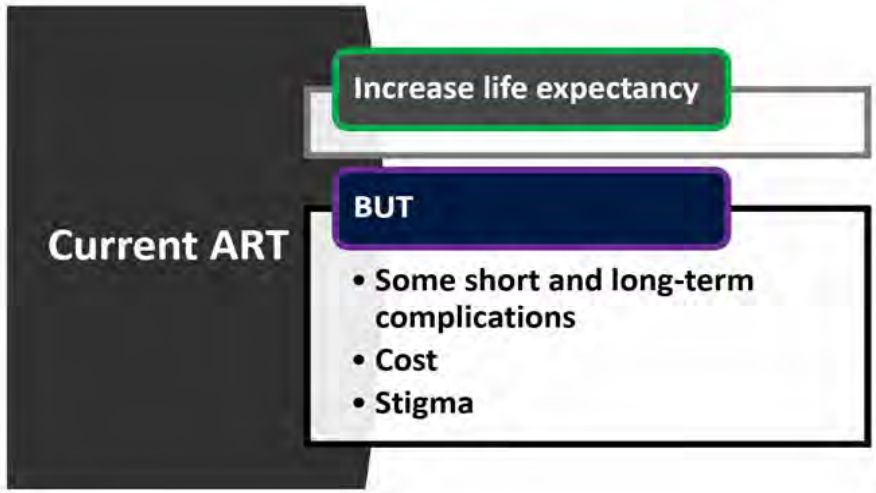
How long could the patients survive after initiation of HIV treatment ?



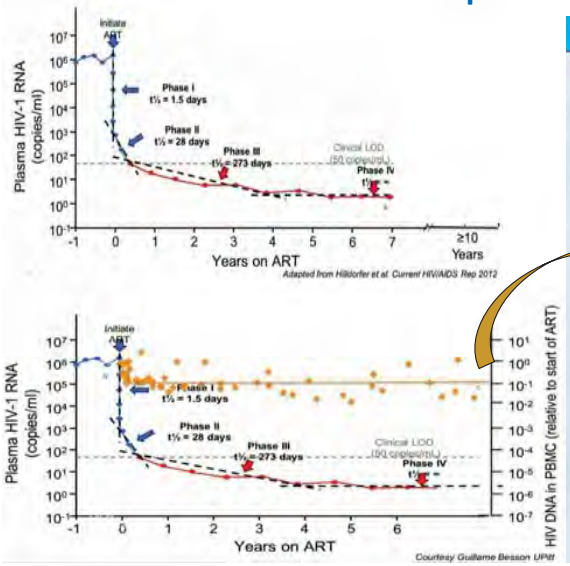
HIV: Infinity War or End Game?
Updates on HIV Cure and Remission

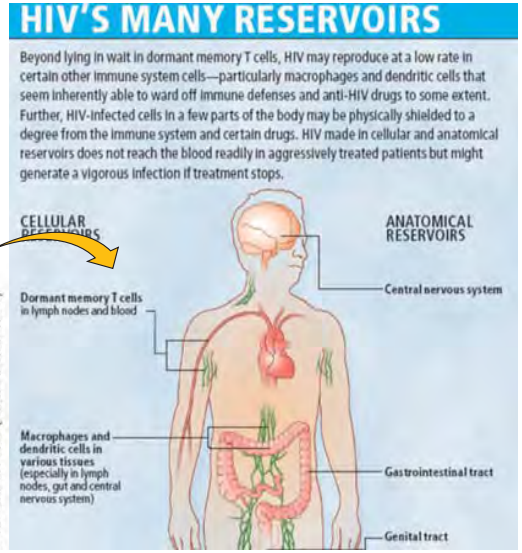
Opass Putcharoen MD, MSc
Division of Infectious Diseases, Department of Medicine,
Faculty of Medicine, Chulalongkorn University
Emerging Infectious Diseases Clinical Center, Thai Red Cross
Bangkok, Thailand

I'll begin with the end
My summary

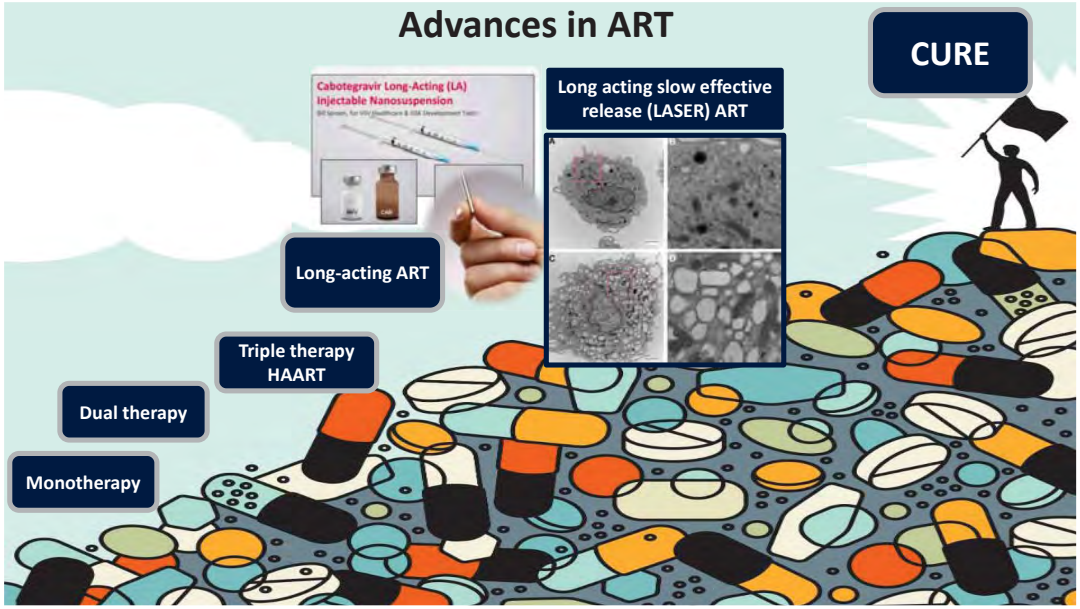
- HIV eradication is difficult but may be possible
  - –Attack at HIV reservoir
  - –Enhance human immune response
  - –Make human cells resistant to HIV infection

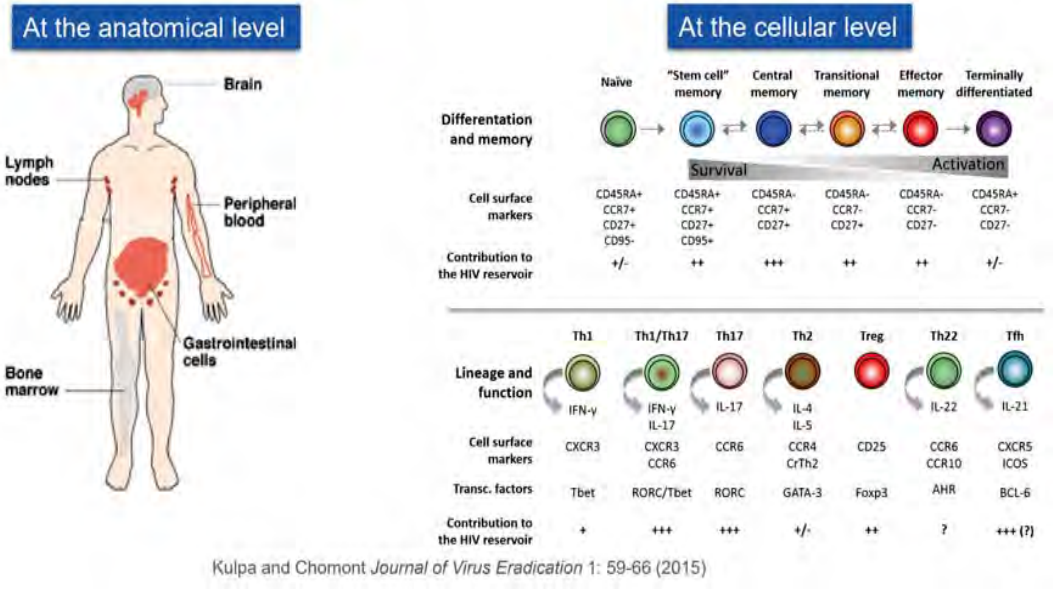
Effects of current ART on plasma viral load and HIV reservoirs





