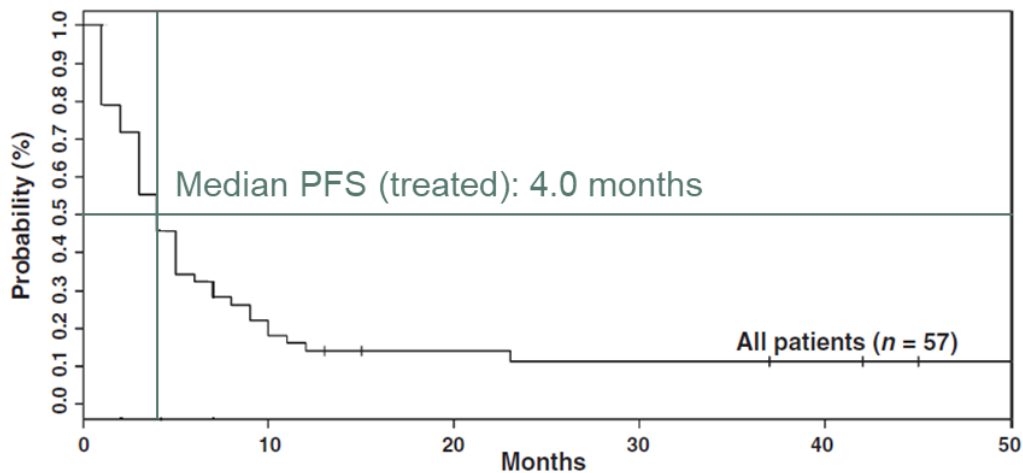
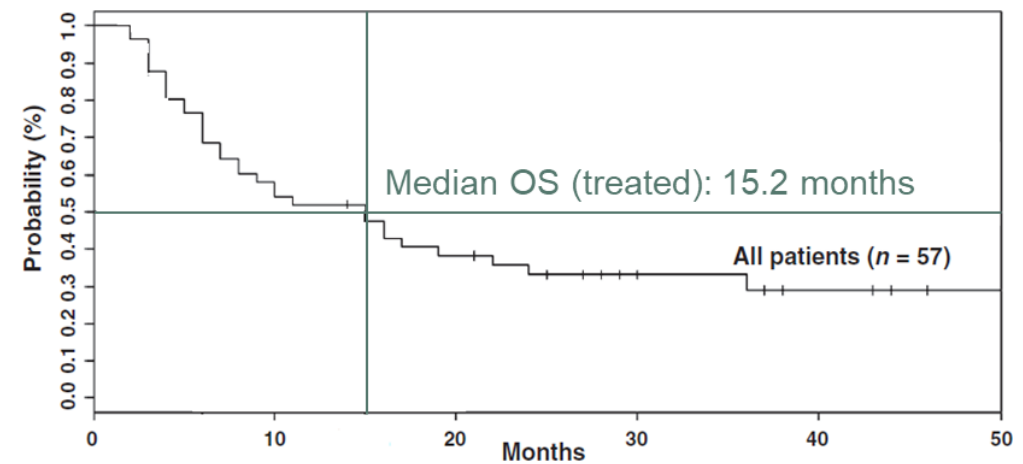
ORR

Median follow-up: 28 months

Response, n (%)	Treated patients (N=57)
ORR	23 (40)
CR	5 (9)
PR	18 (32)
SD	14 (25)
PD	20 (35)

From the 80 enrolled patients:

- 8 (10%) underwent surgery but TILs could not be grown
 - 3 (4%) refused to enter the study
- 11 (14%) experienced clinical deterioration before TIL infusion
 - 1 (1%) could not be evaluated



CR, complete response; IL, interleukin; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD stable disease; TIL, tumor-infiltrating lymphocyte.
Besser MJ, et al. Clin Cancer Res. 2013;19:4792.



Safety of TILs in patients with metastatic melanoma (NCT00287131)

Clinical study of TIL infusion with prior lymphodepletion (cyclophosphamide + fludarabine) and subsequent IL-2 treatment

Median follow-up: 28 months

Grade 3-4 AEs, n (%)	Treated patients (N=57)
Pulmonary congestion	27 (47)
Renal failure	11 (19)
Prolonged hypotension	13 (23)
Hyperbilirubinemia	8 (14)
Diarrhea	7 (12)
Cardiac toxicity	1 (2)
Confusion	4 (7)
Skin rash	2 (4)
Autoimmunity	1 (2)

IL, interleukin; TIL, tumor-infiltrating lymphocyte.
Besser MJ, et al. *Clin Cancer Res.* 2013;19:4792.

Lifileucel in patients with unresectable or metastatic melanoma (NCT02360579): study design and endpoints

Clinical study of lifileucel (lovance Biotherapeutics) with prior lymphodepletion and subsequent IL-2 treatment¹⁻³

Phase 2 study (N=178)

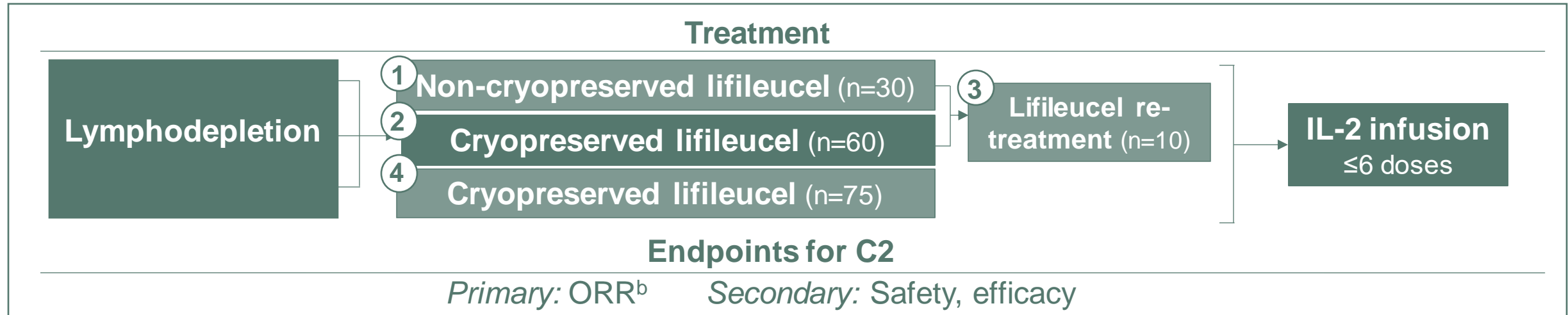
Inclusion criteria

- Aged ≥ 18 years with stage IIIc-IV unresectable or metastatic melanoma
- ECOG PS ≤ 1
- Prior systemic therapy

**Surgical
resection of
tumor**

**Bulk TIL
production and
cryopreservation^a**
22 days

Treatment



BIRC, blinded independent review committee; C, cohort; ECOG PS, Eastern Cooperative Oncology Group performance status; IL, interleukin; ORR, overall response rate; TIL, tumor-infiltrating lymphocyte.

^aBulk TIL refers to non-selected TILs. ^bInvestigator-assessed ORR in cohort 2; ORR by BIRC in cohort 4.

1. Sarnaik A, et al. *J Clin Oncol*. 2020;38(Suppl.):Abstract 10006. 2. Sarnaik A, et al. Poster presented at ASCO Annual Meeting, May 31-June 4, 2019, Chicago, IL, USA. 3. Clinicaltrials.gov.

<https://clinicaltrials.gov/ct2/show/NCT02360579>. Accessed March 17, 2021.

Efficacy and safety of lifileucel in patients with unresectable or metastatic melanoma (NCT02360579)

Clinical study of lifileucel (lovance Biotherapeutics) with prior lymphodepletion and subsequent IL-2 treatment^{1,2}

Cohort 2 efficacy
Median follow-up: 8.8 months

Response, n (%)	Cohort 2 (N=66)
ORR	25 (38)
CR	2 (3)
PR	23 (35)
SD	28 (42)
PD	9 (14)
NE	4 (6)
DCR	53 (80)

DOR (range): NR (1.4+ to 19.8+) months


Cohort 2 safety
Median follow-up: 8.8 months

n (%)	Any Gr	Gr 3/4	Gr 5
Any TEAEs	65 (98.5)	63 (95.5)	2 (3.0)
Thrombocytopenia	59 (89.4)	53 (80.3)	0
Chills	52 (78.8)	4 (6.1)	0
Anemia	44 (66.7)	36 (54.5)	0
Pyrexia	39 (59.1)	11 (16.7)	0
Febrile neutropenia	36 (54.5)	35 (53.0)	0
Neutropenia	36 (54.5)	25 (37.9)	0

AE profile was generally consistent with advanced disease, lymphodepletion, and IL-2 treatment

AE, adverse event; CR, complete response; DCR, disease control rate; DOR, duration of response; Gr, grade; IL, interleukin; NE, not evaluable; NR, not reached; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease; TEAE, treatment-emergent adverse event.

