TEHRAN UNIVERSITY OF MEDICAL SCIENCES

Gene, Cell & Tissue Research Institute

Tumor Infiltrating Lymphocyte (TIL) Cell Therapy in Pediatric Malignancy

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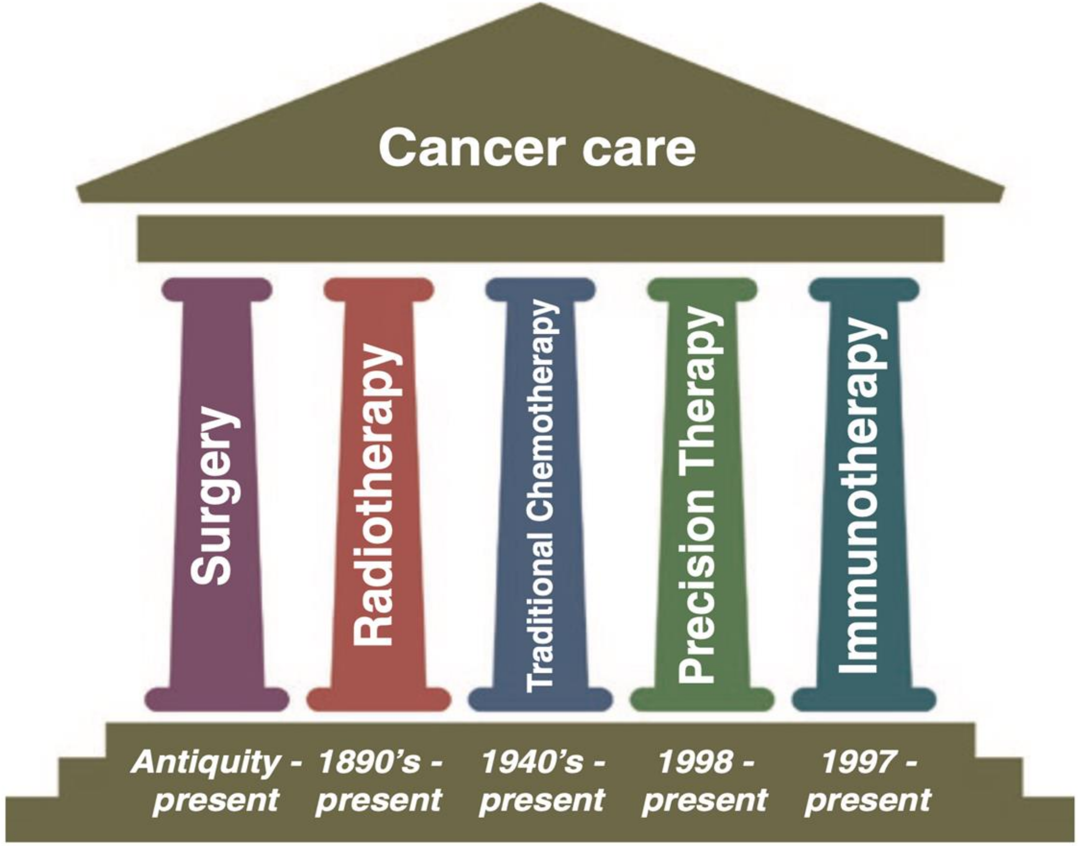
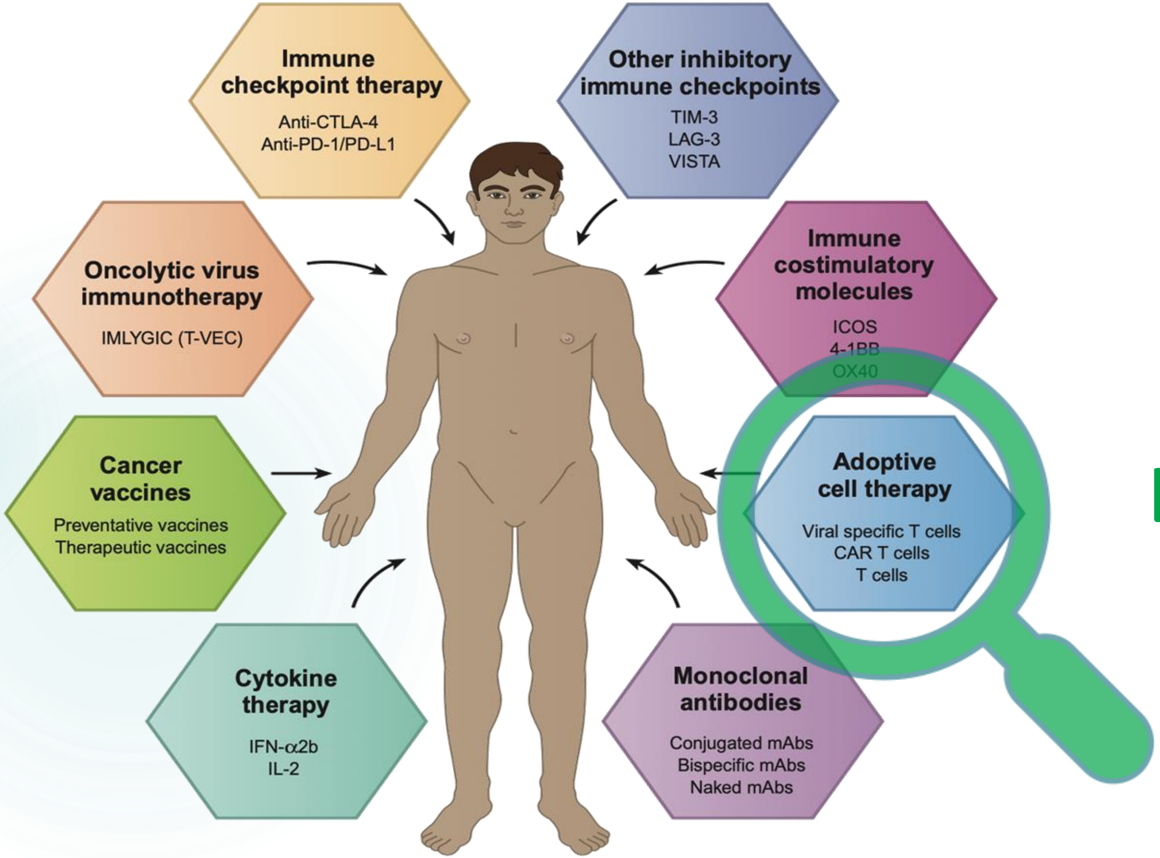
Science
20 December 2013 | $10
Breakthrough of the Year
Cancer Immunotherapy
T cells on the attack
AAAS


Cancer care
Surgery
Radiotherapy
Traditional Chemotherapy
Precision Therapy
Immunotherapy
Antiquity - present
1890's - present
1940's - present
1998 - present
1997 - present

Anticancer immunotherapeutic strategies



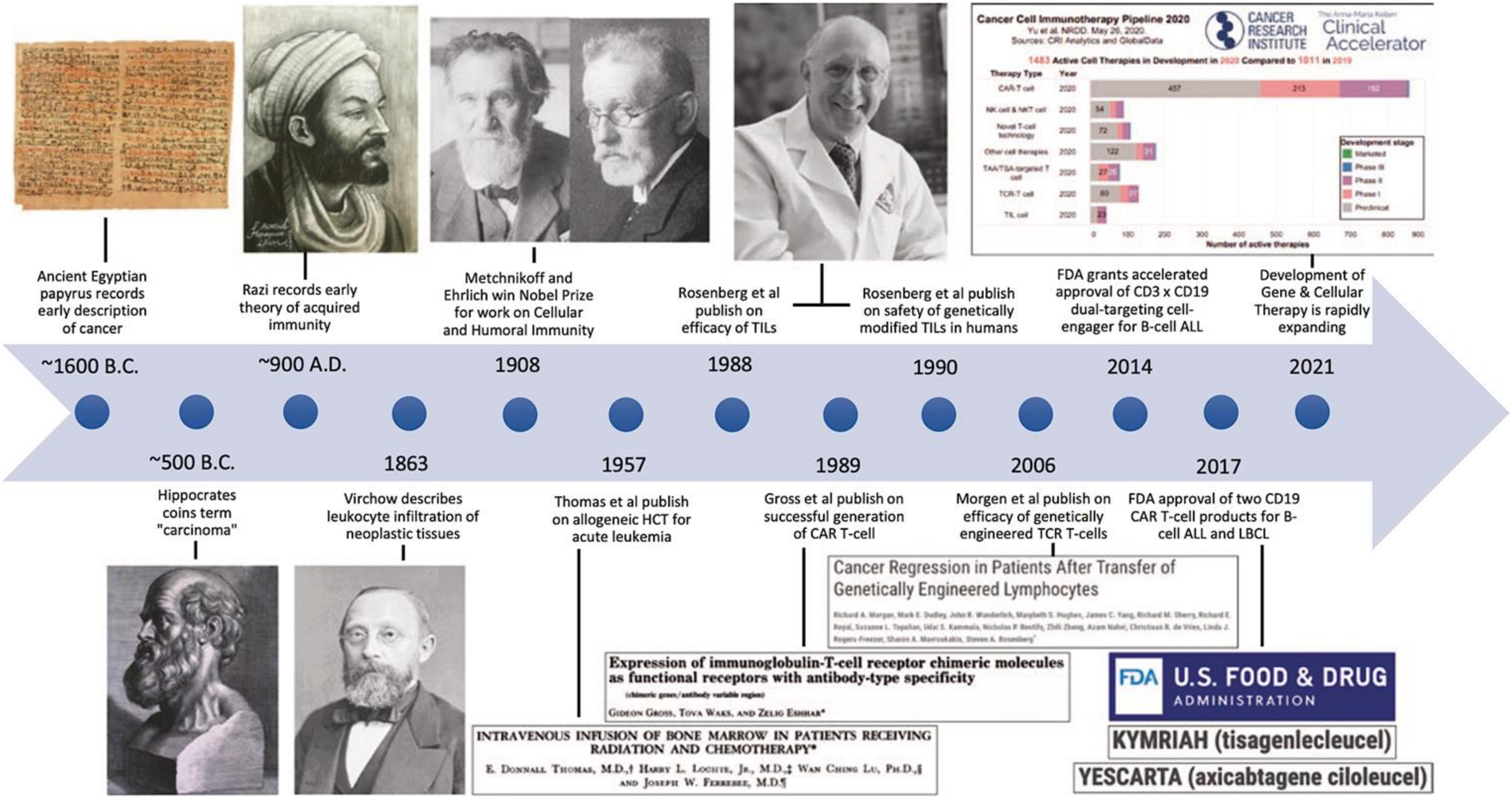
Living Drugs!

