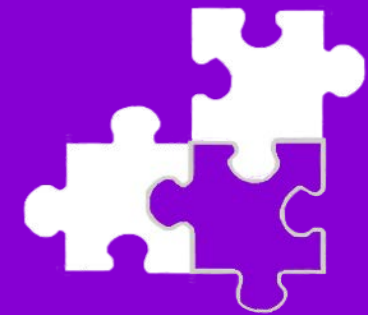


Introduction to HIV Cure-Related Research

DEEPER DIVE



CUREiculum

This research training curriculum is a collaborative project aimed at making the science of HIV cure-related research accessible to the community and the HIV research field.



**If you can read this
sign, You are in the
Deeper Dive!**

**This module is for anyone
exploring a deeper understanding
of the immune-system and HIV**



Why take a deeper dive?

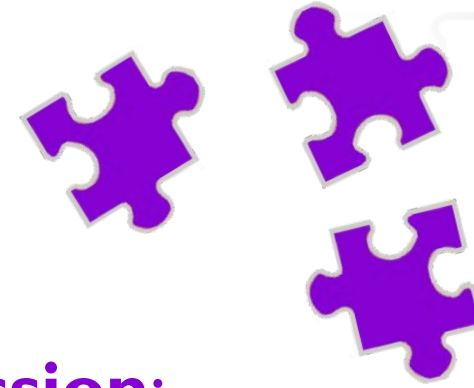
- Following set of slides afford greater detail on some of the complex interaction between HIV and our immune system.
- Designed by **community for community**
- Hope it will lead to meaningful community-centered conversations

6 modules:

1. Introduction to HIV cure-related research
2. Analytical treatment interruptions (ATIs)
3. HIV persistence
4. HIV cure-related research strategies
5. Ethics of HIV cure-related research
6. Community, patient & stakeholder engagement



Glossary of Key Terms



Antiretroviral (ART)-free HIV suppression:

*Control of virus in the absence of HIV treatment
(also: ‘post-treatment control’ or ‘ART-free durable control’)*

Latency:

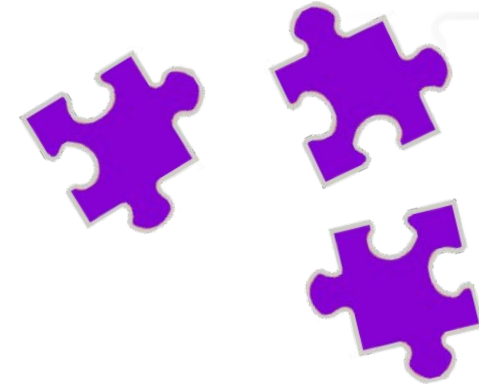
The ability of a virus to stay dormant (inactive) within a cell

Provirus:

*A virus’ genetic material that has become integrated inside the DNA
(deoxyribonucleic acid) of a cell*



Glossary of Key Terms



Reservoirs:

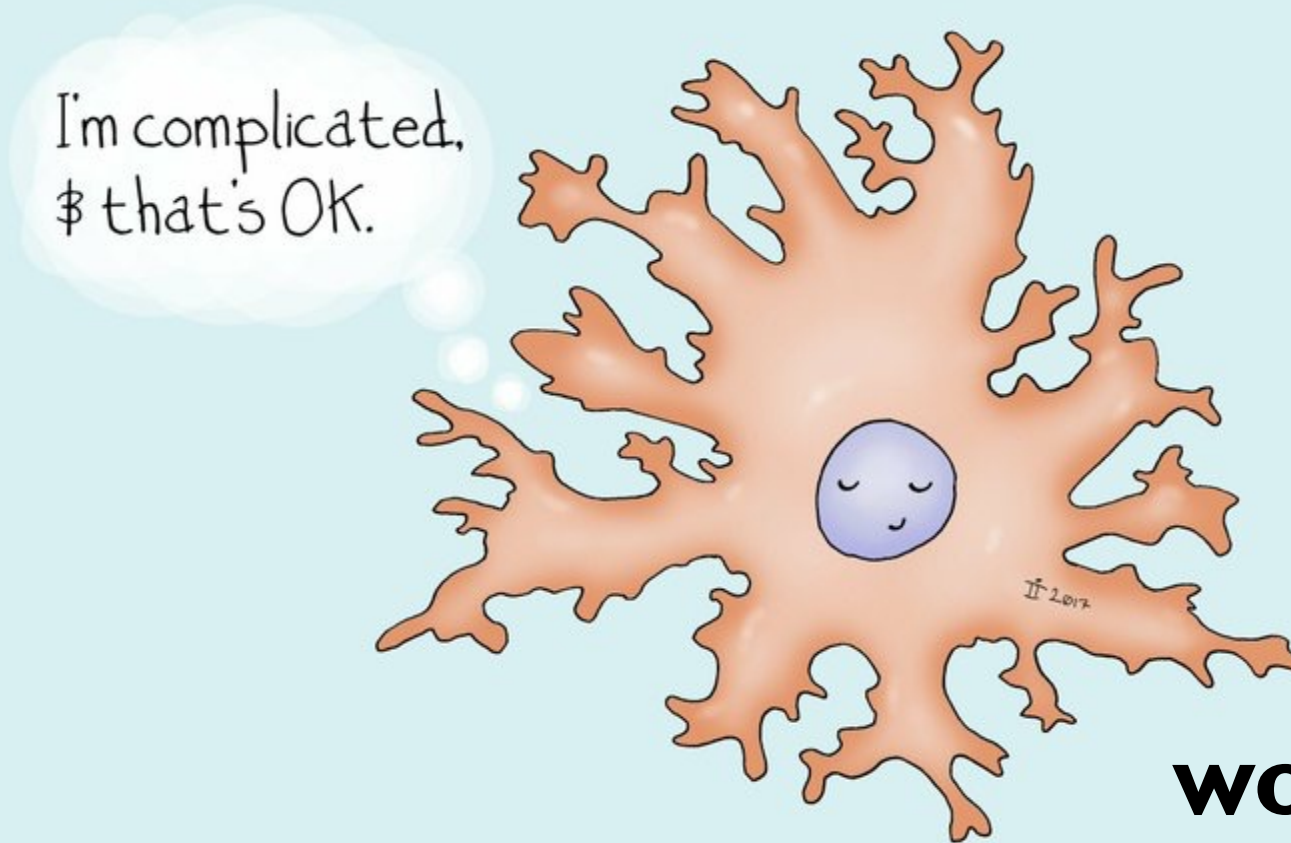
Cells and compartments in the body where HIV can hide and not be found, even in the presence of antiretroviral therapy

Viral rebound:

When a person has detectable levels of HIV in the blood after a period of having undetectable levels



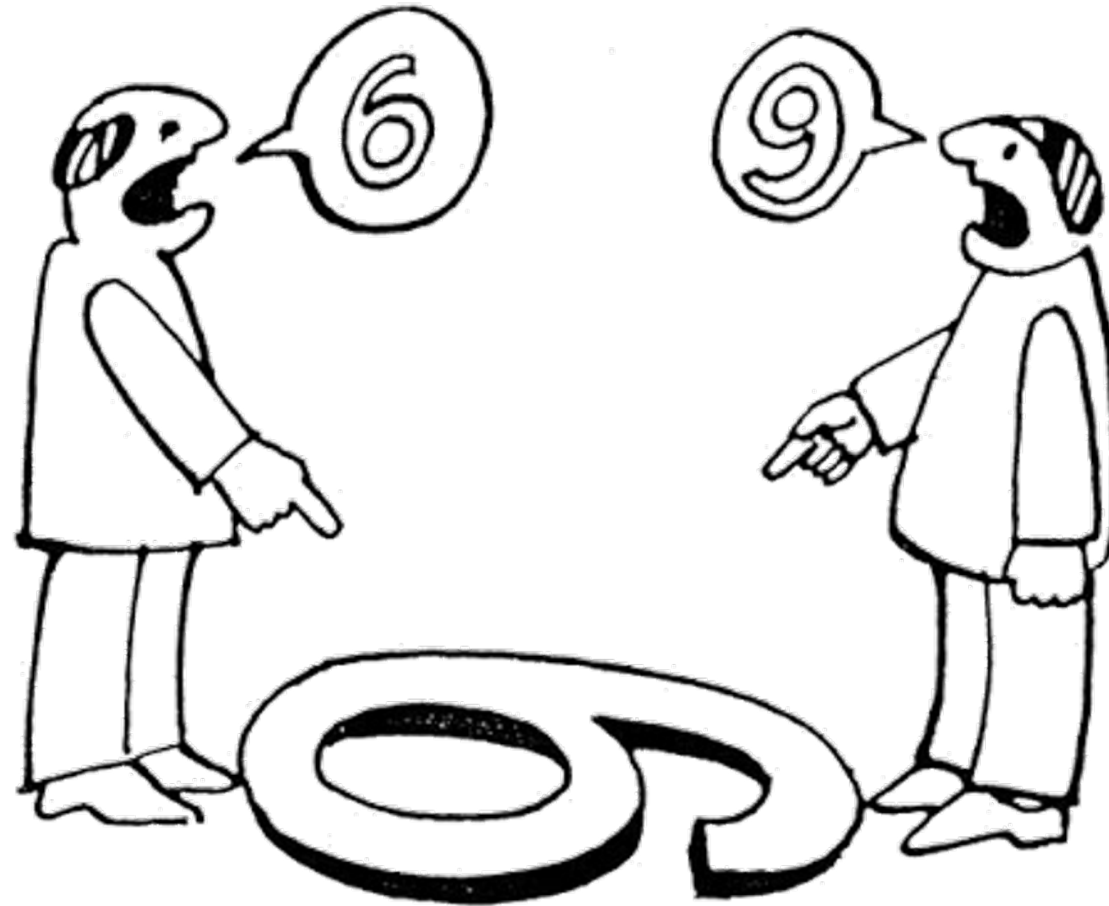
Understanding HIV cure-related research can be **complicated...**



Complicated cell is complicated.

**...but we are
working hard to
help you understand it!**

Perception plays a role in HIV cure research because every individual comes with their own views and experiences.

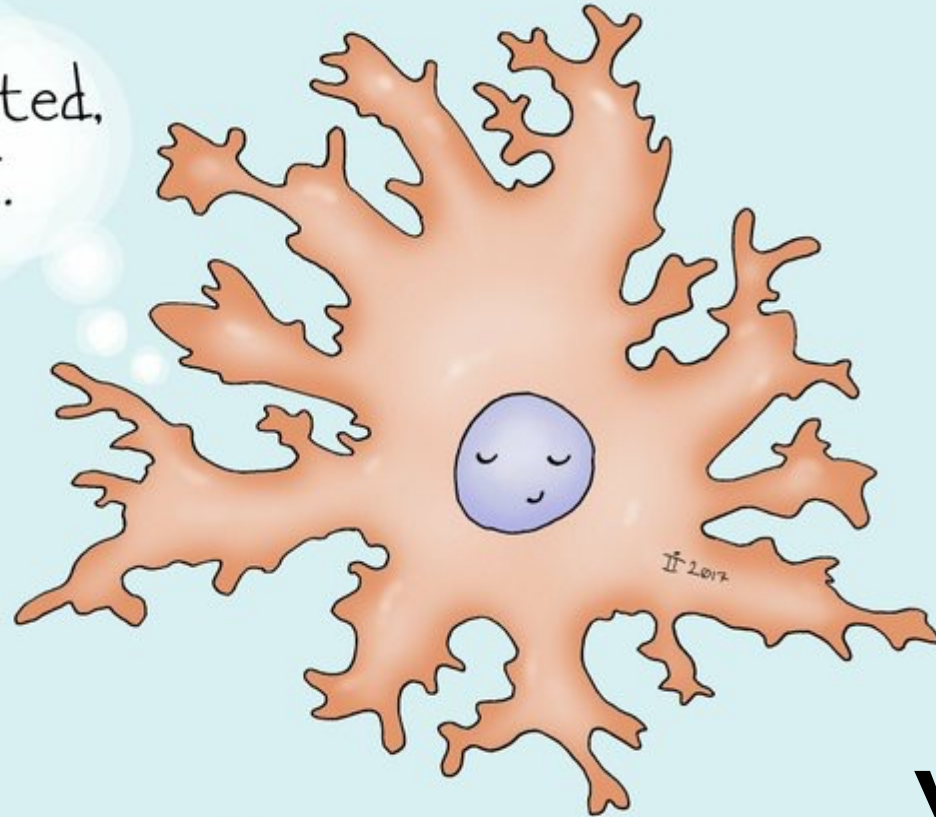


The background of the slide features a light blue gradient. On the left and right sides, there are numerous stylized hands of various skin tones (white, light brown, dark brown, and black) reaching out towards the center. The hands are depicted in a simple, flat style with visible fingers and palms. In the center, the text 'Finding Common Ground' is written in a large, bold, blue sans-serif font. The word 'Finding' is on the top line, 'Common' is on the second line, and 'Ground' is on the third line. The text has a slight drop shadow effect.

Finding Common Ground

Understanding HIV cure-related research can be complicated...

I'm complicated,
& that's OK.



Complicated cell is complicated.

**...but we are
working hard to
help you understand it!**

**ONE DAY, I WOULD
LOVE TO SAY**

“

**I USED TO
HAVE HIV**

”

ANONYMOUS 1998



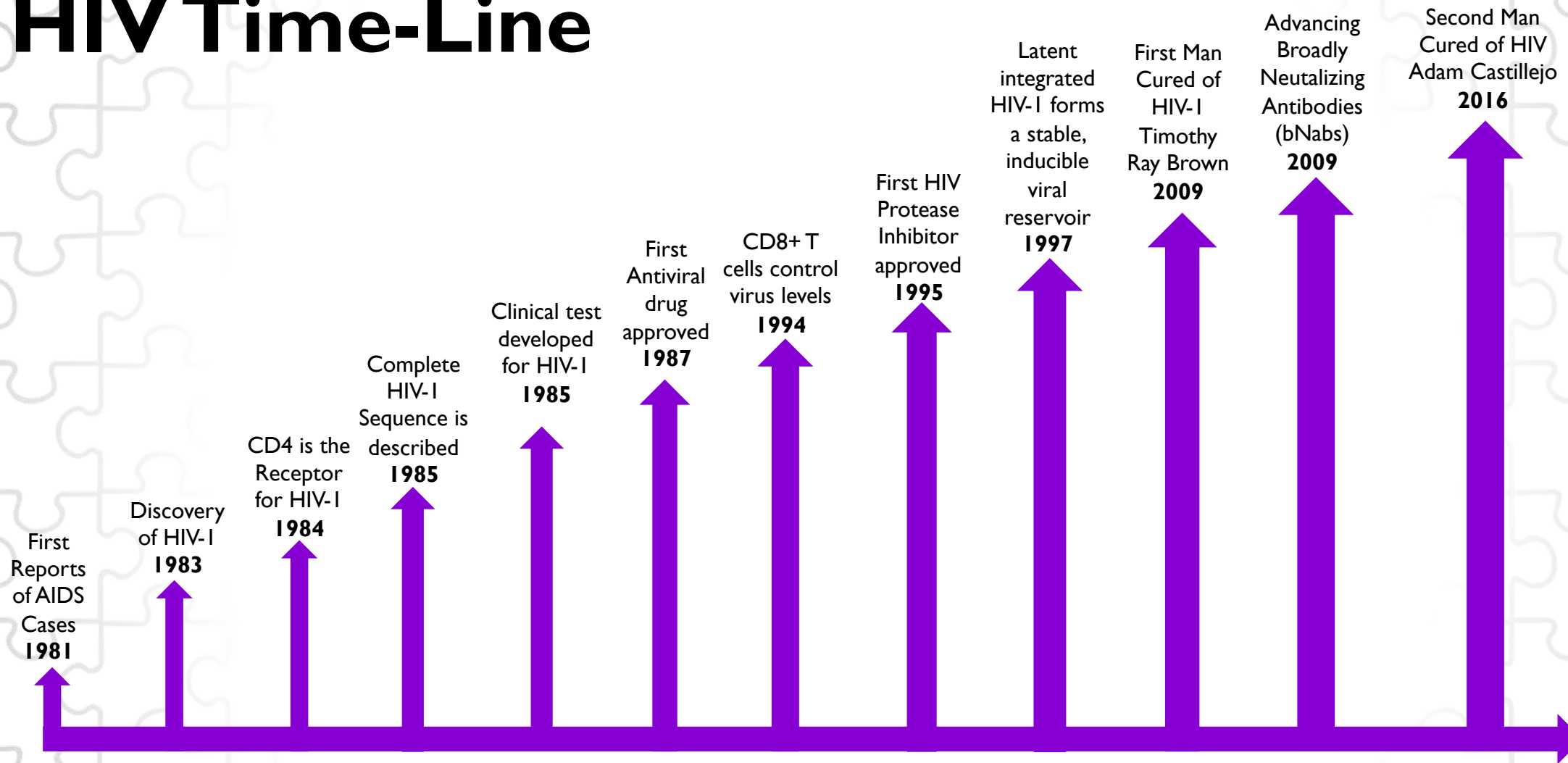
***Stopping the loss of life to AIDS
needs a cure that is safe
and scalable to reach
everyone living with HIV***

https://en.wikipedia.org/wiki/NAMES_Project_AIDS_Memorial_Quilt

HIV Time-Line



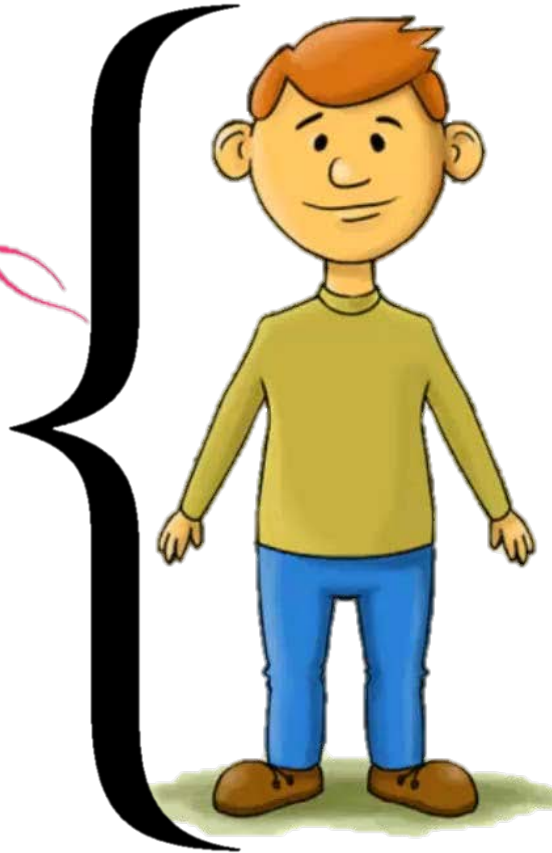
HIV Time-Line



Our Immune System



Pathogens



Immune System

* Made up of...

- ~ Organs
- ~ Tissues
- ~ Cells
- ~ Molecules

* Can...

- ~ Identify a threat
- ~ Mount an attack
- ~ Eliminate a pathogen
- ~ Remember

..... In 10 days!

* Prolonged reactions.....