1. Sarnaik A, et al. *J Clin Oncol*. 2020;38(Suppl.):Abstract 10006. 2. Sarnaik A, et al. Poster presented at ASCO Annual Meeting, May 31-June 4, 2019, Chicago, IL, USA.



TIL
CELL THERAPY
WORKING GROUP

Active/recruiting TIL cell therapy clinical trials (1 of 4)

Study ^a	Phase	Sponsor	N	Treatment ^b	Indication
NCT01045460	2	Sidney Kimmel Comprehensive Cancer Center	36	MIL	Multiple myeloma
NCT01319565	2	NCI	102	Young TIL → ± TBI	Melanoma
NCT01701674	ND	H. Lee Moffitt Cancer Center	13	Ipilimumab → bulk TIL	Melanoma
NCT01659151	2	H. Lee Moffitt Cancer Center	17	Vemurafenib → bulk TIL	Melanoma
NCT01807182	2	Fred Hutchinson Cancer Research Center	30	Chemotherapy → bulk TIL	Melanoma
NCT01993719	2	NCI	33	Young TIL → ± pembrolizumab	Melanoma
NCT02500576	2	MD Anderson Cancer Center	36	Bulk TIL → pembrolizumab ± IL-2	Melanoma
NCT01740557	1/2	MD Anderson Cancer Center	10	CXCR2-transduced TIL	Melanoma

CXCR2, C-X-C motif chemokine receptor 2; IL, interleukin; MIL, marrow-infiltrating lymphocyte; NCI, National Cancer Institute; ND, not disclosed; TBI, total-body irradiation; TIL, tumor-infiltrating lymphocyte.

^aStudies accessed on April 7, 2021. ^bBulk TIL refers to non-selected TILs; young TILs were cultured for ≤2 weeks.

Active/recruiting TIL cell therapy clinical trials (2 of 4)

Study ^a	Phase	Sponsor	N	Treatment ^b	Indication
NCT02652455	1	H. Lee Moffitt Cancer Center	11	± Nivolumab → bulk TIL	Melanoma
NCT03083873	2	Iovance Biotherapeutics	55	LN-145/LN-145-S1	Melanoma
NCT03215810	1	H. Lee Moffitt Cancer Center	20	Nivolumab → bulk TIL	SCCHN
NCT03296137	1/2	Inge Marie Svane	25	Ipilimumab → nivolumab → bulk TIL	NSCLC
NCT02650986	1/2	Roswell Park Cancer Institute	16	± Decitabine → TGF-β-DN-RII-transduced TIL	ND
NCT03073525	2	Gradalis, Inc.	25	± Atezolizumab → vigil → ± atezolizumab	NY-ESO-1–expressing cancers
NCT03347097	1	Huashan Hospital	40	PD-1 TILs	Gynecological cancers
NCT03526185	1	Yale University	30	Young TIL → ± ([nivolumab + ipilimumab] → nivolumab)	Melanoma

CXCR2, C-X-C motif chemokine receptor 2; IL, interleukin; MIL, marrow-infiltrating lymphocyte; ND, not disclosed; NSCLC, non-small cell lung cancer; NY-ESO-1, New York esophageal squamous cell carcinoma 1; PD-1, programmed death-1; SCCHN, squamous cell carcinoma of the head and neck; TGF-β-DN-RII, transcription factor β dominant negative receptor II; TIL, tumor-infiltrating lymphocyte.

^aStudies accessed on April 7, 2021. ^bBulk TIL refers to non-selected TILs; young TILs were cultured for ≤2 weeks.

Active/recruiting TIL cell therapy clinical trials (3 of 4)

Study ^a	Phase	Sponsor	N	Treatment	Indication
NCT04538313	1/2	CAR-T (Shanghai) Cell Biotechnology	40	TIL	Hepatocellular carcinoma
NCT03108495	2	Iovance Biotherapeutics	138	LN-145	Cervical carcinoma
NCT03610490	2	MD Anderson Cancer Center	60	MDA-TIL	Ovarian cancer, colorectal cancer, PDA
NCT03449108	2	MD Anderson Cancer Center	80	LN-145/LN-145-S1	Ovarian cancer, thyroid gland carcinoma, bone carcinoma
NCT03645928	2	Iovance Biotherapeutics	135	Lifileucel ± pembrolizumab, LN-145 ± pembrolizumab, LN-145 + nivolumab + ipilimumab, LN-145-S1	Melanoma, SCCHN, NSCLC
NCT04111510	2	Yale University	10	LN-145	TNBC
NCT04052334	1	H. Lee Moffitt Cancer Center	15	Moffit-TIL	Soft tissue sarcoma

NSCLC, non-small cell lung cancer; PDA, pancreatic ductal adenocarcinoma; SCCHN, squamous cell carcinoma of the head and neck; TIL, tumor-infiltrating lymphocyte; TNBC, triple negative breast cancer.

^aStudies accessed on April 7, 2021.

Active/recruiting TIL cell therapy clinical trials (4 of 4)

Study ^a	Phase	Sponsor	N	Treatment ^b	Indication
NCT03997474	1/2	Achilles Therapeutics	40	ATL001	Melanoma
NCT04032847	1/2	Achilles Therapeutics	50	ATL001 ± CPI	NSCLC
NCT04069936	2	WindMIL Therapeutics	23	MILs ± nivolumab	NSCLC
NCT02278887	3	The Netherlands Cancer Institute	168	Bulk TIL	Melanoma
NCT04614103	2	Iovance Biotherapeutics	95	LN-145	NSCLC

CPI, checkpoint inhibitor; MIL, marrow-infiltrating lymphocyte; NSCLC, non-small cell lung cancer; TIL, tumor-infiltrating lymphocyte.

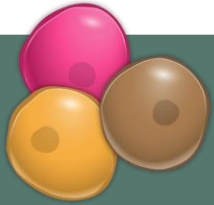
^aStudies accessed on April 7, 2021. ^bBulk TIL refers to non-selected TILs.

Summary

- Completed TIL cell therapy trials in patients with melanoma to date have confirmed
 - Importance of lymphodepletion for treatment success
 - Possibility of limiting IL-2 doses to reduce the incidence of AEs
 - TIL cell therapy is effective in patients previously treated with checkpoint inhibitors
- TIL cell therapy trials that are currently active or enrolling patients
 - Expand the applicability of TIL cell therapy beyond patients with melanoma
 - Incorporate checkpoint inhibitors in treatment paradigm to improve response
 - Explore the opportunity to genetically manipulate TILs to target different cancers

Requirements for Delivery of TIL Cell Therapy

TIL cell therapy infrastructure elements



TIL production

TIL production requires

1. Surgical tumor tissue procurement and processing
2. Consideration of patient recovery time from surgery and timing of TIL availability for subsequent treatment
3. Relationship with TIL manufacturing center or capacity to produce TILs
4. Management of TIL product once returned to treatment center

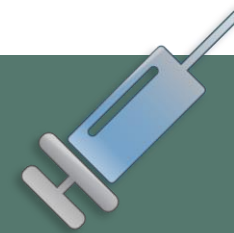


Multidisciplinary expertise

Several HCPs are involved in administering TIL cell therapy and managing patients, including

1. Surgical oncologists
2. Cell therapy experts
3. Medical oncologists
4. Advanced nurse practitioners and physician assistants
5. Subspecialists for toxicity management

Workflows and SOPs are needed to ensure the success of the interdisciplinary team



Treatment and patient experience

Some considerations in TIL cell therapy administration and handling of patient experience include

1. Management of AEs related to lymphodepletion and IL-2 treatment
2. Capability for extended follow-up of patients
3. Improvement of patient QoL
4. Patient education and advocacy

Summary

- The infrastructure elements of TIL cell therapy include
 - Management of TIL production
 - Multidisciplinary team to manage TIL administration and management of AEs
 - Efforts to improve patient experience and treatment satisfaction