History

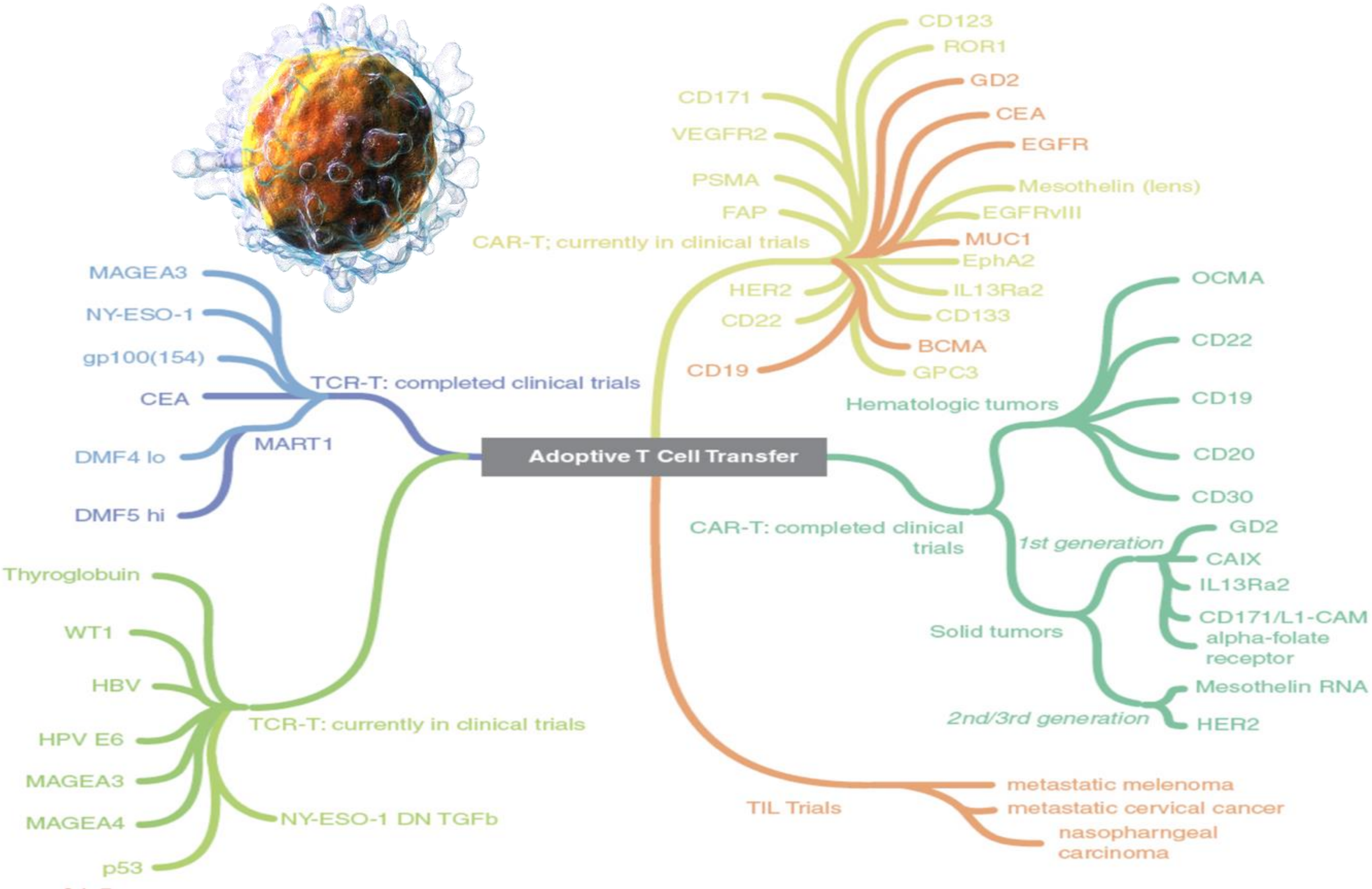
In **1891**, a bone carcinoma surgeon named **William B. Coley** first experimented with this approach, injecting bacteria directly into the tumor of one of his patients.

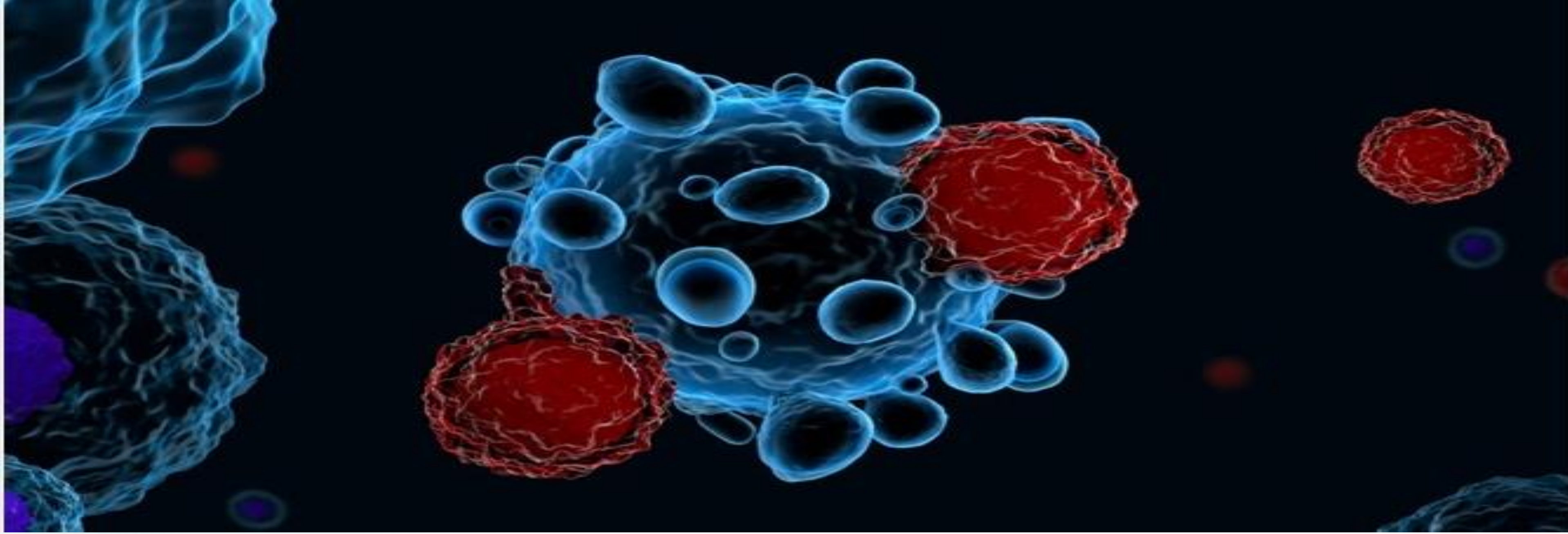
Several decades later **Bellingham** first coined the term **“adoptive immunity”** to describe the transfer of lymphocytes to mediate an effector function in addressing mechanisms of skin allograft rejection.

In the **1950s**, the concept of **“adoptive immune therapy” (ACT)** for tumor allografts was first reported in rodents by **Mitchison**.

