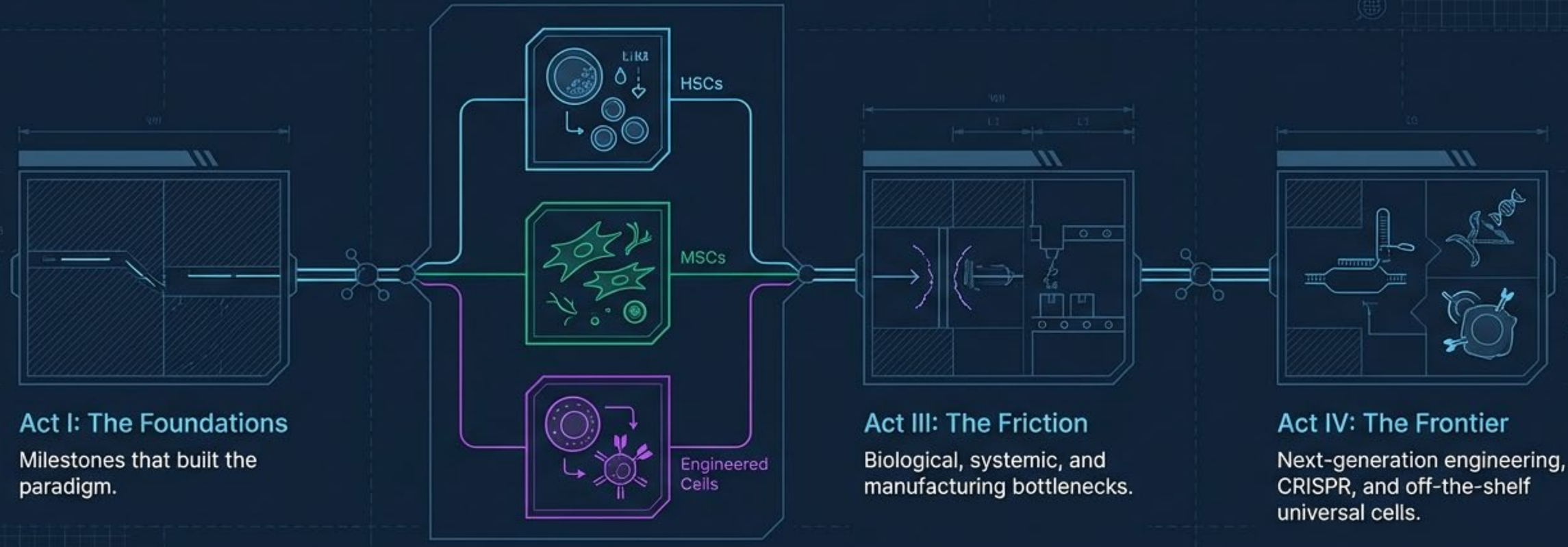
The Architecture of Cellular Medicine



Act I: The Foundations

Milestones that built the paradigm.

Act II: Formal Application

Standard of care, laboratory mechanics, and clinical applications.

Act III: The Friction

Biological, systemic, and manufacturing bottlenecks.

Act IV: The Frontier

Next-generation engineering, CRISPR, and off-the-shelf universal cells.

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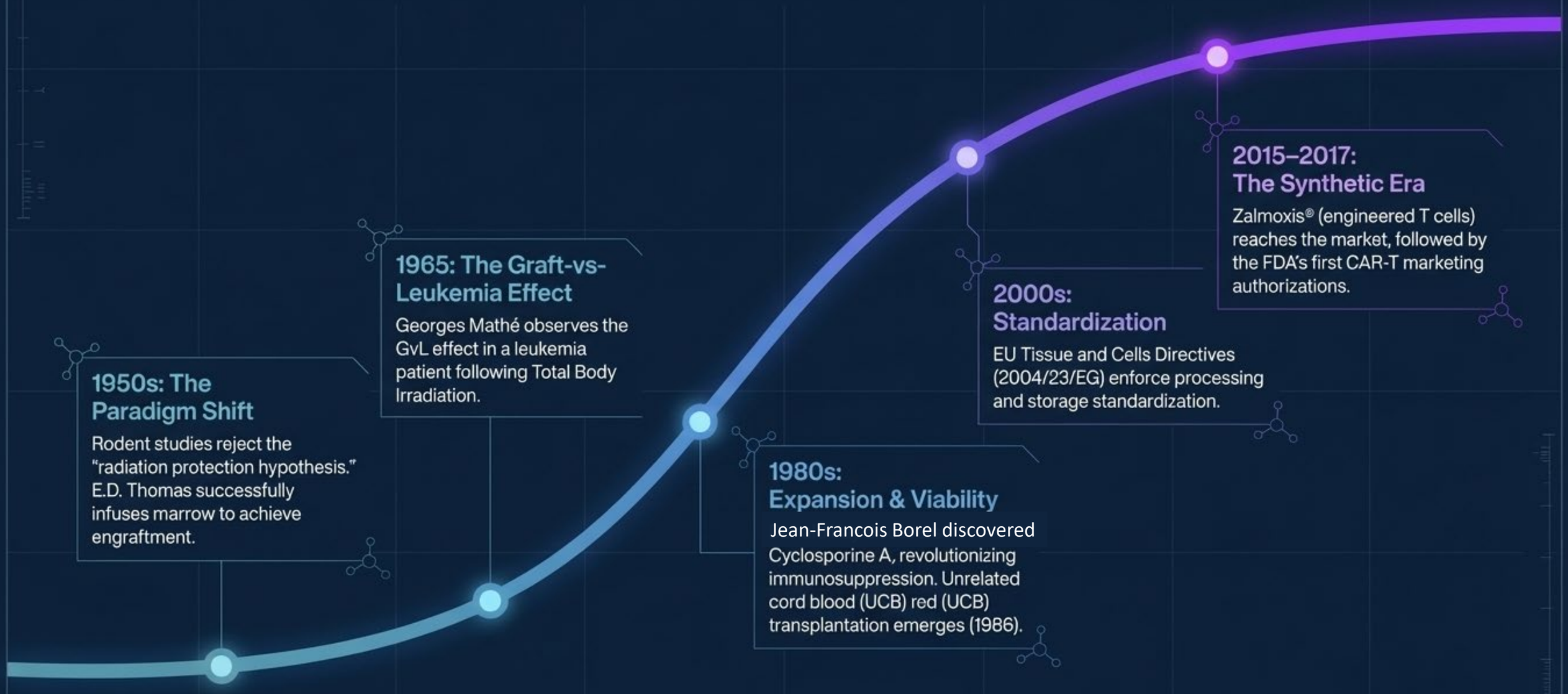
The Evolutionary Curve of Cellular Therapy