Advances in ART

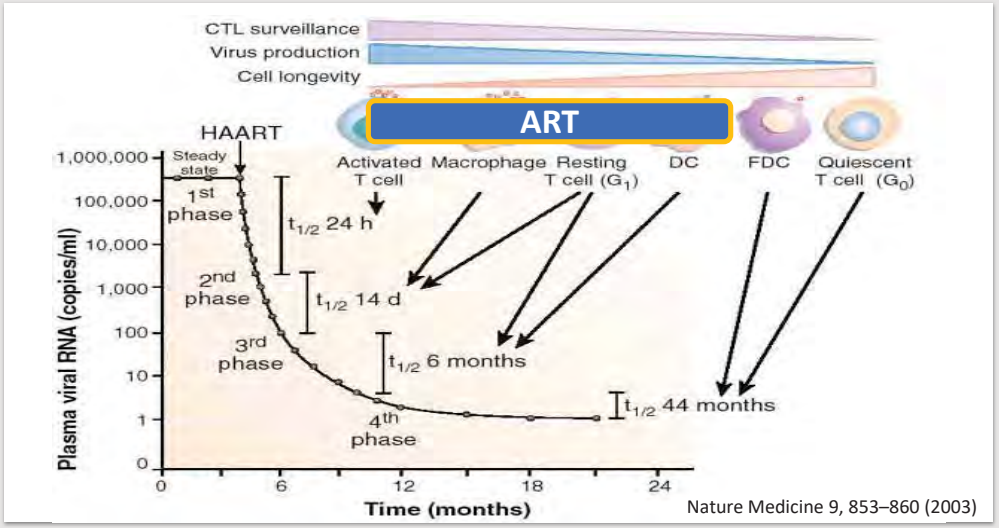




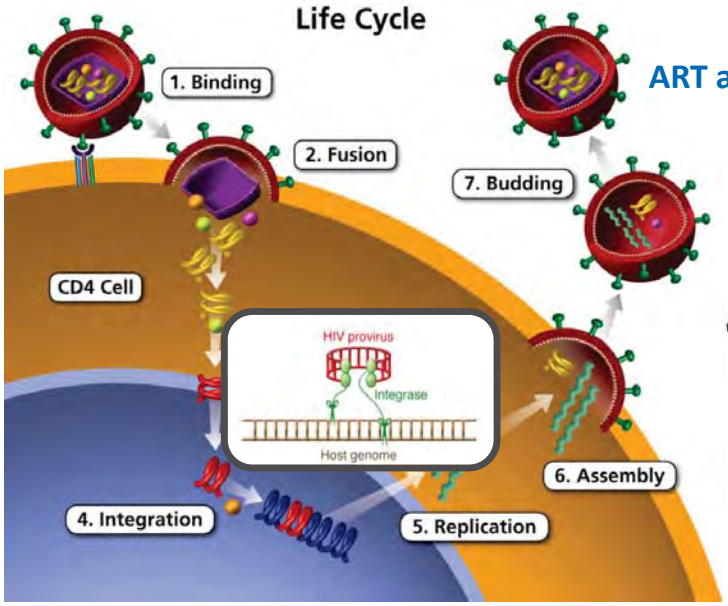
Kulpa and Chomont *Journal of Virus Eradication* 1: 59-66 (2015)

Basics

- **Why HIV eradication is very difficult?**
- Concepts of HIV Cure
- Some people have self immunity to protect from HIV infection



Nature Medicine 9, 853–860 (2003)

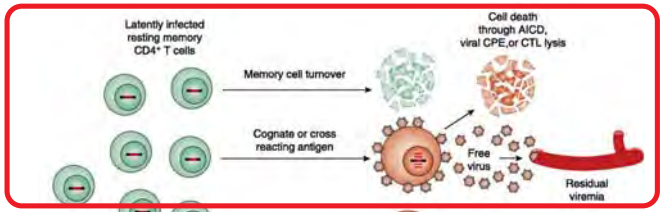


ART and obstacles for cure

- Integrated DNA
- Latent CD4 T cells

Basics

- Why HIV eradication is very difficult?
- **Concepts of HIV Cure**
- Some people have self immunity to protect from HIV infection



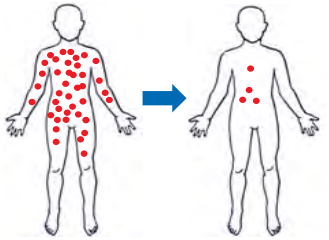
Fate of latently infected T cells

- Activated by antigen or drugs → destroyed by CTL or CPE
- Resting and clonal expansion

Type and principle of HIV Cure

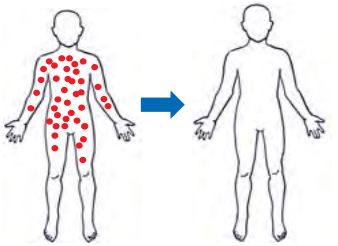
Functional Cure

When the level of HIV particles in an infected person's body has been reduced to such an **extremely low level** that person can stop treatment and not worry about viral rebound and damaging immune system



Sterilizing cure

When every last particle of HIV has been destroyed from an infected person's body (eradication)

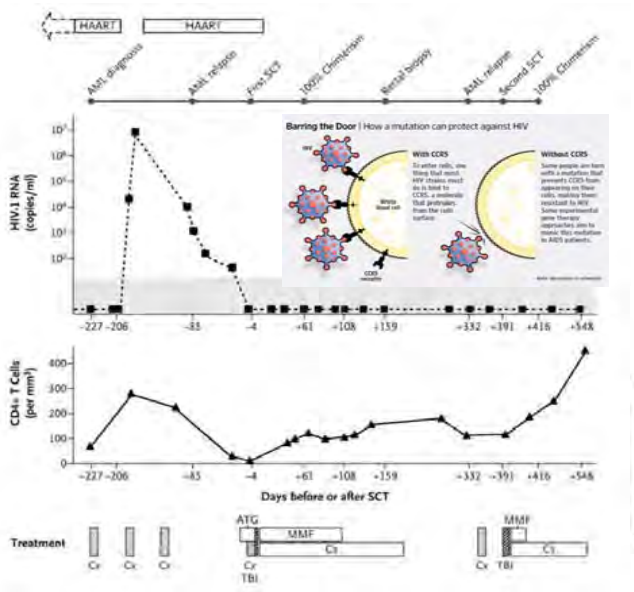


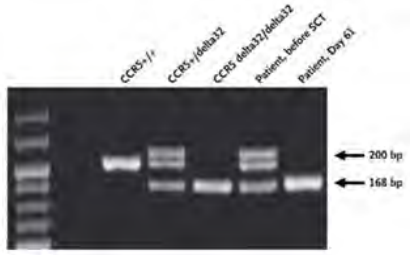
Somethings that you need to know

- Some people got HIV but then viruses disappeared by interventions
- Some people got HIV but then viruses were maintained at low level without drug
- Some people have self immunity to protect from HIV infection

Somethings that you need to know

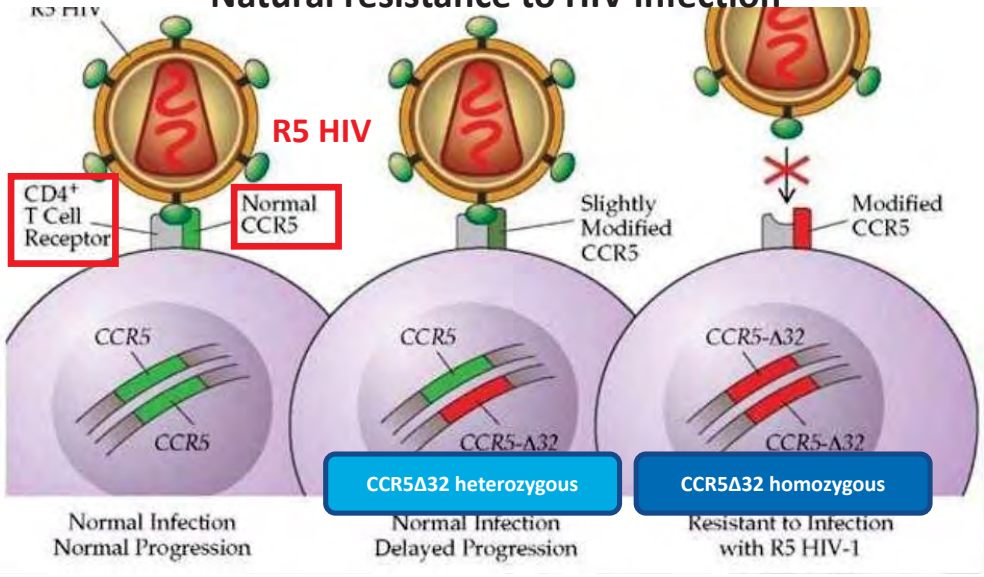
- Some people got HIV but then viruses disappeared by interventions
- Some people got HIV but then viruses were contained at very low level "without" drug
- Some people have self immunity to protect from HIV infection