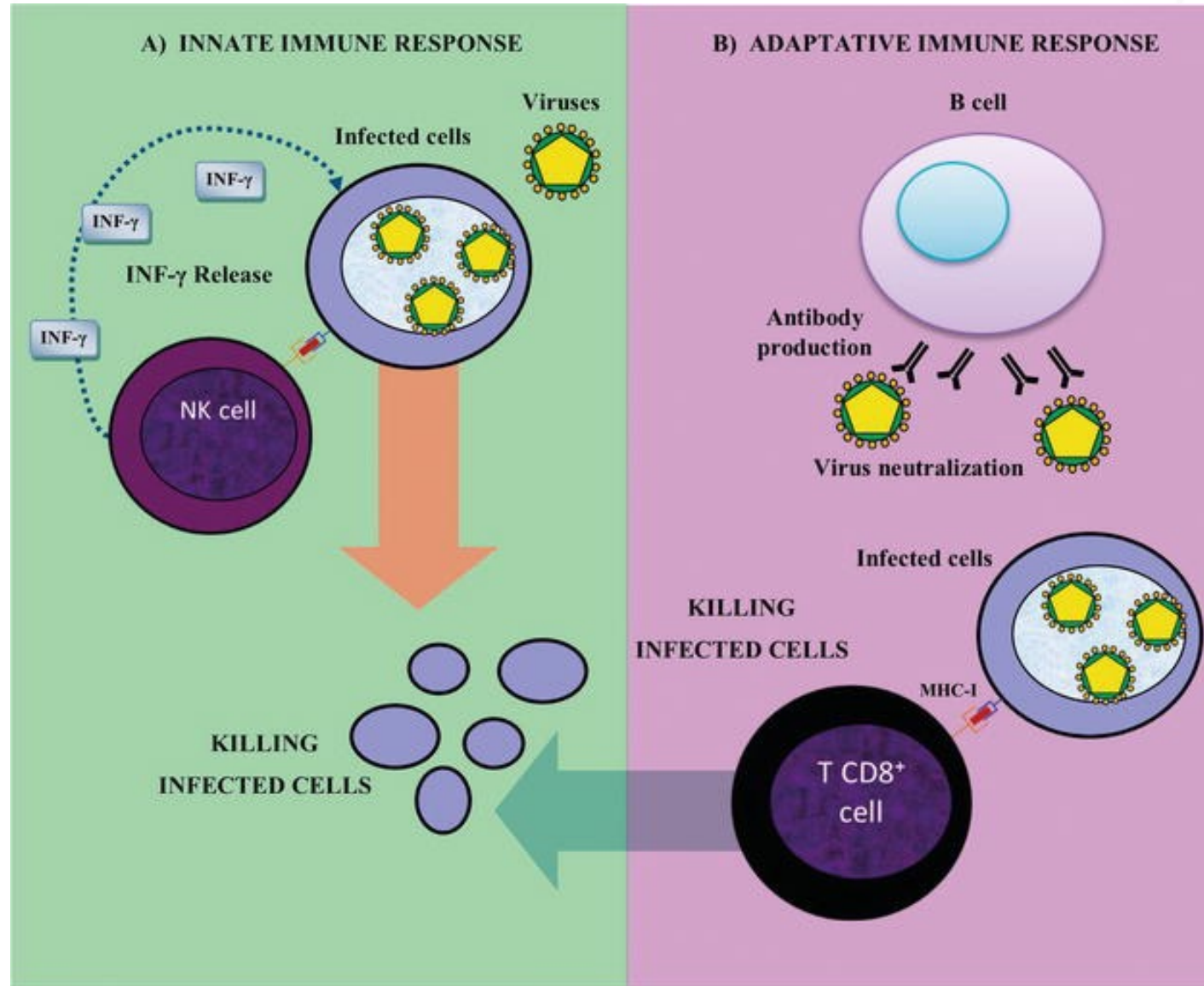
Chronic Inflammation



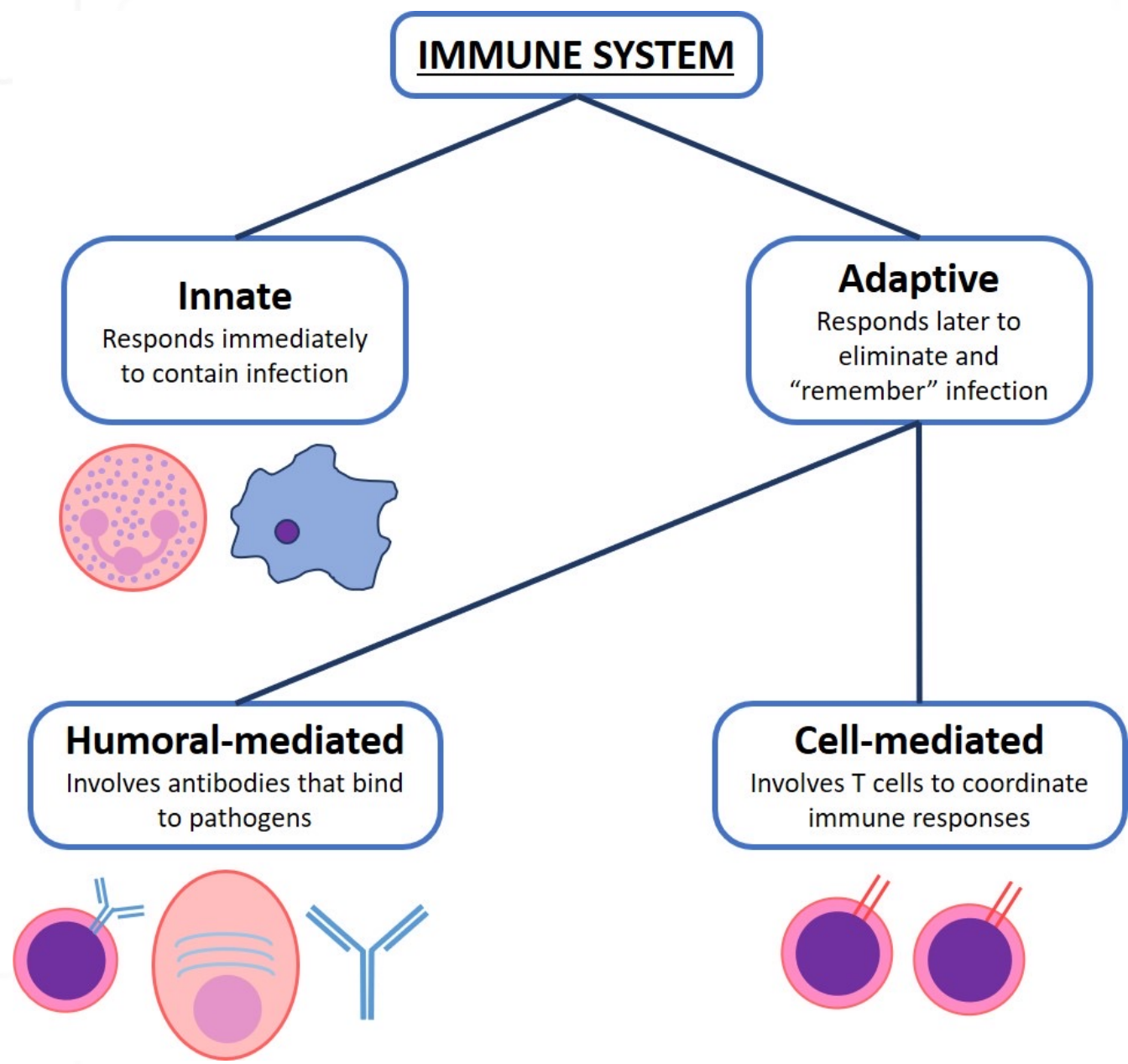
Two main arms of the immune system.

Vertebrate Immunity		
Innate Immune System		Adaptive Immune System
Physical Barriers	Internal Defenses	
<ul style="list-style-type: none">• Skin, hair, cilia• Mucus membranes• Mucus and chemical secretions• Digestive enzymes in mouth• Stomach acid	<ul style="list-style-type: none">• Inflammatory response• Complement proteins• Phagocytic cells• Natural killer (NK) cells	<ul style="list-style-type: none">• Antibodies and the humoral immune response• Cell-mediated immune response• Memory response

Two arms of the immune system.



<https://www.intechopen.com/books/physiology-and-pathology-of-immunology/physiology-and-pathology-of-innate-immune-response-against-pathogens>

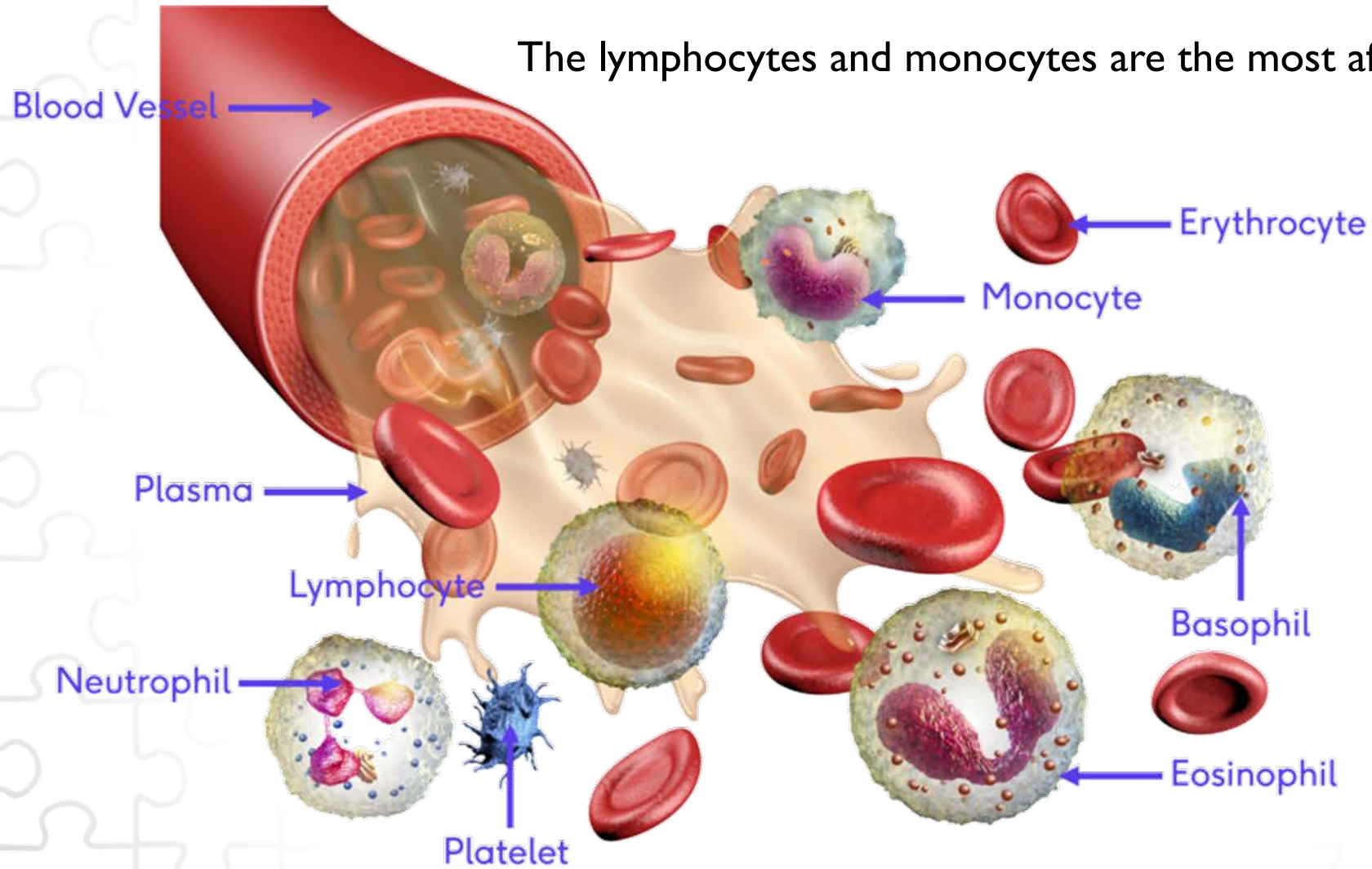


<https://theactivescientist.com/journal-club-001/>

**What are the key components
of the immune system?**



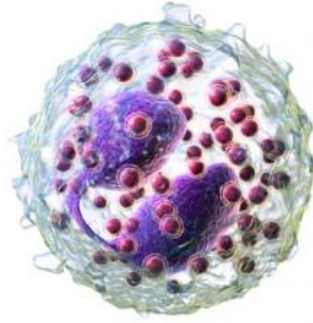
Components of the Peripheral Blood System



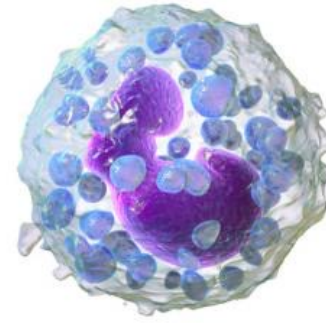
White Blood Cells



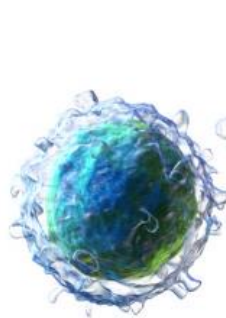
monocyte



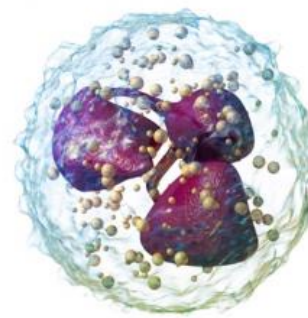
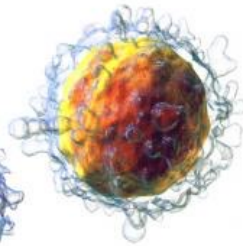
eosinophil



basophil



lymphocytes

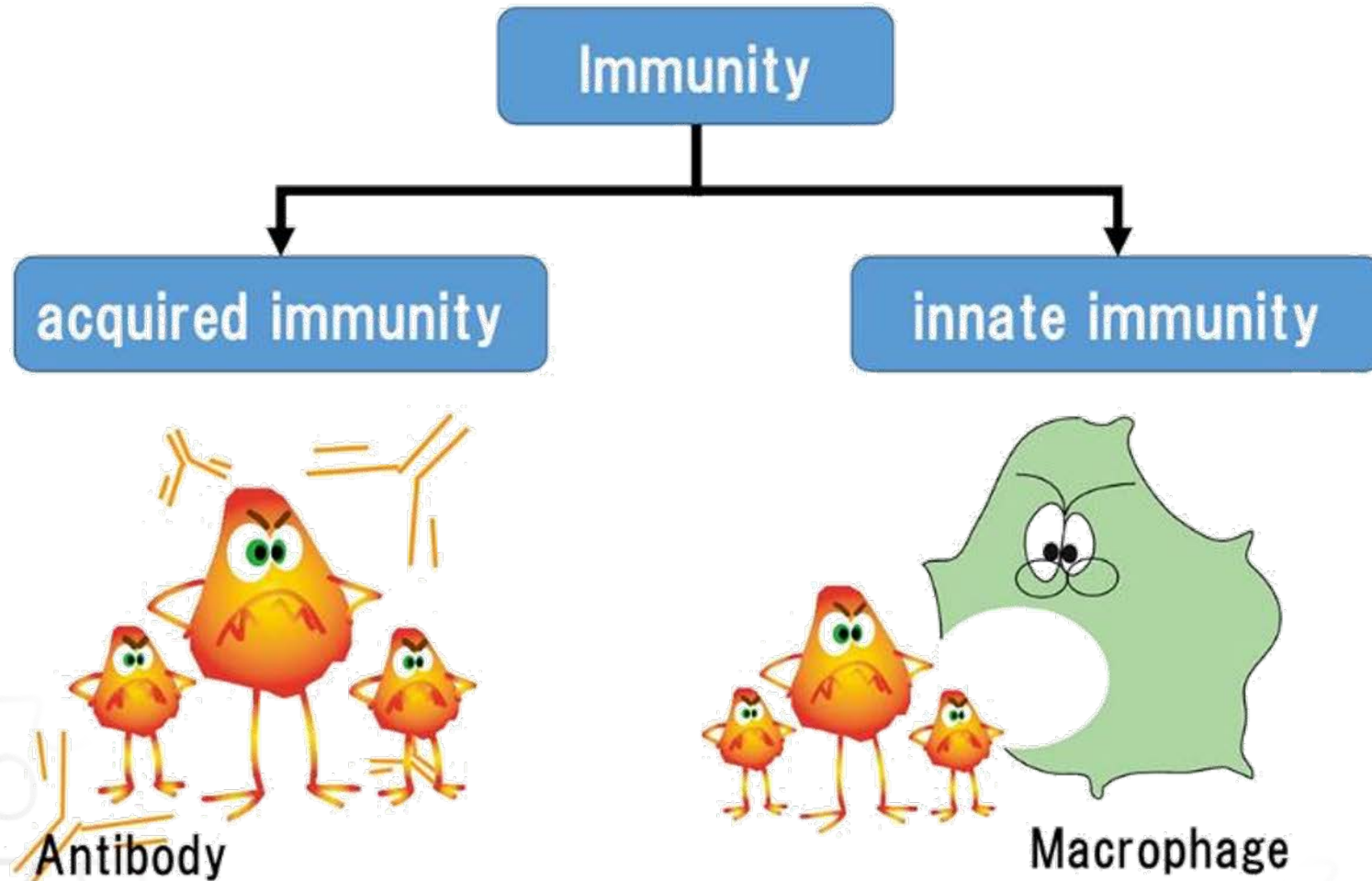


neutrophil

<https://www.medicalnewstoday.com/articles/327446>

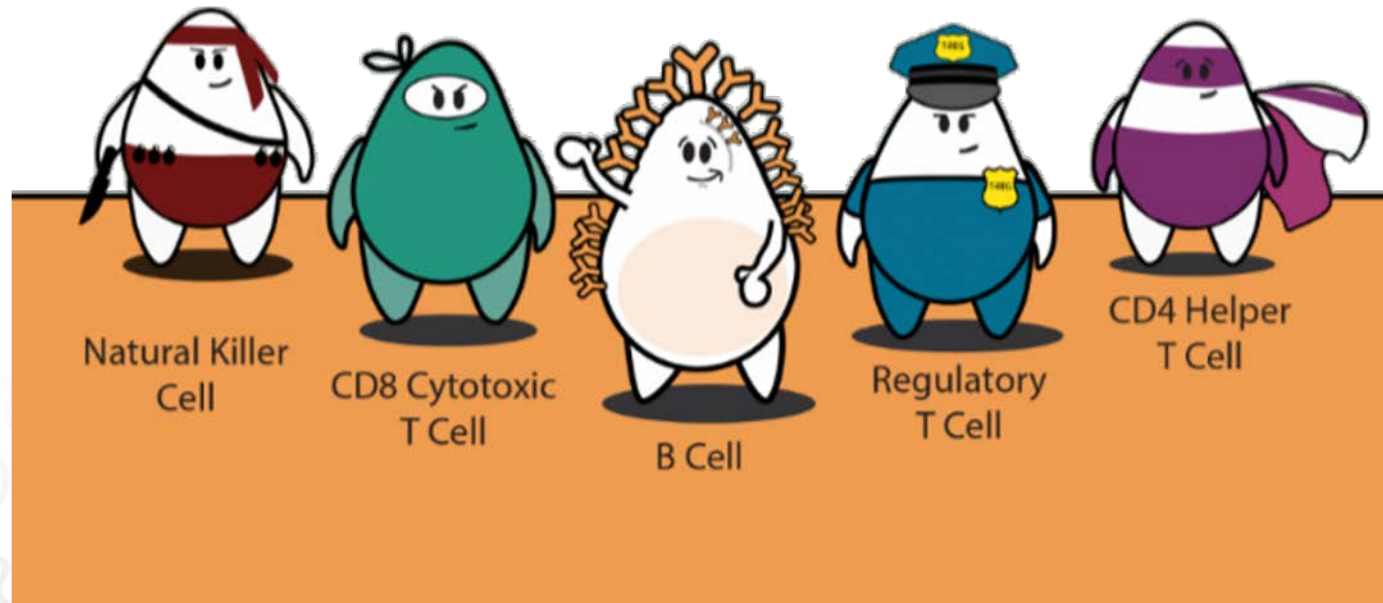


A glimpse at the adaptive (acquired) & the innate arms of the immune system



A functioning immune system requires all cells
working together

Lymphocytes



The CD4 T cell is an important player in a 'League of Superhero Immune Cells'

THERE IS NOTHING TO FEAR! IF ANY VILLAINS DARE TO INVADE I WILL COME TO THE RESCUE!