Summary

Summary

Immunotherapy	T cells recognize antigens on the surface of foreign or abnormal cells, including tumors, and are activated and regulated by other cells in the immune system
TIL cell therapy	TIL cell therapy is a type of adoptive cell transfer immunotherapy in which TILs are harvested from resected tumors, grown in vitro, and transferred to patients after lymphodepletion
Completed TIL cell therapy clinical trials	Completed TIL cell therapy trials in patients with melanoma to date have confirmed the importance of lymphodepletion, the advantages of limiting IL-2 doses post TIL infusion, and treatment efficacy in patients previously treated with checkpoint inhibitors
Future outlook	TIL cell therapy trials that are currently active or enrolling patients expand the applicability of therapy to other indications, incorporate checkpoint inhibitors in the treatment paradigm to improve response, and explore the opportunity to genetically manipulate TILs to target different cancers

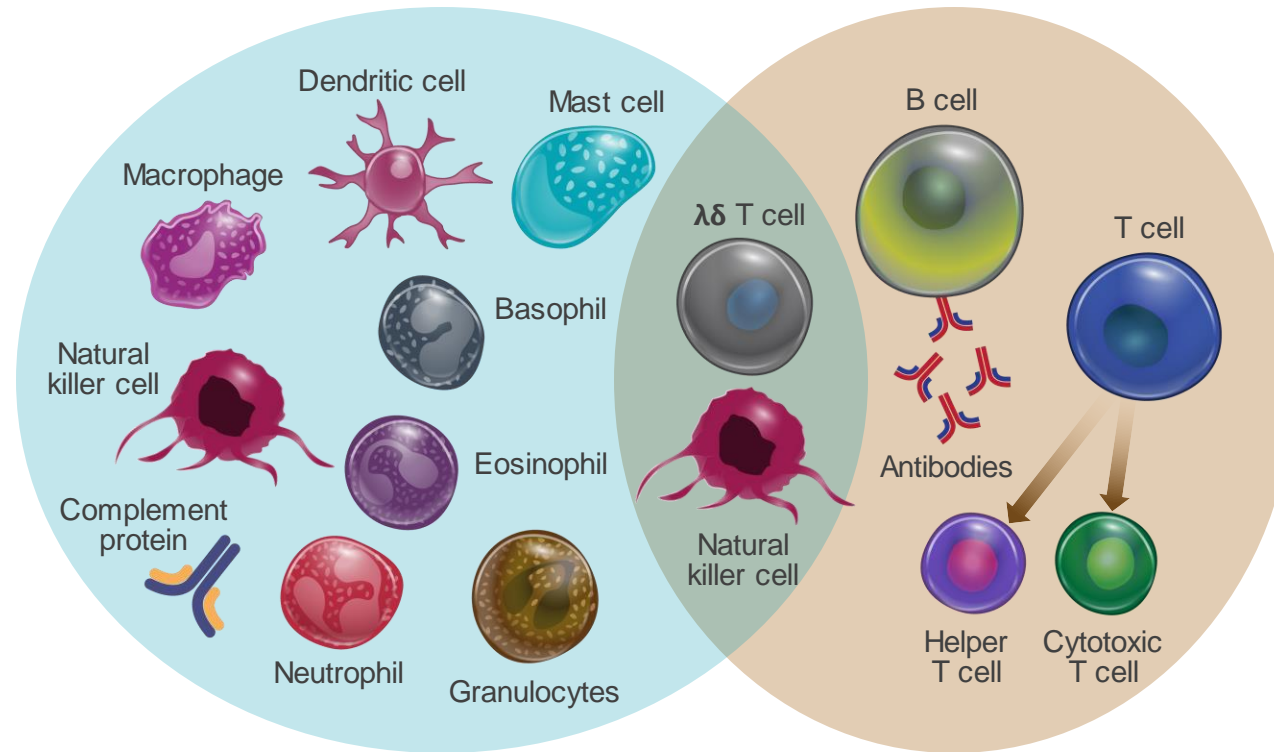
Backup

Backup Slides

- Slides have been included in the backup section that are simplified versions of those found in the Immunology Background section
- Simplified slides may be presented to audiences who are knowledgeable in immune surveillance and according to the presenter's preference