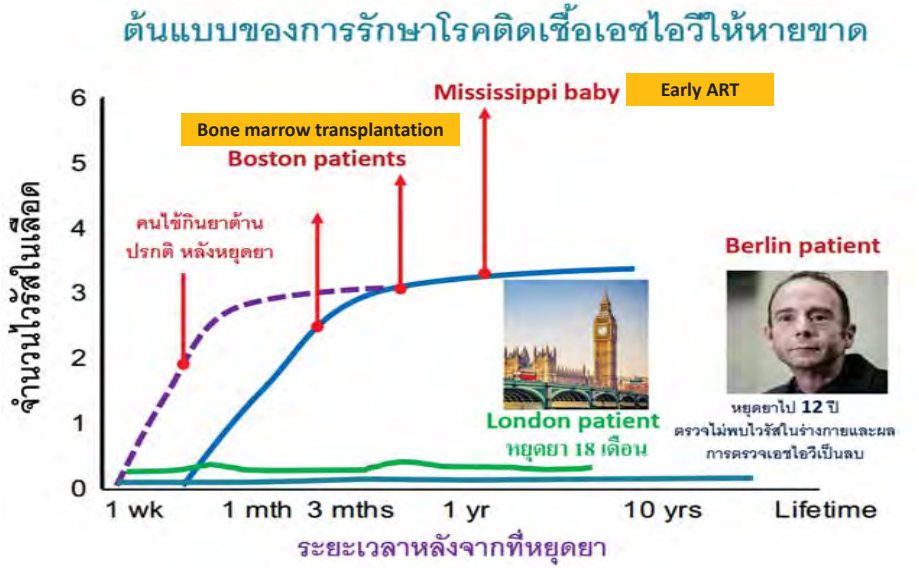


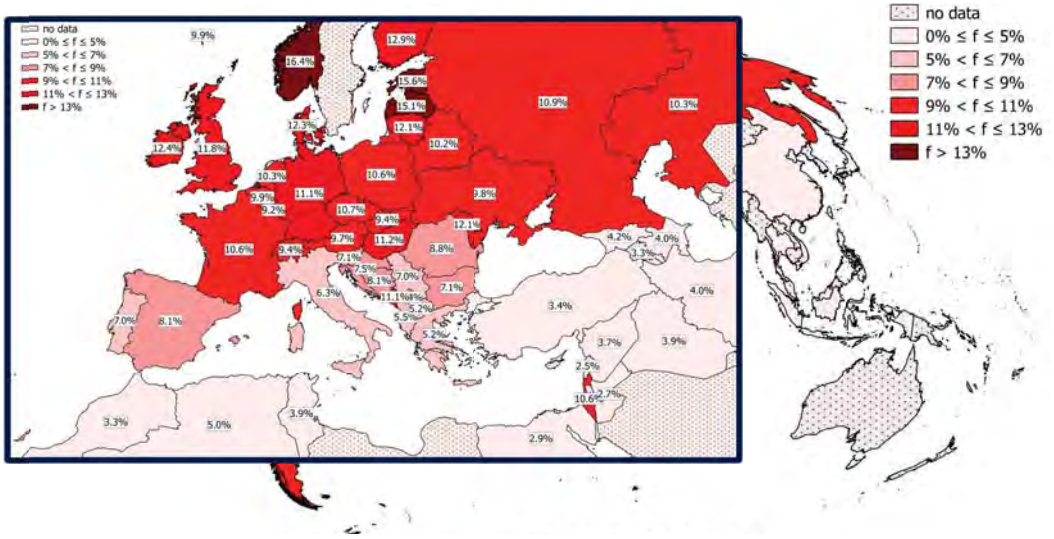


N Engl J Med 2009; 360:692-698

Natural resistance to HIV infection



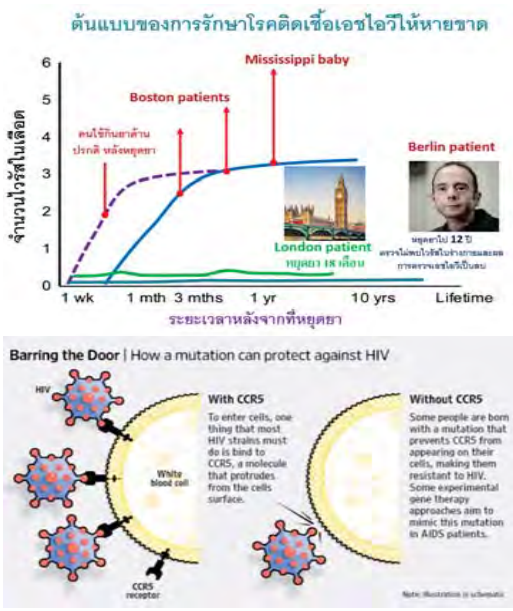




Frequencies of gene variant CCR5-Δ32 in 87 countries based on next-generation sequencing of 1.3 million individuals sampled from 3 national DKMS donor centers *Human Immunology 78 (2017) 710–717*

Basics

- Why HIV eradication is very difficult?
- **Concepts of HIV Cure**
- Some people have self immunity to protect from HIV infection



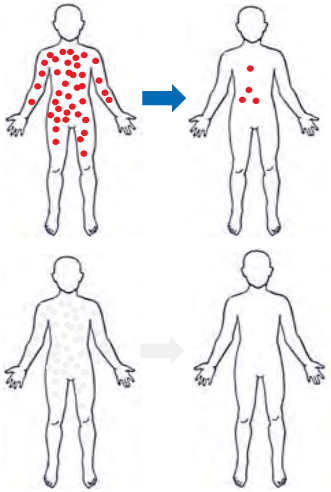
	London patient	Berlin patient
เสต็มเซลล์ของผู้ให้	ปลูกถ่ายเสต็มเซลล์จากคนที่เป็น CCR5Δ32 homozygous	ปลูกถ่ายเสต็มเซลล์จากคนที่เป็น CCR5Δ32 homozygous
ลักษณะของเซลล์ของผู้รับ	มี CCR5 ปรกติ	มี CCR5 แบบ heterozygous
สายพันธ์ของเชื้อเอชไอวี	ติดเชื้อไวรัสสายพันธ์ R5	ติดเชื้อไวรัสสายพันธ์ R5
โรคมะเร็งที่ผู้ป่วยเป็น	Hodgkin lymphoma	Acute myelogenous leukemia
จำนวนครั้งของการปลูกถ่าย	ทำการปลูกถ่ายเสต็มเซลล์ครั้งเดียว	ทำการปลูกถ่ายเสต็มเซลล์สองครั้ง
การฉายรังสีเพื่อกำจัดเซลล์มะเร็ง	ไม่ได้รับการฉายแสงเพื่อกำจัดเซลล์มะเร็ง	ได้รับการฉายแสงเพื่อกำจัดเซลล์มะเร็ง
สูตรยา conditioning regimen	Reduced intensity	Full intensity
ยากดภูมิชนิดกด T cell	ได้รับ antiCD52	ได้รับ ATG
การเกิดภาวะ Graft versus host disease	เล็กน้อย	เล็กน้อย
Chimerism	100% chimerism	100% chimerism
ระยะเวลาหลังจากหยุดยาต้านและไม่ตรวจพบไวรัสอีกเลย	1 ปีครึ่ง **REMISSON**	12 ปี **CURE**
ไวรัสในแหล่งต่างๆ	ยังตรวจไม่ครบ	ตรวจไม่พบไวรัสซุกซ่อนในอวัยวะต่างๆ เช่น สมอง ลำไส้ ต่อมน้ำเหลือง

CCR5 เป็นโปรตีนบนผิวเซลล์ที่เชื้อเอชไอวีใช้เป็นตัวพาเข้าไปในเซลล์ของคน CCR5Δ32 homozygous = คนที่ไม่มีโปรตีนนี้เลยบนเซลล์ ทำให้เชื้อไม่สามารถเข้าไปได้
Conditioning regimen = การให้ยาหรือวิธีการต่างๆที่ทำให้เซลล์ในไขกระดูกผู้รับเซลล์ลดลงเพื่อให้เสต็มเซลล์ใหม่เข้าไปแทนที่
Graft versus host disease = กลไกที่ร่างกายของผู้รับเซลล์มีการต่านเสต็มเซลล์ที่รับเข้ามา ซึ่งมีข้อดีคือเซลล์ที่มีไวรัสซ่อนอยู่ในผู้รับอาจจะถูกกำจัดได้ด้วยวิธีนี้
Chimerism = การที่เซลล์ของผู้รับและผู้ให้เข้ากันได้ดี หลังจากมีการปลูกถ่ายเสต็มเซลล์

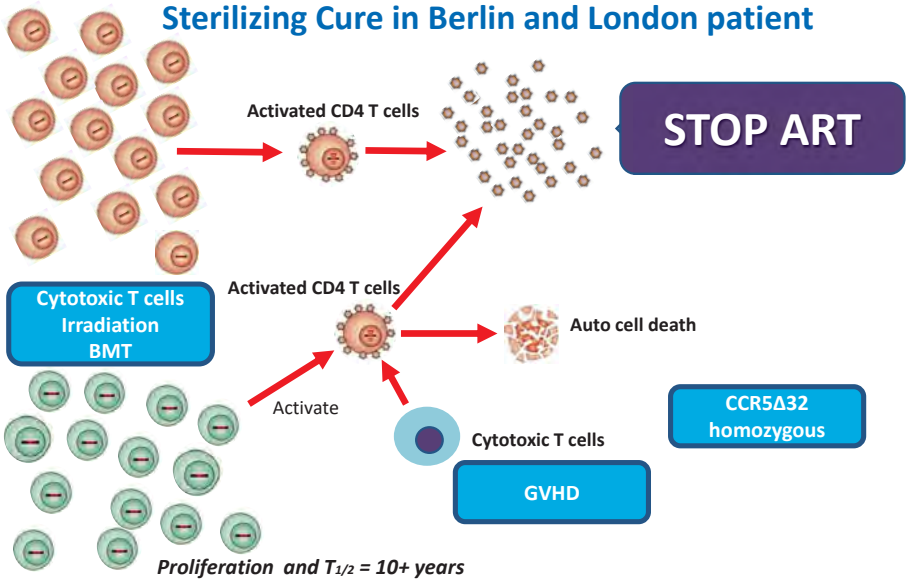
Type and principle of HIV Cure

Functional Cure
When the level of HIV particles in an infected person's body has been reduced to such an **extremely low level** that person can stop treatment and not worry about viral rebound and damaging immune system

Sterilizing cure
When every last particle of HIV has been destroyed from an infected person's body (eradication)



Sterilizing Cure in Berlin and London patient



How many have been on ART?