CD4 T Cell



CellCartoons.net

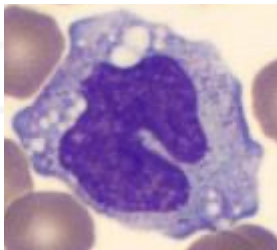


Monocytes and macrophages are a type of white blood cell that love to eat (phagocytize) pathogens with their ferocious appetites

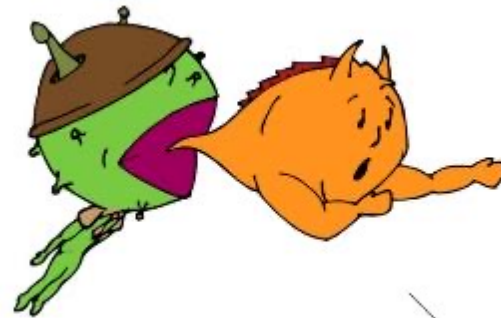
These cells can act as a reservoir of HIV

They express both CD4 and CCR5

Monocyte

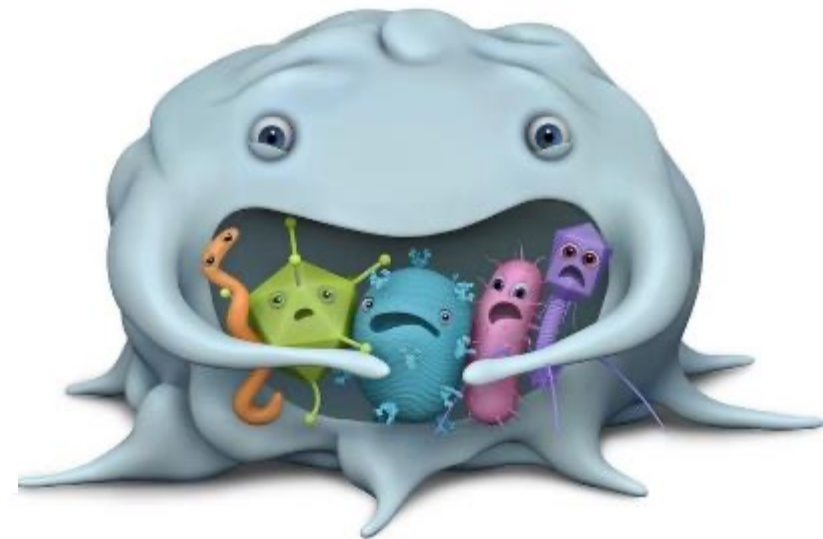


Monocyte



phagocytosis

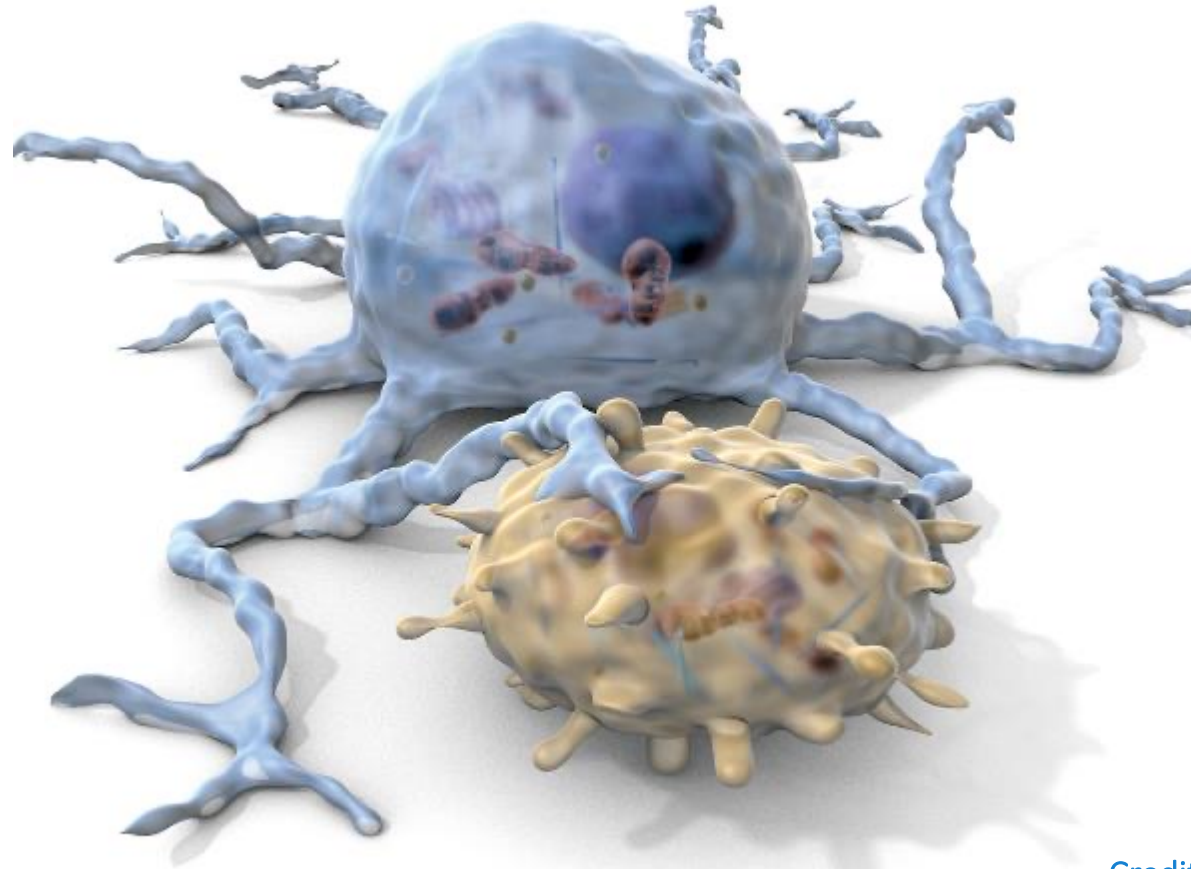
Macrophage



phagocytize



A Dendritic Cell (blue) Engages a T cell (yellow)

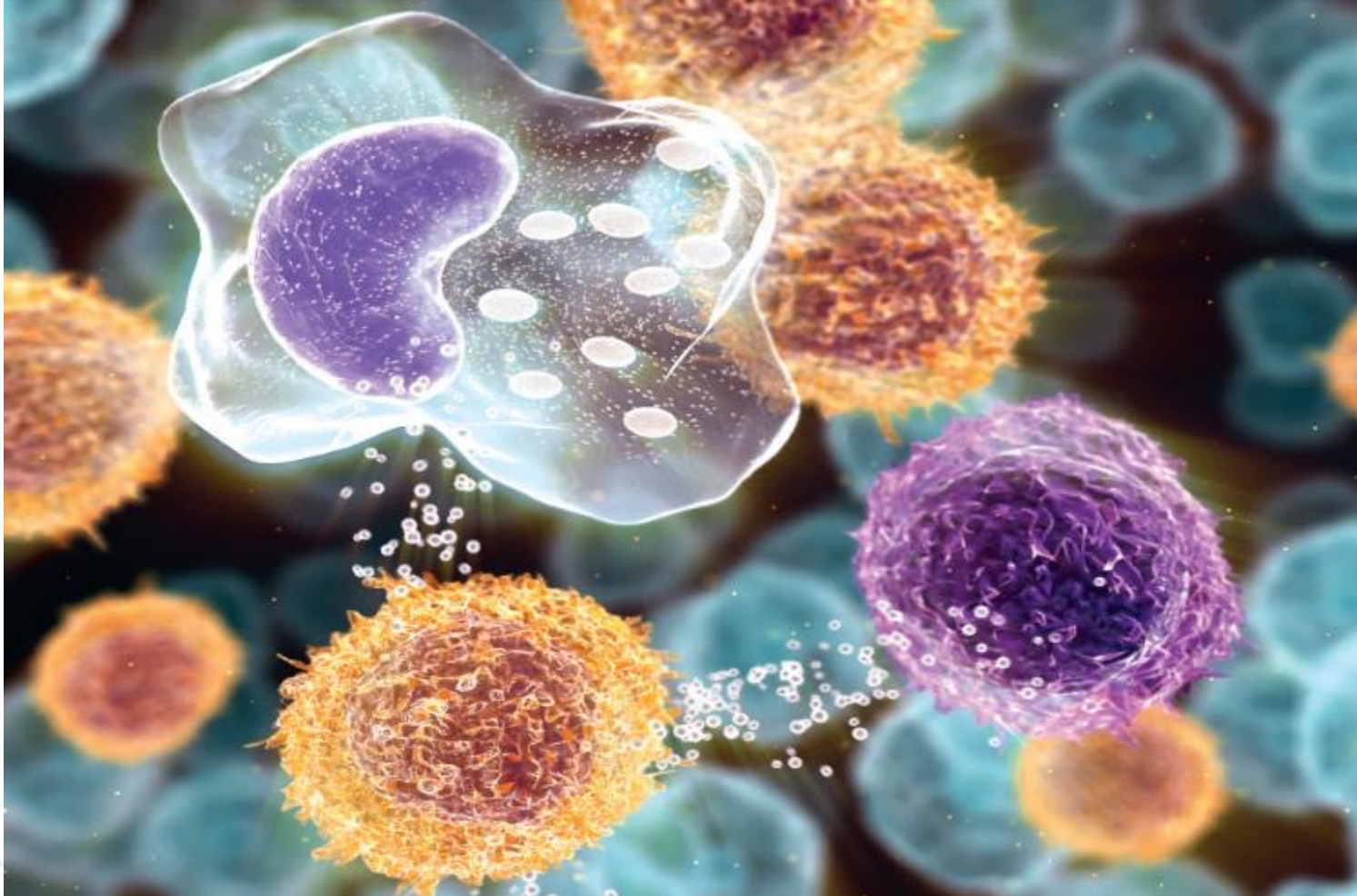


Credit: C. BICKEL/SCIENCE

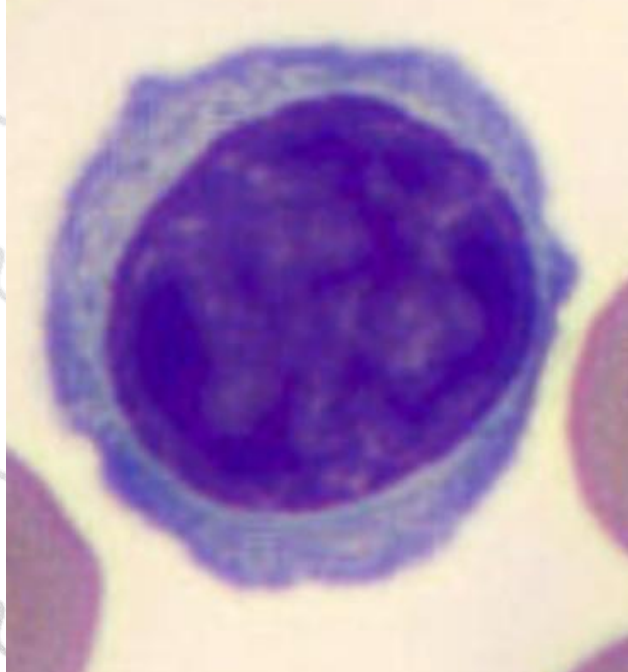
All the cells of the immune system work in perfect concert together under normal conditions making music for our ears



Cells communicate through chemical signals much like we do through words



Lymphocyte



LYMPHOCYTES

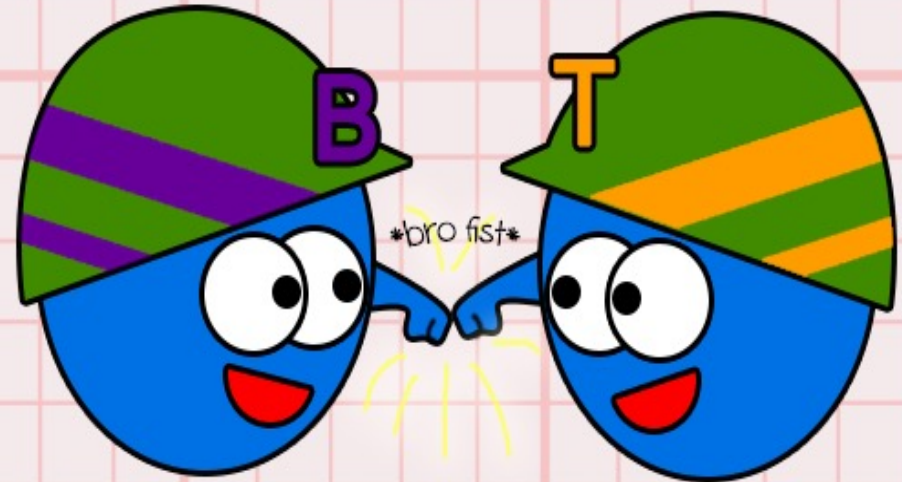
B CELLS & T CELLS

IMMENSE IMMUNOLOGY INSIGHT

Count
20% - 40% in
peripheral blood

Round nucleus

- Large
- Dark staining



Scanty cytoplasm

- Thin rim around the nucleus
- Pale eosinophilic
- Barely visible

Killing strategy
Coming soon!

To be continued...

B CELL

IMMENSE IMMUNOLOGY INSIGHT

Count
20% of total
lymphocytes

War strategy



① Recognize enemy (B cell receptor)

② Receive signal for activation:

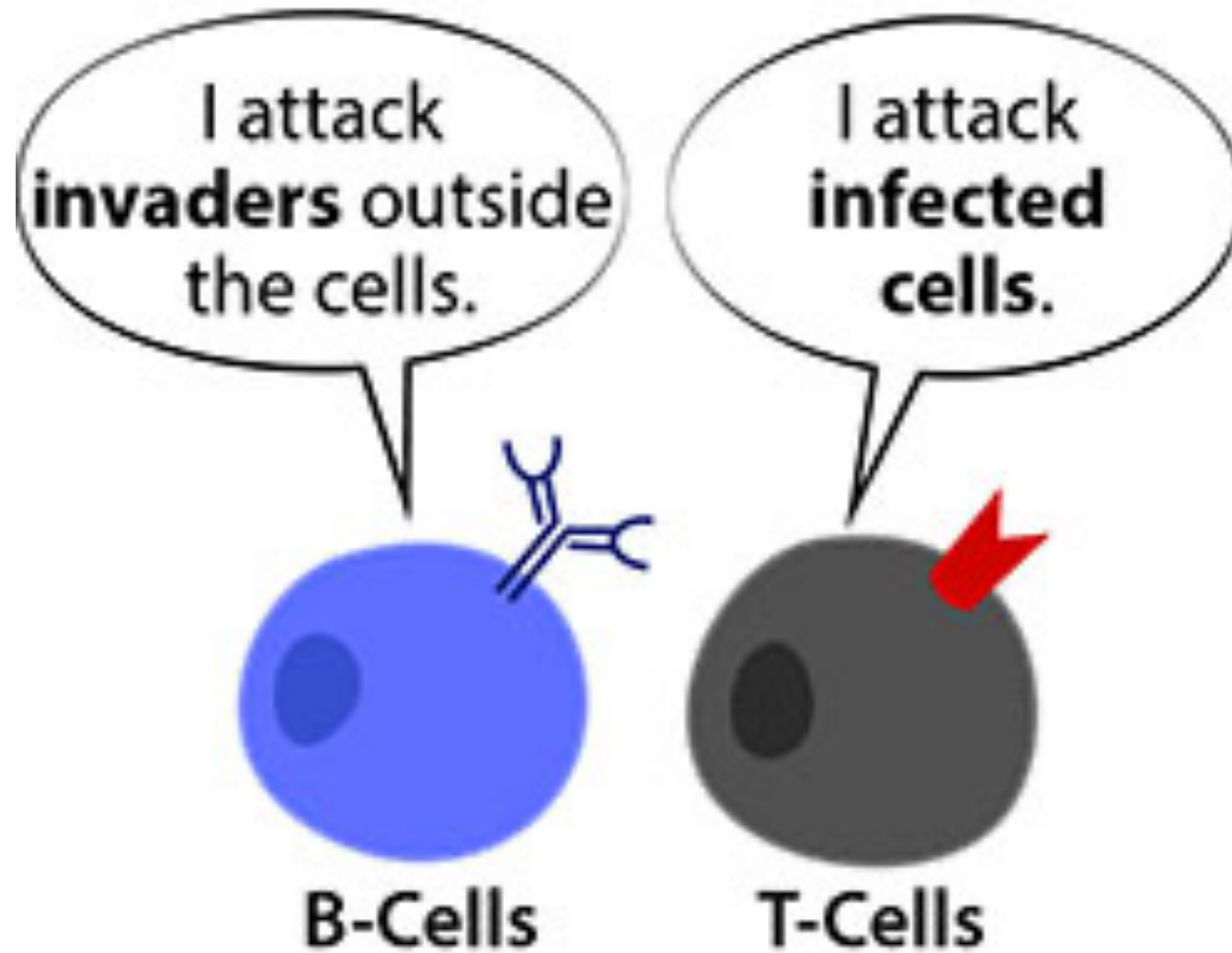
- Show antigen to CD4 T cells (Antigen presentation)
- Recognize multiple crosslinking (Thymus independent activation)

③ Transform into:

- Plasma cell (Secretes antibodies)
- Memory B cell (For faster recognition of the same enemy in the future)



Unique differences between B and T cells.



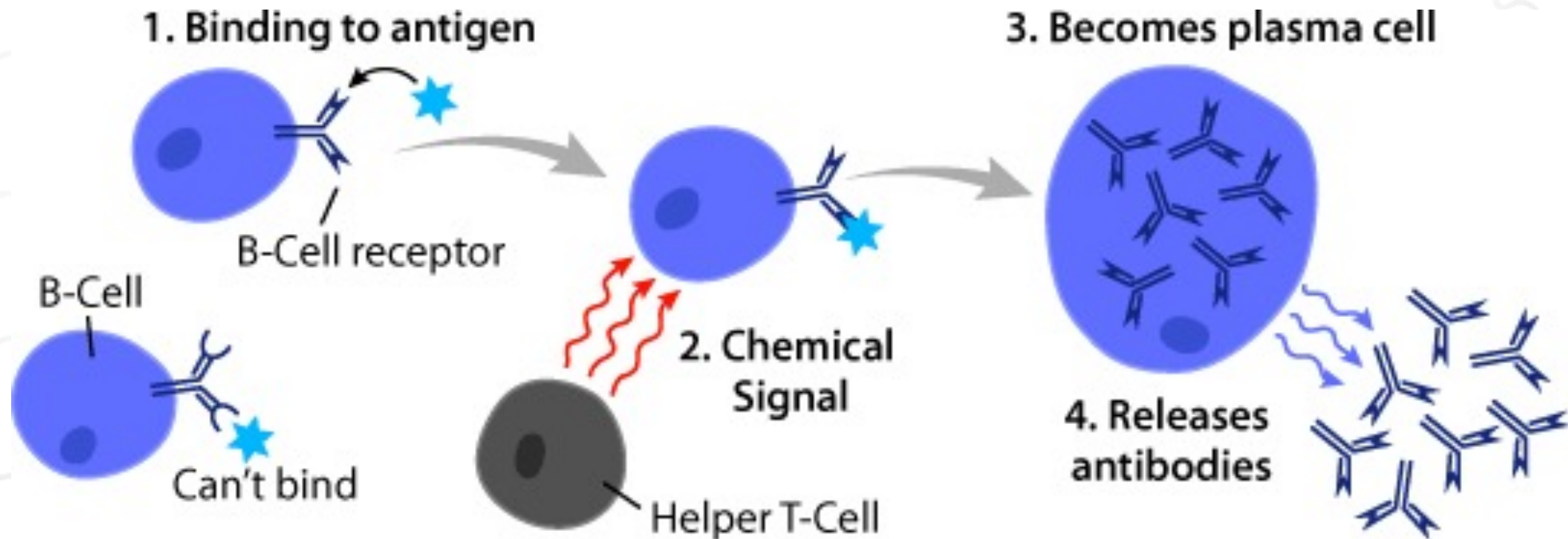
<https://askabiologist.asu.edu/b-cell>

T and B Lymphocyte distribution in different organ systems

Lymphoid Organ	T Lymphocytes (%)	B Lymphocytes (%)
Thymus	100	0
Bone marrow	10	90
Spleen	45	55
Lymph nodes	60	40
Blood	70	30