1950s: The Paradigm Shift

Rodent studies reject the "radiation protection hypothesis." E.D. Thomas successfully infuses marrow to achieve engraftment.

1965: The Graft-vs-Leukemia Effect

Georges Mathé observes the GvL effect in a leukemia patient following Total Body Irradiation.

1980s: Expansion & Viability

Jean-Francois Borel discovered Cyclosporine A, revolutionizing immunosuppression. Unrelated cord blood (UCB) red (UCB) transplantation emerges (1986).

2000s: Standardization

EU Tissue and Cells Directives (2004/23/EG) enforce processing and storage standardization.

2015–2017: The Synthetic Era

Zalmoxis® (engineered T cells) reaches the market, followed by the FDA's first CAR-T marketing authorizations.

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The Cellular Taxonomy Matrix

	Hematopoietic (HSCs)	Mesenchymal (MSCs)	Induced Pluripotent (iPSCs)
Origin	Bone Marrow, Peripheral Blood, Cord Blood.	Bone Marrow, Adipose Tissue, Umbilical Cord.	Reprogrammed somatic cells (e.g., fibroblasts).
Primary Function	Systemic Reconstitution (Blood/Immune system).	Immunomodulation and regenerative repair.	Generating patient-specific, customized immune cells.
Key Advantage	Demonstrated clinical success in hematological malignancies.	Natural tumor-homing ability; serves as a drug/virus carrier.	Avoids embryonic ethical issues; highly scalable.
Major Risk	Graft-Versus-Host Disease (GVHD).	Undesired differentiation; potential tumor promotion.	Genomic instability; teratoma formation.

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Current Applications I: HSC Graft Sourcing and Dosing

Peripheral Blood Stem Cells (PBSC)

Profile: Fastest engraftment; mobilized via G-CSF and collected via leukapheresis.

Dosing Standard: Minimum 2.0 × 10^6 CD34+ cells/kg. Optimal >4–5 × 10^6 CD34+ cells/kg for rapid neutrophil/platelet recovery.

Dosing Threshold

Bone Marrow (BM)

Profile: Harvested via iliac crest aspiration. Slower engraftment but significantly lower rates of chronic GVHD.

Ideal Patient: Mandatory for pediatric cases and non-malignant diseases (e.g., Aplastic Anemia).

Haploidentical Transplants

Profile: Half-matched donors (parents, offspring, siblings), expanding access to >90% of patients.

Key Advance: Post-Transplantation Cyclophosphamide (PT-CY) and T-cell depletion effectively manage fatal GVHD risks.

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Current Applications II: The Laboratory Pipeline

Vein-to-Vein Flowchart

Donor Leukapheresis

Mobilized collection (≤ 5 hours, central venous access for pediatrics).

Volume / Plasma Reduction

Processing large volumes to achieve ≥ 85% Total Nucleated Cell Count (TNCC) recovery.

RBC Depletion

Mandatory for major ABO incompatibility. Target: acceptable residual RBC (≤ 0.3 cc/kg) with ≥ 80% TNCC recovery.

T-Cell Depletion

Advanced CD34+ selection to prevent GVHD.

Cryopreservation & Release

Precise calculation verifications prior to patient infusion.

Thawing/Infusion

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Current Applications III: The Dual Function of MSCs

Regenerative & Autoimmune

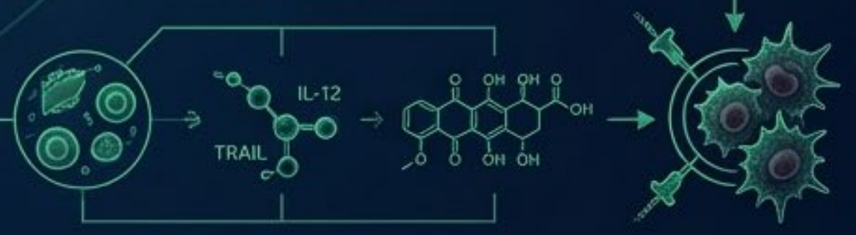
Mechanism: Potent immunomodulation without triggering allogeneic rejection (lack of co-stimulatory molecules).

Clinical Targets: Refractory perianal fistulas in Crohn's Disease, severe acute GVHD, and acute myocardial infarction repair.

The Oncology Vector

Mechanism: Natural migration and homing to sites of injury and tumor microenvironments.

Clinical Targets: Engineered payload delivery. Delivering pro-apoptotic proteins (TRAIL), IL-12, or chemotherapy (Doxorubicin) directly to metastatic sites.