23 million (WHO 2018)

How many have been cured?

At least (2+1)+14

2018

37.9 million people

Long-term control of viremia and stable CD4 T cells in 14 cases after stop ART!!

Post-Treatment HIV-1 Controllers with a Long-Term Virological Remission after the Interruption of Early Initiated Antiretroviral Therapy ANRS VISCONTI Study

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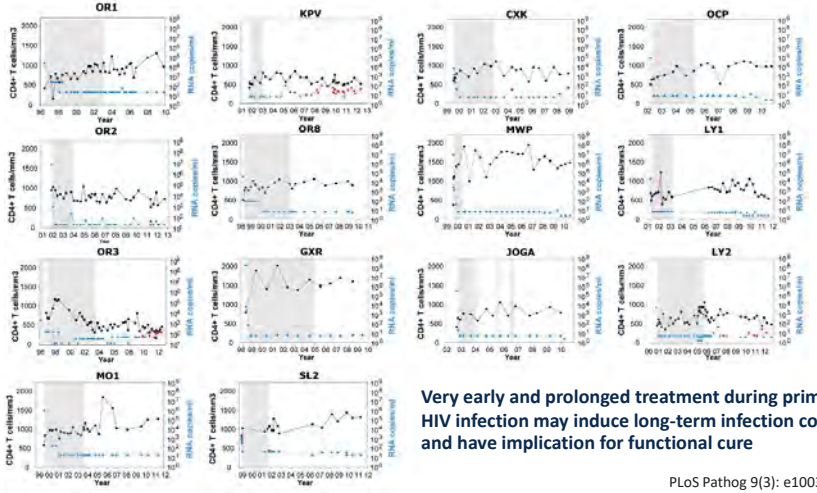
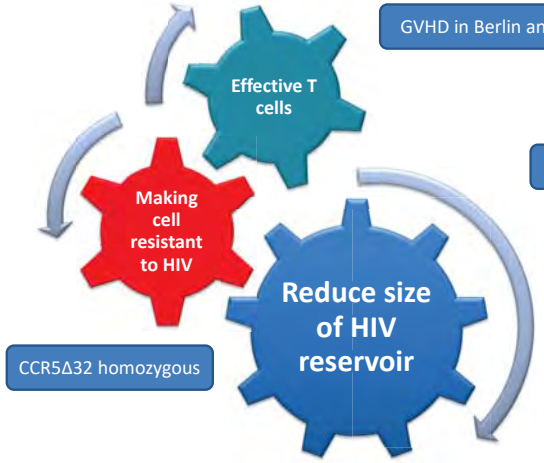


Very early and prolonged treatment during primary HIV infection may induce long-term infection control and have implication for functional cure

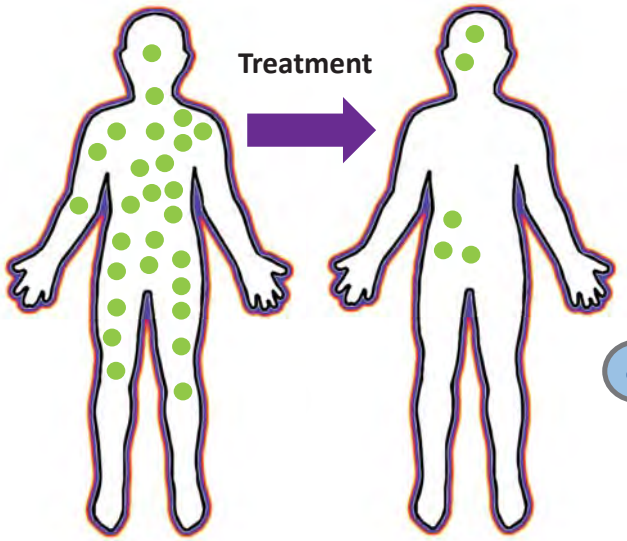
PLoS Pathog 9(3): e1003211

How to replicate this kind of treatment and make more applicable for a large-scale treatment

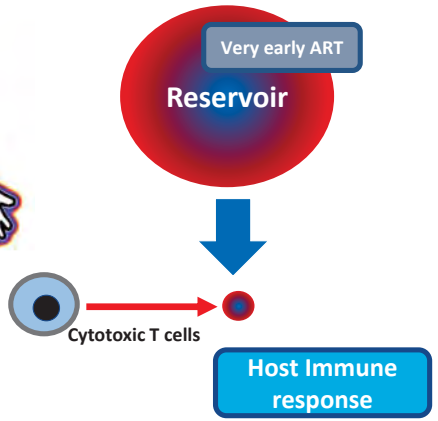


- GVHD in Berlin and London patients
- Chemotherapy
- Total body irradiation
- CCR5Δ32 homozygous