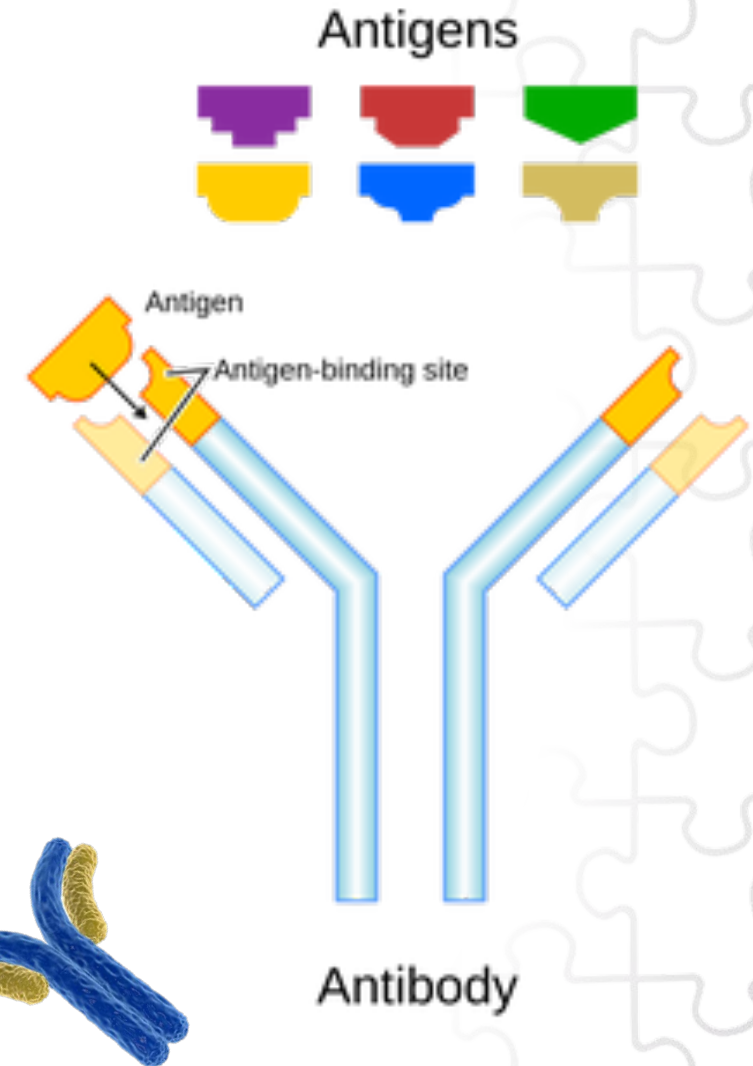
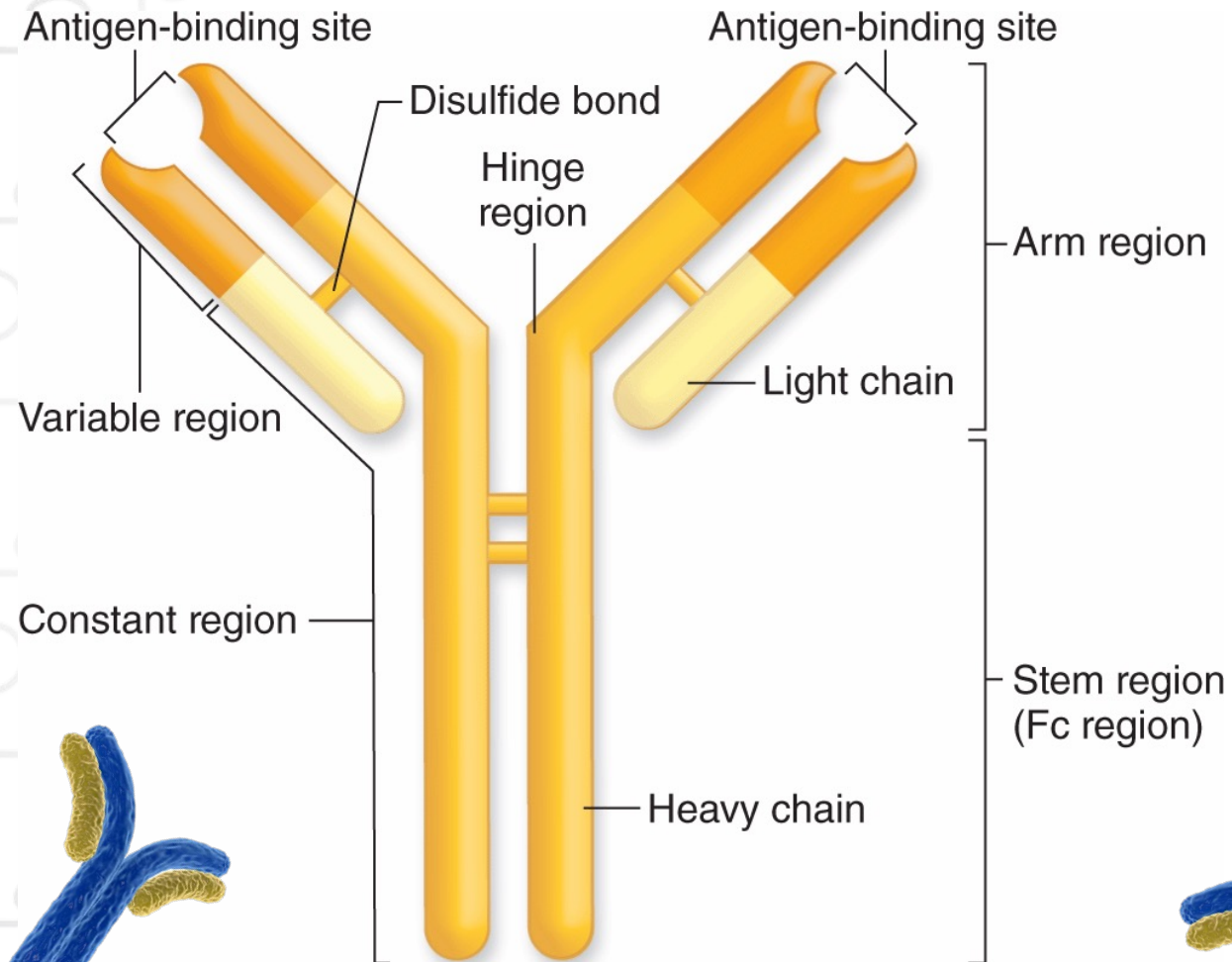
<https://basicmedicalkey.com/the-immune-system-lymphoid-organs/>

T helper cells provide an important link to B cells.



<https://askabiologist.asu.edu/b-cell>

Immunoglobulin G (IgG)



<https://basicmedicalkey.com/the-immune-system-lymphoid-organs/>

<https://en.wikipedia.org/wiki/Antibody>



HELPER T CELL

IMMENSE IMMUNOLOGY INSIGHT

Expresses
CD4 glycoprotein
(CD4+ T cells)

Warrior types



Th1 cells

Activates:

- Macrophages
- Killer T cells
- B cells

Secretes:

- IFN - γ
- IL - 2
- TNF - α

Th2 cells

Activates:

- Eosinophils
- Mast cells
- Basophils

Secretes:

- IL - 4
- IL - 5
- IL - 13

T_{FH} cells

Activates B cells for:

- Isotype switching
- Affinity maturation

Th17 cells

Recruits:

- Neutrophils

Secretes:

- IL - 17

T_{reg} cells

Inhibits

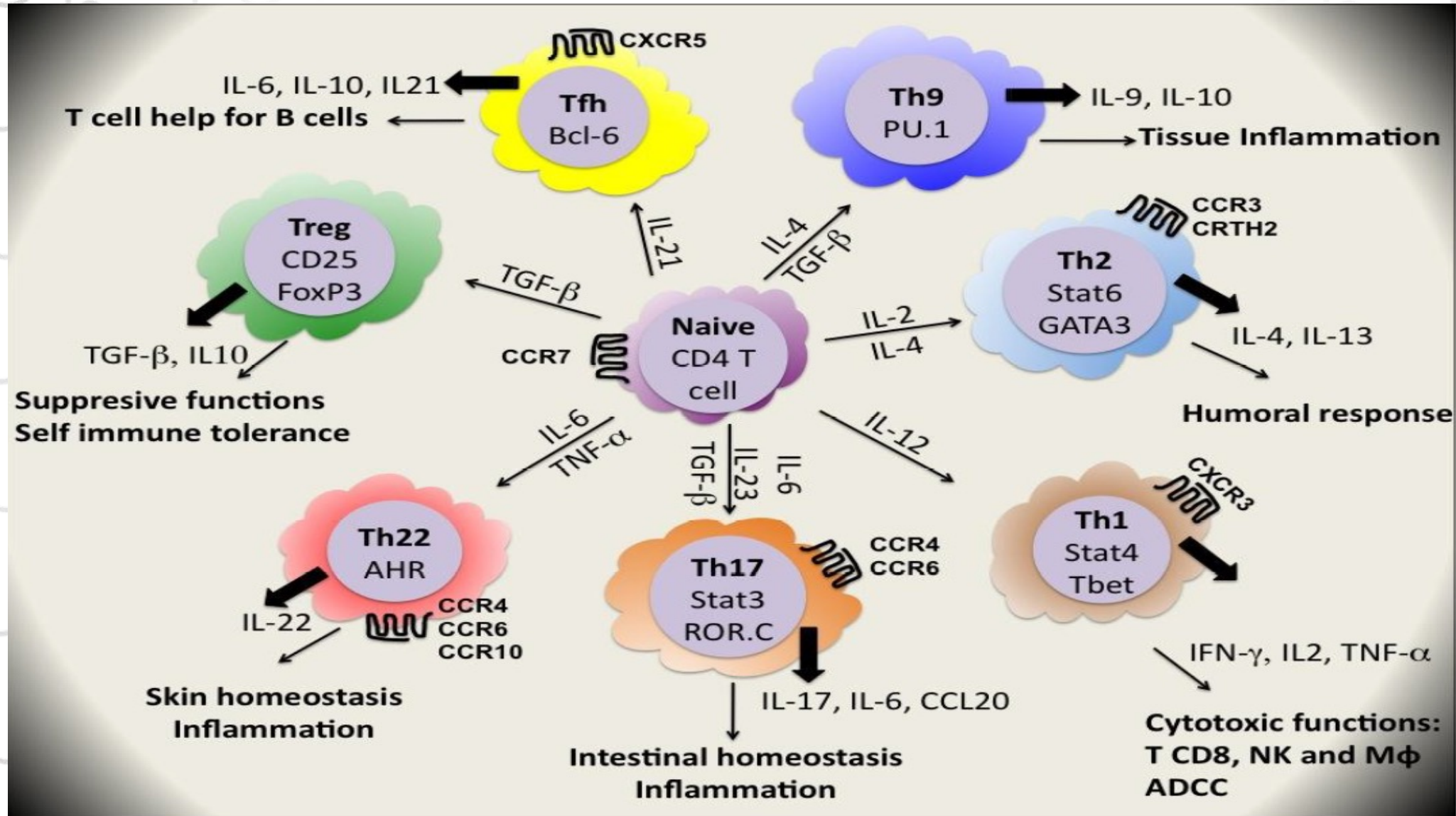
dendritic cells:

- Prevents autoimmunity
- Suppresses T cell

ronwendyheal.com

#AskStorm

The many functions of CD4 T Helper Cells.



<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3185763/>

"I am a
killer T cell"

Expresses
CD8 glycoprotein
(CD8+ T cells)



① Recognize antigen combined
with class I MHC (altered self cells)

② Recieve signal for activation:
• With CD4 T cell help: Cytokines (IL-2)
• Without help: Activation by potent APCs

③ Releases cytotoxic proteins:

• Perforin: Forms a pore for delivery of granzymes

• Granzymes: Programmes cell to die (Induces apoptosis)

• Granulysin: Stabs the cell (Creates holes
in target membrane & destroys it)

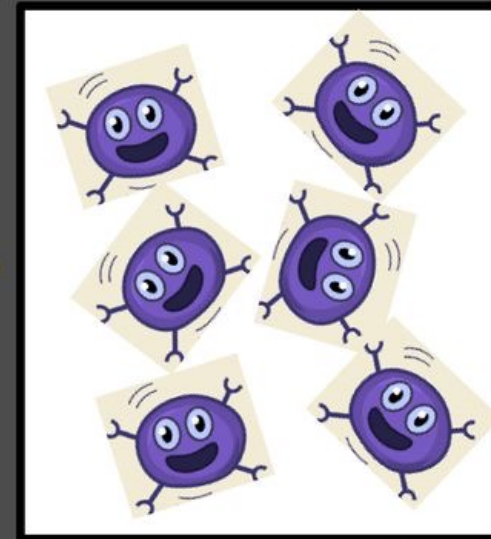
- The selection of the correct B and T lymphocytes is known as clonal selection

Clonal Selection



Followed
by...

Clonal Expansion



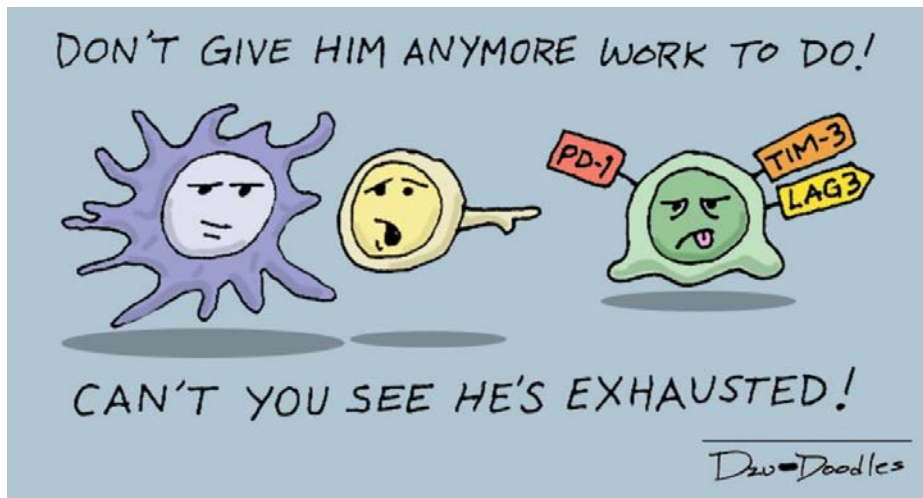
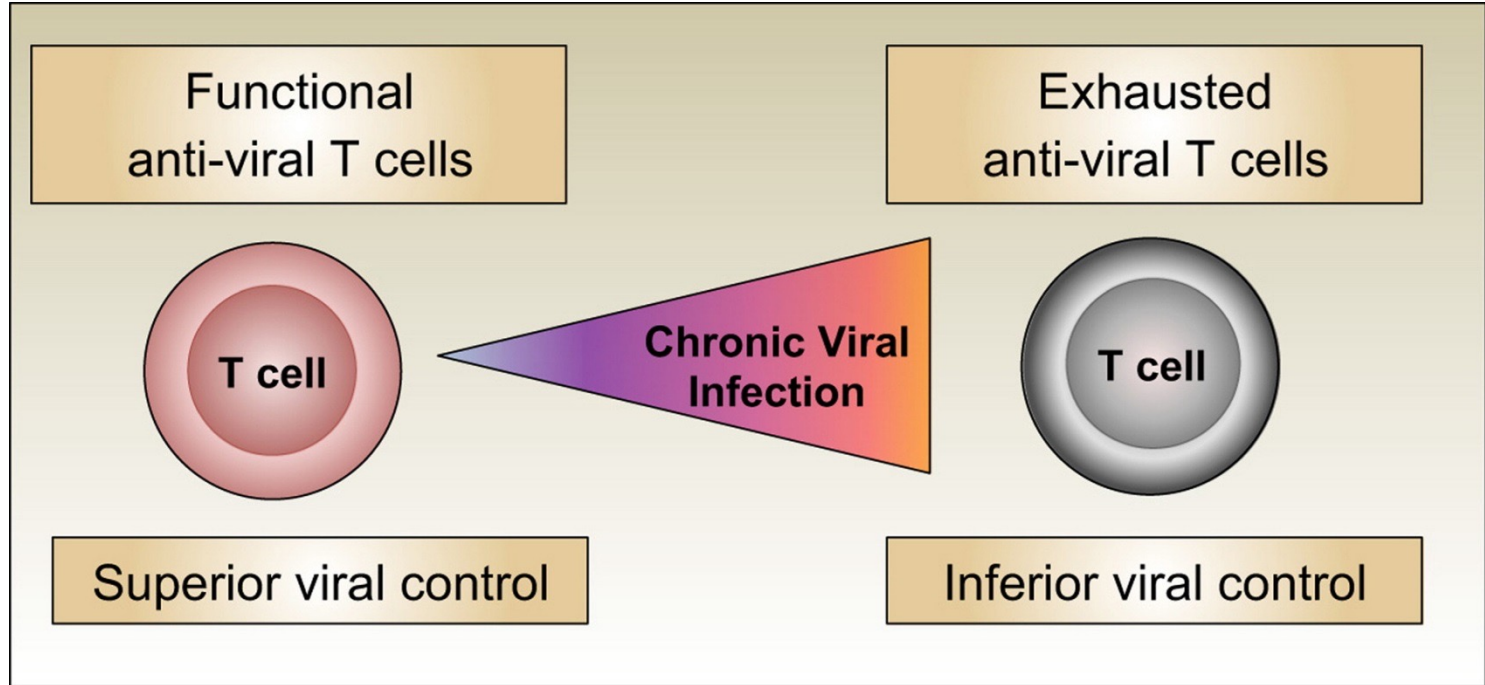
- To become effective at fighting the pathogen the lymphocytes must increase in number, this is clonal expansion

A sample of some important cytokines.

GM-CSF, M-CSF	Growth and differentiation factors for leukocyte progenitor cells in bone marrow
TNF- α , TGF- β , IL-1	Stimulation of inflammation and fever
IL-12	Stimulation of growth in T lymphocytes and NK cells
IL-2, IL-4	Growth factors for T helper cells and B lymphocytes
IL-5	Eosinophil proliferation, differentiation, and activation
Interferon- γ , IL-4	Activation of macrophages
IL-10	Inhibition of macrophages and specific adaptive immune responses
Interferon- α , interferon- β	Antiviral activity
IL-8	Chemokine for neutrophils and T lymphocytes

^aGM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; TGF, transforming growth factor; TNF, tumor necrosis factor.

The effect T cell exhaustion in fight against HIV.



Why Do We Love Dendritic Cells so Much?

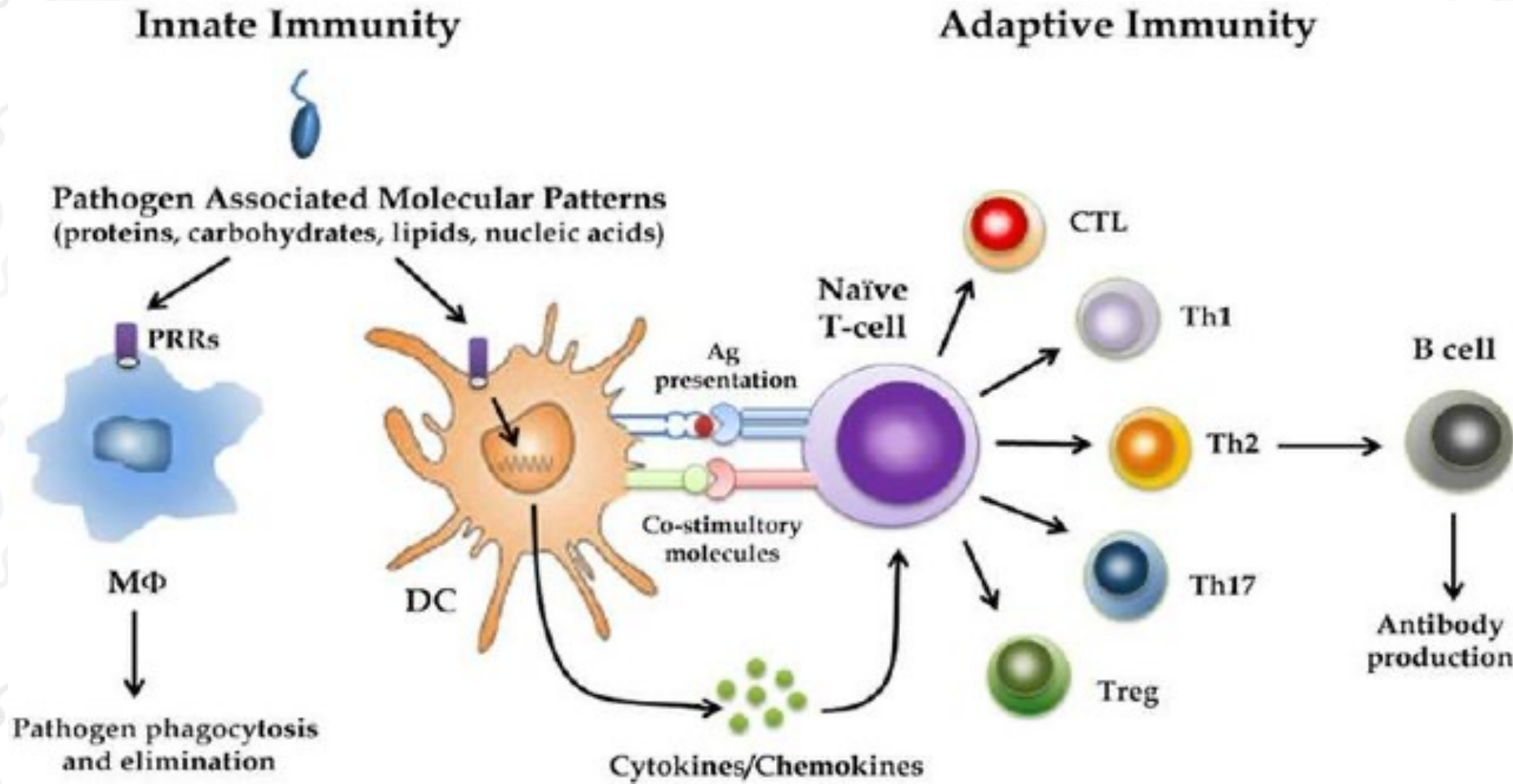
- Present antigens to the adaptive immune system
- Act as messenger cells



WHERE ARE
DENDRITIC CELLS
FOUND?