Components of the immune system

Innate Immunity



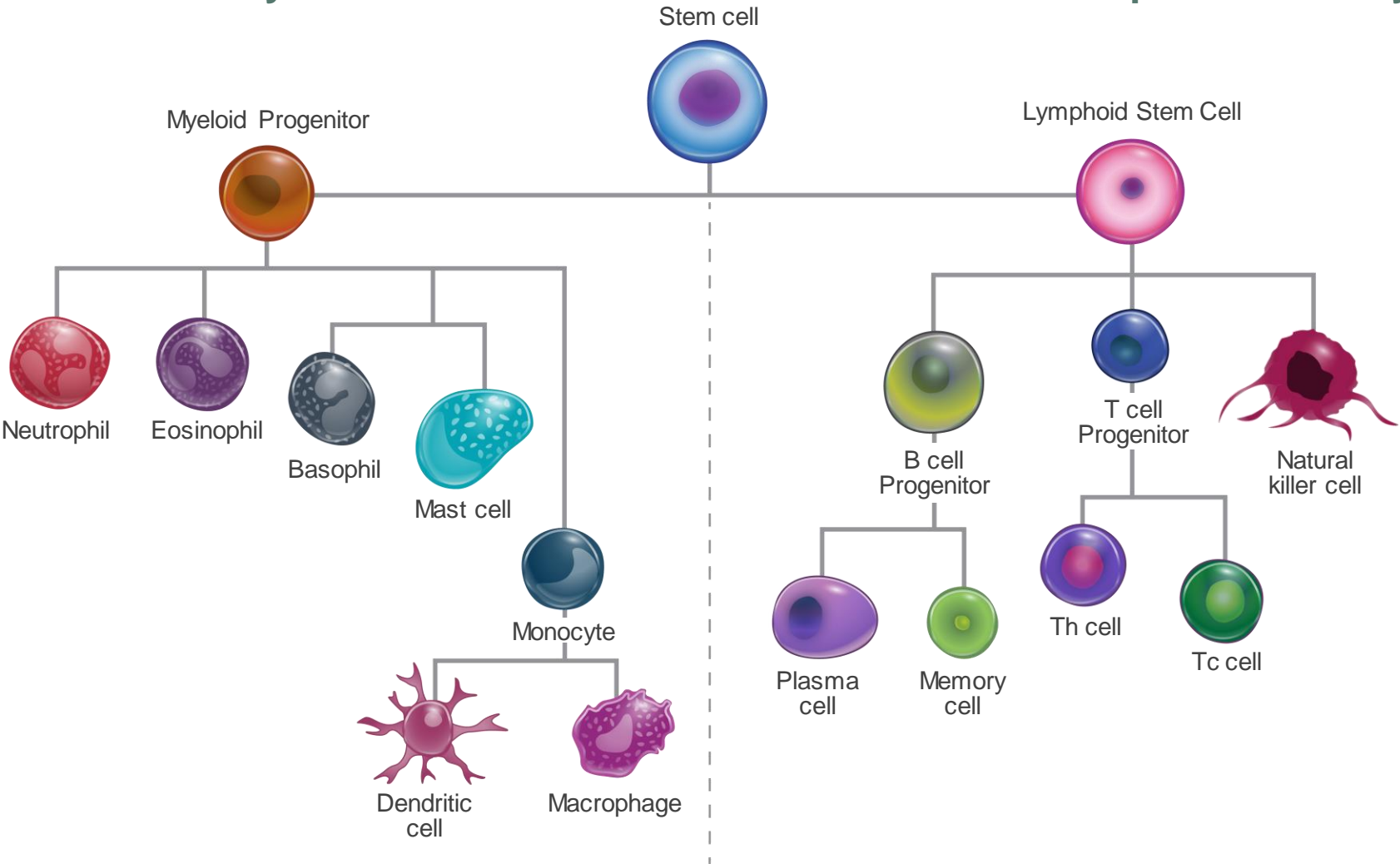
Adaptive Immunity

Immune Surveillance

Cells of the immune system

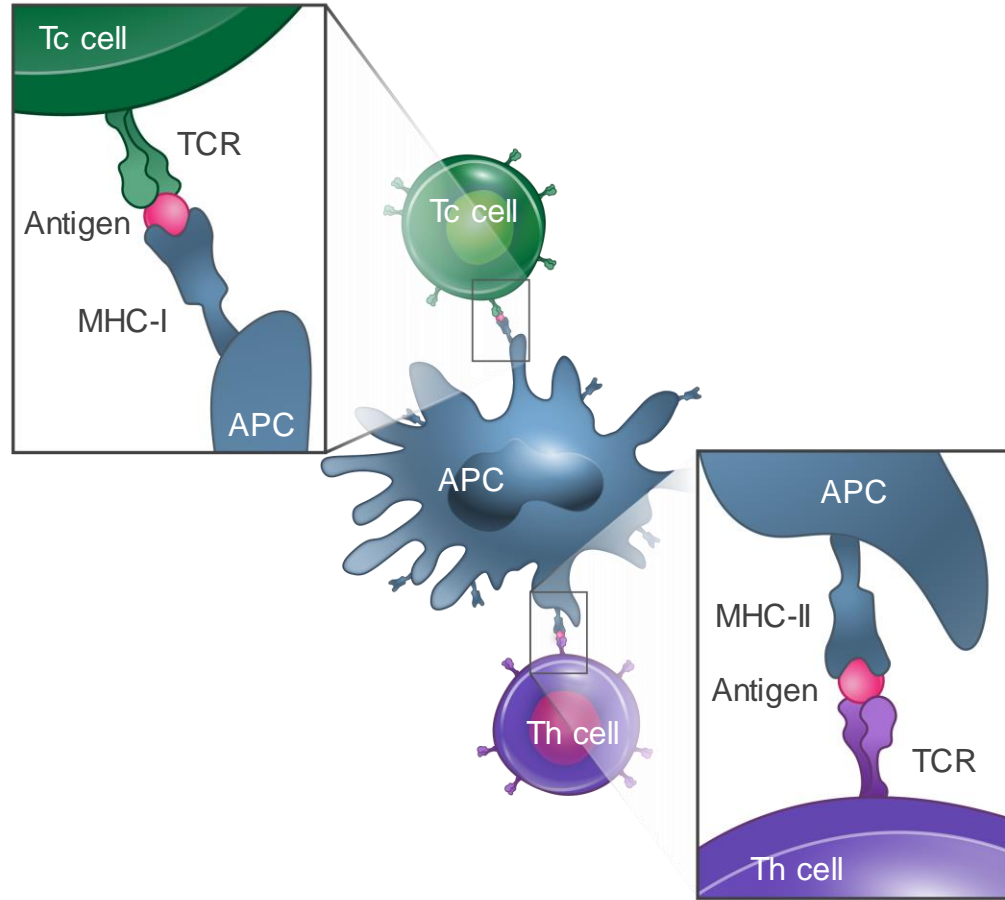
Innate Immunity

Adaptive Immunity