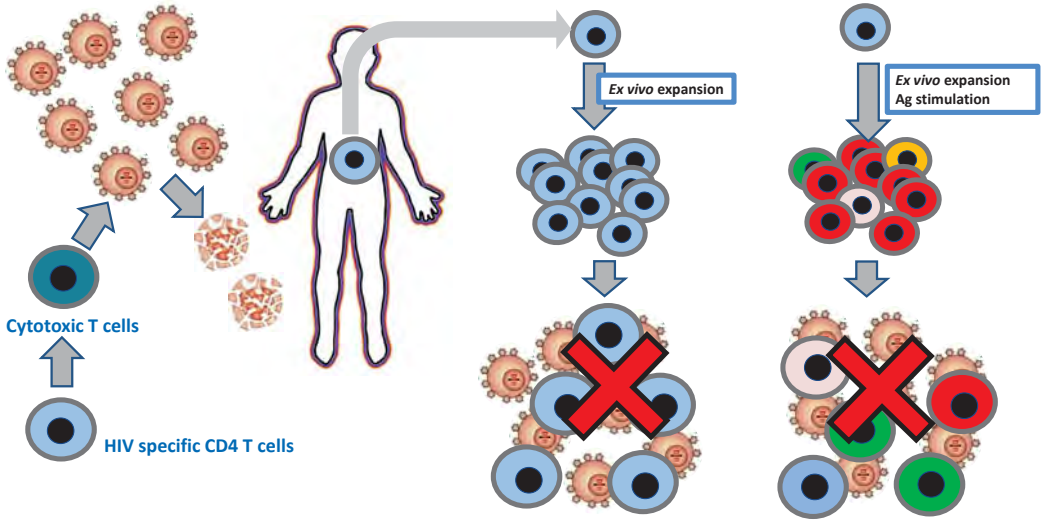
Functional cure



Size of reservoir

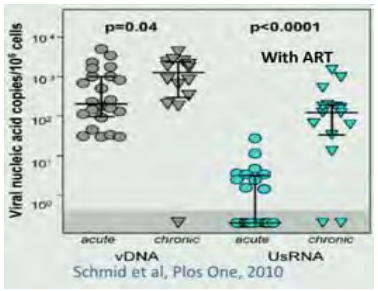


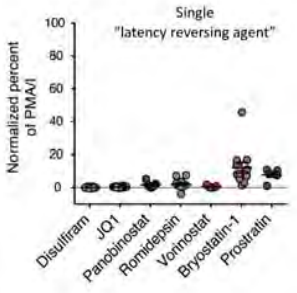
We need T cells for HIV control

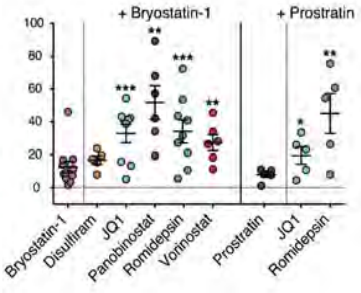


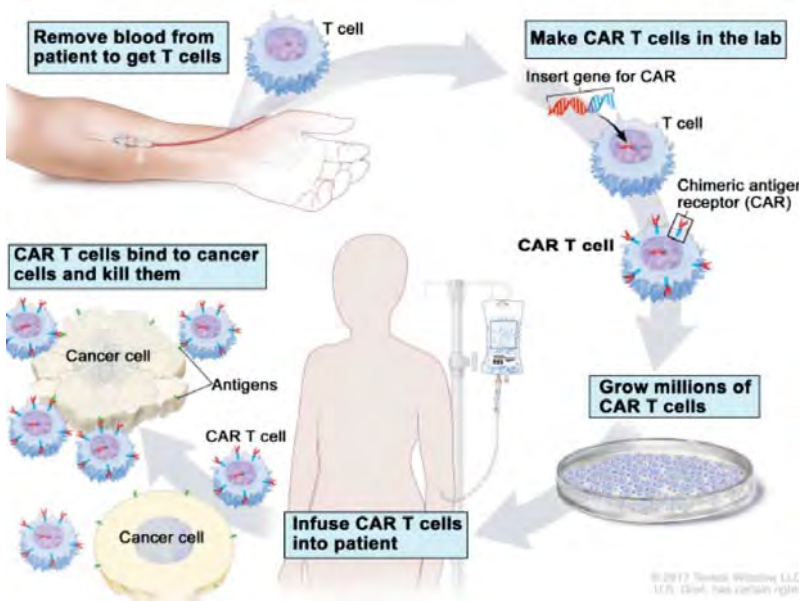
Reduce size of HIV reservoir

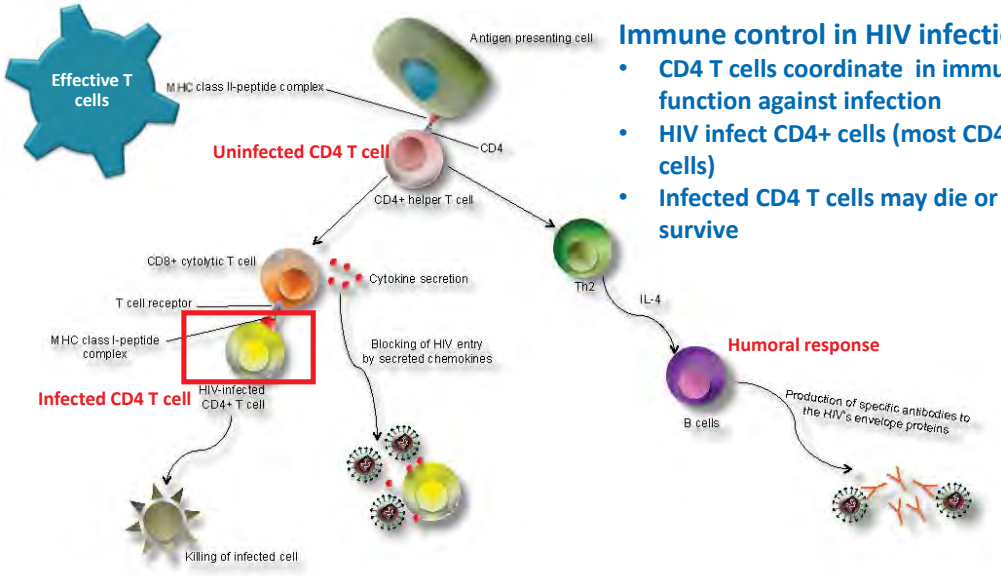
- Very early treatment during acute infection
- Shock and kill → Latency reversing agents +/- immunotherapy





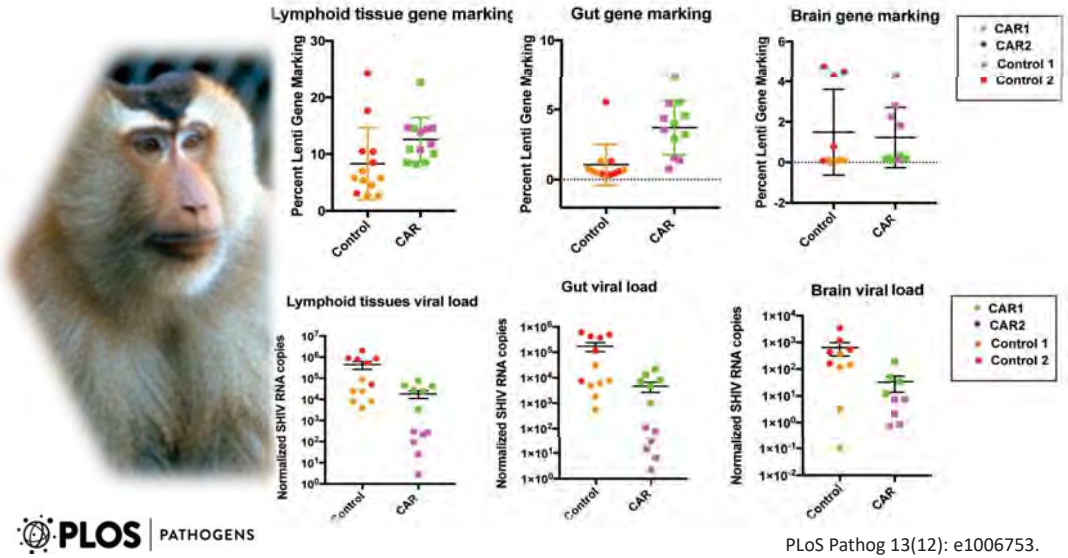






Immune control in HIV infection

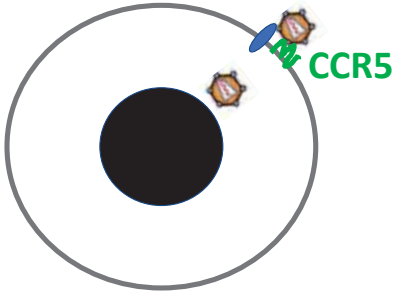
- CD4 T cells coordinate in immune function against infection
- HIV infect CD4+ cells (most CD4+T cells)
- Infected CD4 T cells may die or survive

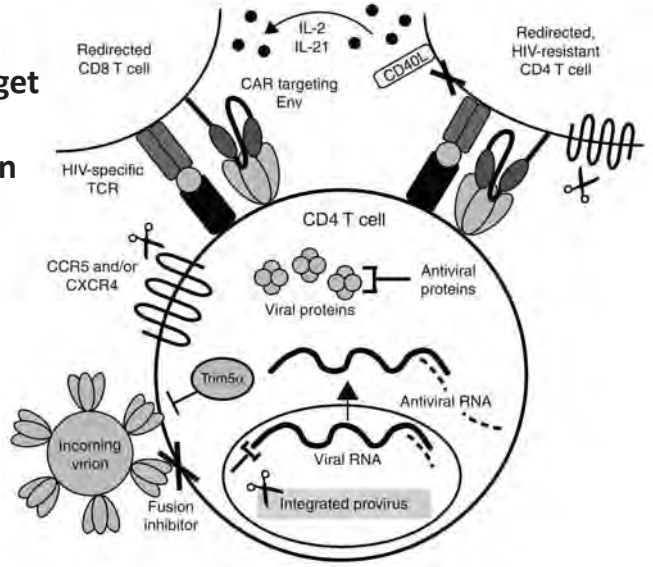


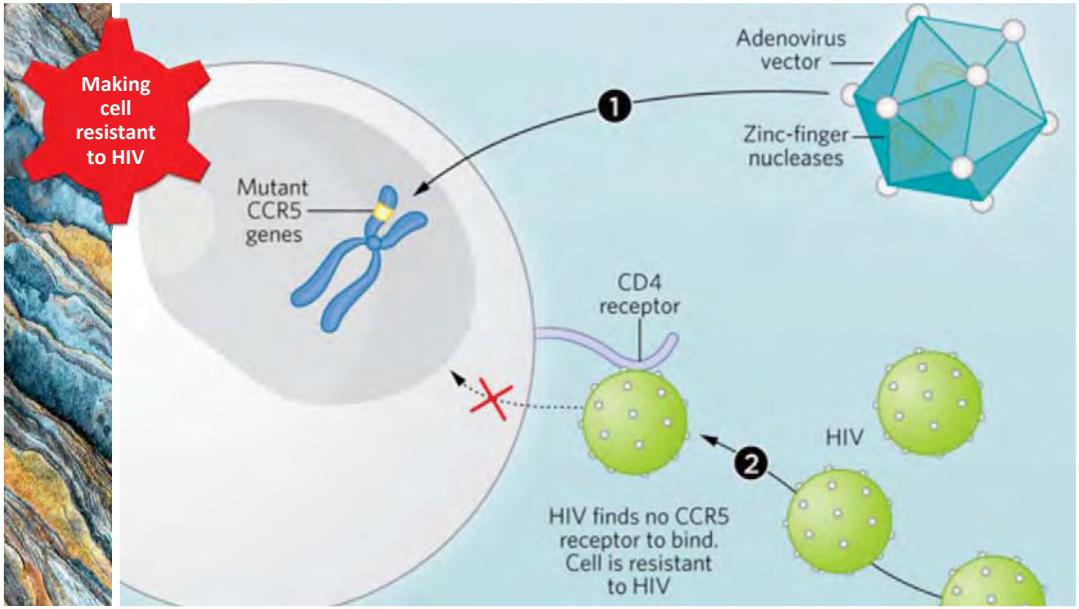
PLoS Pathog 13(12): e1006753.