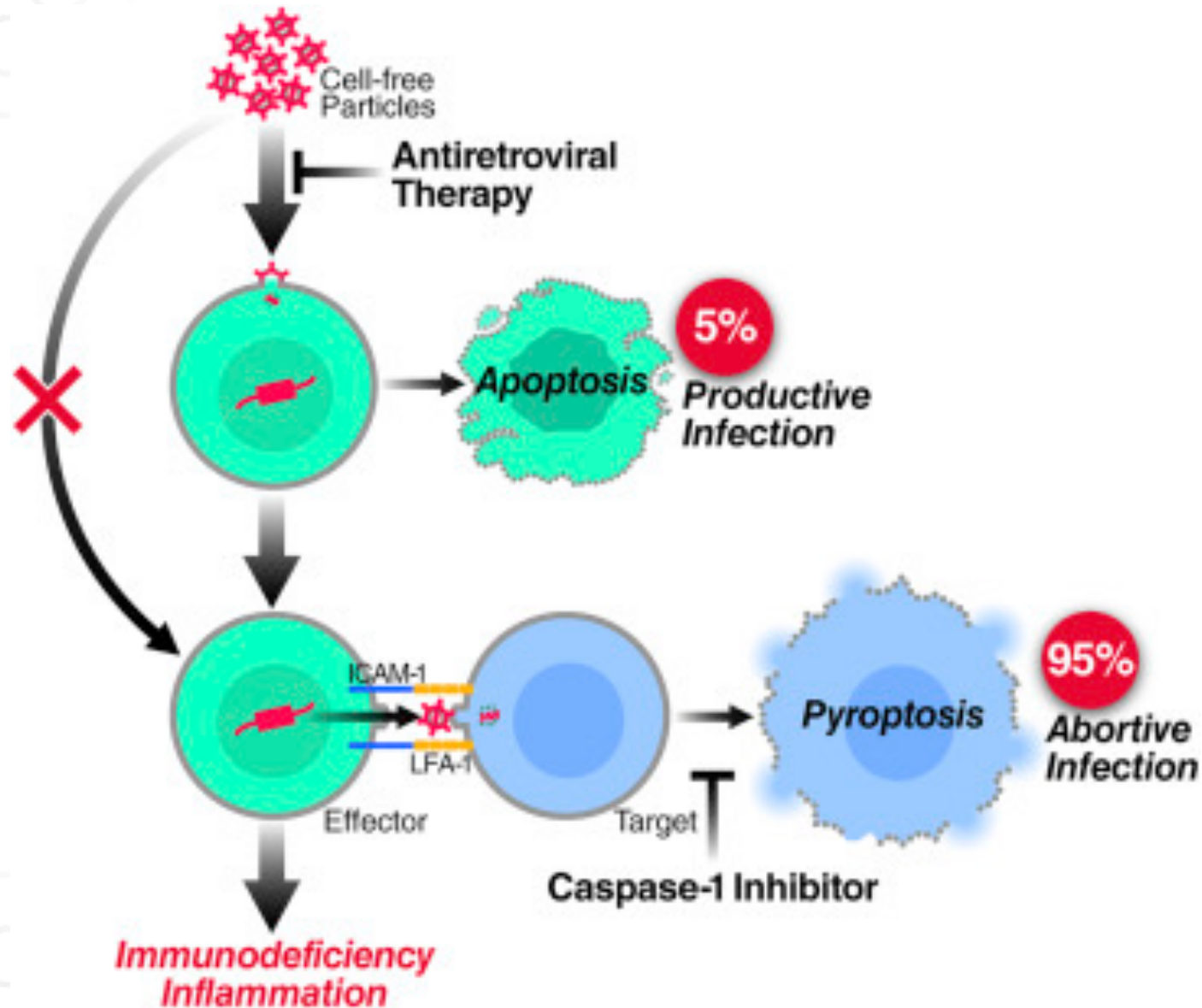
Most of the dendritic cells are found in the tissues of the external environment, such as skin contact (in this area Dendritic cells are also called *Langerhans Cells*) and also found in the nose, lungs and digestive tract.

The important role of dendritic (DC) cells.

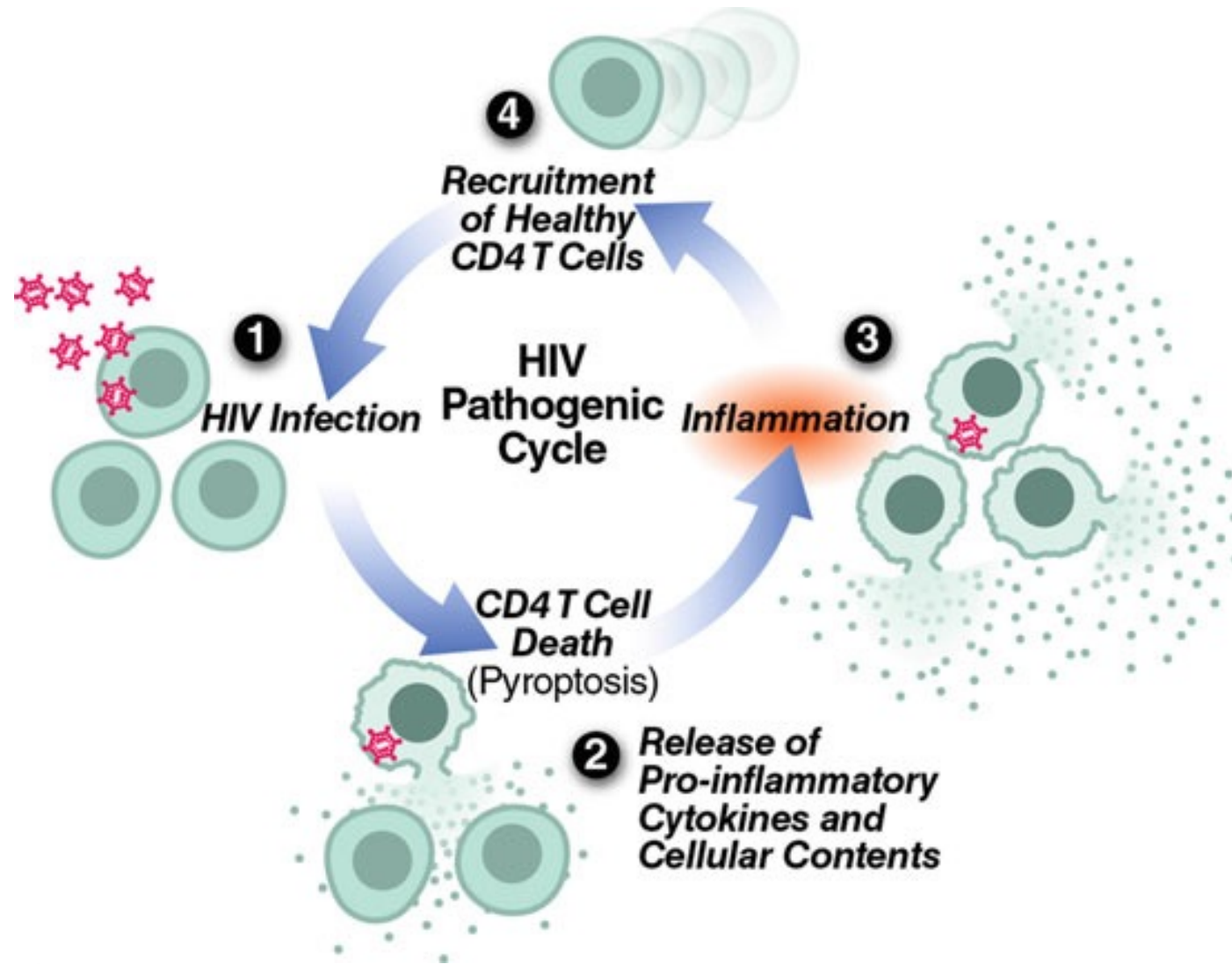


https://www.researchgate.net/publication/256257651_Pathogen_Strategies_to_Evade_Innate_Immune_Response_A_Signaling_Point_of_View/figures?lo=1

Two of three ways HIV causes the death of CD4 T Helper Cells.



The role of HIV and pyroptosis in the death of CD4 T Helper Cells.



https://link.springer.com/referenceworkentry/10.1007%2F978-1-4614-9610-6_392-1

Cell mediated killing of HIV infected cells.

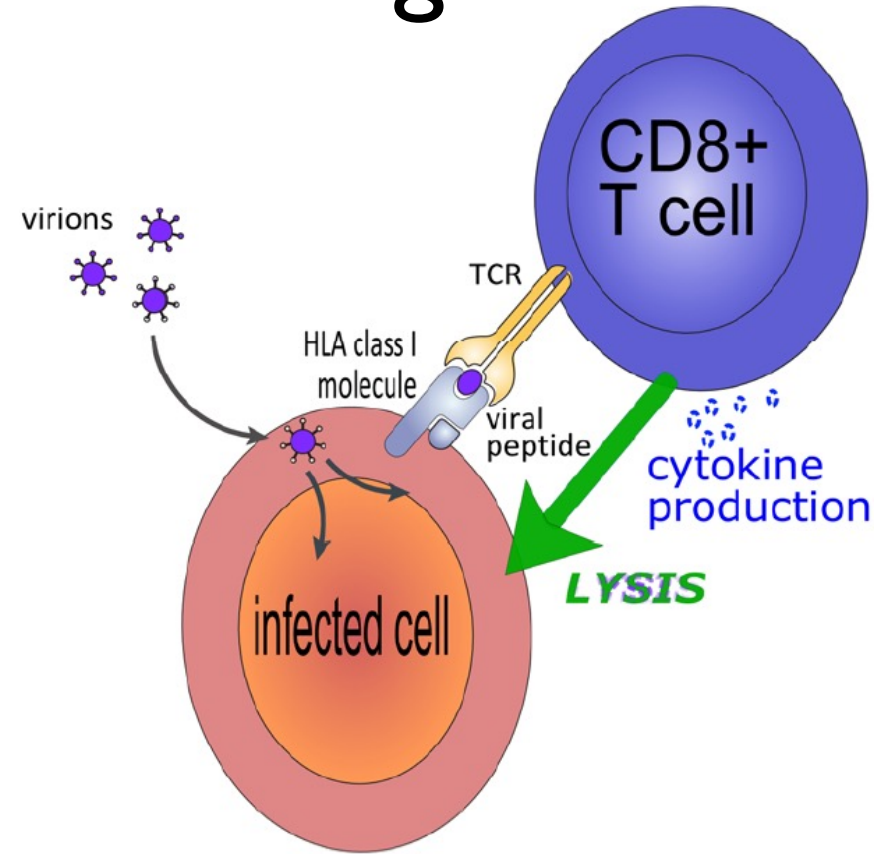


Figure 1. CD8+ T cells recognise virally infected cells via their T cell receptor (TCR). The TCR binds complexes of viral peptides and HLA class I molecules at the surface of virus-infected cells. Following CD8+ T cell recognition of the infected cell, the CD8+ T cell effector mechanisms are triggered. CD8+ effector mechanisms can be lytic (killing of the infected cell) and/or non-lytic (secretion of cytokines such as IFN γ and TNF α which reduce the probability of cell infection and viral production).

doi:10.1371/journal.pcbi.1002381.g001

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3285570/>

**Why do we think a cure for
HIV is possible?**



Loreen Willenberg: An Exceptional Elite Control

- Exceptional **spontaneous** response

Test	Cell number	Cell type	Intact virus
Sequencing	>1.5 billion cells	PBMC	No
Viral outgrowth	340 million cells	Resting CD4	No
Intact DNA (PCR)	14 million cells	Resting CD4	No
	4 million cells	GI tract	No



Loreen Willenberg

Article

Distinct viral reservoirs in individuals with spontaneous control of HIV-1

<https://doi.org/10.1038/s41586-020-2651-8>

Received: 2 October 2019

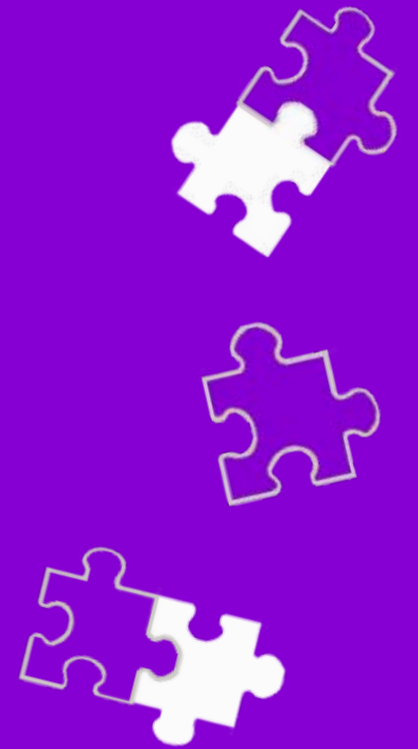
Accepted: 15 July 2020

Published online: 26 August 2020

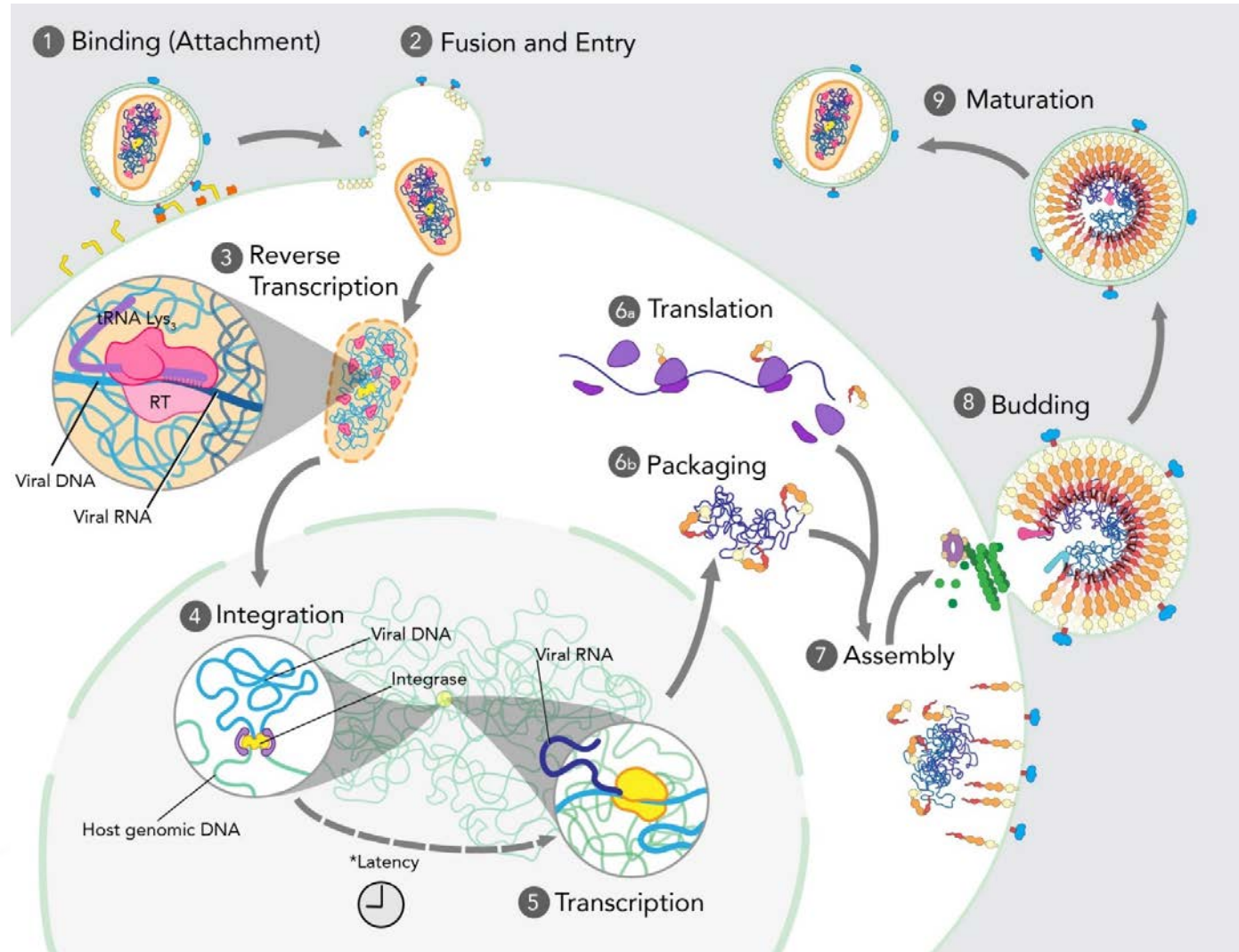
 Check for updates

Chenyang Jiang^{1,2,5}, Xiaodong Lian^{1,2,15}, Ce Gao^{1,15}, Xiaoming Sun¹, Kevin B. Einkauff^{1,2}, Joshua M. Chevalier^{1,2}, Samantha M. Y. Chen¹, Stephane Hua¹, Ben Rhee^{1,2}, Kaylee Chang¹, Jane E. Blackmer¹, Matthew Osborn¹, Michael J. Peluso³, Rebecca Hoh³, Ma Somsouk³, Jeffrey Milush³, Lynn N. Bertagnoli⁴, Sarah E. Sweet⁴, Joseph A. Varriale⁴, Peter D. Burbelo⁵, Tae-Wook Chun⁶, Gregory M. Laird⁷, Erik Serrao^{8,9}, Alan N. Engelman^{8,9}, Mary Carrington¹⁰, Robert F. Siliciano^{4,11}, Janet M. Siliciano^{4,11}, Steven G. Deeks³, Bruce D. Walker^{1,11,12,13}, Mathias Lichterfeld^{1,2,14} & Xu G. Yu^{1,2}✉

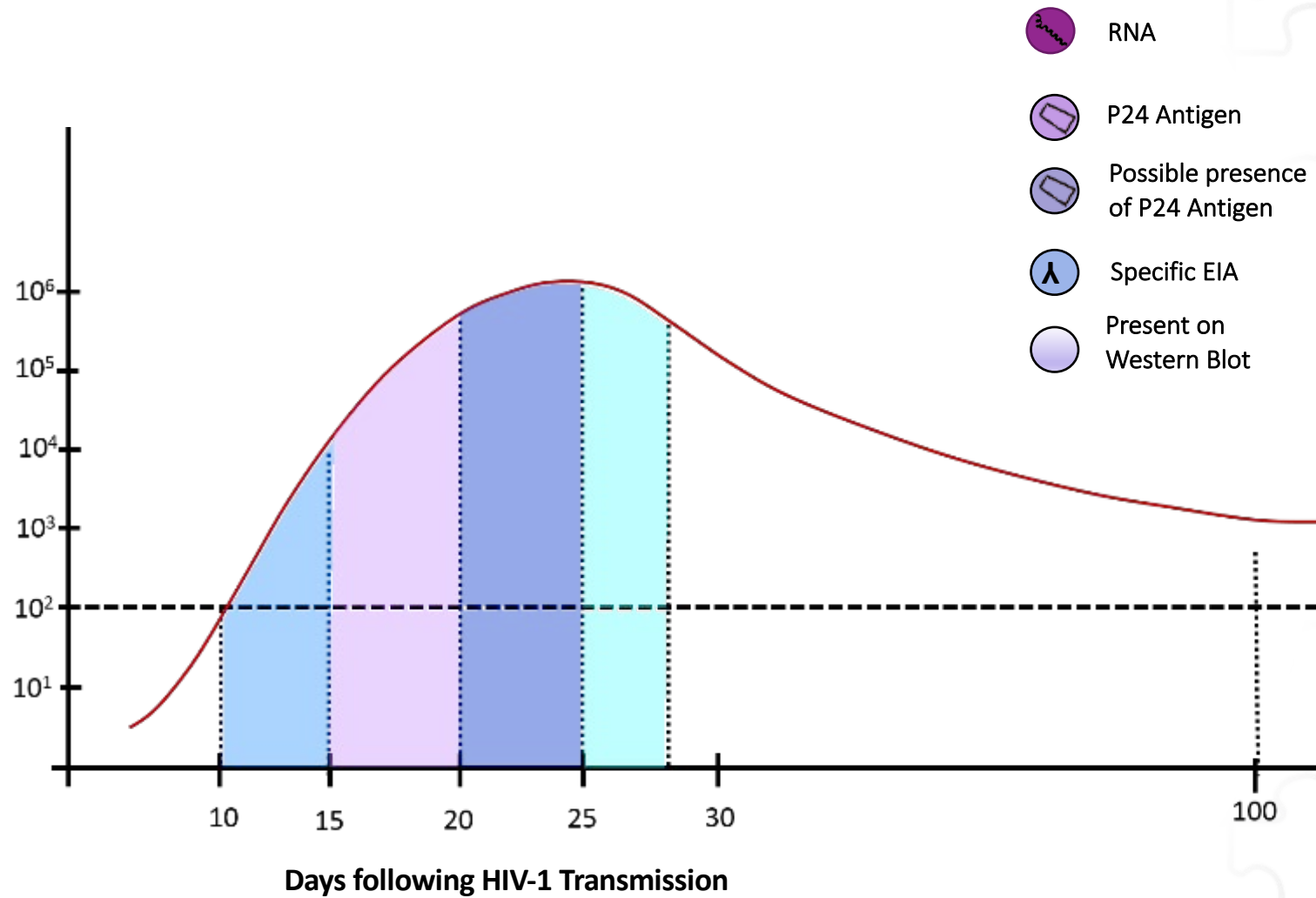
**How can HIV medications
be helpful to a cure?**