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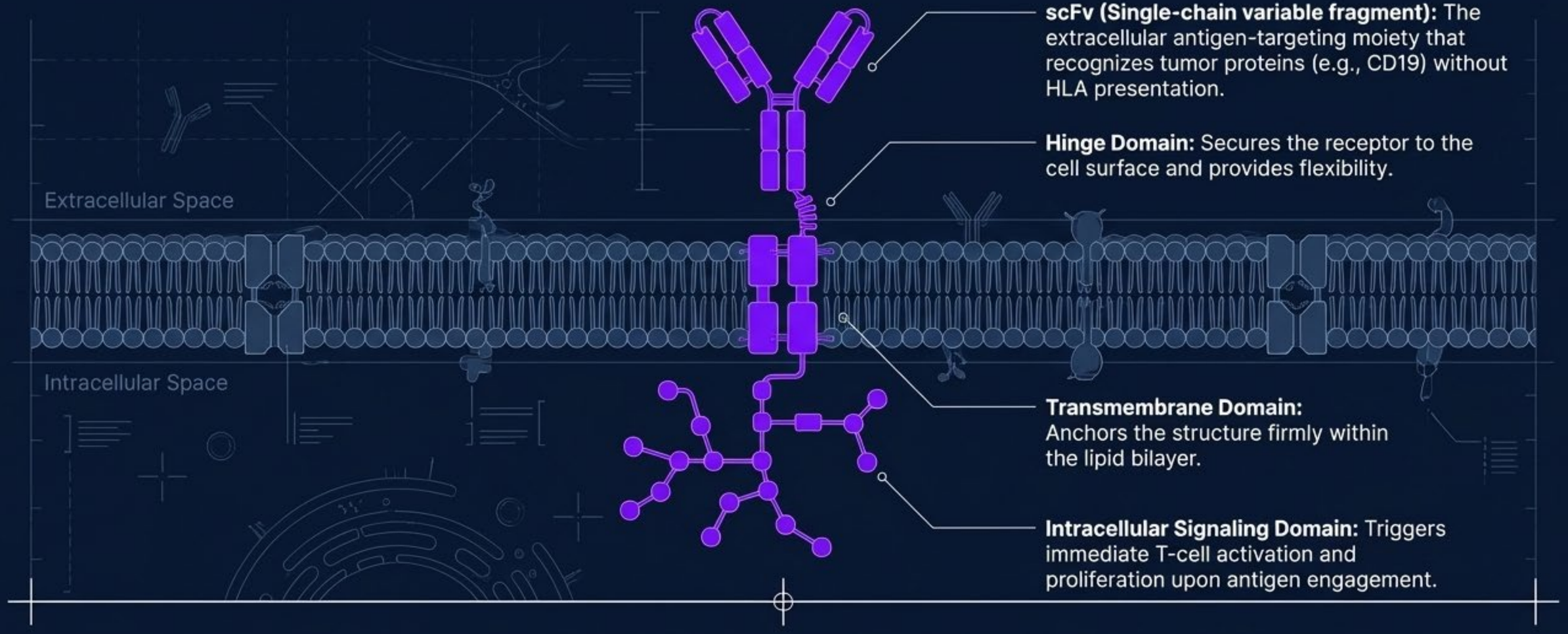
Current Applications IV: Engineered Immune Effectors

Chimeric Antigen Receptor (CAR) T-Cells: Bypassing HLA-restricted targeting to achieve >80% remission rates in B-cell acute lymphoblastic leukemia (B-ALL).



scFv (Single-chain variable fragment): The extracellular antigen-targeting moiety that recognizes tumor proteins (e.g., CD19) without HLA presentation.

Hinge Domain: Secures the receptor to the cell surface and provides flexibility.

Transmembrane Domain: Anchors the structure firmly within the lipid bilayer.

Intracellular Signaling Domain: Triggers immediate T-cell activation and proliferation upon antigen engagement.

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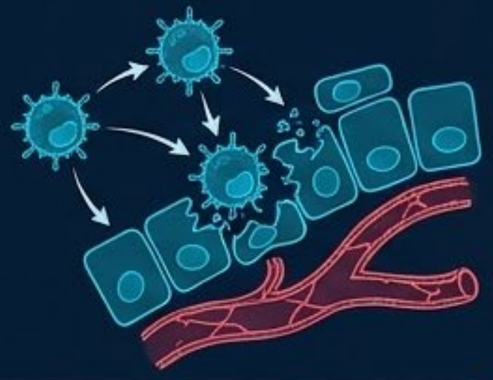
Clinical Friction I: The Double-Edged Sword of Safety

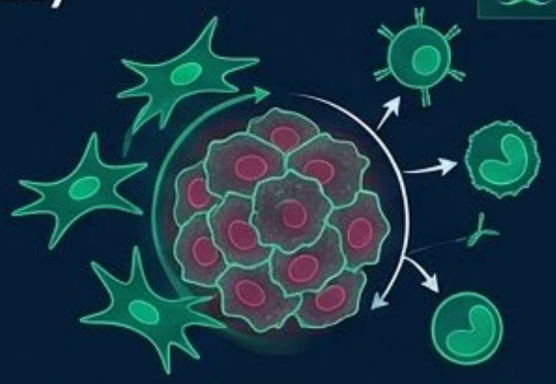
Graft-vs-Host Disease (GVHD)

The primary threat in allogeneic HSCT, where donor T-cells attack recipient tissues.



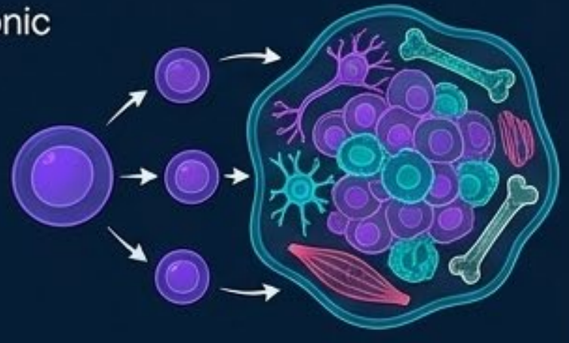
Tumor Promotion (MSCs)

MSCs' potent immunosuppressive capabilities can inadvertently protect cancer cells from immune surveillance, creating a "tumor-friendly" environment.