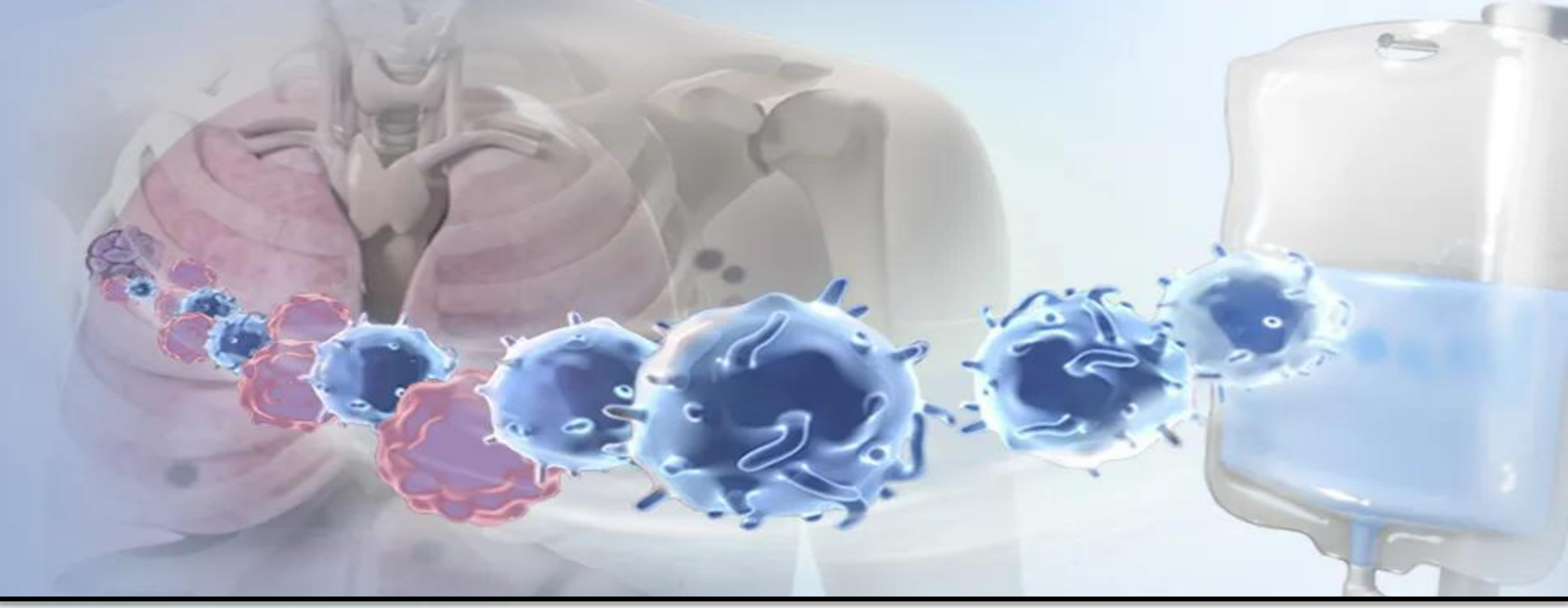


Razi's portrait courtesy of master artist Homayoun Alvand


Adoptive T Cell Transfer
TCR-T: completed clinical trials
MAGEA3
NY-ESO-1
gp100(154)
CEA
MART1
DMF4 lo
DMF5 hi
TCR-T: currently in clinical trials
Thyroglobuin
WT1
HBV
HPV E6
MAGEA3
MAGEA4
p53
NY-ESO-1 DN TGFb
CAR-T; currently in clinical trials
CD123
ROR1
GD2
CEA
EGFR
Mesothelin (lens)
EGFRvIII
MUC1
EphA2
IL13Ra2
CD133
BCMA
GPC3
CD171
VEGFR2
PSMA
FAP
HER2
CD22
CD19
CAR-T: completed clinical trials
Hematologic tumors
OCMA
CD22
CD19
CD20
CD30
1st generation
Solid tumors
GD2
CAIX
IL13Ra2
CD171/L1-CAM
alpha-folate receptor
2nd/3rd generation
Mesothelin RNA
HER2
TIL Trials
metastatic melenoma
metastatic cervical cancer
nasopharngeal carcinoma



- Adoptive cell therapy (ACT) is a treatment concept that uses immune cells to kill cancer cells, and includes chimeric antigen receptor engineered T (CAR-T) therapy, T-cell receptor-engineered T (TCR-T) therapy, natural killer (NK) cell therapy, and tumor-infiltrating lymphocyte (TIL) therapy.



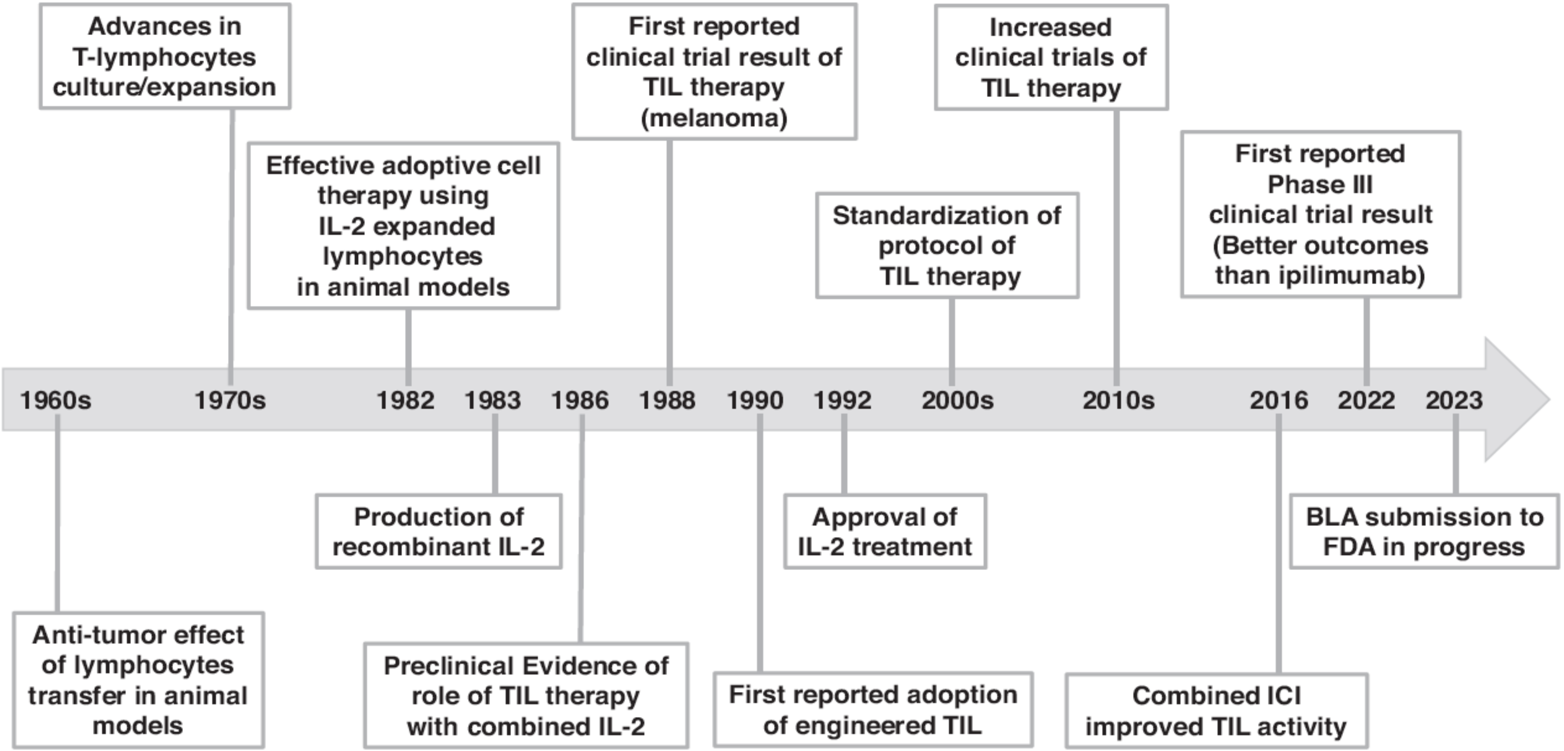
Tumor Infiltrating Lymphocytes (TIL)

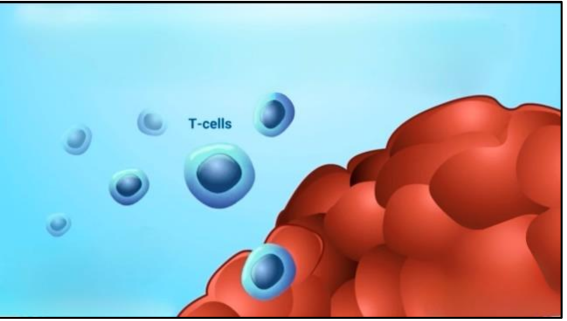
What is TIL Cell Therapy?

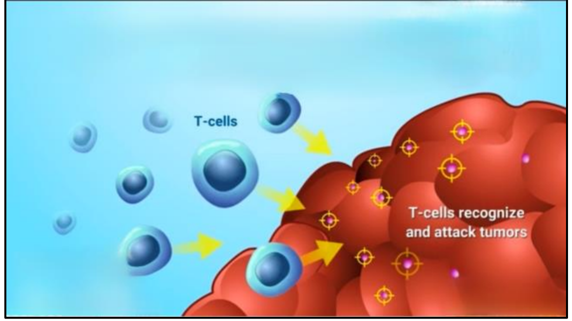
- TIL (tumor-infiltrating lymphocyte) therapy is a type of cellular immunotherapy that uses the patient's own cells to fight tumor-based cancers like advanced melanoma.

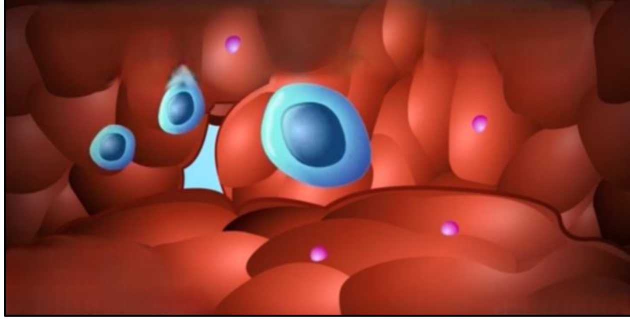
- TIL therapy has also been used to treat head and neck squamous cell carcinoma, lung cancer, genitourinary cancers and a growing list of other malignancies.