Engineering T cells with

- **Enhanced activity to target HIV**
- **Resistant to HIV infection**

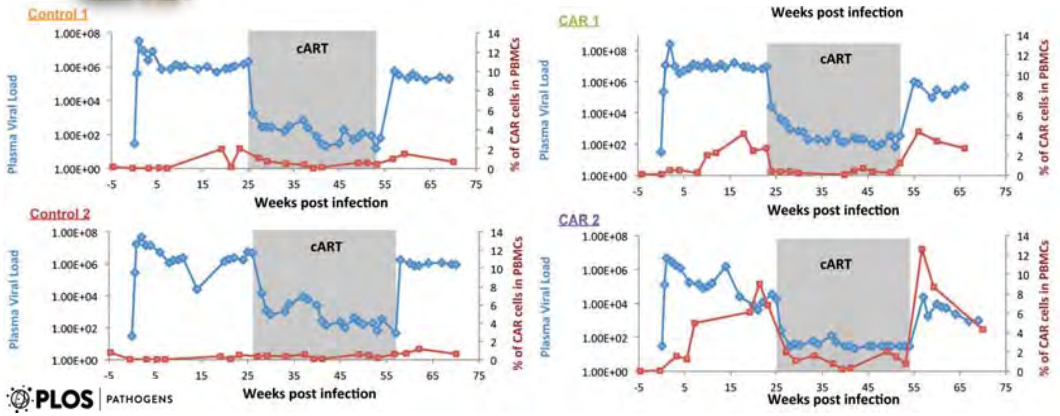






Long-term persistence and function of hematopoietic stem cell-derived chimeric antigen receptor T cells in a nonhuman primate model of HIV/AIDS

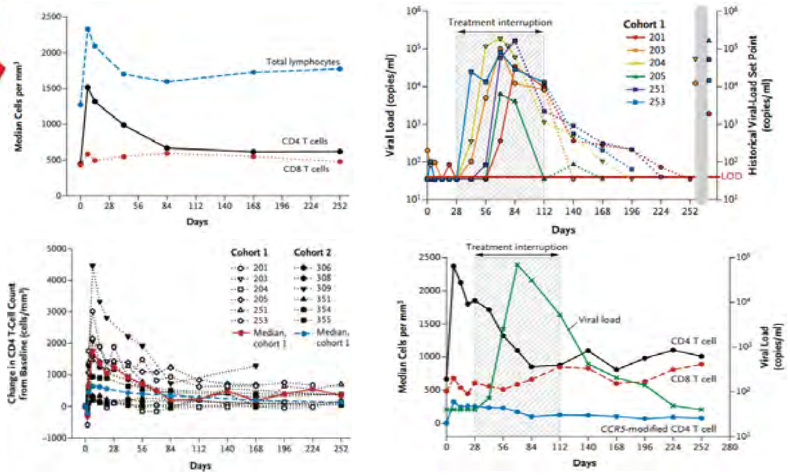
PLoS Pathog 13(12): e1006753.



Making cell resistant to HIV

Gene Editing of CCR5 in Autologous CD4 T Cells of Persons Infected with HIV

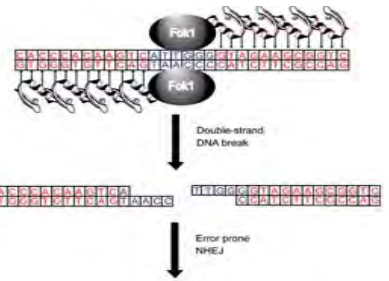
The NEW ENGLAND JOURNAL of MEDICINE



N Engl J Med 2014;370:901-10

Making cell resistant to HIV

Engineering HIV-Resistant Human CD4+ T Cells with CXCR4 or CCR5 -Specific Zinc-Finger Nucleases



ACTGGAACACAACCACCCACAAGTCATTGGGGTAGAAGCGGTCACAGATATATC	Reference
ACTGGAACACAACCACCCACAAGTCATT GGTAGAAGCGGTCACAGATATATC	2bp deletion (1.4%)
ACTGGAACACAACCACCCACAA GTAGAAGCGGTCACAGATATATC	9bp deletion (4.5%)
ACTGGAACACAACCACCCACAA GAAGCGGTCACAGATATATC	12bp deletion (2.6%)
ACTGGAACACAACCACCCACAA GCGGTCACAGATATATC	15bp deletion (5.2%)
ACTGGAACACAACCACCCACAA GTCACAGATATATC	18bp deletion (11.2%)
ACTGGAACACAACCACCCACA GATATATC	25bp deletion (1.6%)
ACTGGAACACAACCACCCACAAGTCATTGGTTGGGGTAGAAGCGGTCACAGATATATC	4bp insertion (2.5%)

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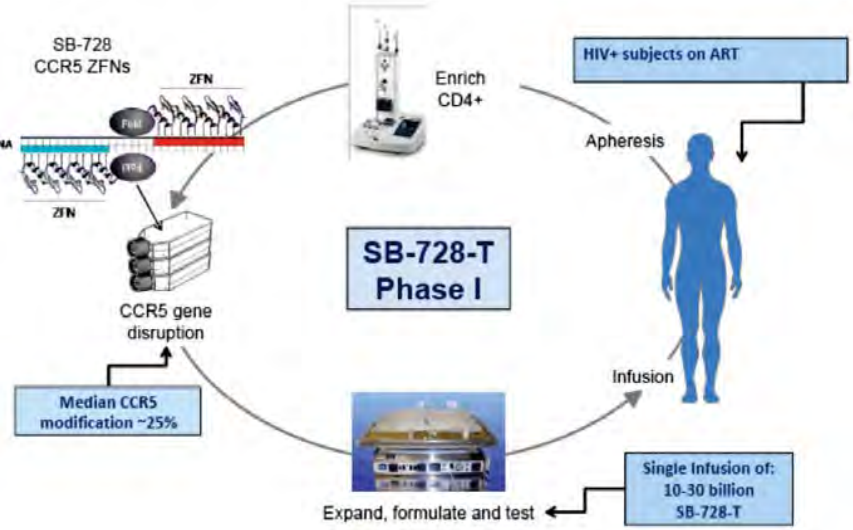
TAKE THE NEXT STEP...

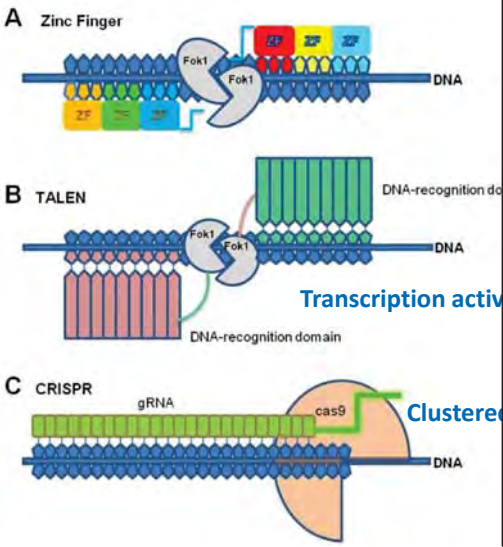
Making cell resistant to HIV



How CRISPR used in HIV Cure?

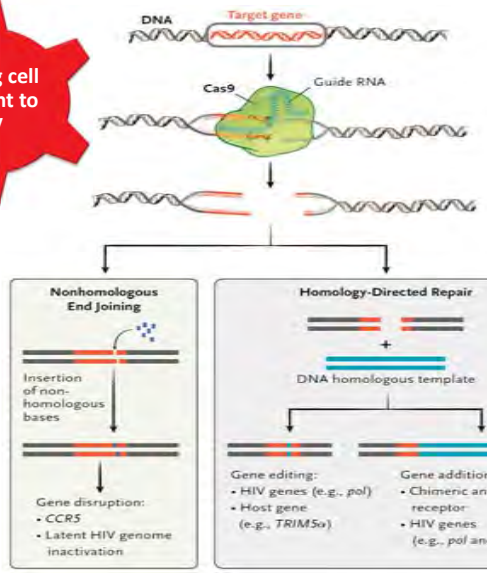
TRC EIDCC

New gene editi



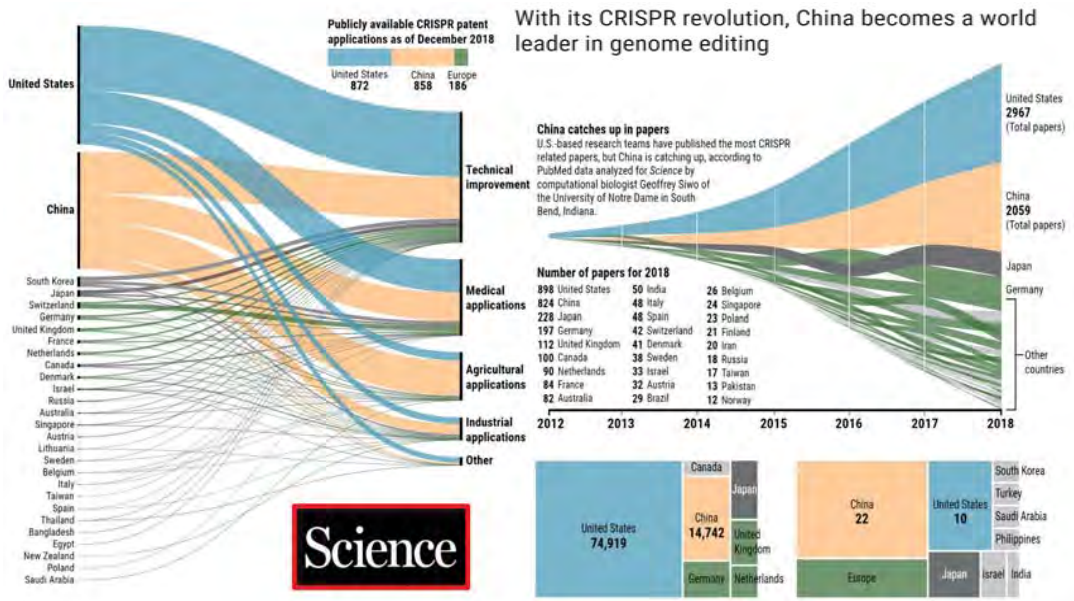
Activity	ZFN	TALEN	CRISPR
Recognition	Protein-DNA	Protein-DNA	RNA-DNA
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	RNA
Nuclease	Fok1	Fok1	Cas9
Target sequences	2 × 12 nucleotide and up	2 × 16 nucleotide and up	Nearly 20 nucleotide
Construct size	(1 kb) 2	(3 kb) 2	4.2 kb (Cas9) + 0.1 kb (RNA)
Construct	Zinc finger sequence specifically recognizing 3 bp sequence linked to Fok1	Protein sequence specific to binding a nucleotide sequence linked to Fok1	A 20nt crRNA fused to tracrRNA and Cas9 endonuclease
Construct	Zinc finger sequence specifically recognizing 3 bp sequence linked to Fok1	Protein sequence specific to binding a nucleotide sequence linked to Fok1	A 20nt crRNA fused to tracrRNA and Cas9 endonuclease
Cost and time involved in assembly	Very expensive and time consuming	Relative expensive and time consuming	Low cost and minimum time
Multiplexing	No	No	Capable
Success Rate	Low	High	High