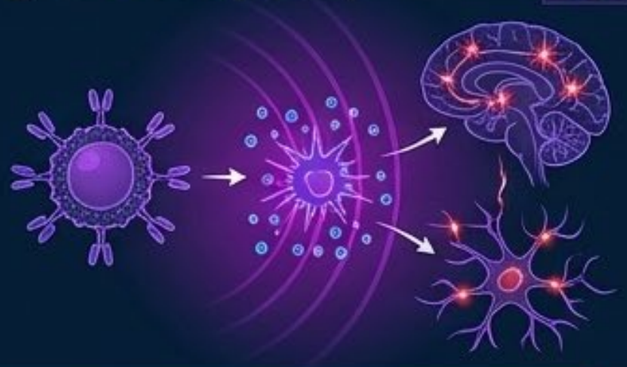
Teratoma Formation (iPSCs)

Undifferentiated embryonic and iPSCs carry a high risk of developing into tumors containing all three germ layers.



Cytokine Release Syndrome (CRS) & Neurotoxicity

The rapid, massive immune activation of CAR-T cell infusions can trigger fatal systemic toxicity.



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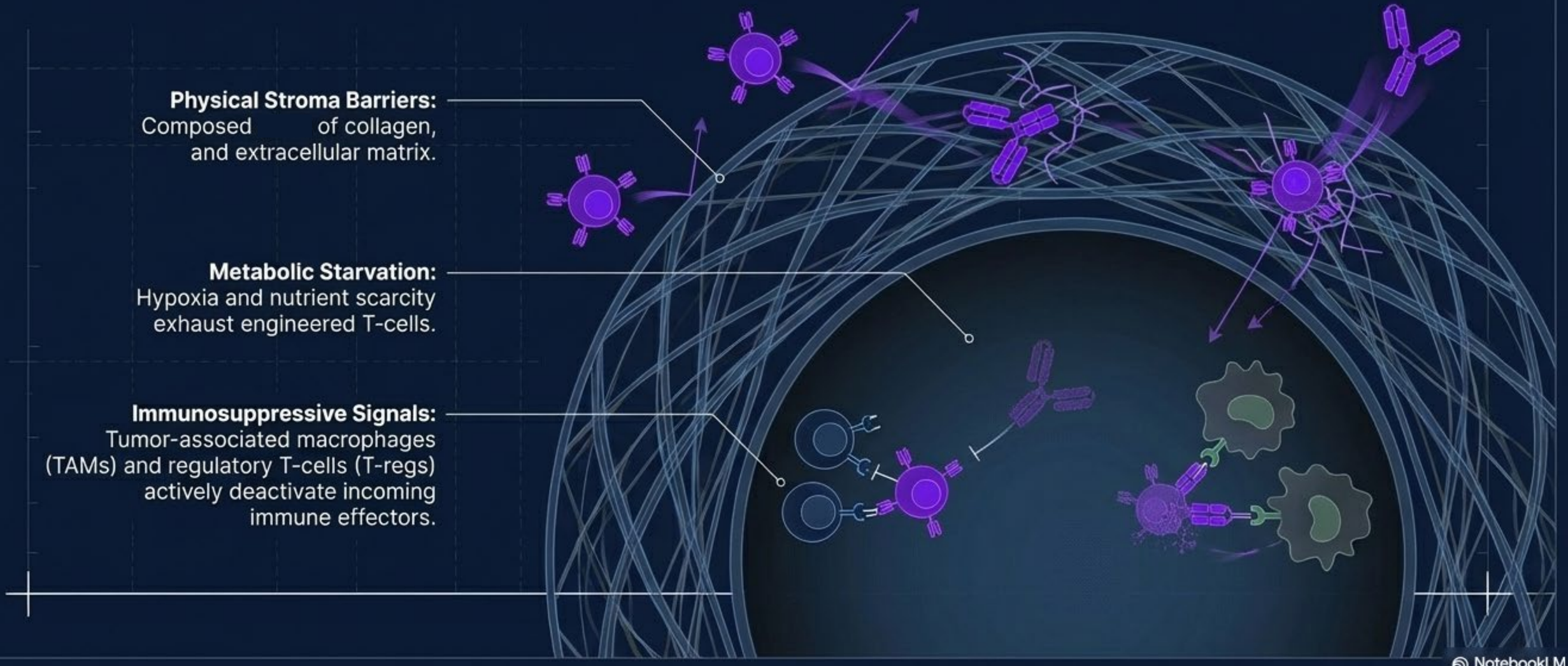
Clinical Friction II: The Solid Tumor Fortress

While highly effective in blood, cellular therapies face a hostile Tumor Microenvironment (TME) in solid tissue. Result: Less than 10% infiltration efficiency in solid tumor trials.