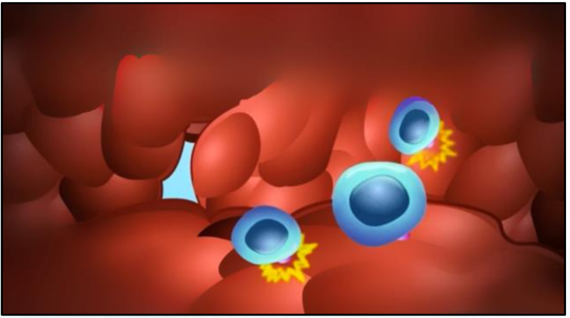
History

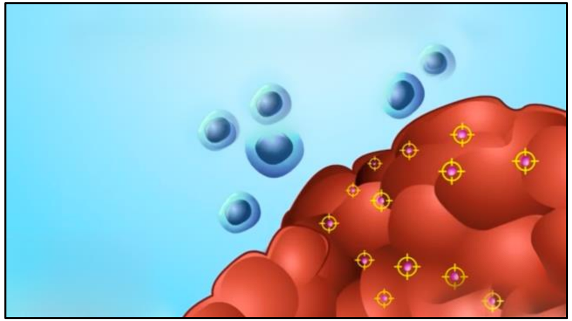

Advances in T-lymphocytes culture/expansion
Effective adoptive cell therapy using IL-2 expanded lymphocytes in animal models
First reported clinical trial result of TIL therapy (melanoma)
Standardization of protocol of TIL therapy
Increased clinical trials of TIL therapy
First reported Phase III clinical trial result (Better outcomes than ipilimumab)
1960s
1970s
1982
1983
1986
1988
1990
1992
2000s
2010s
2016
2022
2023
Production of recombinant IL-2
Approval of IL-2 treatment
BLA submission to FDA in progress
Anti-tumor effect of lymphocytes transfer in animal models
Preclinical Evidence of role of TIL therapy with combined IL-2
First reported adoption of engineered TIL
Combined ICI improved TIL activity

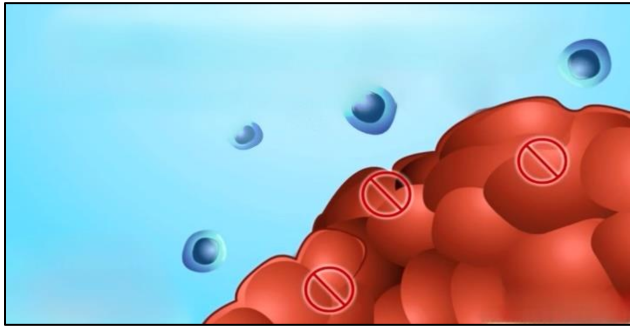

T-cells

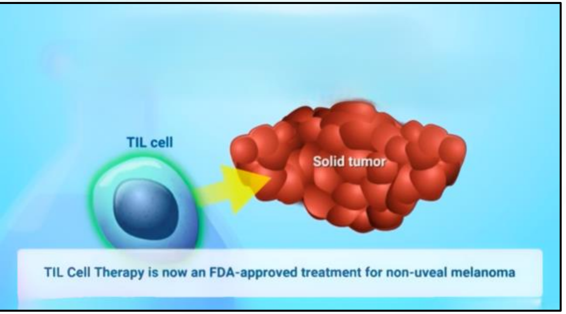

T-cells
T-cells recognize and attack tumors

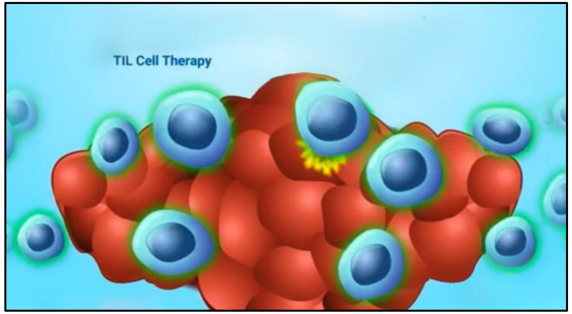


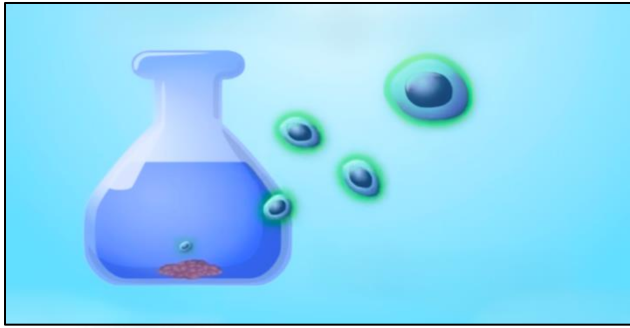


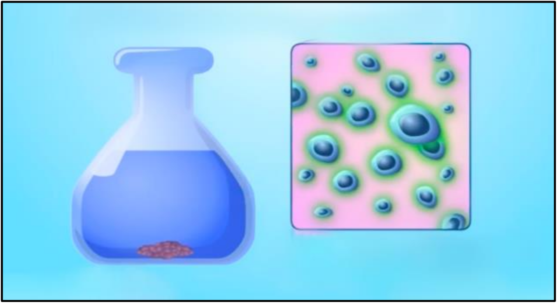


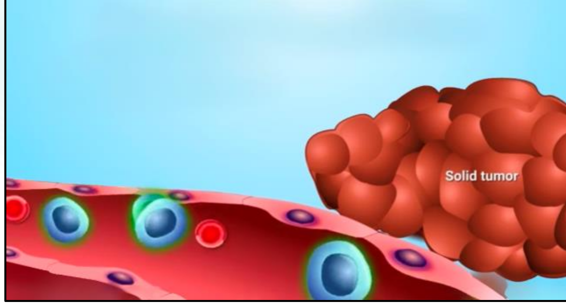


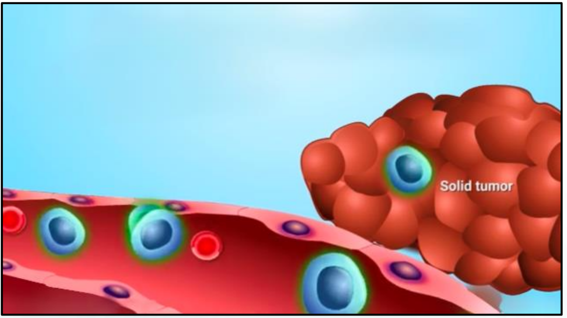

TIL cell
Solid tumor
TIL Cell Therapy is now an FDA-approved treatment for non-uveal melanoma