Making cell resistant to HIV



Outcomes

- Gene disruption
- Gene editing
- Gene addition
- Off target

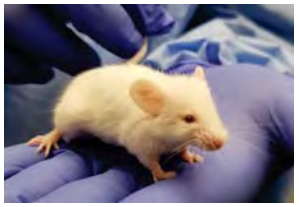


ARTICLE

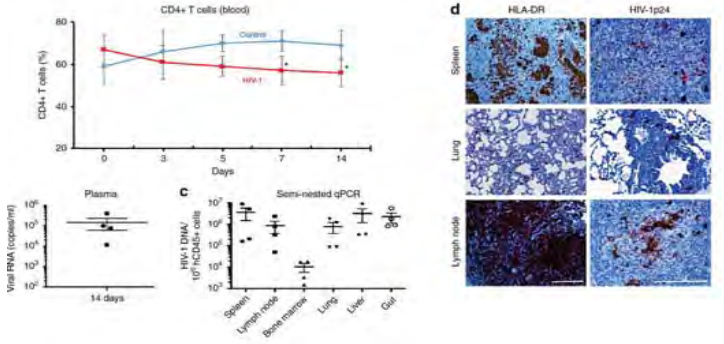
OPEN

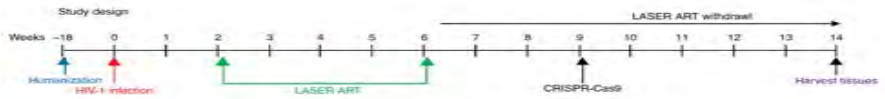
Sequential LASER ART and CRISPR Treatments Eliminate HIV-1 in a Subset of Infected Humanized Mice

nature COMMUNICATIONS | NATURE COMMUNICATIONS | (2019)10:2753

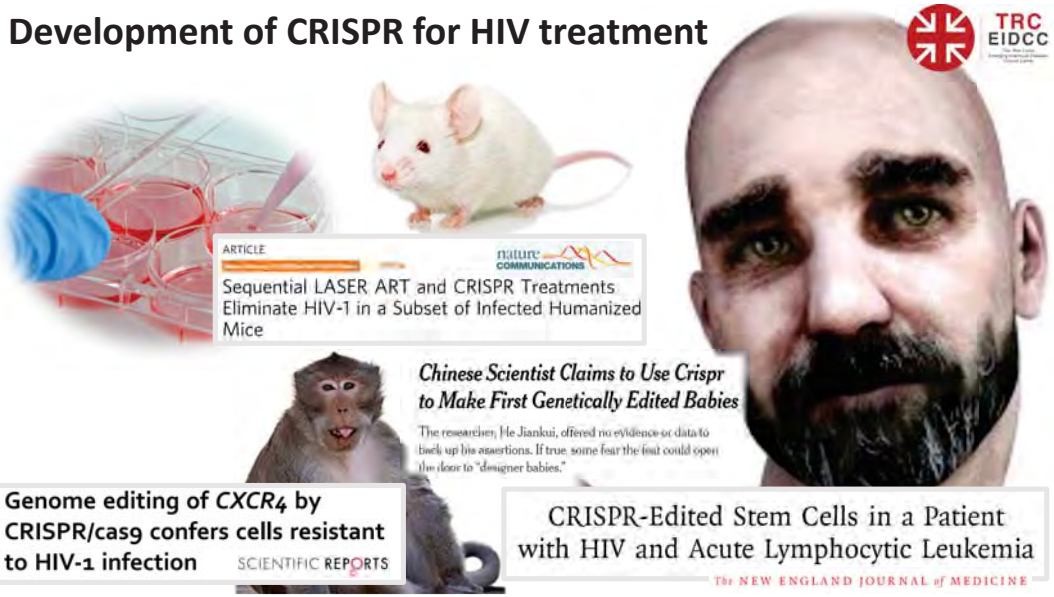


Mice with human T cells, that are broadly susceptible to HIV-1 infection





Development of CRISPR for HIV treatment



ARTICLE

nature COMMUNICATIONS

Sequential LASER ART and CRISPR Treatments Eliminate HIV-1 in a Subset of Infected Humanized Mice

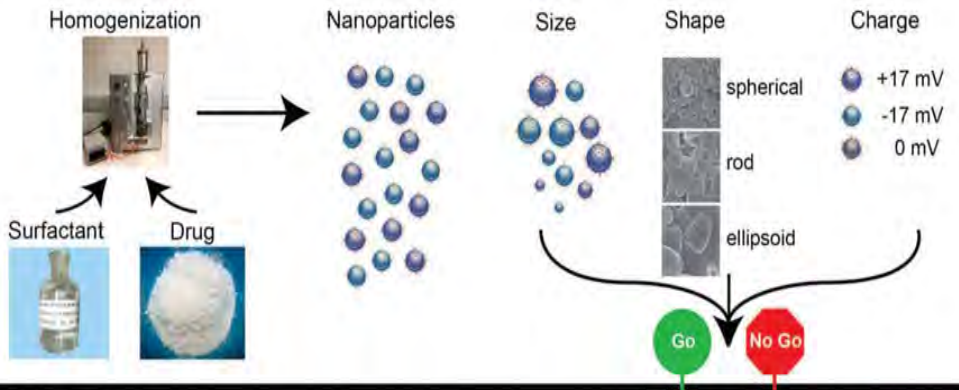
Chinese Scientist Claims to Use Crispr to Make First Genetically Edited Babies

The researcher, He Jiankui, offered no evidence or data to back up his assertions. If true, some fear the feat could open the door to "designer babies."

Genome editing of *CXCR4* by CRISPR/cas9 confers cells resistant to HIV-1 infection SCIENTIFIC REPORTS

CRISPR-Edited Stem Cells in a Patient with HIV and Acute Lymphocytic Leukemia

The NEW ENGLAND JOURNAL of MEDICINE



Cellular Testing

Drug	Formulation	Cell Uptake		Cell Retention		Cell Release		Antiretroviral		Toxicity		Final Score	
		AUC	Score	AUC	Score	AUC	Score	AUC	Score	Y/N	Score	Total	Score
DTG	H433	185	10.0	500	10.0	23	7.5	15000	10.0	N	10.0	47.5	9.5
	H333	150	8.6	350	8.4	16	5.0	20000	9.2	N	10.0	41.2	8.2
	M412	70	4.5	88	2.0	29	8.1	43000	3.6	Y	5.0	23.2	4.6
	H134	36	2.5	30	1.0	35	10.0	66500	2.5	N	10.0	26.0	5.2

Elimination of HIV-1 Genomes from Human T-lymphoid Cells by CRISPR/Cas9 Gene Editing

Rafal Kaminski[1,2], Yilan Chen[1,2], Tracy Fischer[1,2], Ellen Tedaldi[2,3], Alessandro Napoli[1,2], Yonggang Zhang[1,2], Jonathan Karn[4], Wenhui Hu[1,2] & Kamel Khalili[1,2]