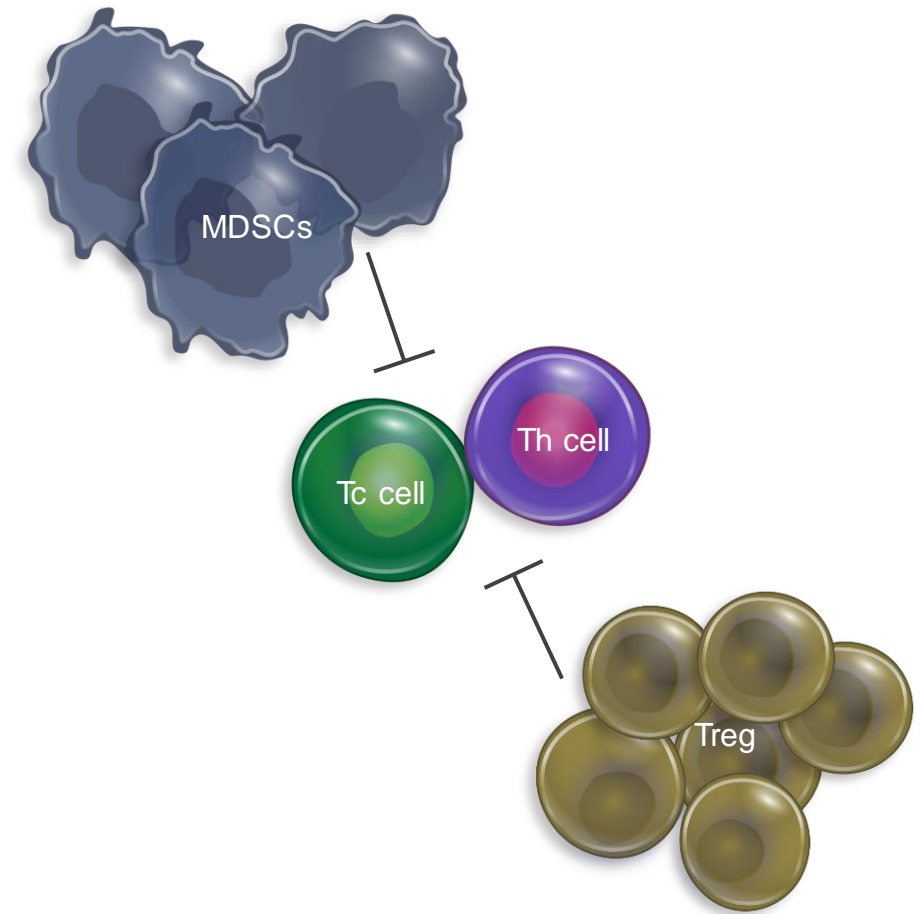


T-cell activation and regulation

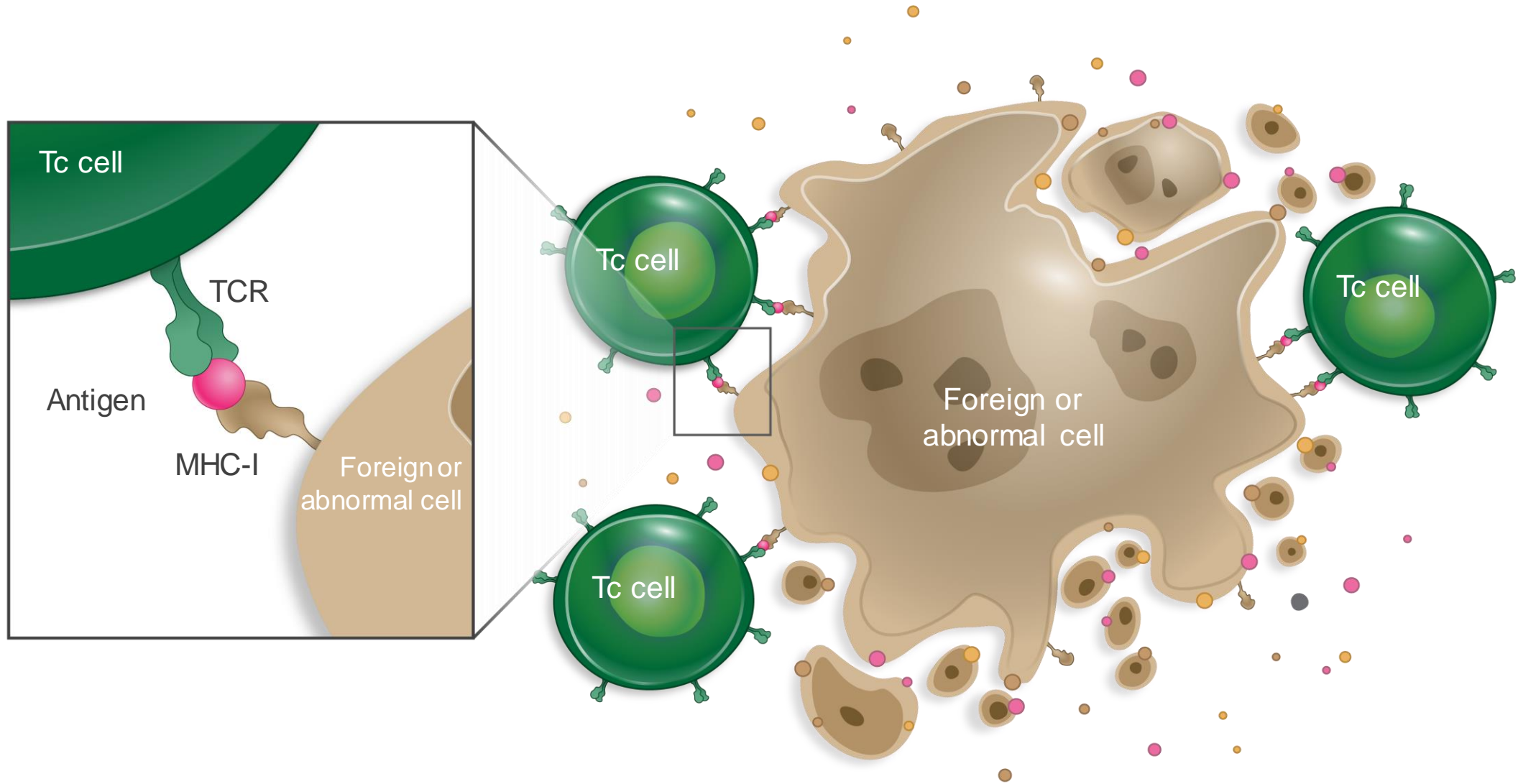
T-cell activation



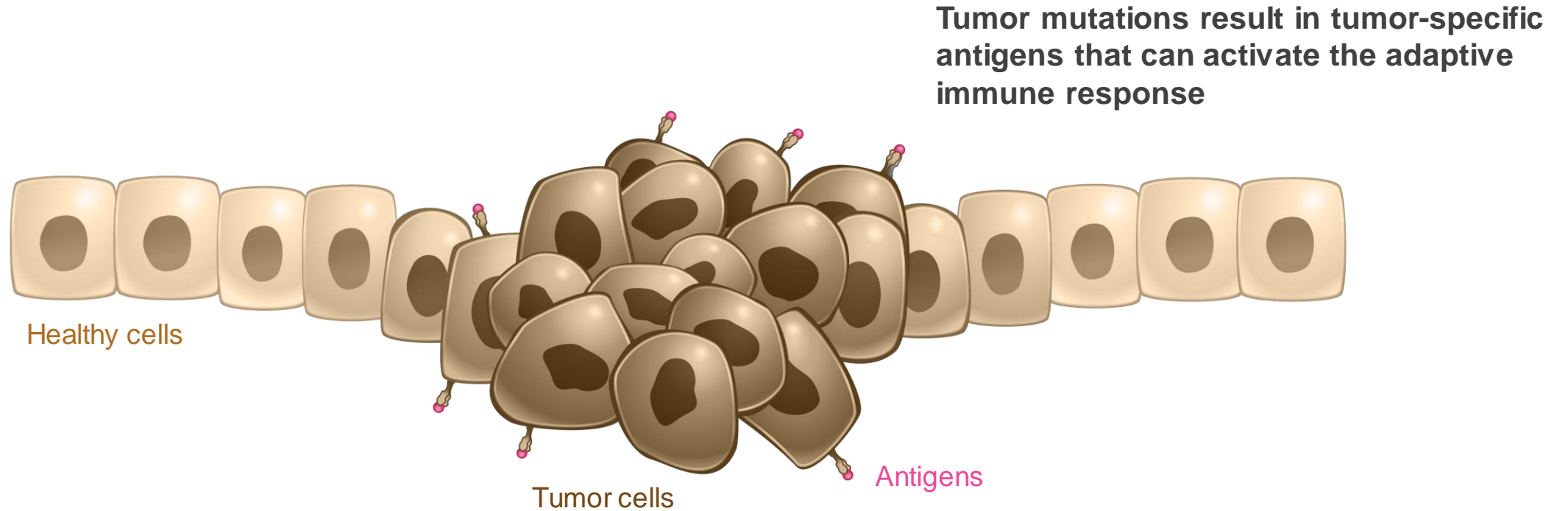
T-cell regulation



T-cell elimination of foreign cells

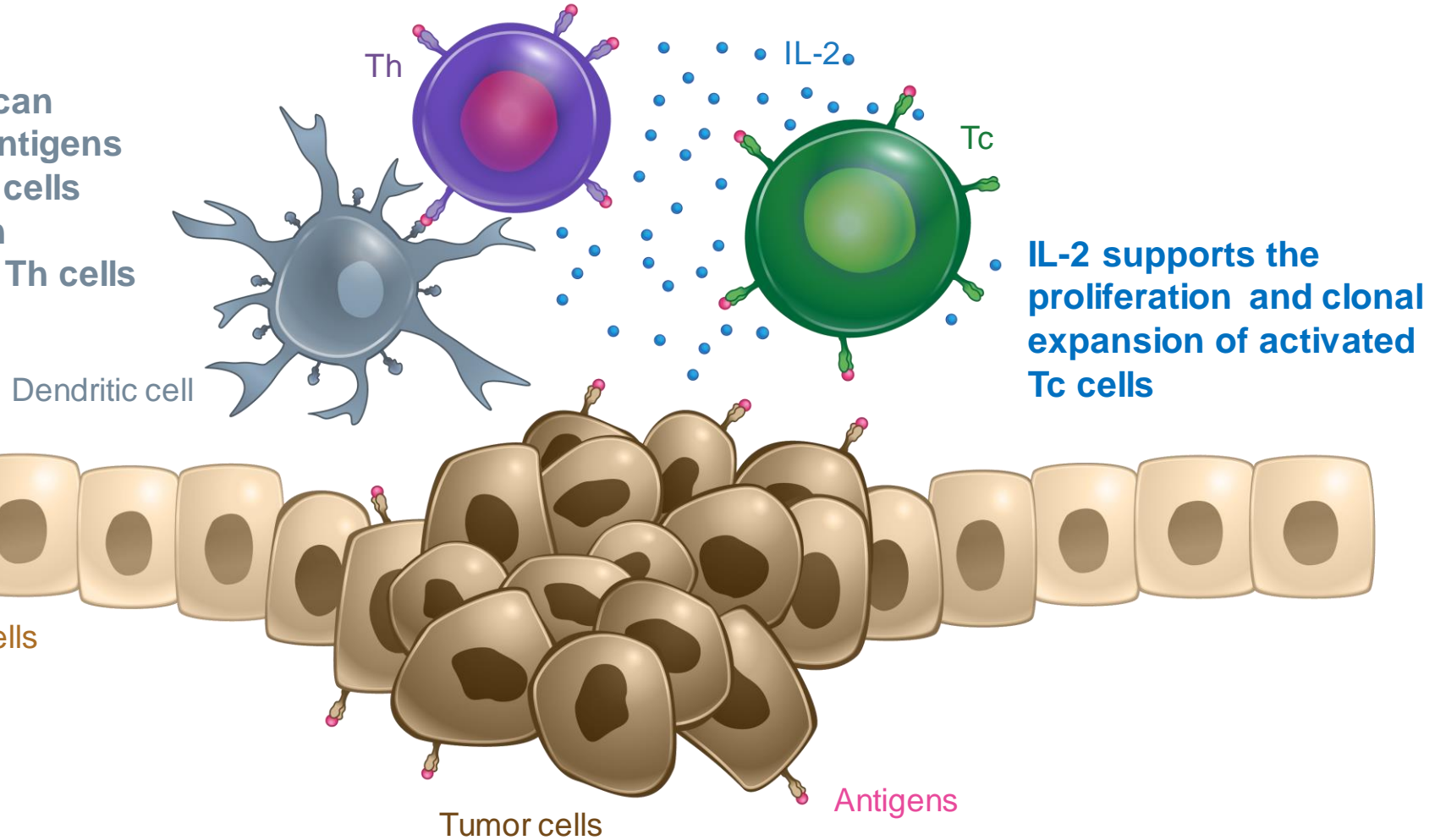