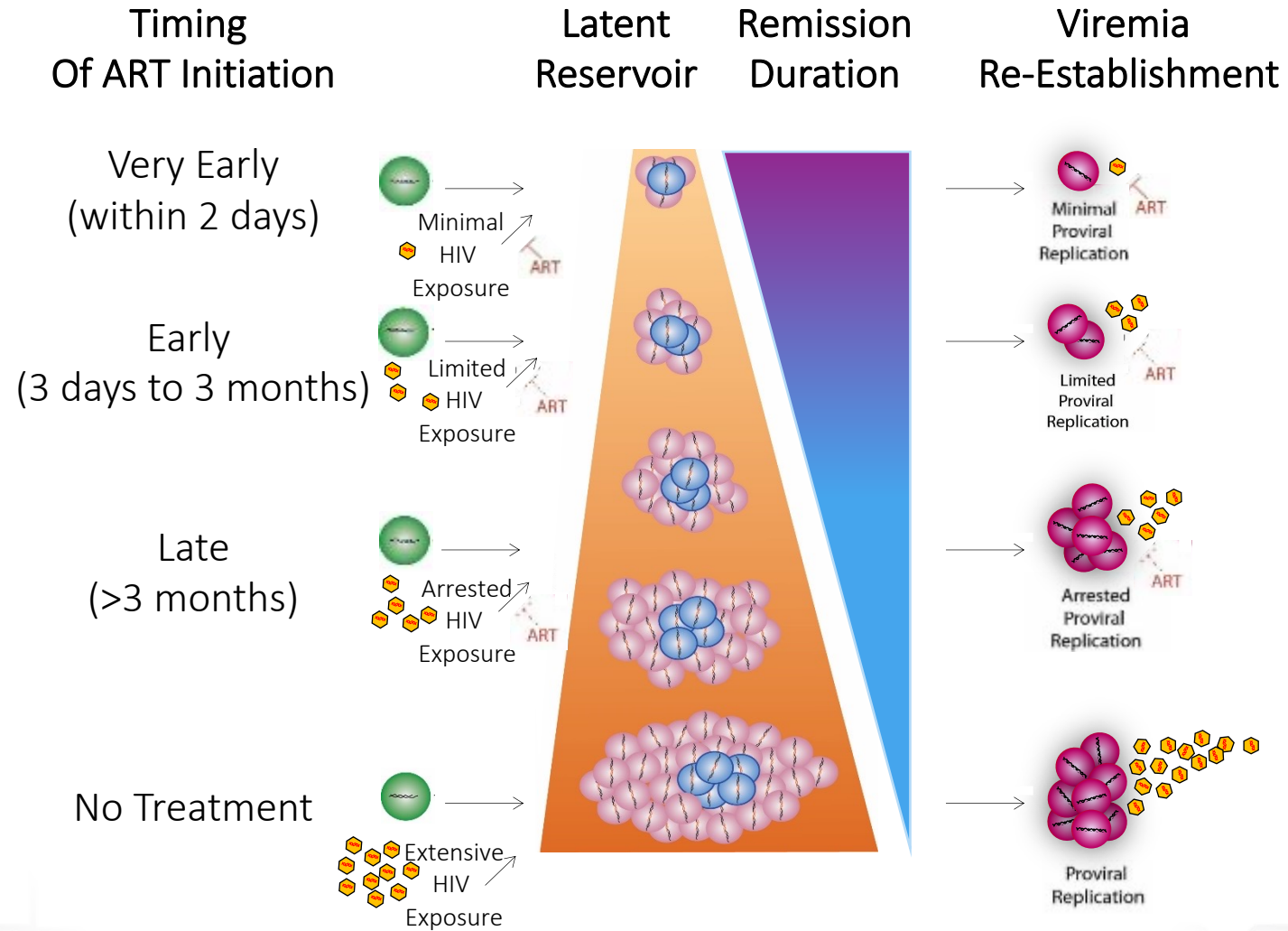
HIV Life Cycle and Drug Targets



What are the Fiebig Stages?



Early ART in Infants



Common Medications Used to Treat HIV

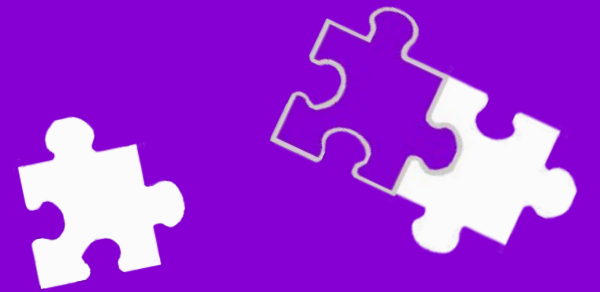
HIV Medication Chart

Combination Antiretrovirals					Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI)		
Atripla (EFV/TDF/FTC) 	Biktarvy (BIC/TAF/FTC) 	Combivir [†] (ZDV/3TC) 	Complera (RPV/TDF/FTC) 	Delstrigo (DOR/TDF/3TC) 	Emtriva [*] (emtricitabine, FTC) 	Epivir ^{†*} (lamivudine, 3TC) 	Retrovir ^{†*} (zidovudine, ZDV)
Descovy (TAF/FTC) 	Dovato (DTG/3TC) 	Epzicom [†] (ABC/3TC) 	Genvoya (EVG/COBI/TAF/FTC) 	Juluca (DTG/RPV) 	Viread ^{†*} (tenofovir DF, TDF) 	Ziagen ^{†*} (abacavir, ABC) 	Vemlidy (tenofovir alafenamide, TAF) FDA approved for HBV only
Odefsey (RPV/TAF/FTC) 	Stribild (EVG/COBI/TDF/FTC) 	Symtuza (DRV/COBI/TAF/FTC) 	Triumeq (DTG/ABC/3TC) 	Truvada (TDF/FTC) 			
Protease Inhibitors (PI)					Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)		
Evotaz (ATV/COBI) 	Kaletra [*] (lopinavir/ritonavir, LPV/RTV) 	Lexiva [*] (fosamprenavir, FPV) 	Prezcobix (DRV/COBI) 		Edurant (rilpivirine, RPV) 	Intelence (etravirine, ETR) 	Pifeltro (dorzavirine, DOR)
Prezista [*] (darunavir, DRV) 	Reyataz ^{†*} (atazanavir, ATV) 		Viracept [*] (nelfinavir, NFV) 		Sustiva [†] (efavirenz, EFV) 	Viramune ^{†*} (nevirapine, NVP) 	
Entry Inhibitors			Integrase Inhibitors (INSTI)			Boosting Agents	
Fuzeon (enfuvirtide, T-20) Fusion Inhibitor 	Selzentry (maraviroc, MVC) CCR5 Antagonist 	Trogarzo (ibalizumab, IBA) Post-Attachment Inhibitor 	Isentress ^{*▲} (raltegravir, RAL) 	Isentress HD (raltegravir, RAL) 	Tivicay (dolutegravir, DTG) 	Norvir ^{†*} (ritonavir, RTV) 	Tybost (cobicistat, COBI)

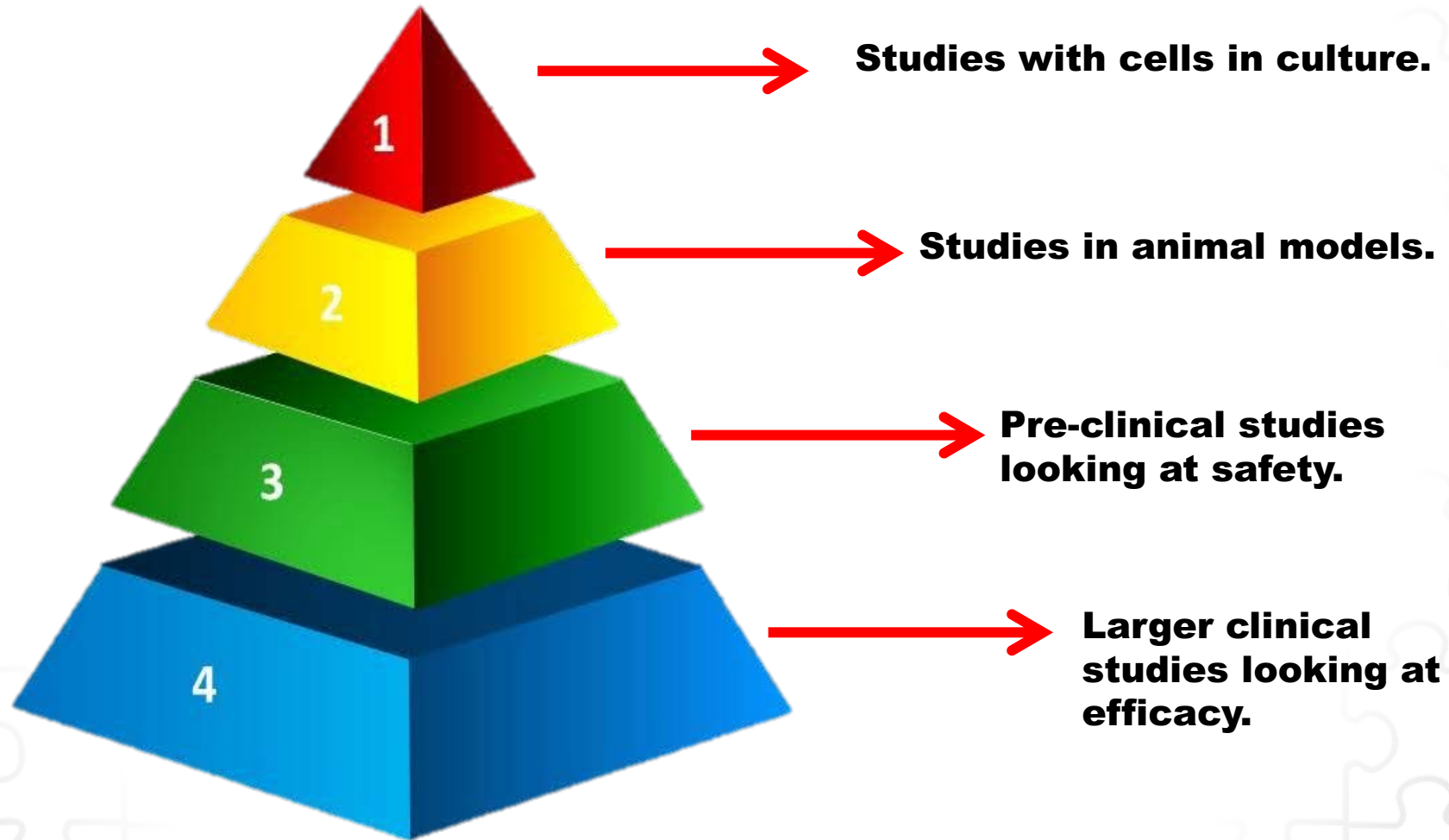
All pills shown in relative size/scale. Medication brand names appear in bold. Generic names and commonly used abbreviations appear in parentheses.

*Also available in liquid or powder form. †Generic formulation available. ▲Chewable form available.

**What has been the pathway
to an HIV cure?**



Stages of Clinical Research



HIV Cure-Related Research

Bench Science

Understanding
Persistence &
Immune control

- Viral subtype
- Genetics
- Tissues
- Intervention Mechanism
- Analysis of preliminary work

Intervention Trials

Animal & Human
Studies



*Single or
Combination
Interventions*

Early
ART

Latency
modifying
agents

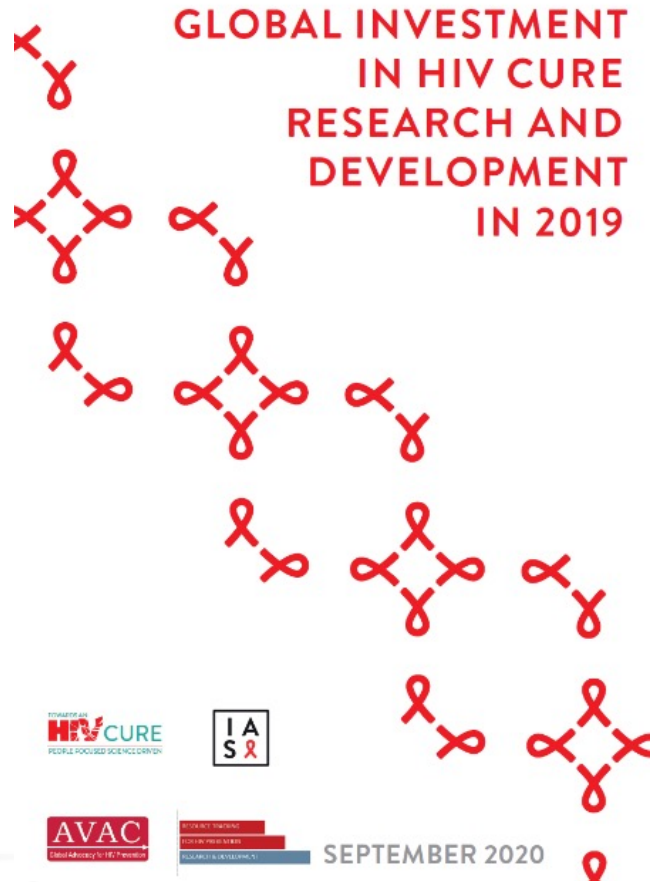
Immune
Therapies

Cell &
Gene
Therapies

Ethics & Social-Behavioral Sciences

- Decision-making
- Perceptions of risks and benefits
- Attitudes about research
- Individual and societal impact

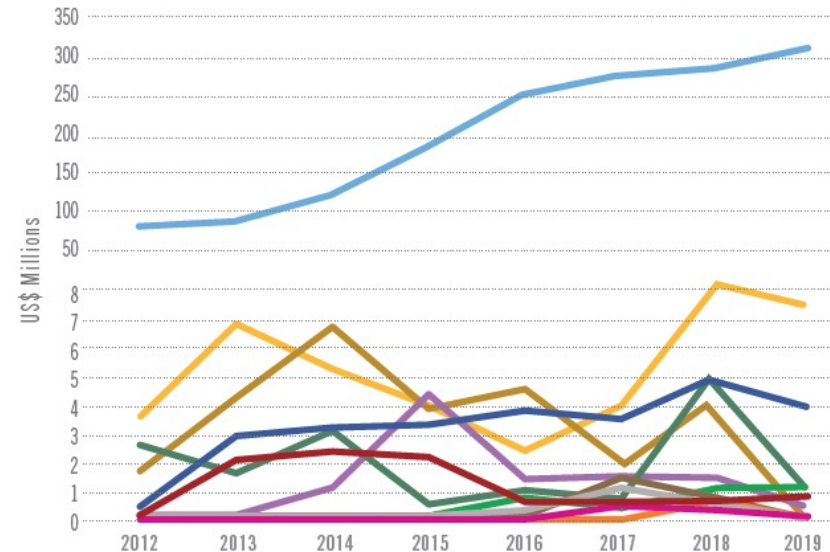
Global Investment in HIV Cure R & D (2019)

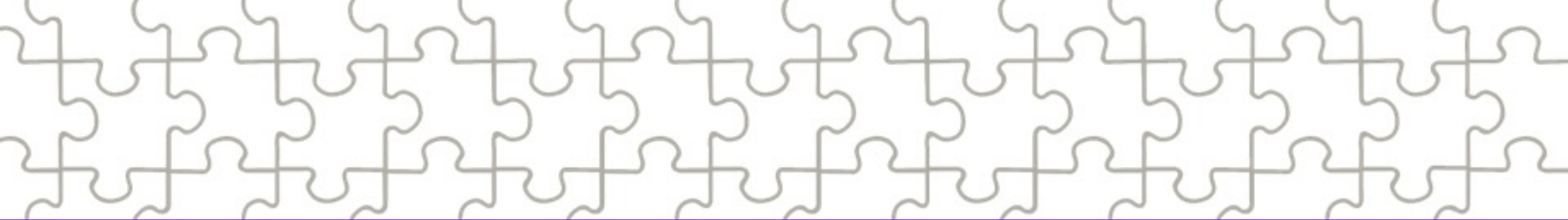


Global investment:
approximately **US \$323.9 million**

HIV Cure R&D Investments by Country, 2012–2019

	2012	2013	2014	2015	2016	2017	2018	2019
Australia	2.6	1.6	3.1	0.5	1.0	0.7	5.0	1.1
Canada	0.4	2.9	3.2	3.3	3.8	3.5	4.9	4.0
Cuba	0	0	0	0	0	0.5	0.4	0.1
European Commission	1.6	4.2	6.7	3.8	4.5	2.0	4.0	0
France	3.5	6.8	5.2	3.9	2.3	3.9	8.2	7.5
Germany	0	0	0	0	0.7	0.3	1.2	1.1
Italy	0	0	0	0	0	1.4	0.5	0
Spain	0	0	0	0	0	0	0.8	0
Switzerland	0.03	2.0	2.3	2.1	0.5	0.5	0.6	0.9
The Netherlands	0	0	0	0	0.2	1.0	0.4	0
United Kingdom	0.03	0.03	1.0	4.3	1.3	1.4	1.6	0.7
United States	79.4	85.9	120.4	183.2	252.1	273.6	296.0	312.8





Why is it difficult to cure HIV?

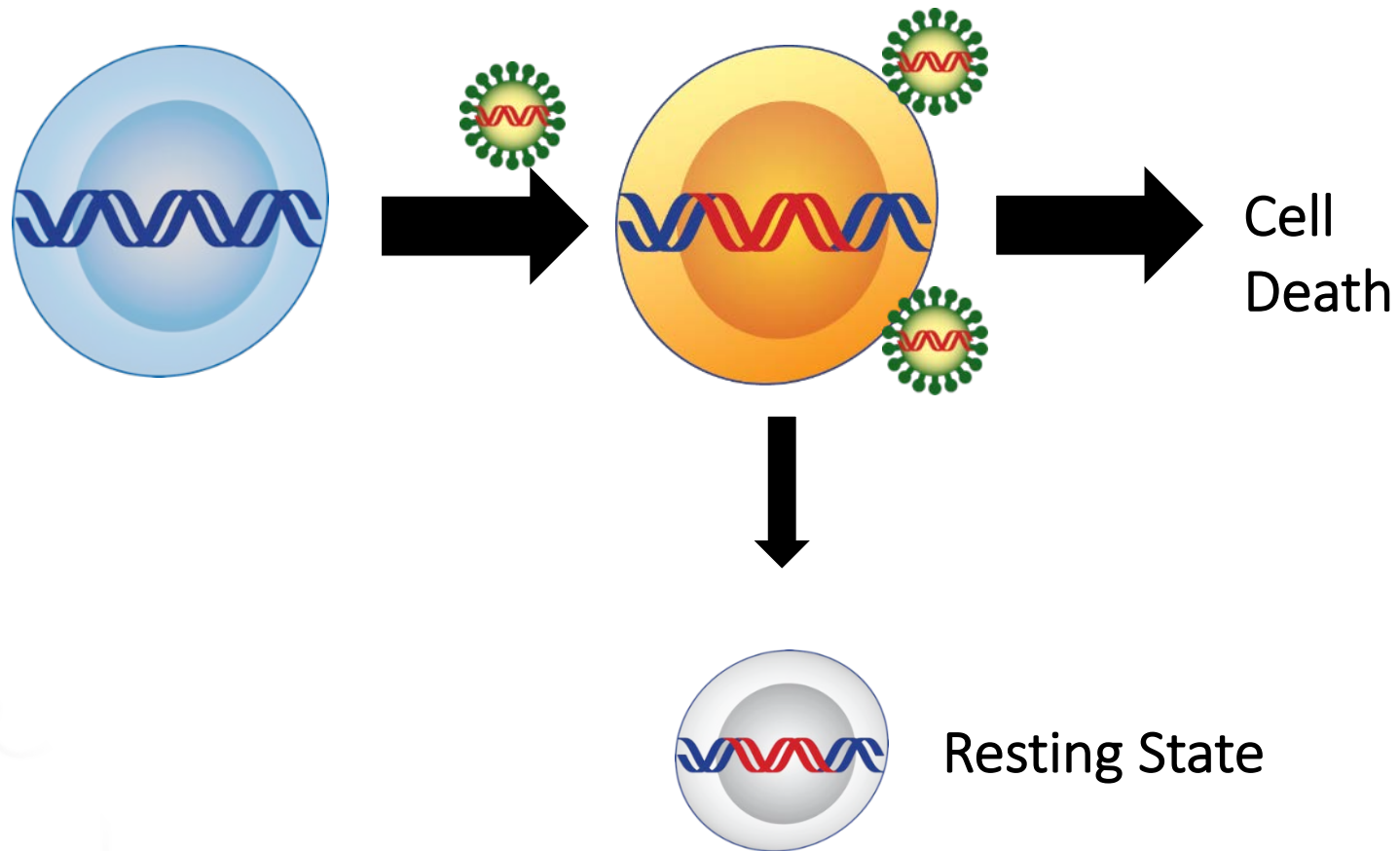


Why is HIV **so hard** to cure?