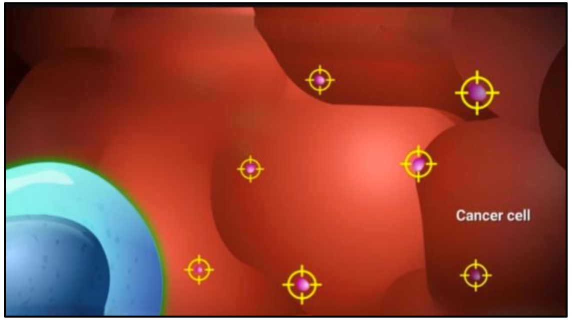

TIL Cell Therapy

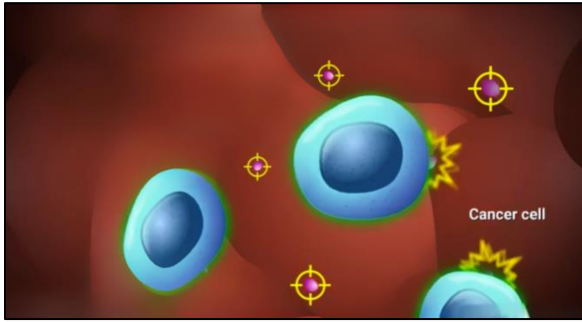


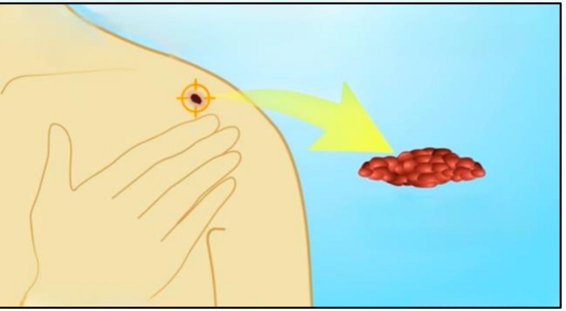


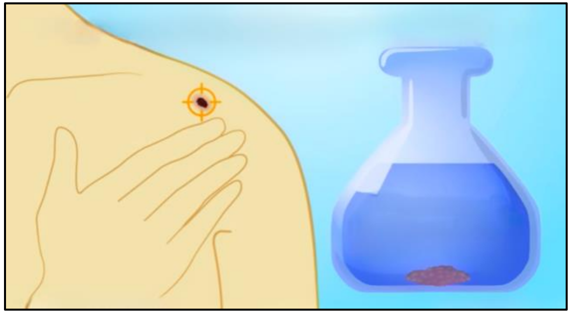

Solid tumor

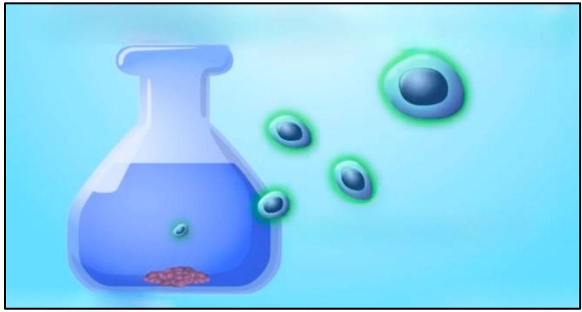

Solid tumor

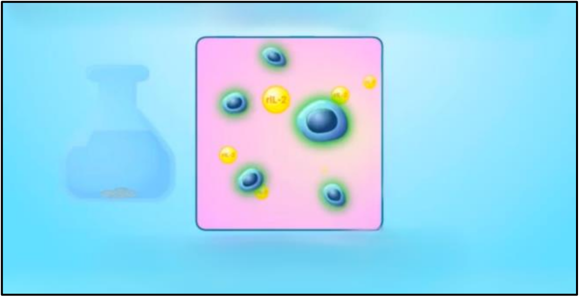

Cancer cell

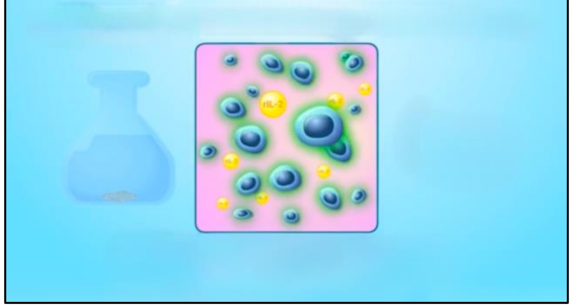

Cancer cell

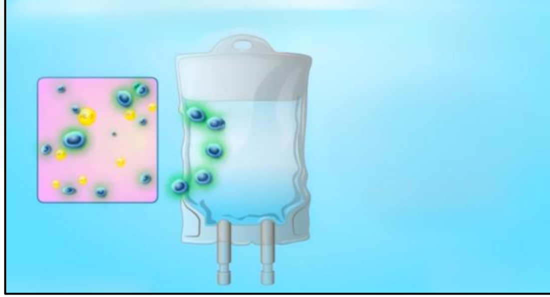


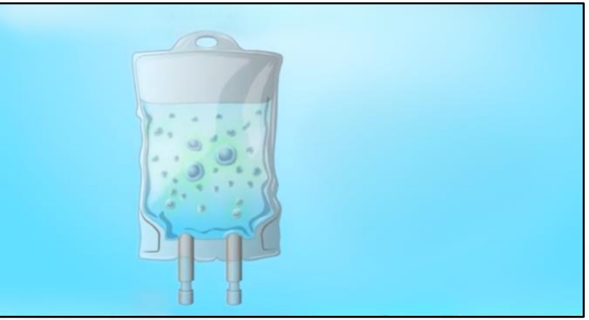




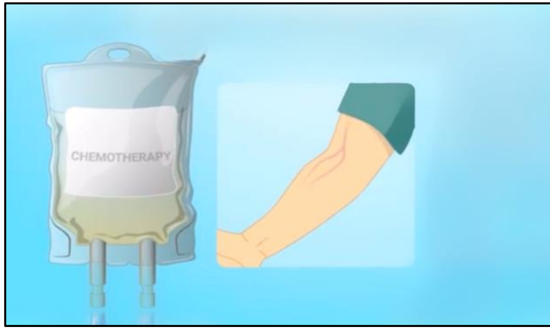

rIL-2

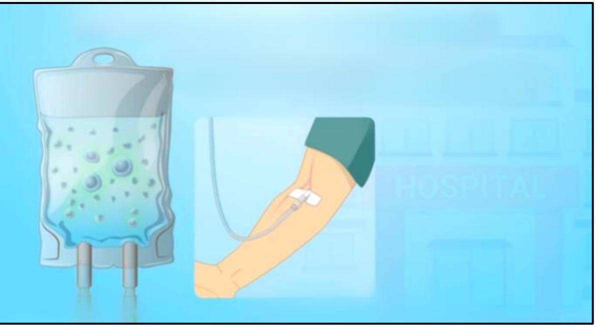

rIL-2

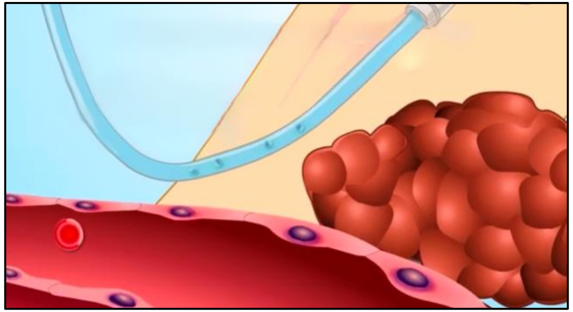


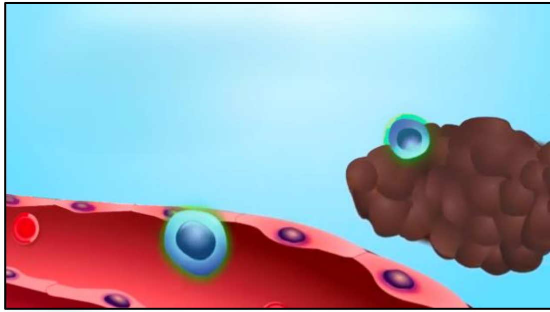


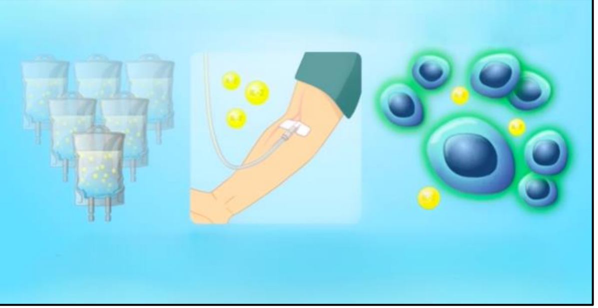

MAY
S M T W T F S
1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18 19
20 21 22 23 24 25 26
27 28 29 30 31
H
HOSPITAL

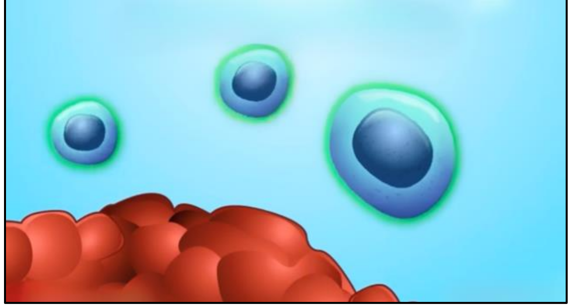

CHEMOTHERAPY

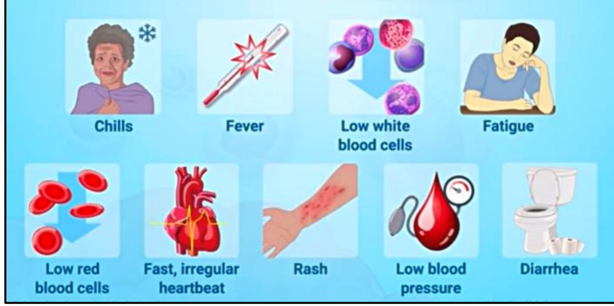

HOSPITAL



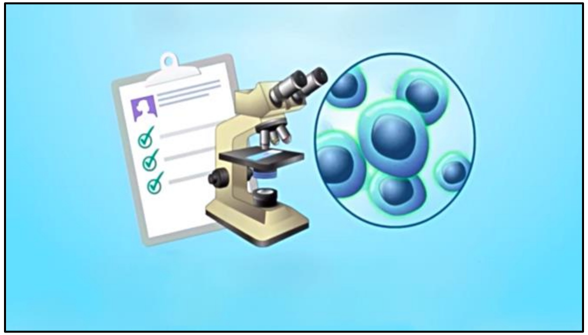


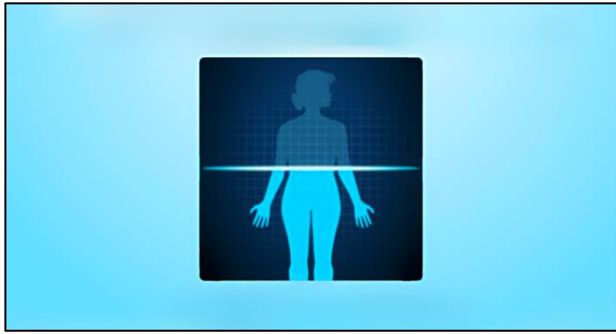


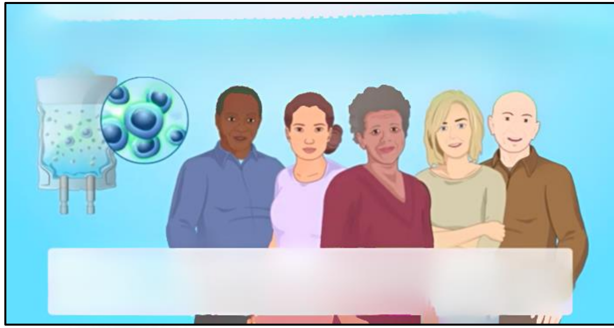



Chills
Fever
Low white blood cells
Fatigue
Low red blood cells
Fast, irregular heartbeat
Rash
Low blood pressure
Diarrhea