T-cell detection of tumor cells

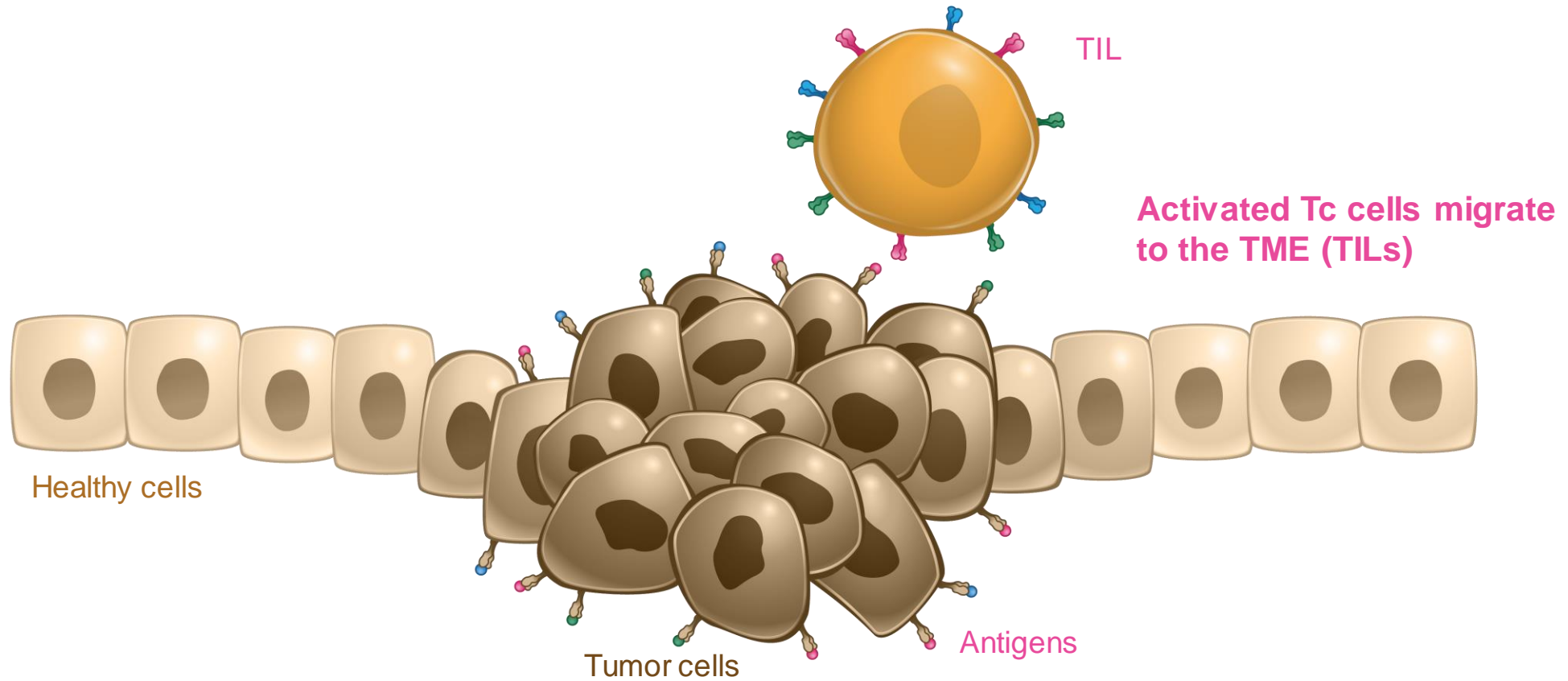


T-cell detection of tumor cells

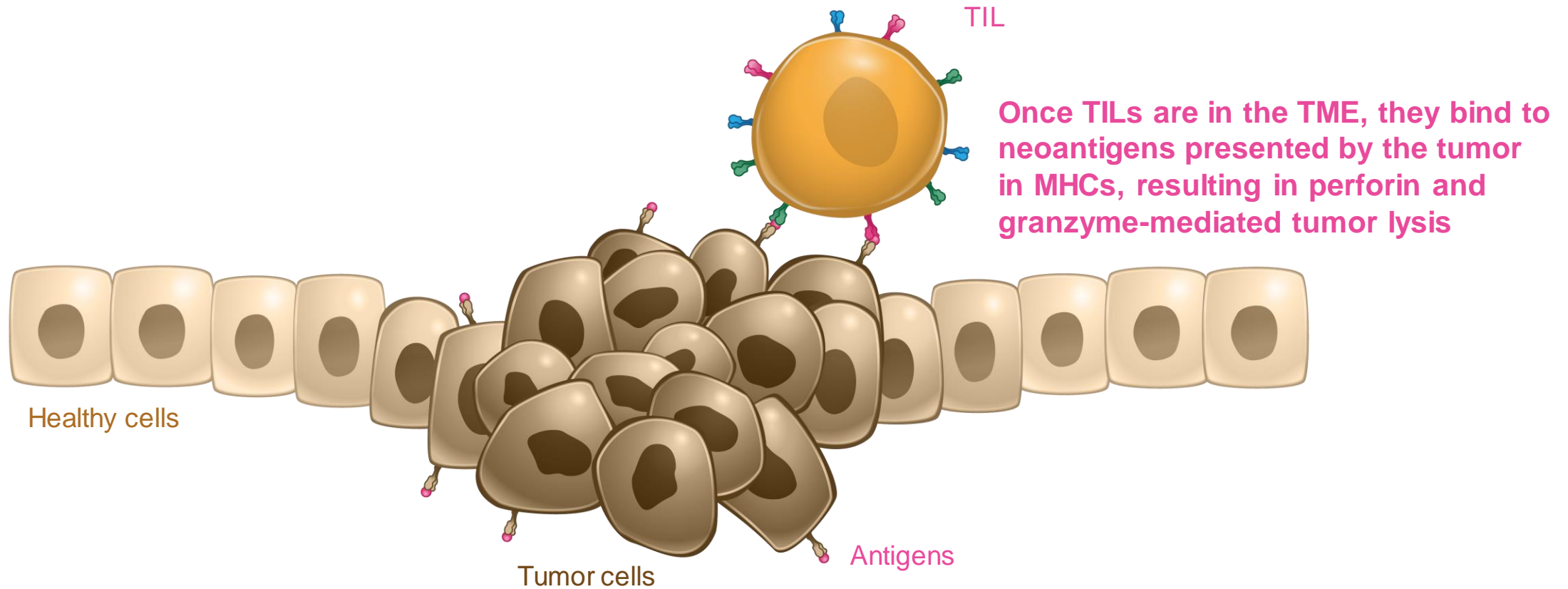
Dendritic cells can recognize neoantigens and activate Tc cells through antigen presentation to Th cells