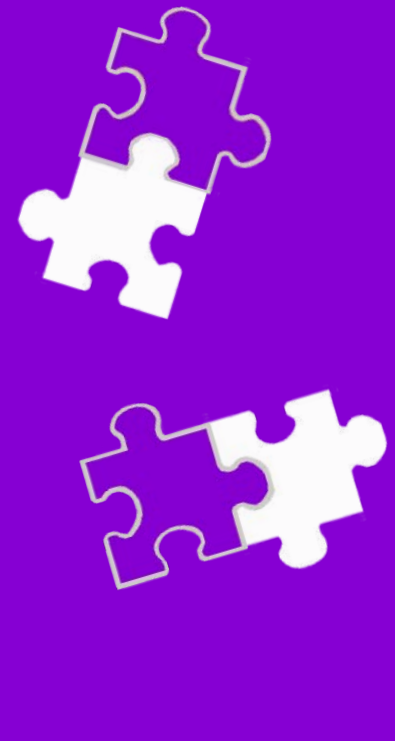
- HIV enters a cell and integrates into the cell's DNA
- **Most** cells recognize infection – causing cell death
- **A few** infected cells become “long-lived” memory cells or “resting memory” cells
- The collection of long-lived memory cells is called the **Latent Reservoir** = main barrier to curing HIV



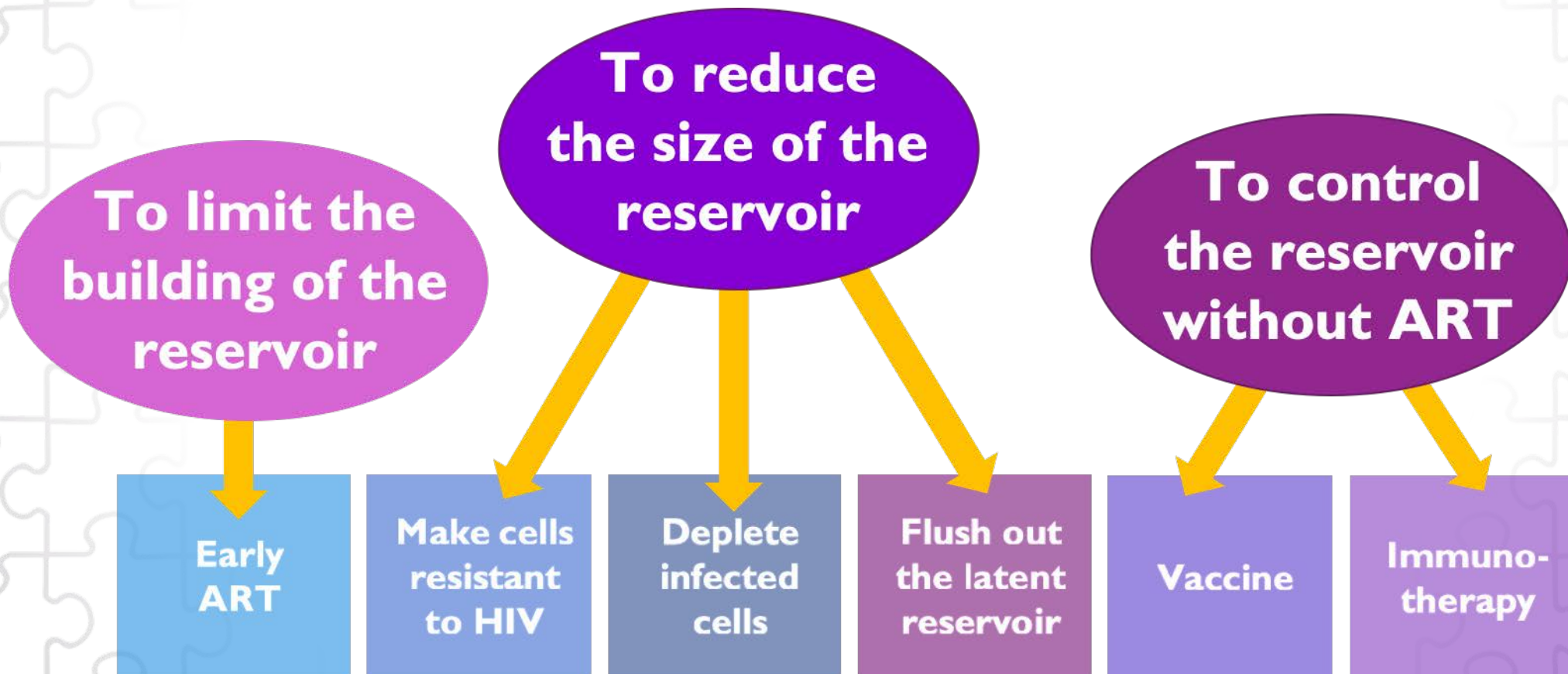
Establishing the Reservoir



**What pathways are
currently being explored?**



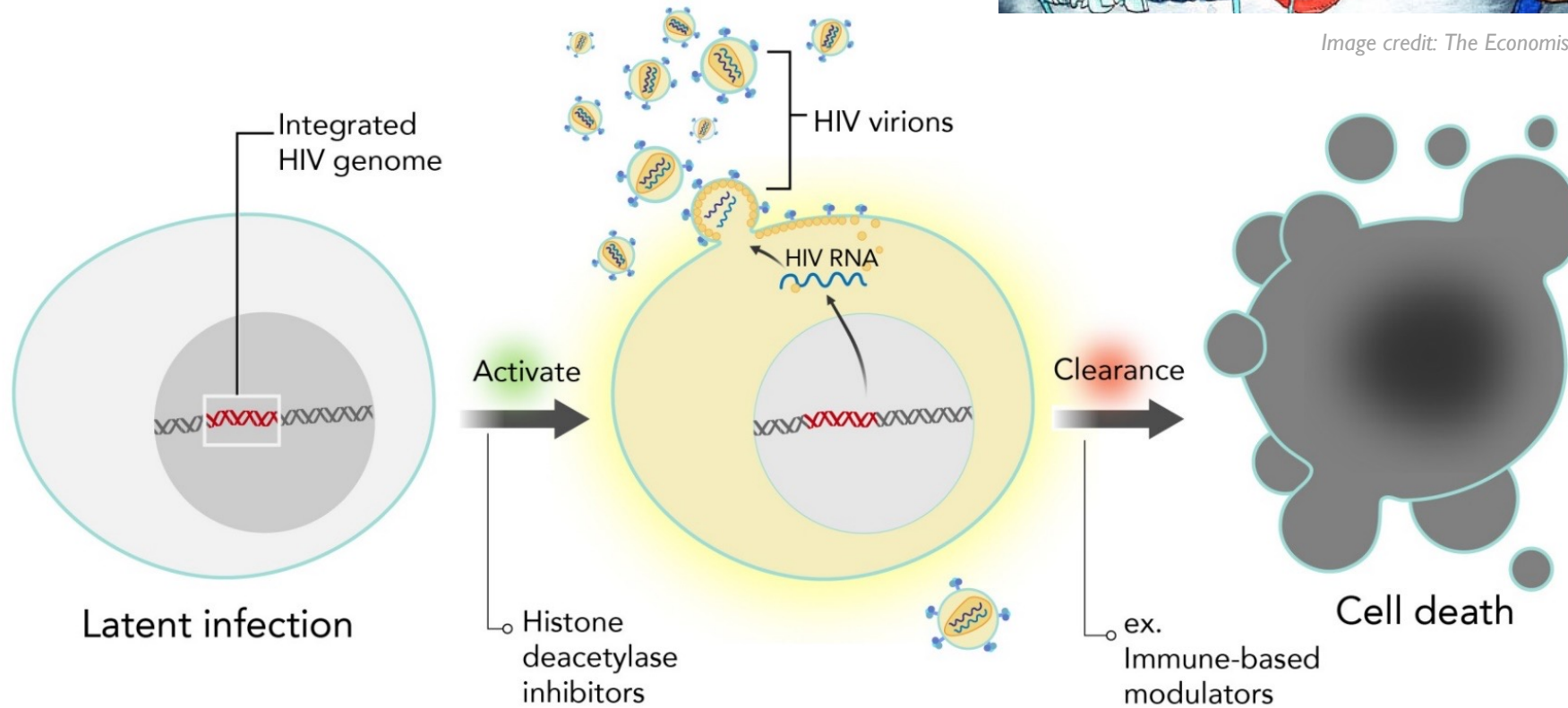
HIV Cure-Related Research Strategies Under Investigation



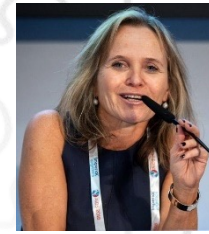
Latency Reversing Agents



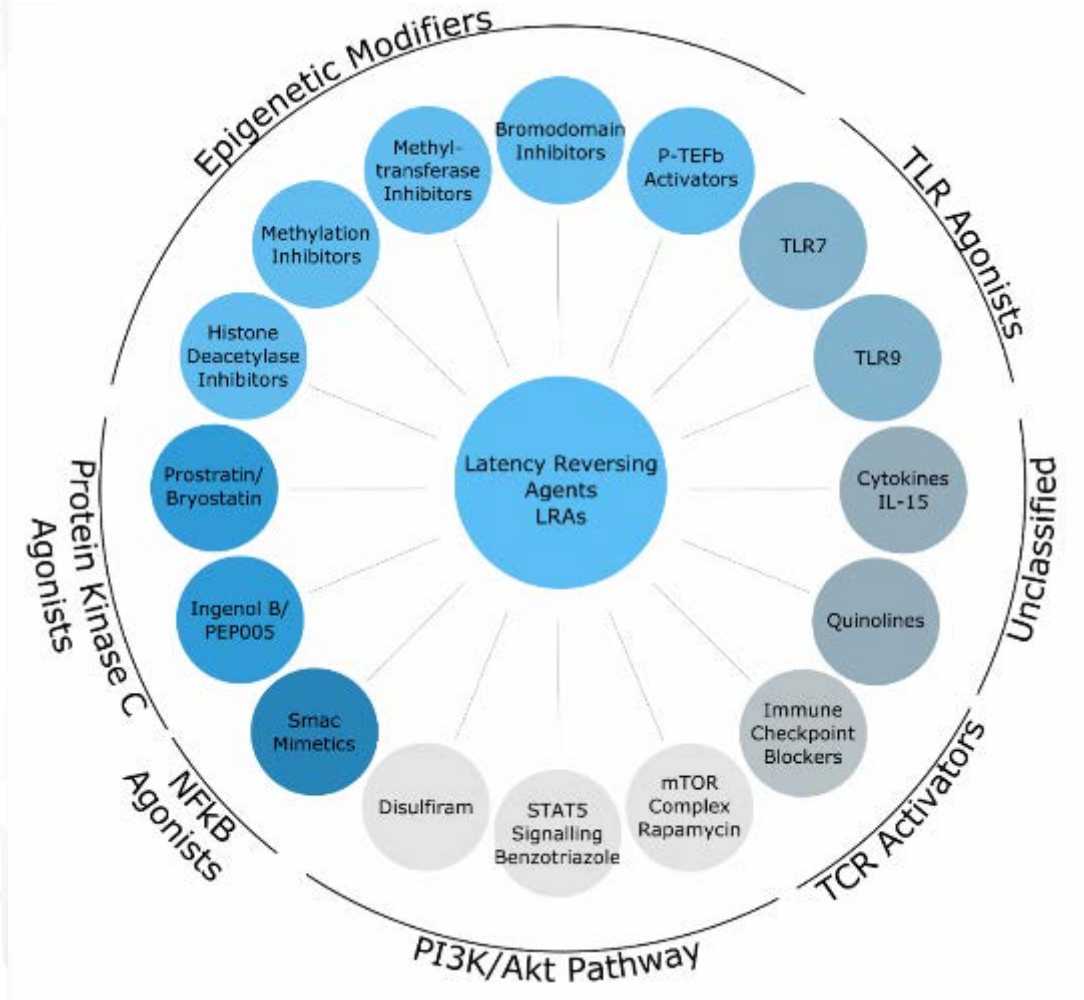
Image credit: The Economist, July 11



Latency-Reversing Agents



Sharon Lewin

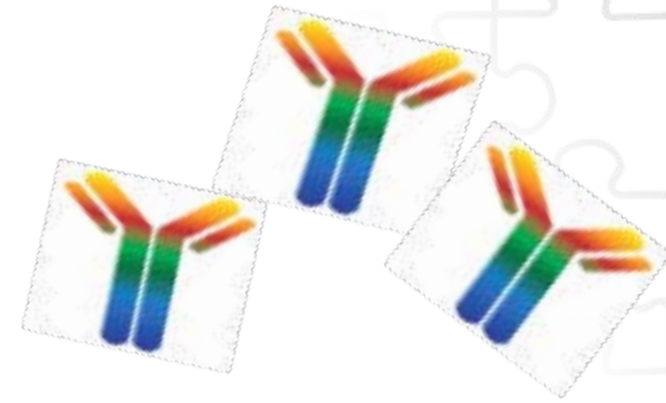
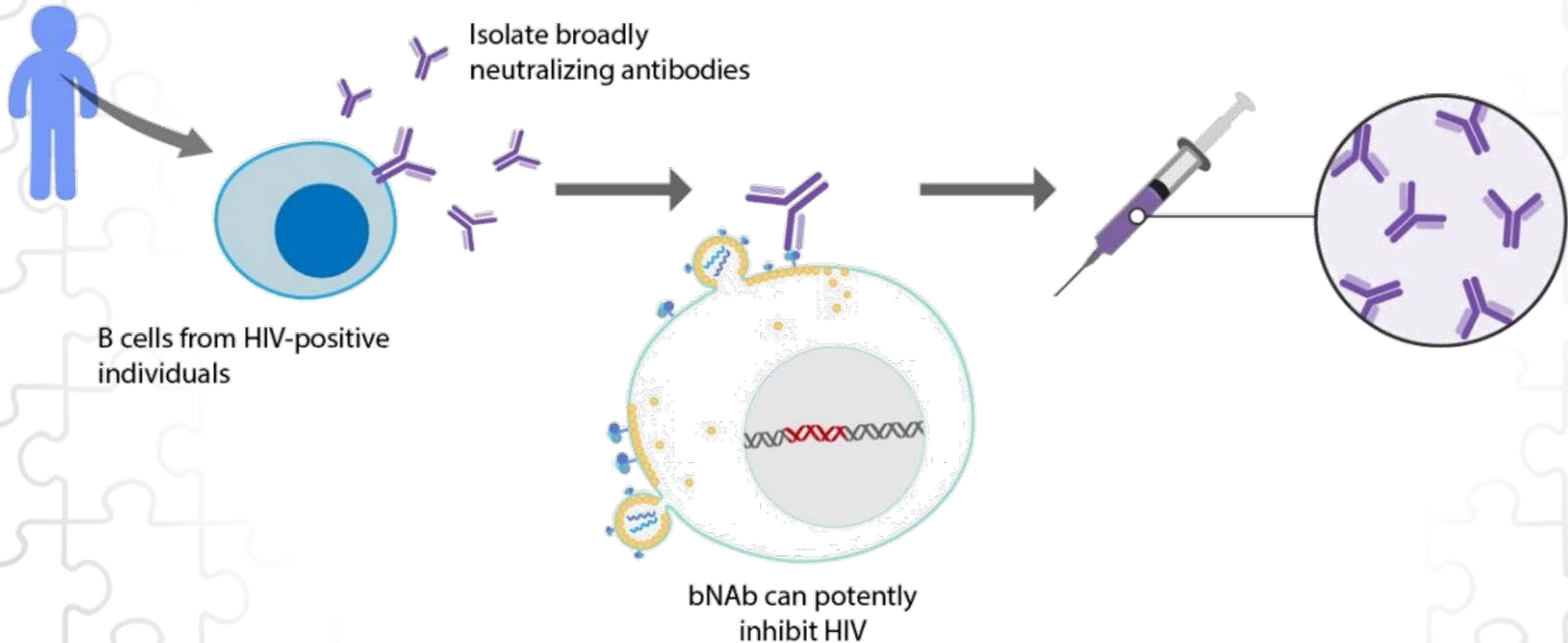


- None are HIV specific
- None are as potent as T-cell activation
- Unclear if virus is coming from all cells or just a few
- Still need better latency-reversing agents

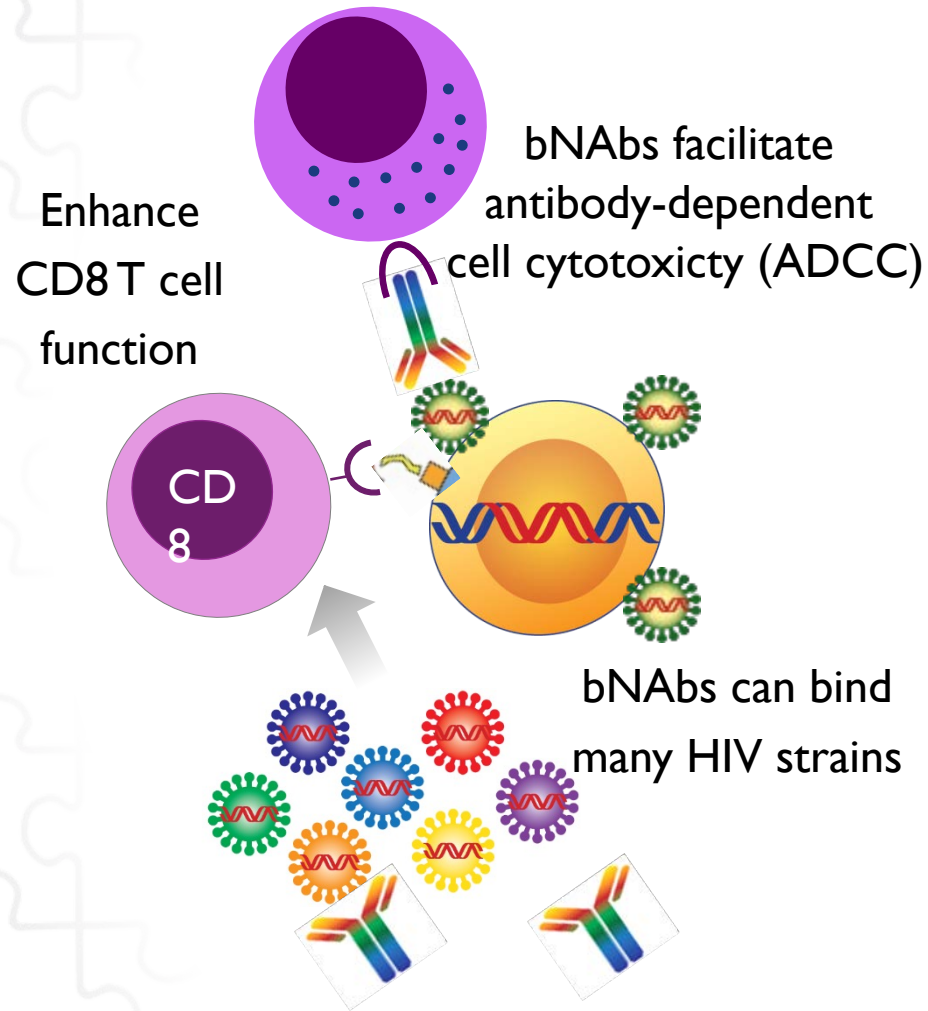
Kim, Anderson and Lewin. *Cell Host Microbe* 2018

Lewin SR. Why Should We 'Shock and Kill'. Community Cure Workshop. Saturday July 21, 2018.