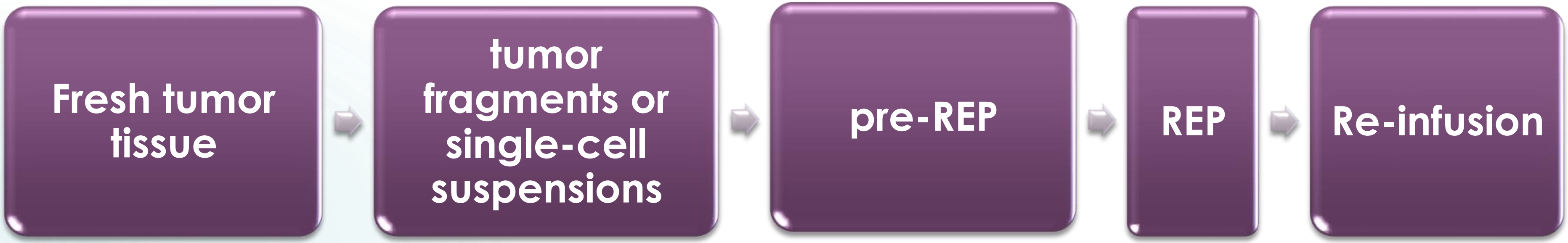

MAY

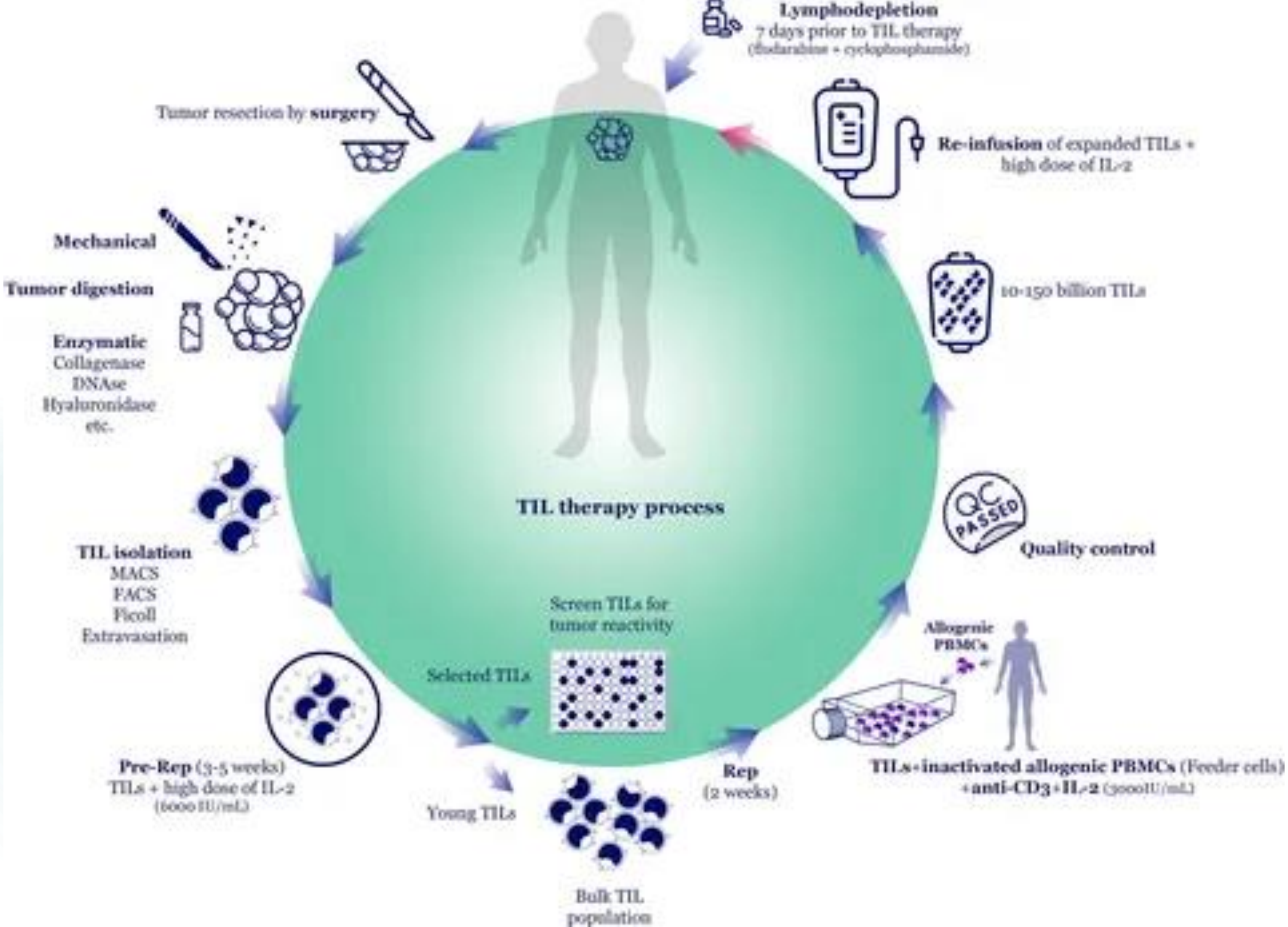






Preparation of TIL




Lymphodepletion
7 days prior to TIL therapy
(fludarabine + cyclophosphamide)
Tumor resection by surgery
Re-infusion of expanded TILs + high dose of IL-2
Mechanical
Tumor digestion
Enzymatic
Collagenase
DNAse
Hyaluronidase
etc.
10-150 billion TILs
TIL therapy process
Quality control
QC PASSED
TIL isolation
MACS
FACS
Ficoll
Extravasation
Screen TILs for tumor reactivity
Selected TILs
Allogenic PBMCs
Pre-Rep (3-5 weeks)
TILs + high dose of IL-2
(6000 IU/mL)
Young TILs
Bulk TIL population
Rep
(2 weeks)
TILs+inactivated allogenic PBMCs (Feeder cells)
+anti-CD3+IL-2 (3000IU/mL)

Consideration

Tissue Requirements: Cancer tissues >1 cm in diameter are typically needed, but needle biopsies can suffice.

Process Duration: TIL production traditionally takes ~4 weeks, now reduced to 14–16 days with recent advancements.

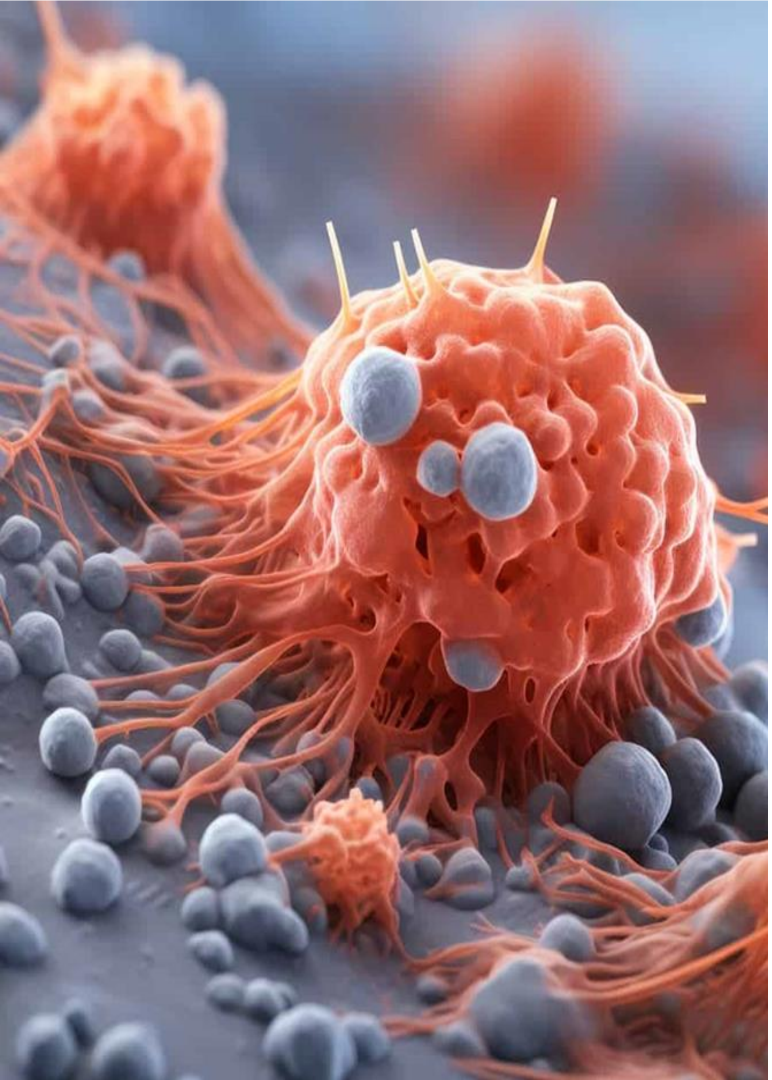
Cost: Over $100,000 per patient (excluding hospital expenses) due to GMP facility maintenance, bioreactors, and supplements. Affordable methods are urgently needed.

Success Rate in expanding: Recent advancements have achieved a >90% success rate in expanding TILs to therapeutic levels.

Cryopreservation: Used in various steps of TIL production for clinical applications.

Pre-REP

- TIL selecting strategies isolating tumor-reactive T cells
  - Selection by Surface Markers
  - Selection by Neoantigens

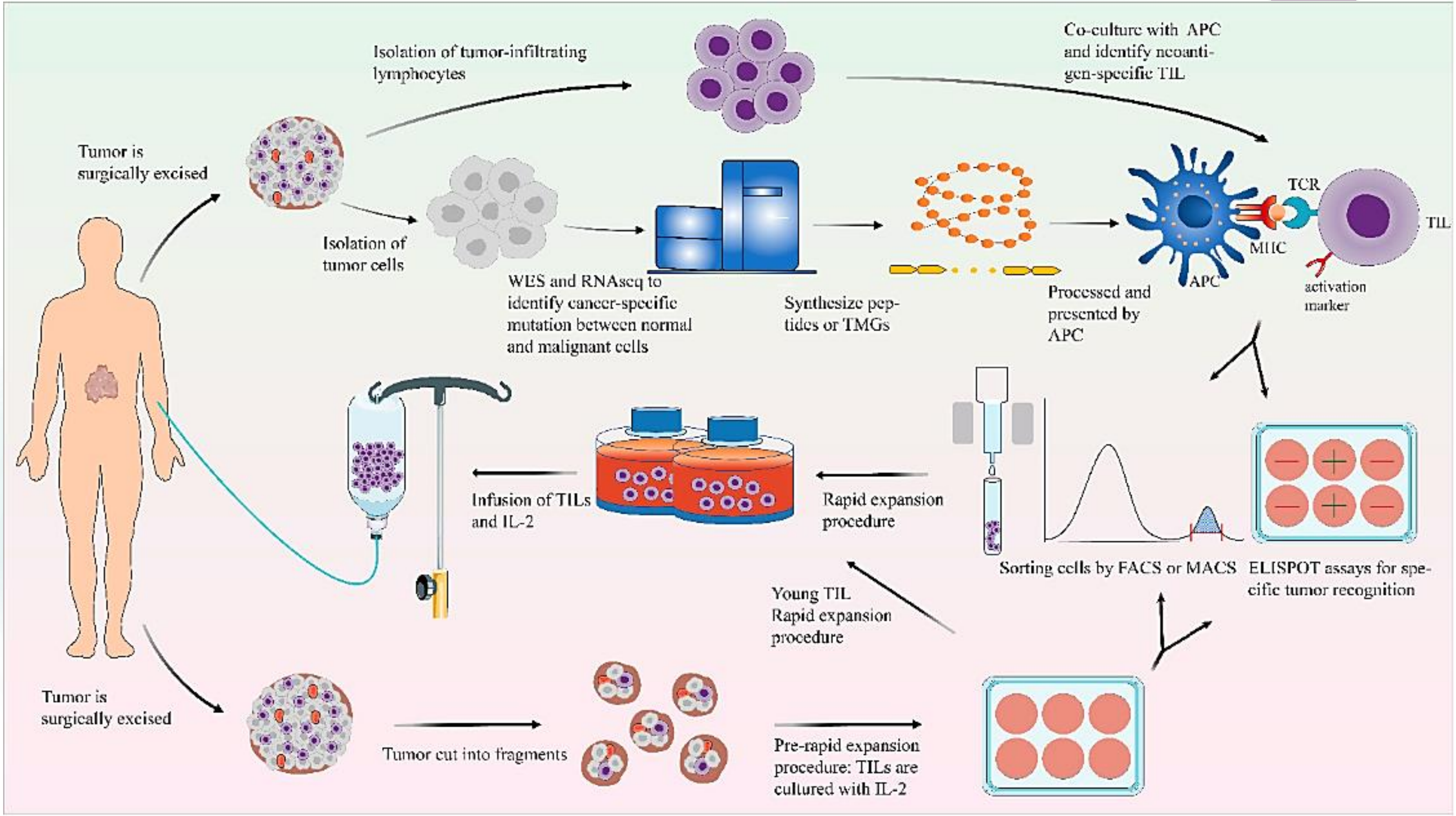


REP

14 days

IL-2, anti-CD3 antibody (which is added only at the start of REP), and irradiated feeder cells