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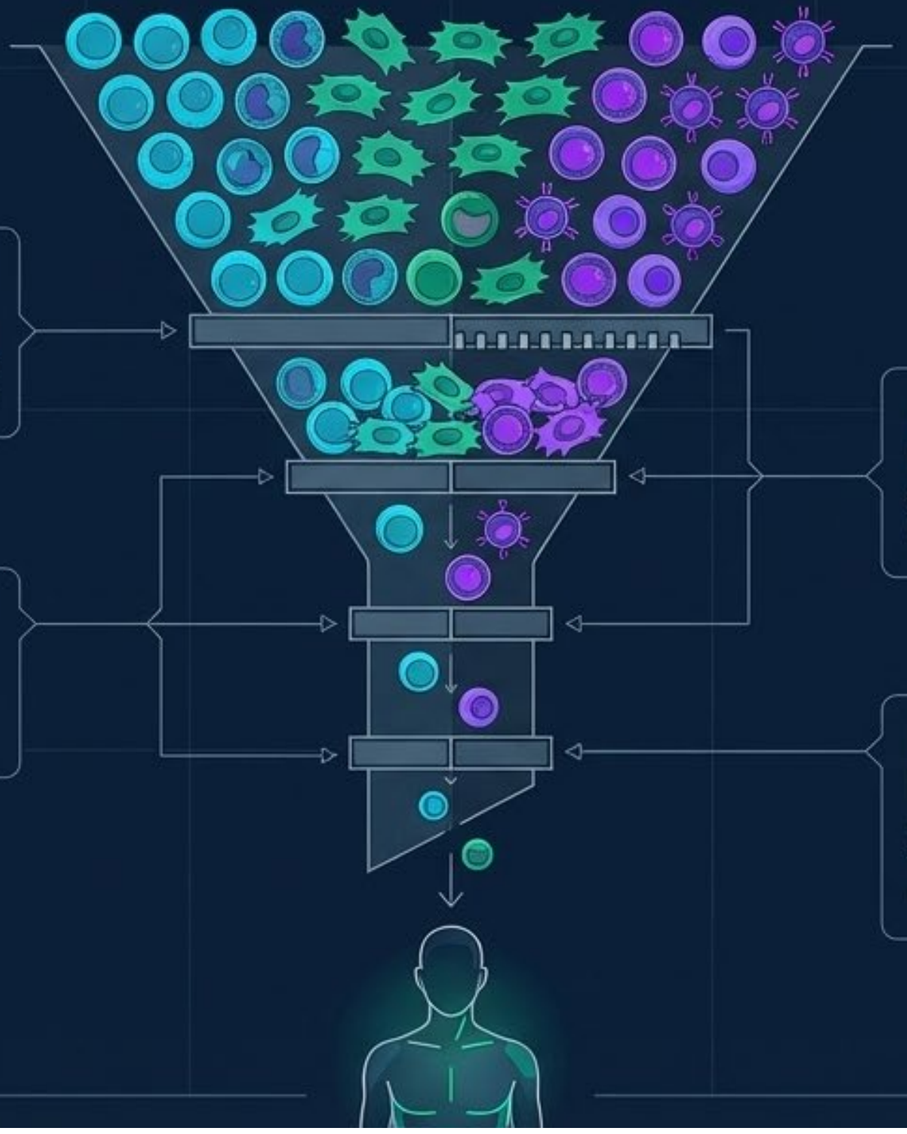
Clinical Friction III: Translational & Systemic Barriers



Manufacturing Bottlenecks

Autologous *ex vivo* expansion is manual, labor-intensive, and prone to T-cell fatigue, causing a 30% expansion failure rate. (Inter

Ethical Constraints

The destruction of human embryos for ESCs continues to restrict specific research pathways and funding.

Economic Exclusion

High cGMP infrastructure costs limit access in Low- and Middle-Income Countries (LMICs).

Regulatory Disparities

Lack of global harmonization fuels unregulated "stem cell tourism," exposing vulnerable patients to unproven and dangerous treatments.

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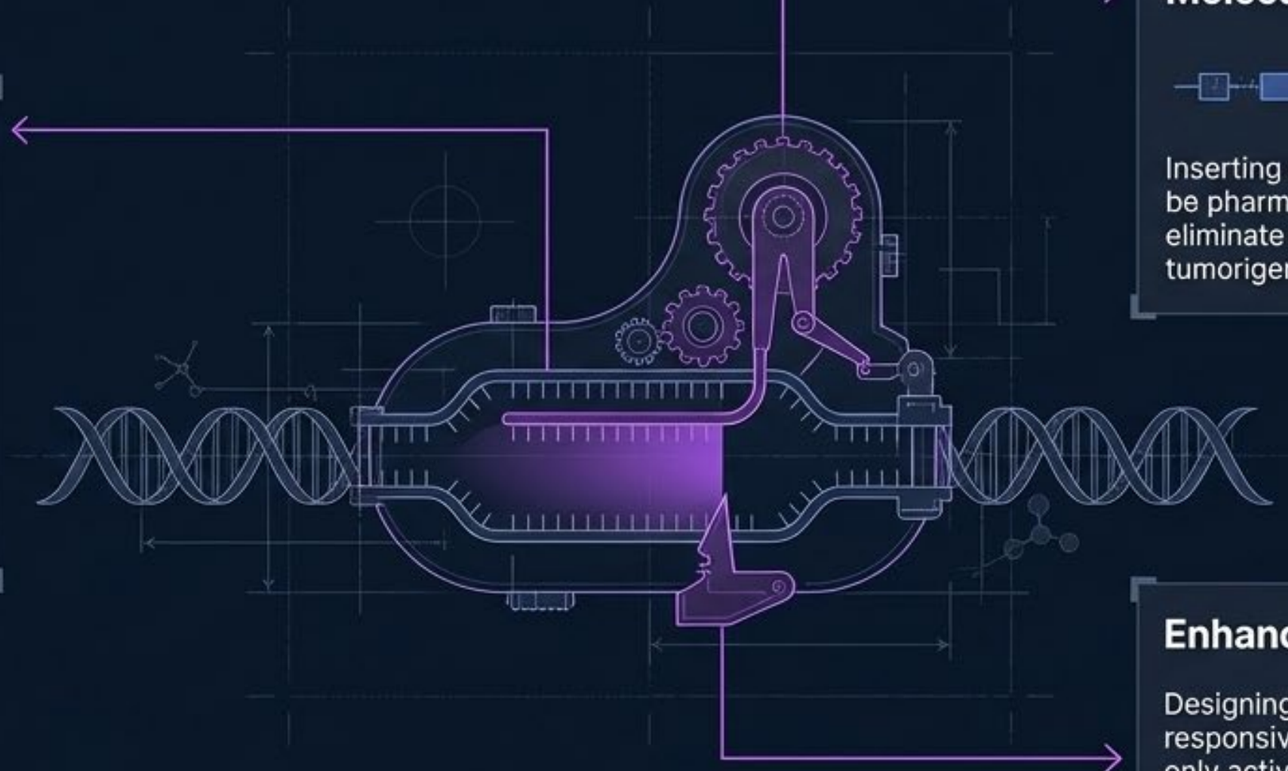
The Frontier I: Next-Gen Engineering via CRISPR

CRISPR-Cas9 is upgrading immune cells from blunt instruments to precision software.