Broadly Neutralizing Antibodies



Broadly Neutralizing Antibodies (bNAbs)



Main obstacle

- Pre-existing resistance

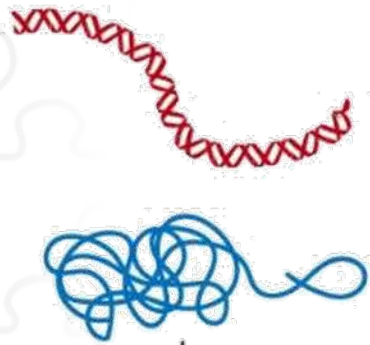
Strategies

- Broad and potent
- Multiple
- Tri-specific
- Long-acting
- Novel delivery platforms
- Early administration
- Combine with other agents

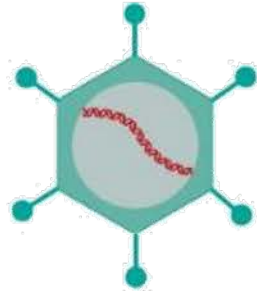
Kong, J Virol 2015; Barr, NEJM 2016; Scheid, Nature 2016; Caskey, Nature Med 2017; Hessel, Nature Med 2016; Liu, Science 2016; Nishimura, Nature 2017; Pardi, Nature Communications 2017; Gardner, Nature 2015; Pitman, Lancet 2018

Immune-Based Strategies

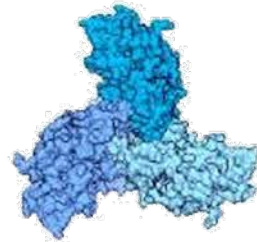
1. DNA and RNA vaccines



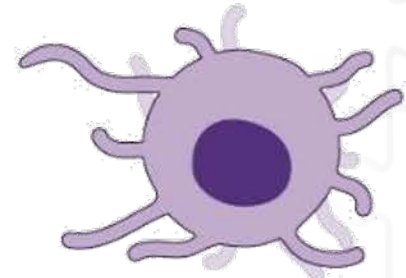
2. Viral vector vaccines



3. Protein or peptide vaccines

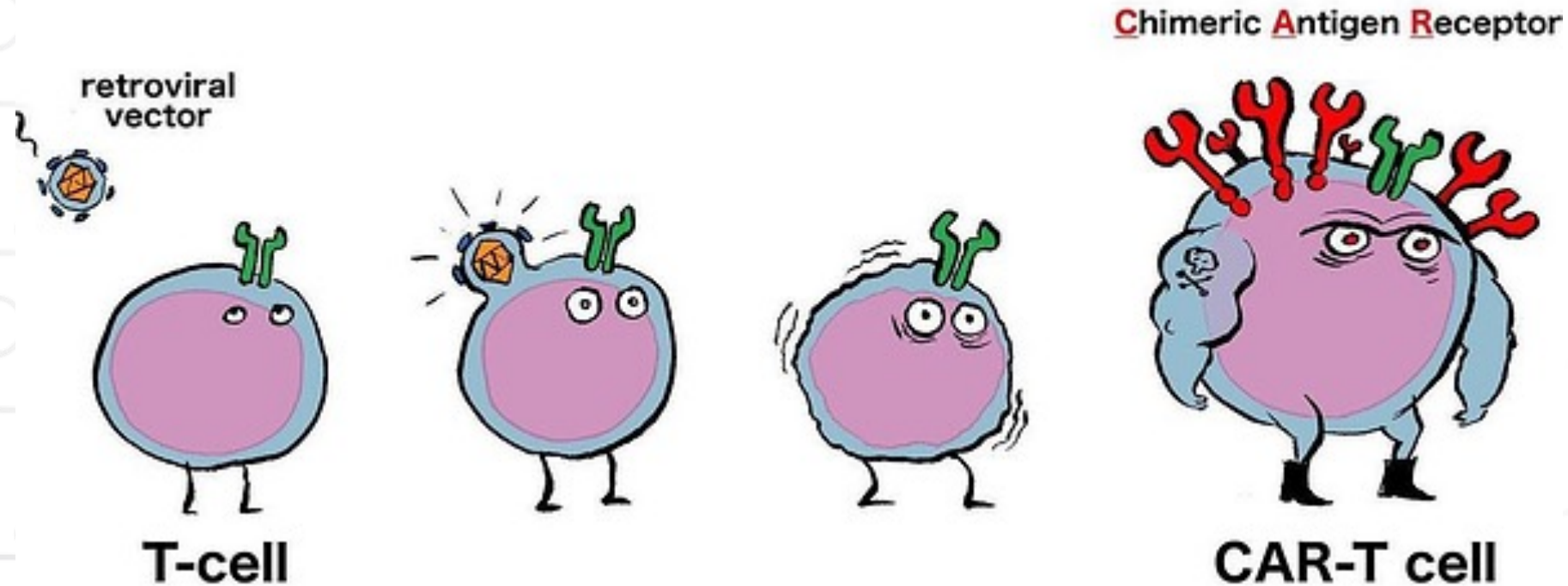


3. Dendritic cell vaccines



Chimeric Antigen T cells (CAR-T cell)

Generating super-soldiers the production of CAR-T cells

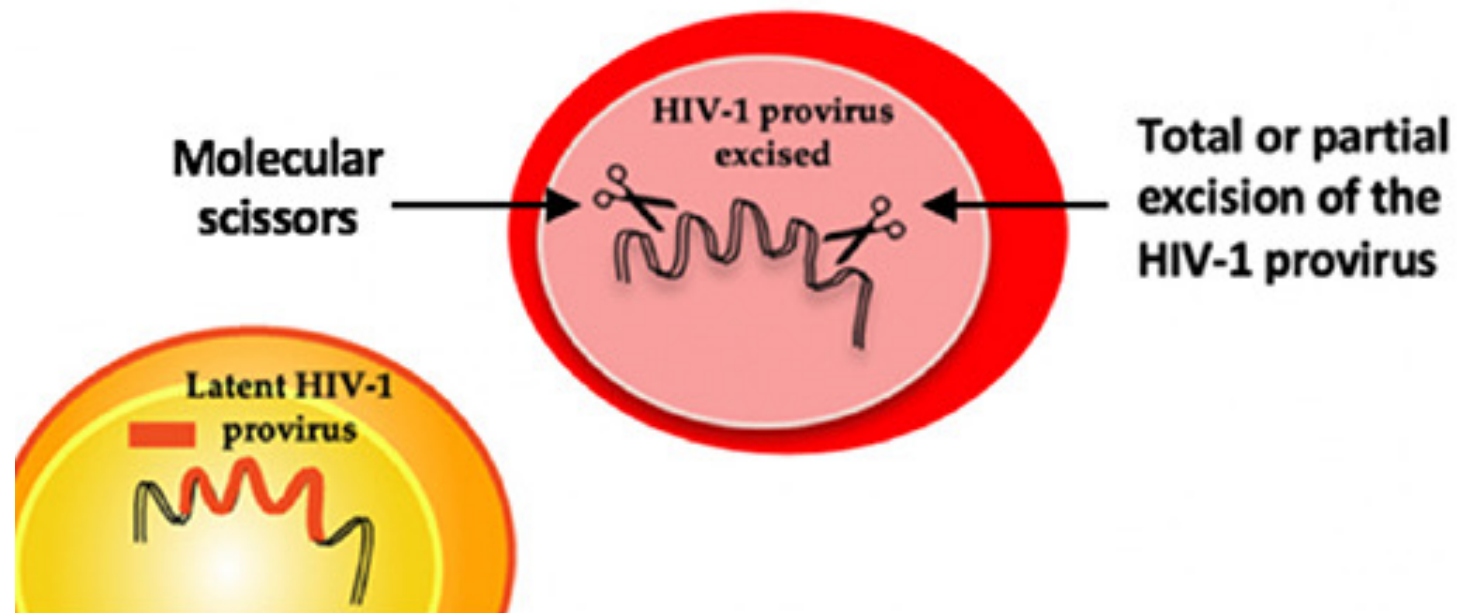


facebook.com/pedromics

https://blog.seracare.com/ngs/aacr-2019-day-one-highlights-next-generation-car-t-cells?utm_medium=social&utm_source=facebook

Cell & Gene Approaches

CRISPR/Cas9 gene editing

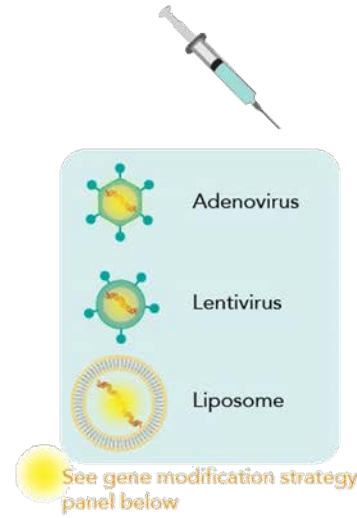


<https://www.frontiersin.org/articles/10.3389/fcimb.2020.00424/full>

Cell & Gene Approaches

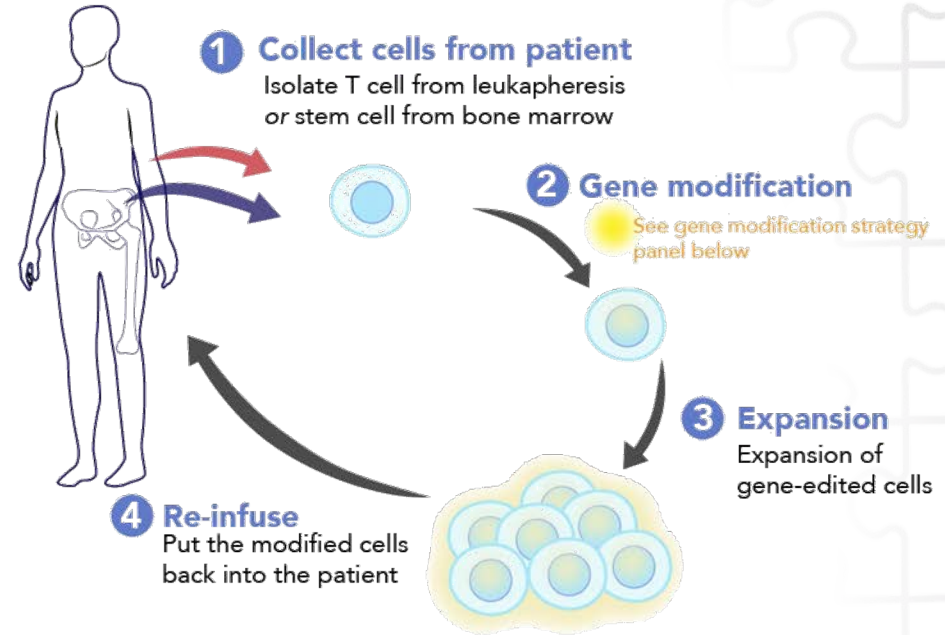
In vivo gene therapy

Vectors are used to carry anti-HIV genes to the target cells *in situ*



Ex vivo gene therapy

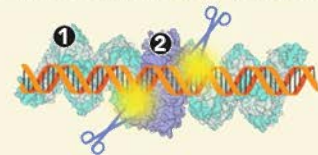
Isolation of desired cell types from the patient, followed by gene modification and reinfusion



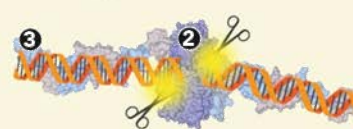
Gene modification strategy

Nucleases and CRISPR/Cas9 are like molecular scissors

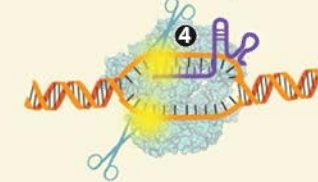
Transcription Activator-like Effector Nucleases (TALENs)



Zinc Finger Nuclease

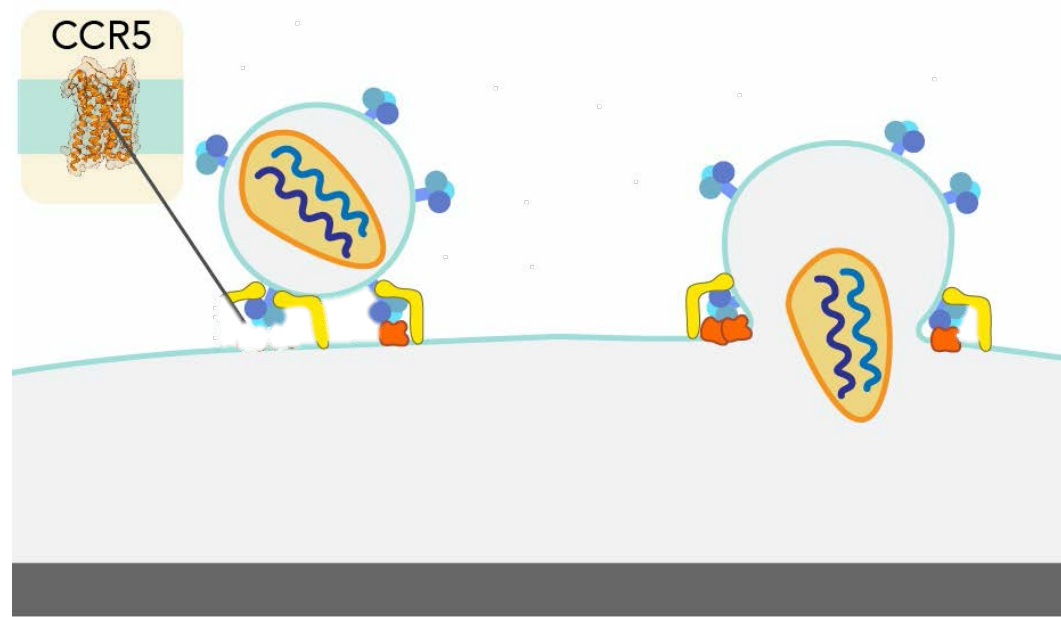


CRISPR-Cas9 complexes

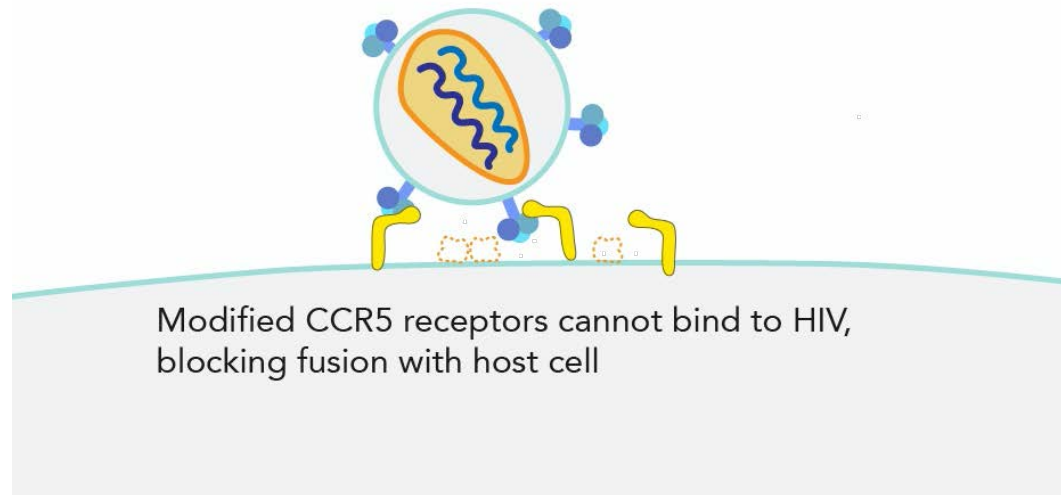


- 1** TAL-effectors
- 2** Nuclease
- 3** Zinc Fingers
- 4** Cas9

Cell & Gene Approaches

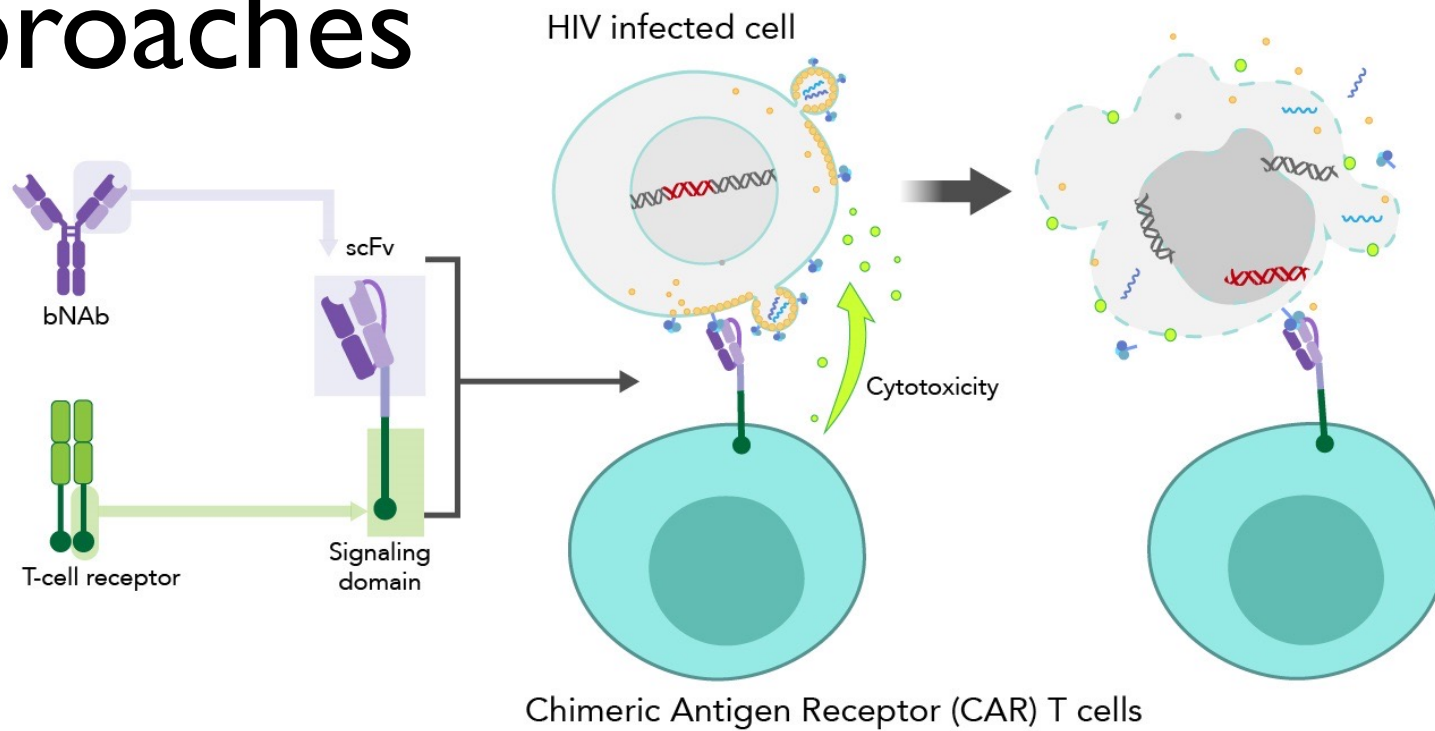


Use gene therapy techniques to disable the gene that encodes CCR5 receptor



Modified CCR5 receptors cannot bind to HIV, blocking fusion with host cell

Cell & Gene Approaches



Useful Video Links

- **Understanding Apoptosis and Pyroptosis:**

<https://www.youtube.com/watch?v=vKi95jdJLhs>

- **Khan Academy - How HIV Kills So Many CD4 T Cells**

<https://www.youtube.com/watch?v=vKi95jdJLhs>

- **Khan Academy - How HIV infects us I: mucous membranes, dendritic cells, and lymph nodes.**

<https://www.youtube.com/watch?v=QEvjvpvLxmQ>

- **What is CAR T-Cell Therapy?**

<https://www.youtube.com/watch?v=LNx-ywebx-0>

- **HIV: Basic Function Of The Immune System**

<https://www.youtube.com/watch?v=YUNVGCQVe2g>

- **HIV Infection Stages, Pathology And Treatment**

<https://www.youtube.com/watch?v=horX8xLVpCg>

- **HIV Infection Mechanism – Dendritic Cells**

<https://www.youtube.com/watch?v=ZroXIBg8keA>

- **Understanding HIV Reservoirs**

<https://www.youtube.com/watch?v=XkQqE02gbVc>

- **Understanding Broadly Neutralizing Antibodies**

<https://www.youtube.com/watch?v=Dr7werW5Or4&t=26s>

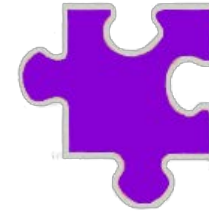


Useful Video Links (cont'd)

- **Gene Editing explained**
https://www.youtube.com/watch?v=E8vi_PdGrKg
- **CAR-T Cells in cancer**
<https://www.youtube.com/watch?v=OadAW99s4Ik>
- **Live Cell Imagine T cells with Macrophages**
<https://www.youtube.com/watch?v=SkTIZxsVSGM>
- **Cytotoxic T cell function**
 - <https://www.youtube.com/watch?v=WdCialS2LV4>



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