Gas-permeable flasks



Isolation of tumor-infiltrating lymphocytes
Co-culture with APC and identify neoantigen-specific TIL
Tumor is surgically excised
Isolation of tumor cells
WES and RNAseq to identify cancer-specific mutation between normal and malignant cells
Synthesize peptides or TMGs
Processed and presented by APC
APC
TCR
MHC
TIL
activation marker
Infusion of TILs and IL-2
Rapid expansion procedure
Sorting cells by FACS or MACS
ELISPOT assays for specific tumor recognition
Young TIL Rapid expansion procedure
Tumor is surgically excised
Tumor cut into fragments
Pre-rapid expansion procedure: TILs are cultured with IL-2

standard supportive treatment

lymphodepletion

Non-myeloablative (NMA) lymphodepletion regimen with chemotherapy or total body irradiation (TBI)

- 7 days
- Potential mechanisms:
  - Elimination of Tregs
  - Increasing host homeostatic cytokines
  - Decreasing endogenous lymphocytes
  - Activation of antigen presenting cells (APC)

IL-2

Stimulates effector T cell growth and survival

High levels of IL-2 upregulate the inhibitory receptors of CD8+ T cells

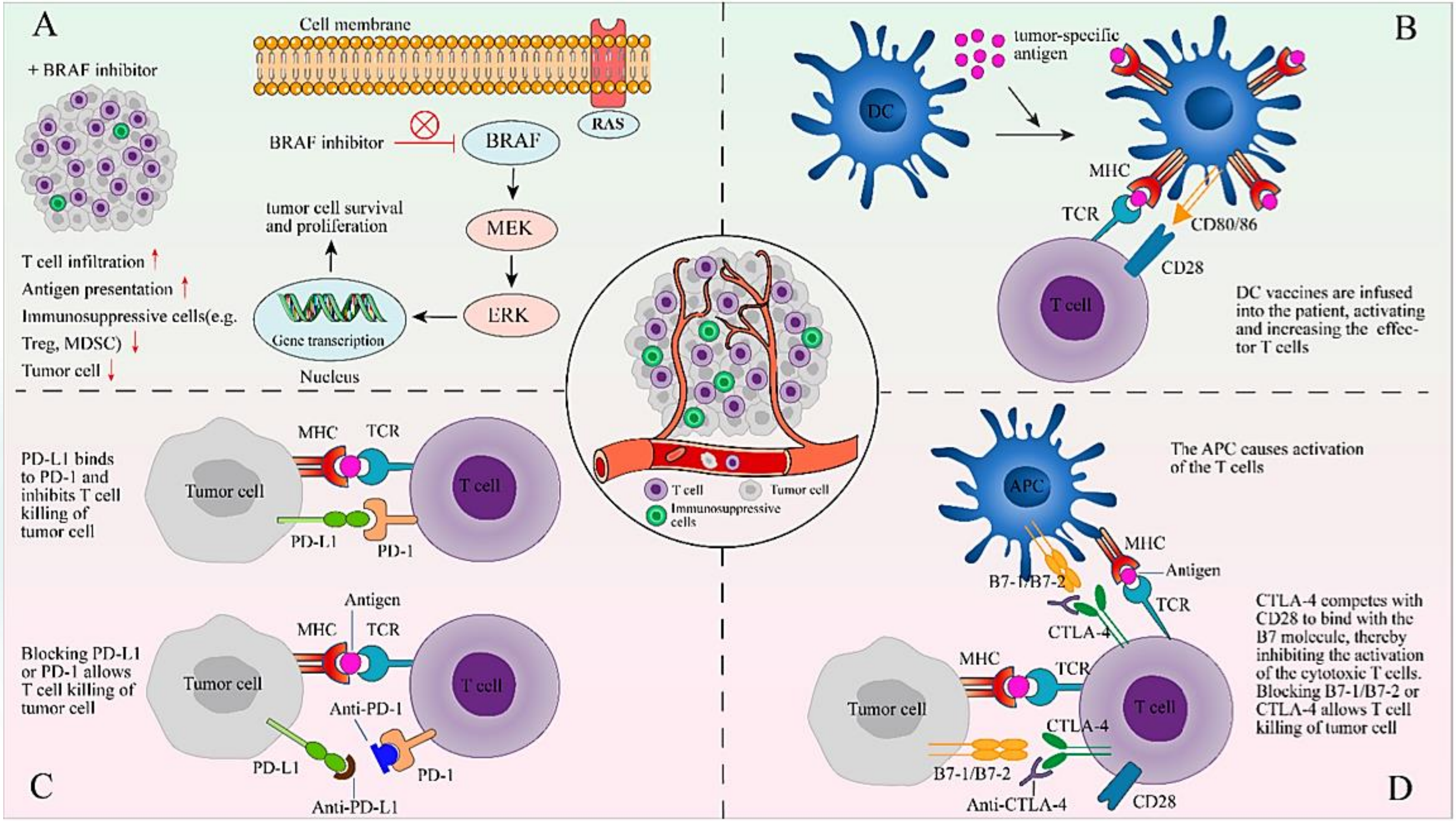
IL-2 could lead to terminal differentiation and T cell exhaustion

IL-7, IL-15, and IL-21 can produce poorly differentiated T cells

Replace intravenous with subcutaneous

Combination Therapy with TILs

- Immune Checkpoint Inhibitors
  - anti-PD-1/PD-L1 antibody therapy
  - anti-CTLA-4
- BRAFF Inhibitor
- DC vaccine
- Oncolytic virus


A
Cell membrane
+ BRAF inhibitor
RAS
BRAF inhibitor
BRAF
MEK
ERK
tumor cell survival
and proliferation
Gene transcription
Nucleus
T cell infiltration ↑
Antigen presentation ↑
Immunosuppressive cells(e.g.
Treg, MDSC) ↓
Tumor cell ↓
B
DC
tumor-specific
antigen
MHC
TCR
CD80/86
CD28
T cell
DC vaccines are infused
into the patient, activating
and increasing the effec-
tor T cells
T cell
Tumor cell
Immunosuppressive
cells
C
PD-L1 binds
to PD-1 and
inhibits T cell
killing of
tumor cell
Tumor cell
MHC
TCR
T cell
PD-L1
PD-1
Blocking PD-L1
or PD-1 allows
T cell killing of
tumor cell
Antigen
Anti-PD-1
Anti-PD-L1
D
APC
The APC causes activation
of the T cells
MHC
Antigen
TCR
B7-1/B7-2
CTLA-4
CTLA-4 competes with
CD28 to bind with the
B7 molecule, thereby
inhibiting the activation
of the cytotoxic T cells.
Blocking B7-1/B7-2 or
CTLA-4 allows T cell
killing of tumor cell
Anti-CTLA-4
CD28

Pediatrics' Solid tumor

Solid Tumors in Pediatrics

Account for ~25% of pediatric cancers (excluding CNS).

- • Common types: Neuroblastoma, sarcomas, Wilms tumor.

Challenges in Immunotherapy

- • Low tumor mutational burden (TMB) → Fewer neoantigens.
- • Minimal T-cell activation; limited success of checkpoint inhibitors.

Clinical Trials

NCT Number	Study Title	Study-Status	Condition	Interventions	Age	Phase	Study-type	Date start/Finish
NCT06047977	Tumor-infiltrating Lymphocyte Therapy for Pediatric High Risk Solid Tumor	Not-Yet-Recruiting	Lymphocytes	Biological: Tumor-infiltrating Lymphocytes, Fludarabine, Cyclophosphamide Interleukin-2	Child. Adult	1	Interventional	2024/2027

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CYTOTHERAPY

journal homepage: www.isct-cytotherapy.org

International Society ISCT Cell & Gene Therapy®

Short Report

Expansion of tumor-infiltrating and marrow-infiltrating lymphocytes from pediatric malignant solid tumors

Jonathan Metts[1,2,*], Madeline Rodriguez-Valentin[2], Jonathan Hensel[3], Alex Alfaro[2], Christopher W. Snyder[4], Odion Binitie[2], Caroline Chebli[5], Hector Monforte[6], Shari Pilon-Thomas[2], John Mullinax[2]