Knocking Out Exhaustion

Deactivating immunosuppressive receptors like PD-1 and CTLA-4 to prevent the TME from switching off T-cells.

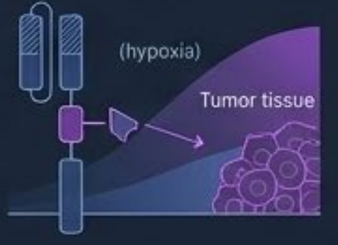


Molecular Safety Switches

Inserting suicide genes (kill switches) that can be pharmacologically activated to instantly eliminate engineered cells if severe CRS or tumorigenesis occurs.

Enhancing Metabolic Resilience

Designing hypoxia-responsive CARs that only activate in the low-oxygen environments specific to tumors.



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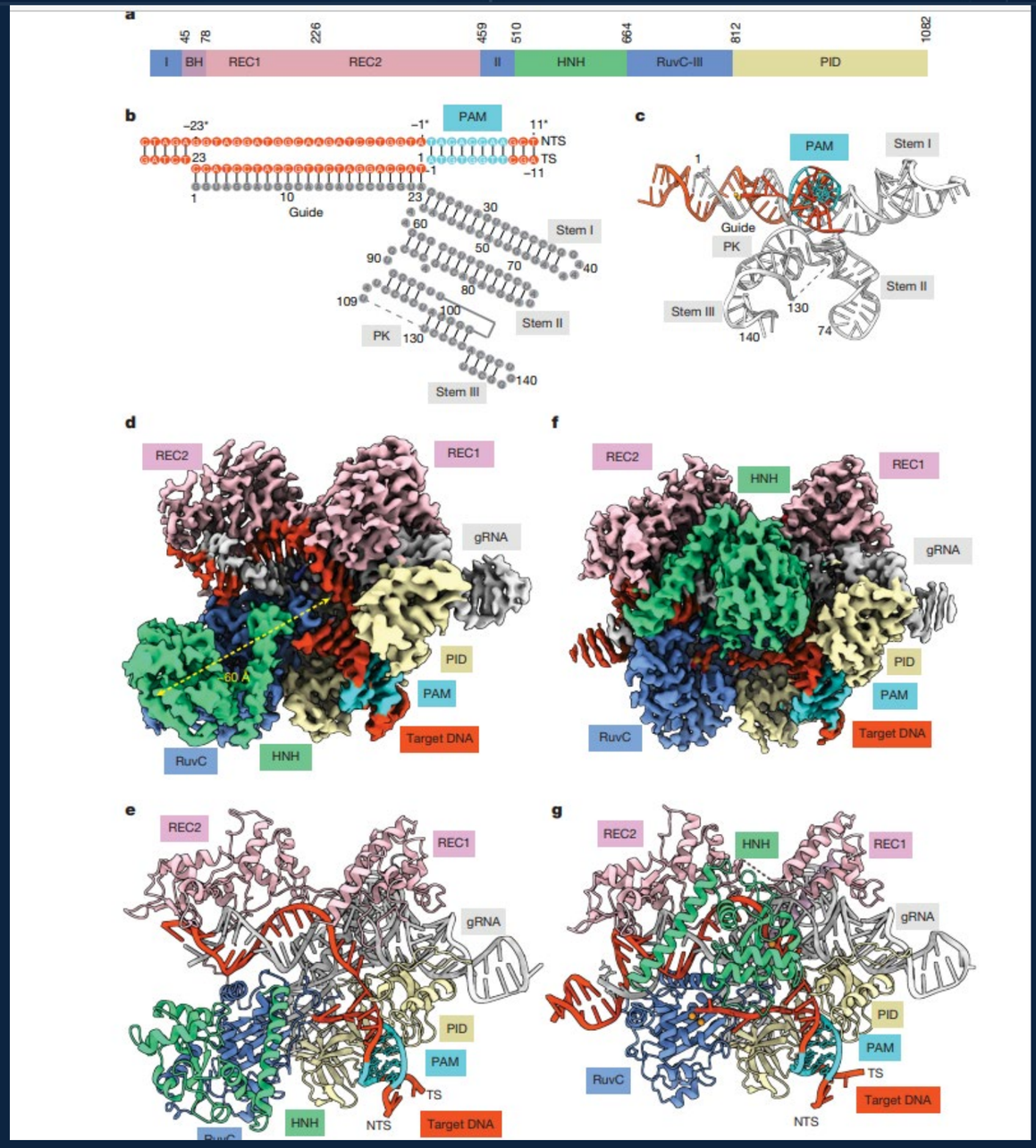
Directed by a complementary guide RNA (gRNA), Cas9 proteins catalyze the cleavage of double-stranded DNA (dsDNA). **A methylation-sensitive Cas9** would serve as a simple enzyme-based tool that can both map epigenetic changes and support highly precise gene-editing applications.



A team led by Van Andel Institute's Dr. Hong Li and Wageningen University & Research's Dr. John van der Oost has used a **CRISPR variant called ThermoCas9 to distinguish tumor DNA from healthy DNA** and selectively cut only the former. The findings are a promising step toward possible future high-precision cancer therapies.