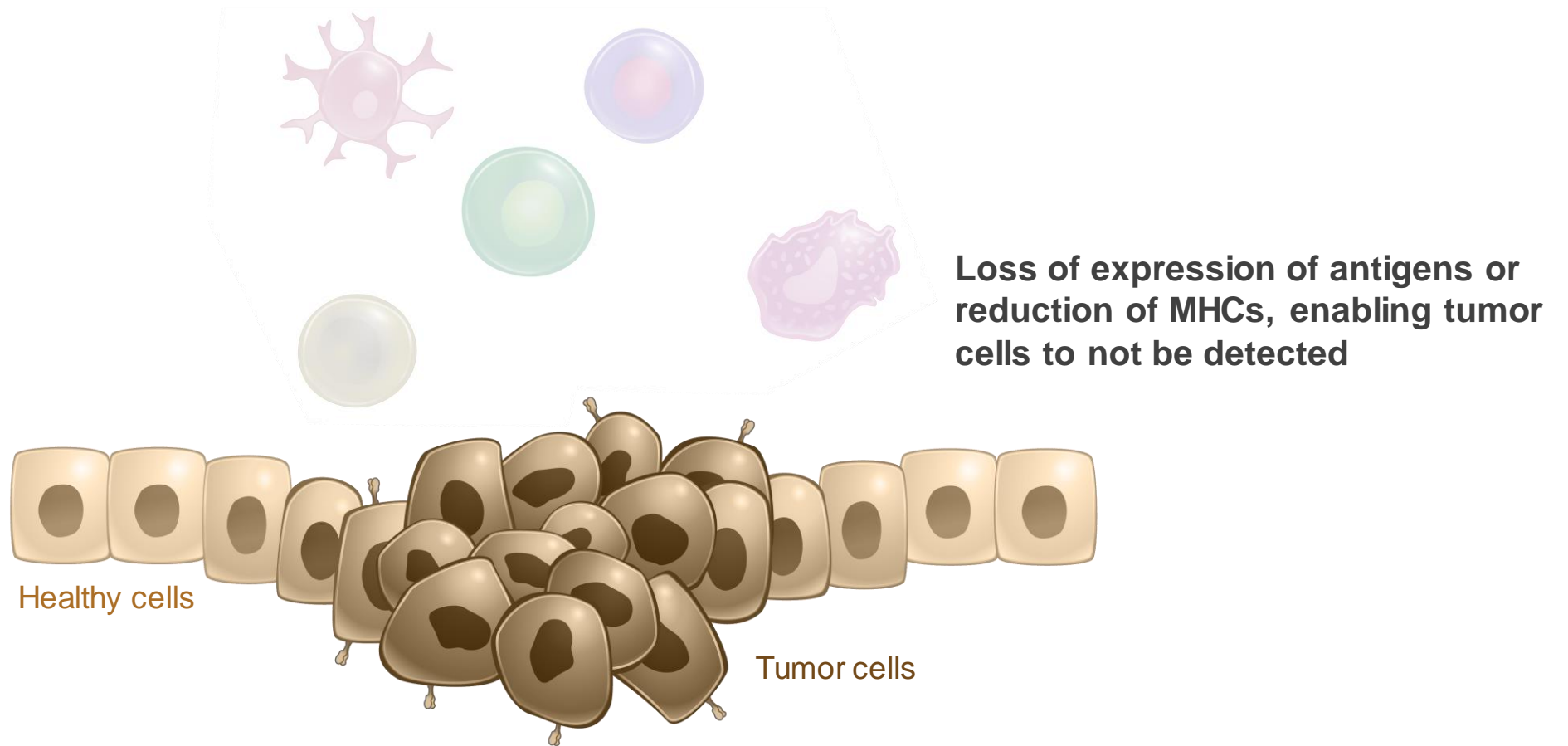
T-cell detection of tumor cells



T-cell detection of tumor cells

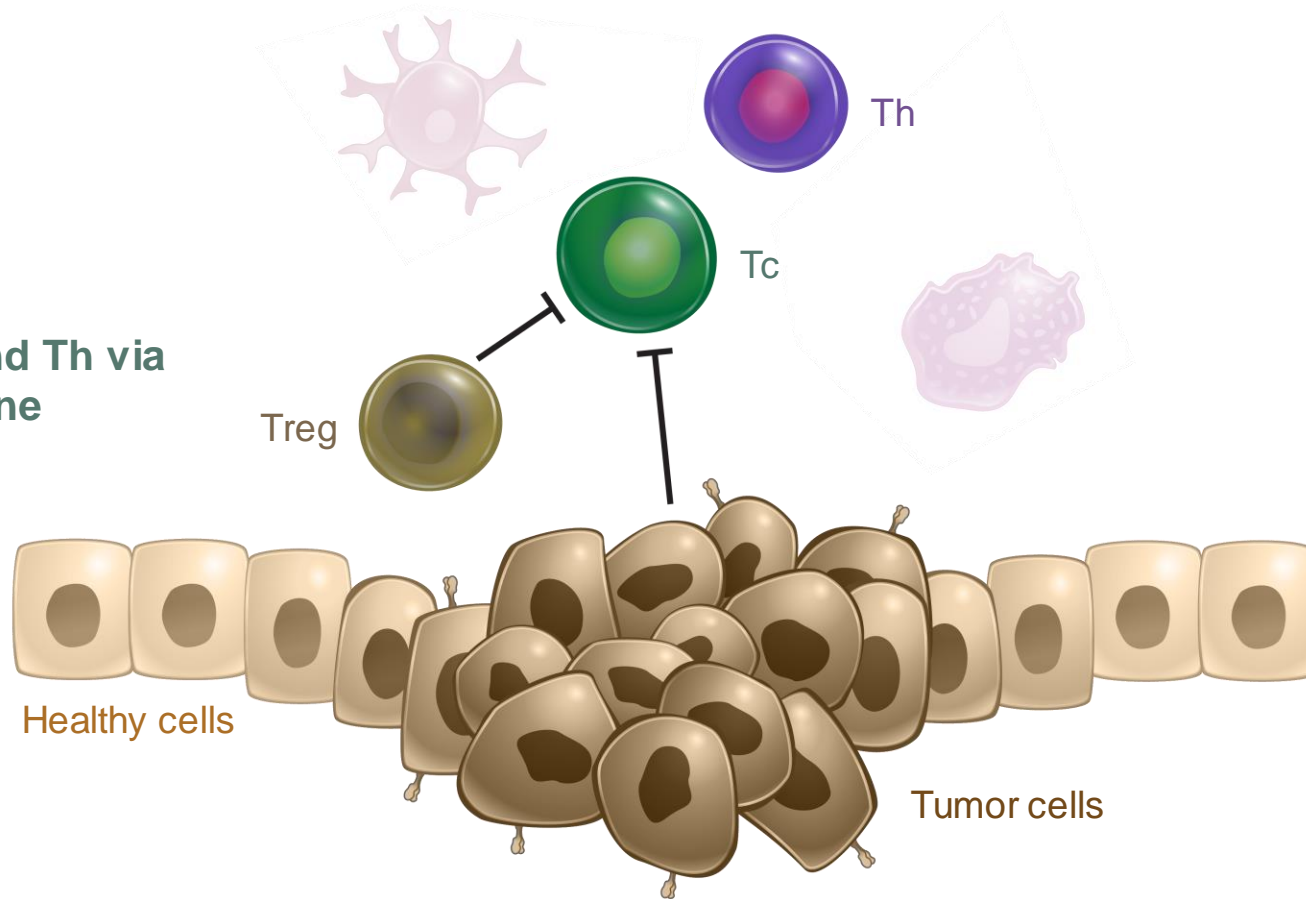


Suppression of TIL activity by the tumor microenvironment

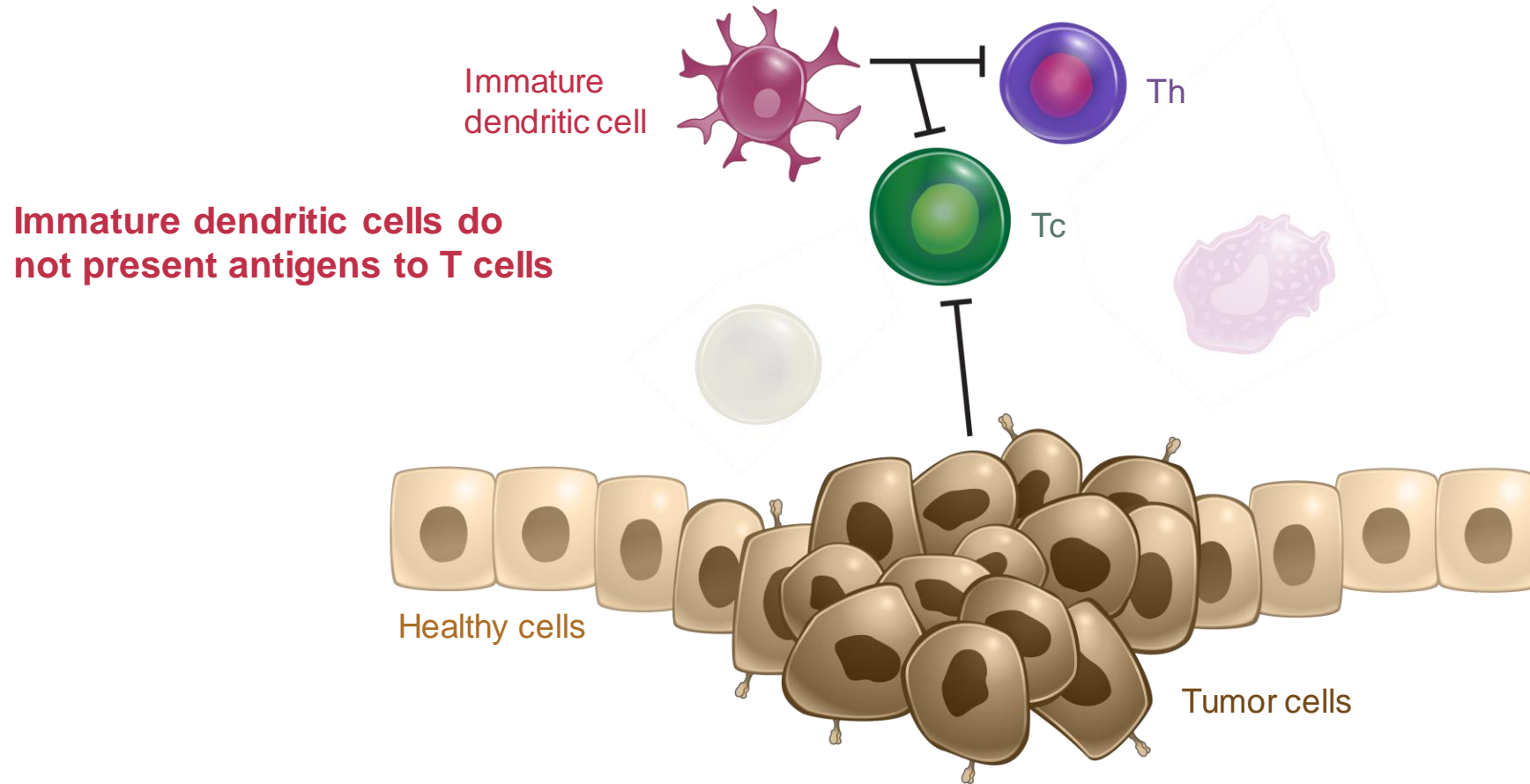


Suppression of TIL activity by the tumor microenvironment

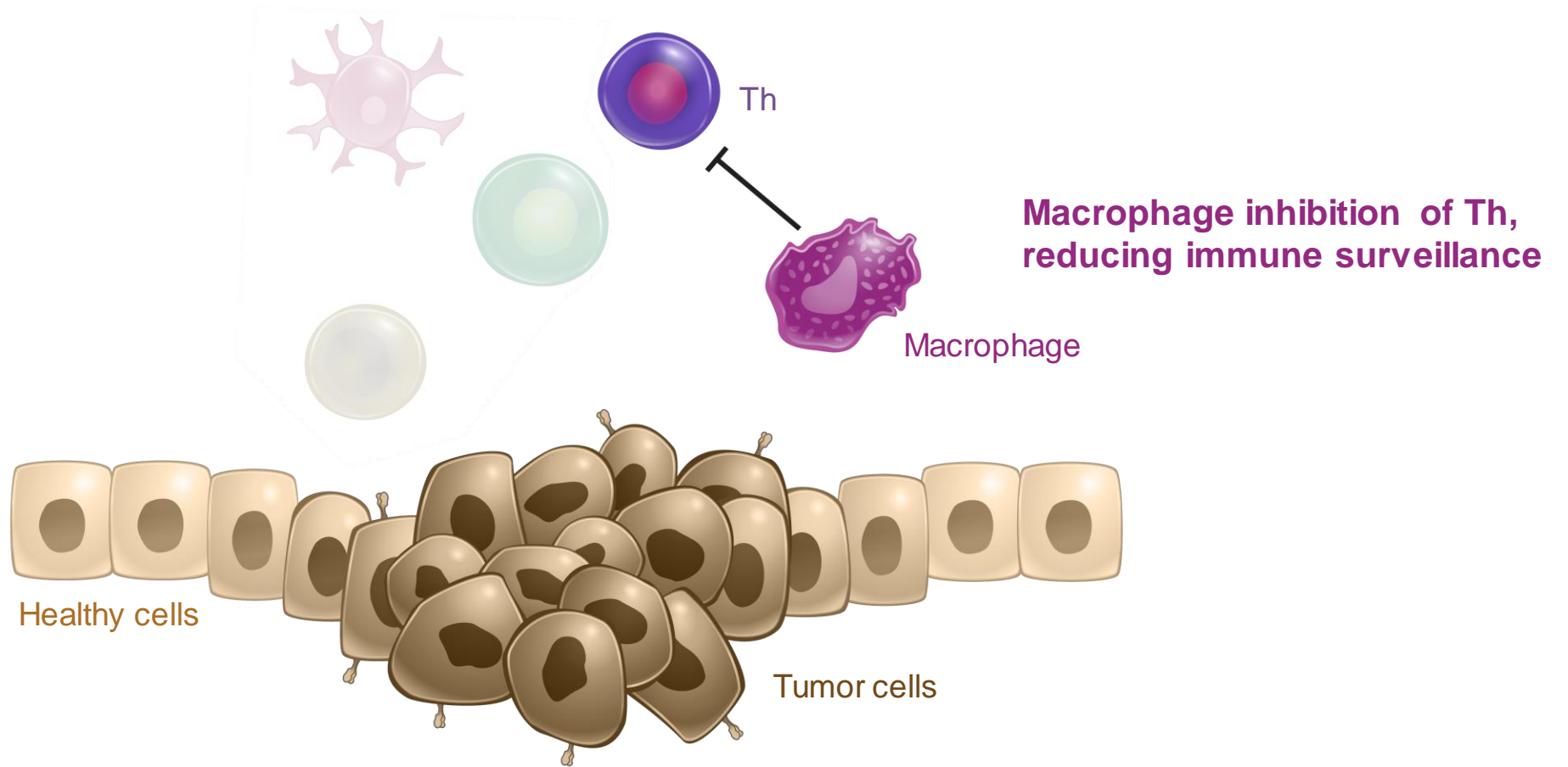