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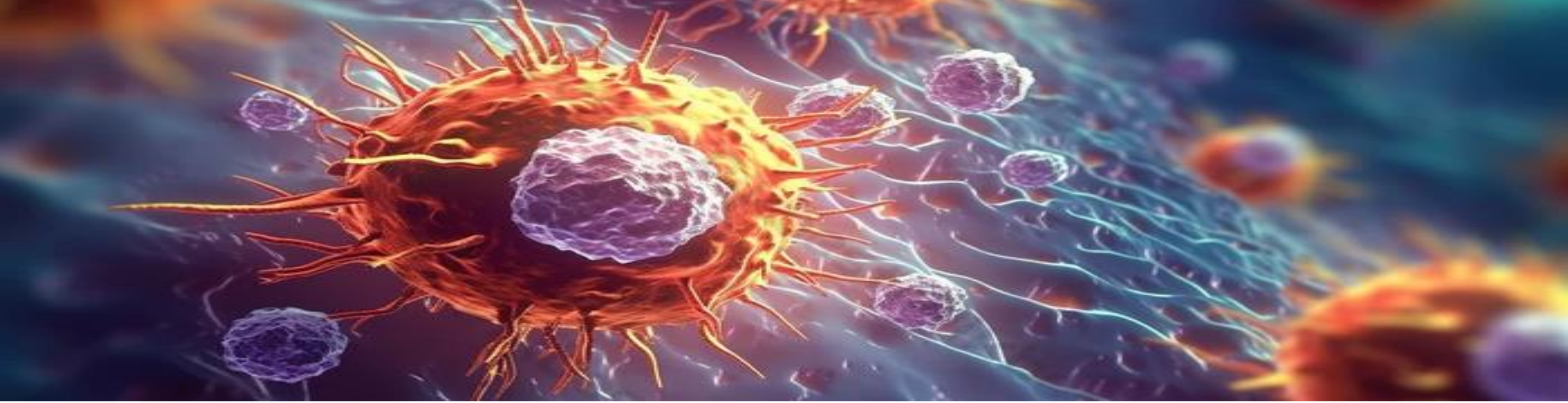
ABSTRACT

Introduction: The expansion of tumor-infiltrating lymphocytes (TIL) for adoptive cellular therapy is under investigation in many solid tumors of adulthood. Marrow-infiltrating lymphocytes (MIL) have demonstrated antitumor reactivity preclinically. Successful expansion of TIL/MIL has not been reported across pediatric solid tumor histologies. The objective of this study was to demonstrate successful expansion of TIL from pediatric solid tumors for translation in an adoptive cell therapy (ACT) treatment strategy.
Methods: A prospective study of TIL/MIL expansion was performed on solid tumors of pediatric patients undergoing standard-of-care procedures. TIL/MIL expansions were performed in the presence of high-dose interleukin 2. To demonstrate a full-scale expansion to clinically-relevant cell doses for TIL therapy, initial TIL culture was followed by a rapid expansion protocol for select patients. Expanded specimens were analyzed for phenotype by flow cytometry and for anti-tumor reactivity by the interferon-gamma release assay.
Results: Eighteen tumor samples were obtained. Initial TIL cultures were successfully generated from 14/18 samples (77.7%). A median of 5.52 × 107 (range: 2.5 × 106–3.23 × 108) cells were produced from initial cultures, with 46.9% expressing a CD3 phenotype (46.9%). Eight samples underwent rapid expansion, demonstrating a median 458-fold expansion and a CD3 phenotype of 98%. Initial MIL cultures were successfully generated from five samples, with a predominantly CD3 phenotype (45.2%). Sufficient tumor tissue was only available for seven TIL samples to be tested for reactivity; none demonstrated responsiveness to autologous tumor.
Conclusions: TIL and MIL expansion from pediatric solid tumors was successful, including the full-scale expansion process. This data supports translation to an ACT-TIL treatment strategy in the pediatric population and thus a Phase I trial of ACT-TIL in pediatric high-risk solid tumors is planned.



- Pediatric extra-cranial malignant tumors (pMST) comprise 30%-40% of pediatric cancers that carry a heavy burden of morbidity and mortality.

- A pMST was defined as noncentral nervous system solid tumor includind Sarcomas, Wilms tumor, Neuroblastoma, Hepatoblastoma, and Germ-Cell tumors.

- Reseach on TIL expansion in pMST has been limited, likely due to unsuccessful early attemps. Aside from one study on neuroblastoms, successful TIL expansion in pMST has not been demonstrated.

Sample	Dx	Stage, risk[c]	Age (years)	Sex	Disease status	Tumor sampling site	Prior therapy[b]	MIL analyzed?
1	NBL/GNBL	L1, low	3	M	Initial treatment	Organ/soft tissue, primary site	N	Yes
2	WT	2, standard	2	M	Initial treatment	Organ/soft tissue, primary site	N	No
3	WT	4, higher	1	M	Initial treatment	Organ/soft tissue, primary site	N	No
4	OS	4	15	M	Relapse	Organ/soft tissue, metastatic site	C	No
5	WT	4, higher	7	M	Initial treatment	Organ/soft tissue, lymph node	N	No
6	NBL/GNBL	M, high	7	M	Relapse	CNS, metastatic site	I	No
7	IMT	1	14	F	Initial treatment	Organ/soft tissue, primary site	N	No
8	ML	1	16	F	Initial treatment	Organ/soft tissue, primary site	N	No
9	OS	4	14	F	Initial treatment	Bone tumor, primary site	C	Yes
10[a]	ASPS	4	20	M	Relapse	CNS, metastatic site	I	No
11	OS	4	13	M	Relapse	Organ/soft tissue, metastatic site	C	No
12[a]	ASPS	4	20	M	Relapse	CNS, metastatic site	I	No
13	NBL/GNBL	M, high	7	M	Initial treatment	Organ/soft tissue, primary site	N	Yes
14	SS	2	13	M	Initial treatment	Organ/soft tissue, primary Site	N	No
15	ERMS	3, intermediate	3	F	Initial treatment	Organ/soft tissue, primary site	N	No
16	OS	2	14	M	Initial treatment	Bone tumor, primary site	C	Yes
17	WT	3, standard	4	M	Initial treatment	Organ/soft tissue, primary site	N	No
18	OS	2	12	M	Initial treatment	Bone tumor, primary site	C	Yes

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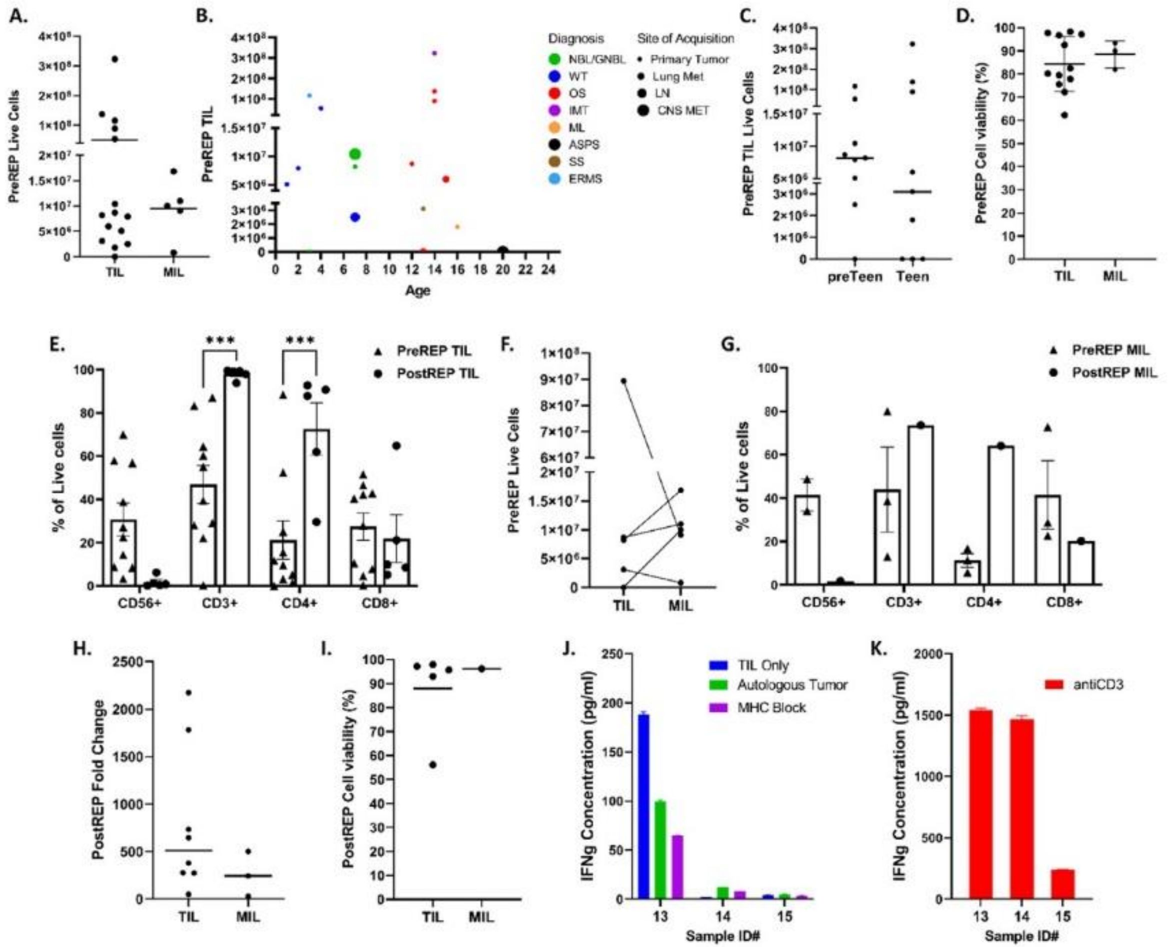

Conclusion

- With the possibility of disease recurrence due to nonimmunogenic tumor cell subclones, TIL-based ACT offers an approach that can limit the refractory response to therapy because of the polyclonal nature of the infusion product. It is therefore an opportune time to fully investigate the utility of TIL-ACT in pMST and also potentially exploit the advantages of targeting multiple TAAs within heterogeneous tumors.
- Hurdles presently exist for pMST TIL-based ACT, and differences between adult and pediatric immune systems will require modifications to achieve greater efficacy. Yet, its potential to address unmet needs among refractory, high-tumor-burden and metastatic pMST patients is far too great to overlook.
- Preclinical work in pMST demonstrates that the hurdle of isolating TILs and expanding them ex vivo to clinically relevant numbers in pMST is certainly surmountable and should undergo further preclinical evaluation as well as clinical investigation in early-phase trials.


thank you