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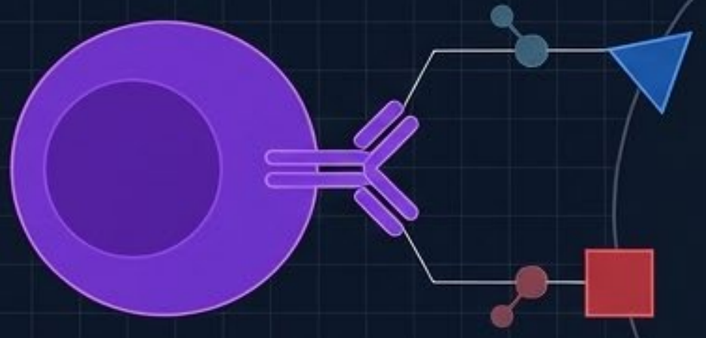
The Frontier II: Breaching the Solid Tumor

Bispecific CARs



Next-generation T-cells engineered with multiple targeting capabilities to recognize different tumor antigens simultaneously, preventing cancer immune escape.

The MSC Trojan Horse

- Harnessing the natural tumor-homing ability of MSCs to deliver Oncolytic Virotherapy.
- Viruses are hidden inside the MSC, protecting them from early immune clearance.
- Once inside the TME, viruses selectively replicate and destroy cancer cells while sparing healthy tissue.

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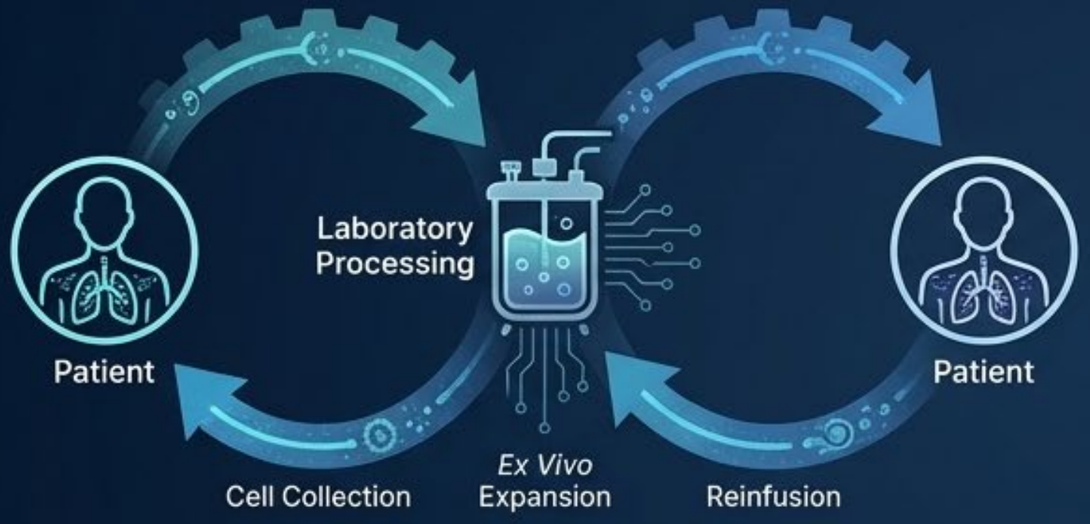
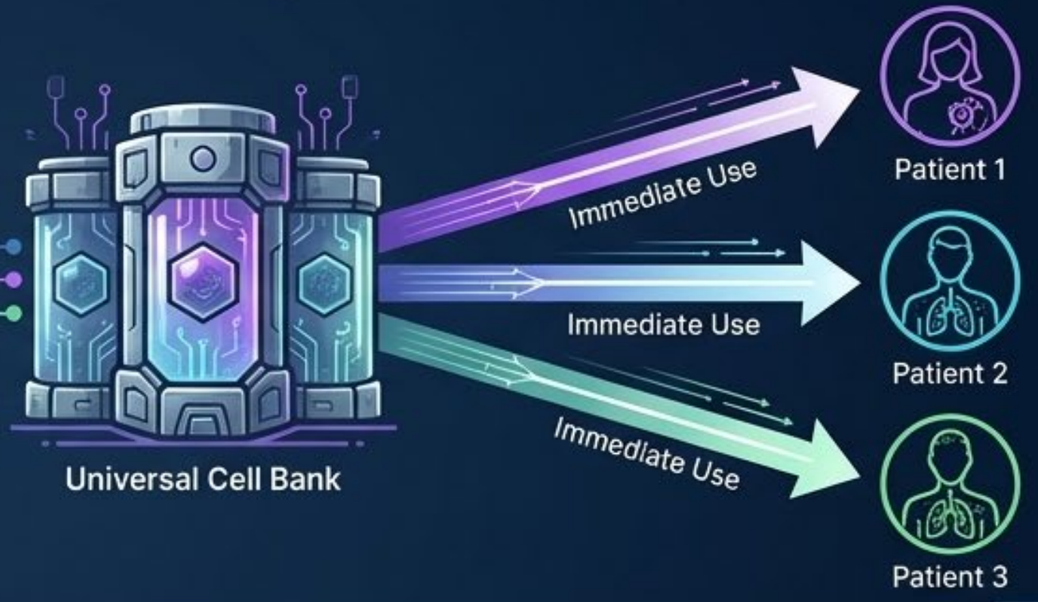
The Frontier III: ‘Off-the-Shelf’ & Cancer Vaccines

Autologous (Bespoke, Slow)



Allogeneic (Universal, Fast)



Allogeneic Universal Cells

Shifting from autologous to ‘off-the-shelf’ products. Utilizing iPSCs or natural universal donor cells (γδ-TCR T cells) to bypass HLA-matching and slash manufacturing time.

Personalized iPSC Vaccines

Because iPSCs share characteristics with cancer stem cells, they can be utilized as customized vaccines. Designed to prime the patient’s cytotoxic T-cells and NK cells to recognize therapy-resistant tumor populations, actively preventing relapse.