Suppression of Tc and Th via
Treg, reducing immune
surveillance



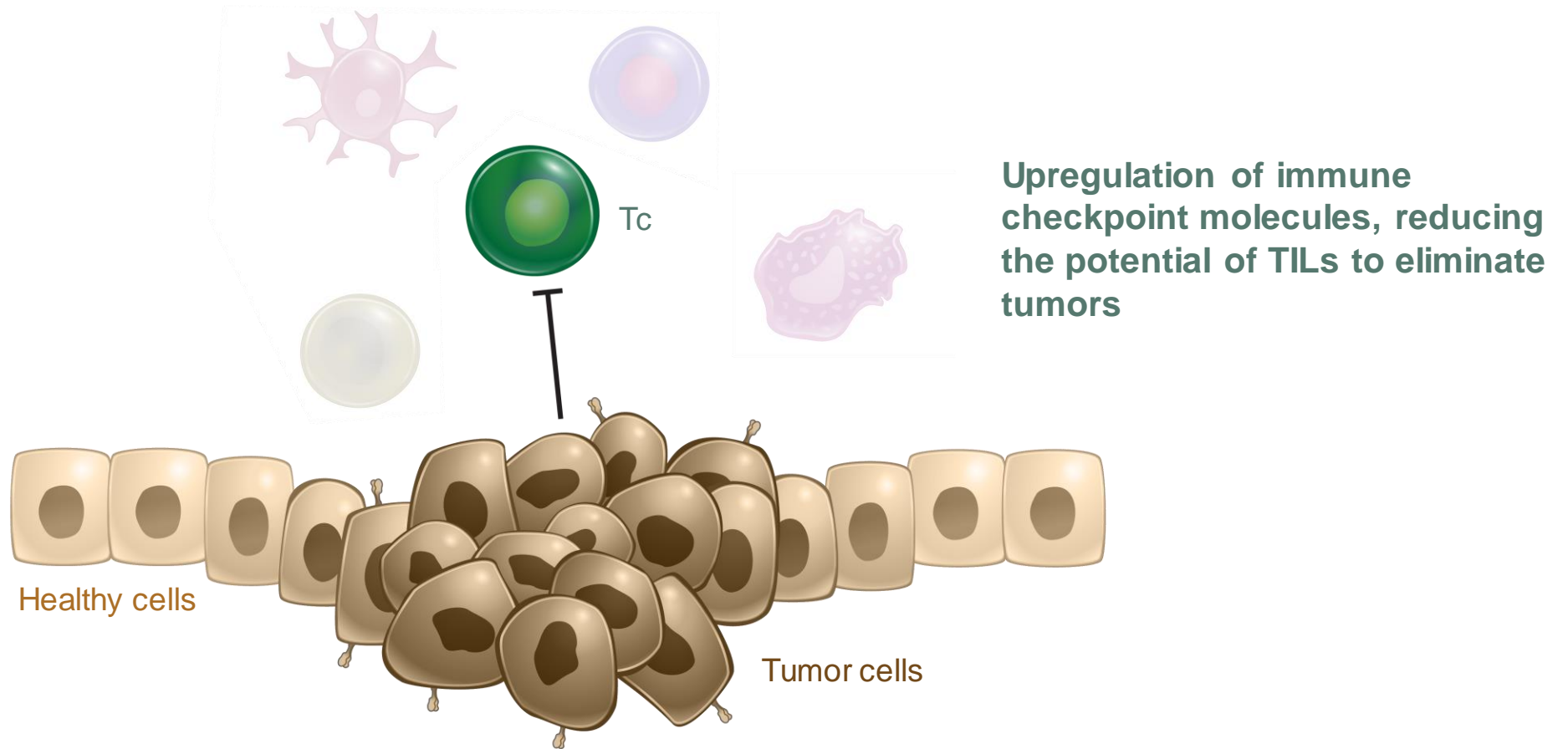
Suppression of TIL activity by the tumor microenvironment



Suppression of TIL activity by the tumor microenvironment



Suppression of TIL activity by the tumor microenvironment



Tumor evasion of TILs through checkpoint pathways