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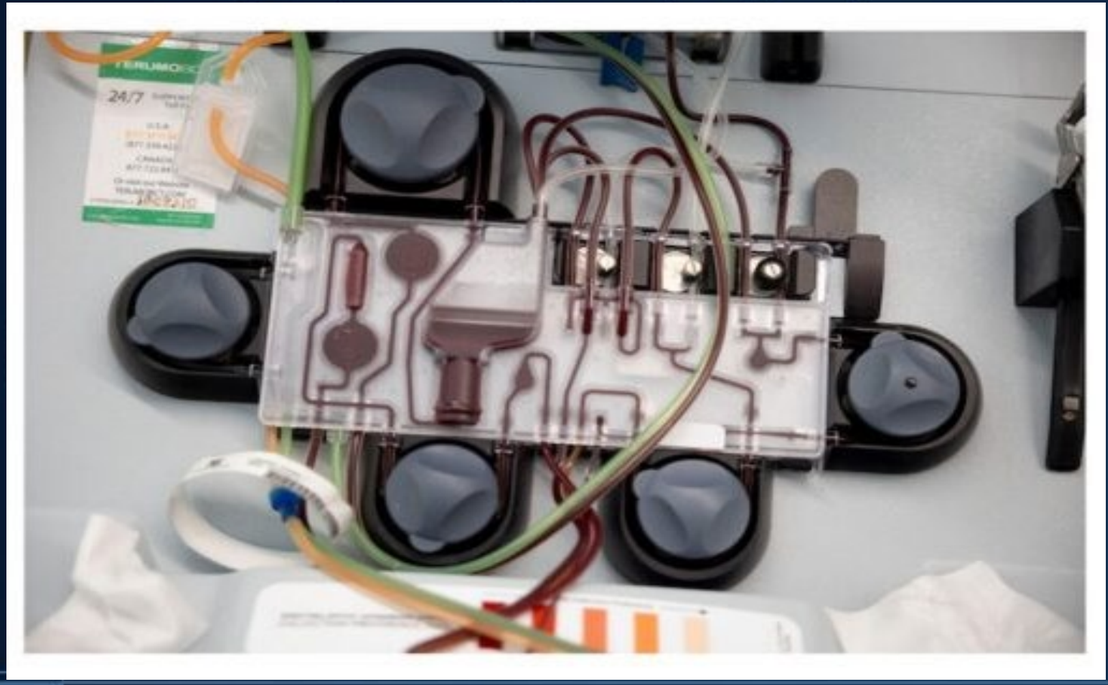
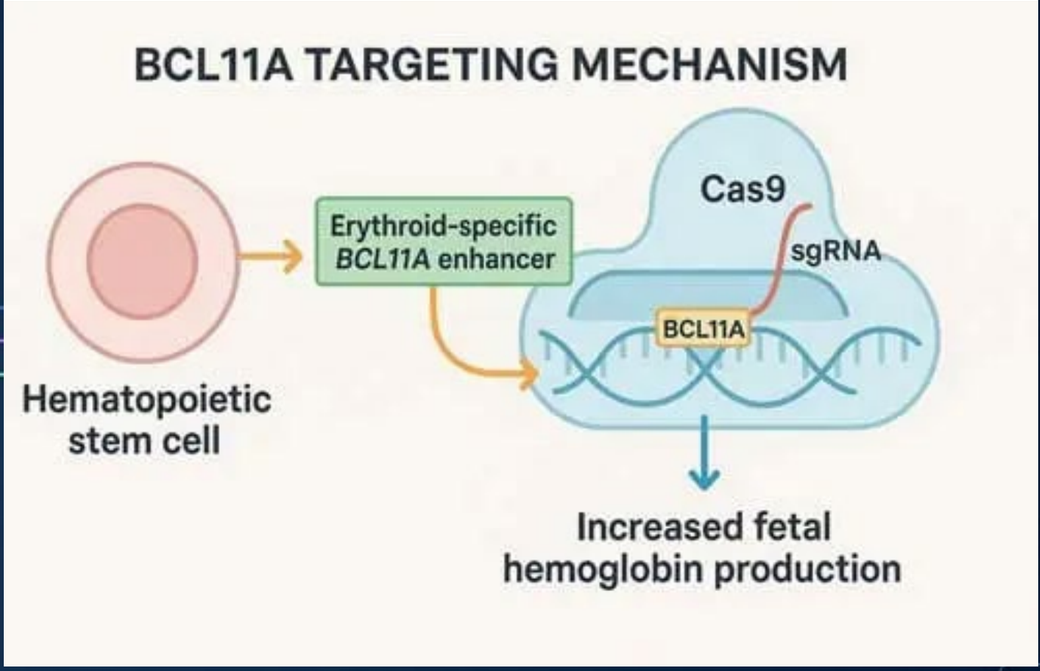
The Frontier IV: Sickle Cell Disease Today !!





The provider will collect blood stem cells from the patient through a procedure/process called mobilization and apheresis. This process takes approximately one week and may need to be repeated to obtain enough cells.

These stem cells will be sent to the manufacturing site where the cells are used to make the therapy. It typically takes 10 to 15 weeks from the time cells are collected to making and testing treatment.

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The Frontier IV: Sickle Cell Disease Today !!

Manning Family Children's
August 11, 2025 ·

Meet Daniel, a 22-year-old from Metairie who has battled sickle cell disease his whole life. Now, he's making history as the first patient in Louisiana to undergo groundbreaking gene therapy that could cure the condition entirely. This transformational cure won't just end years of pain—it could put him in the cockpit, chasing his dream of becoming a pilot.

Last week, Daniel visited us here at Manning Family Children's to begin one of the first steps of this long journey. We're proud to be part of this moment for Daniel, his family, and the future of sickle cell care. 💙

Read more about his journey and this incredible treatment breakthrough at https://bit.ly/45JY86x

VERTEX manufacturing prepare Daniel's infusion using CRISPr to make CASGEVY a HbF inducer.

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THANK YOU, for your attention.

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