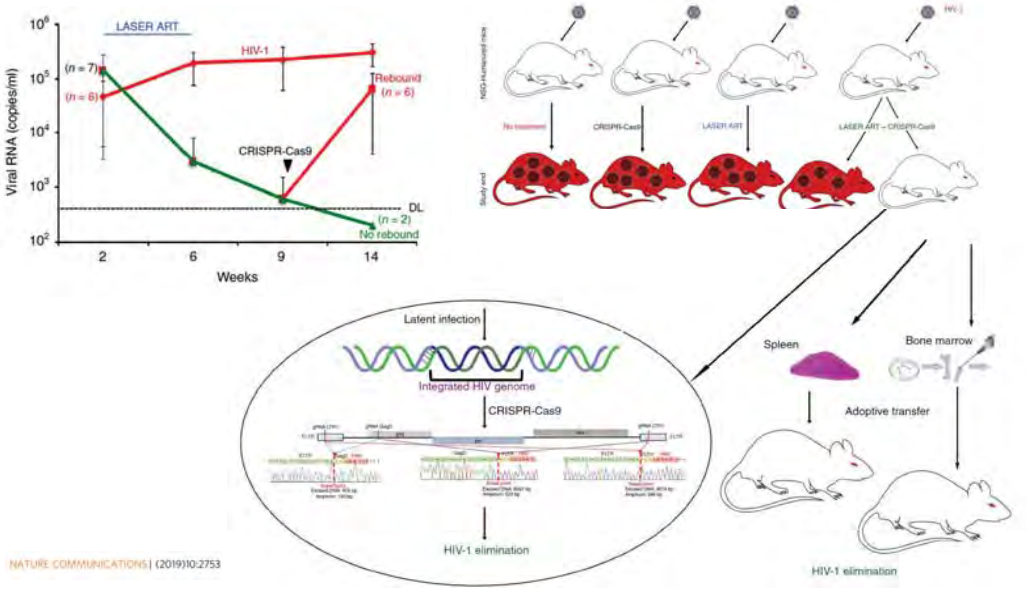
ARTICLE

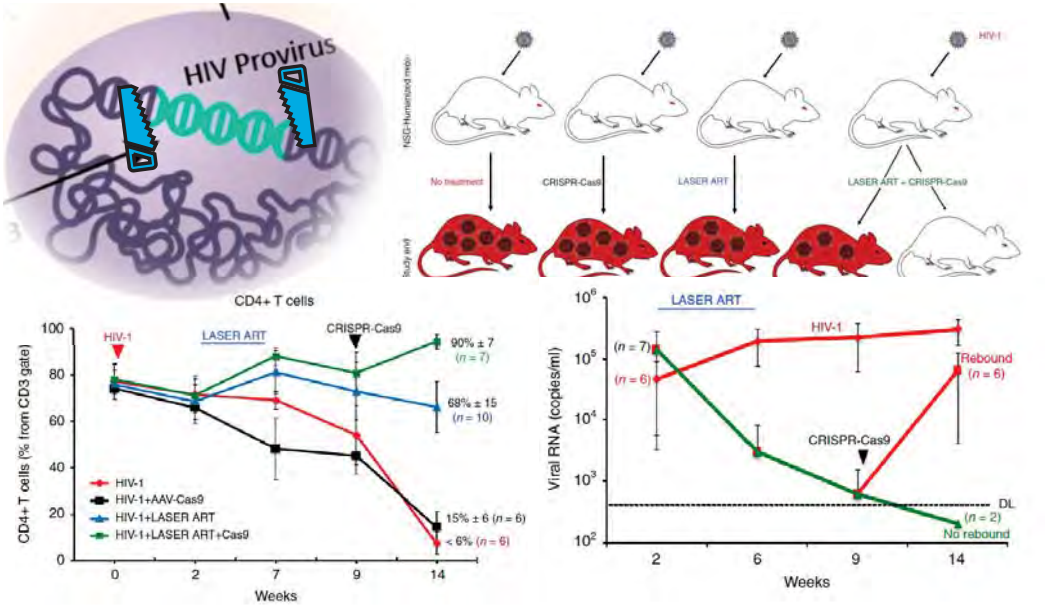
OPEN

Sequential LASER ART and CRISPR Treatments Eliminate HIV-1 in a Subset of Infected Humanized Mice

SCIENTIFIC REPORTS | 6:22555 | DOI: 10.1038/srep22555

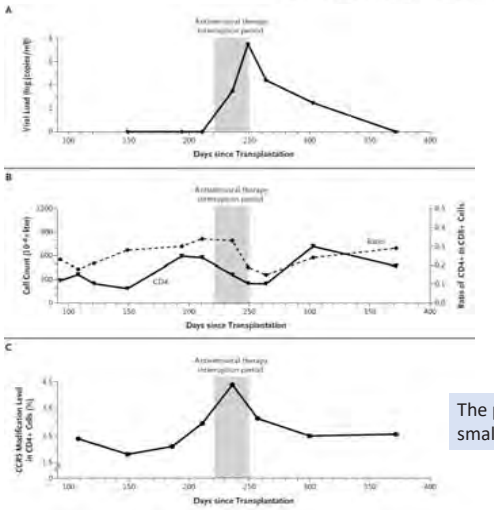

Gene-Edited Babies: What a Chinese Scientist Told an American Mentor
Chinese Scientist Claims to Use Crispr to Make First Genetically Edited Babies
The researcher, He Jiankui, offered no evidence or data to back up his assertions. If true, some fear the feat could open the door to "designer babies."
World's First Gene-Edited Babies Born This Month, Researcher Says


LASER ART
HIV-1
Rebound (n = 6)
CRISPR-Cas9
DL
(n = 2)
No rebound
Viral RNA (copies/ml)
Weeks
Latent infection
Integrated HIV genome
CRISPR-Cas9
HIV-1 elimination
Spleen
Bone marrow
Adoptive transfer
NATURE COMMUNICATIONS | (2019)10:2753

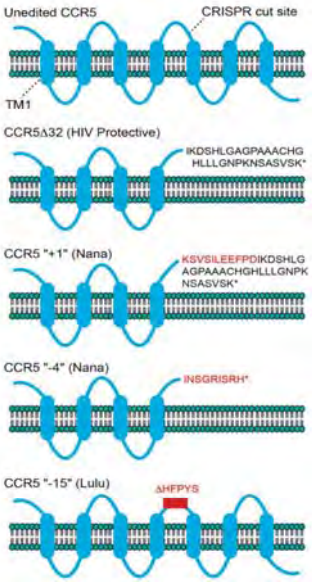

HIV Provirus
CD4+ T cells
LASER ART
CRISPR-Cas9
HIV-1
HIV-1+AAV-Cas9
HIV-1+LASER ART
HIV-1+LASER ART+Cas9
90% ± 7 (n = 7)
68% ± 15 (n = 10)
15% ± 6 (n = 6)
< 6% (n = 6)
CD4+ T cells (% from CD3 gate)
Weeks
Viral RNA (copies/ml)
Rebound (n = 6)
DL
(n = 2)
No rebound

CRISPR-Edited Stem Cells in a Patient with HIV and Acute Lymphocytic Leukemia

N Engl J Med 2019;381:1240-7.



The percentage of CD4+ cells with CCR5 ablation increased by a small degree during a period of antiretroviral-therapy interruption



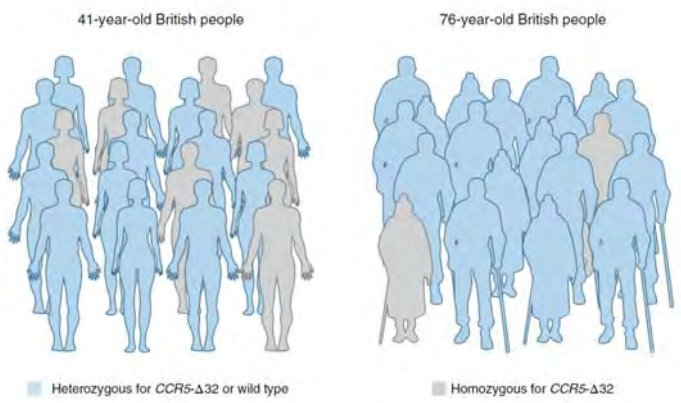
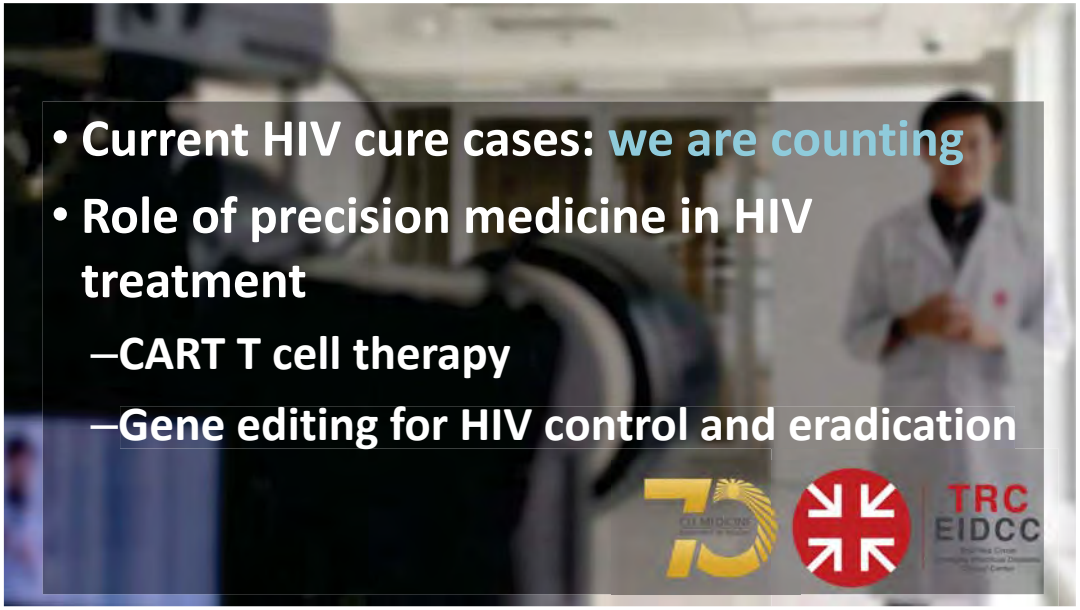


Fig. 1 | **There is an increased mortality rate among people homozygous for *CCR5*-Δ32.** In the UK Biobank, 409,693 people of British ancestry between the ages of 41 and 76 were assessed for the effect of *CCR5*-Δ32 on mortality rate. No difference was seen between people heterozygous for *CCR5*-Δ32 and wild type (both of these groups are shown as blue). In contrast, people homozygous for *CCR5*-Δ32 (gray) had a mortality rate that was increased by 20%. NATURE MEDICINE | VOL 25 | JUNE 2019

The CRISPR Journal
Volume 1, Number 6, 2018



CRISPR-Edited Stem Cells in a Patient with HIV and Acute Lymphocytic Leukemia

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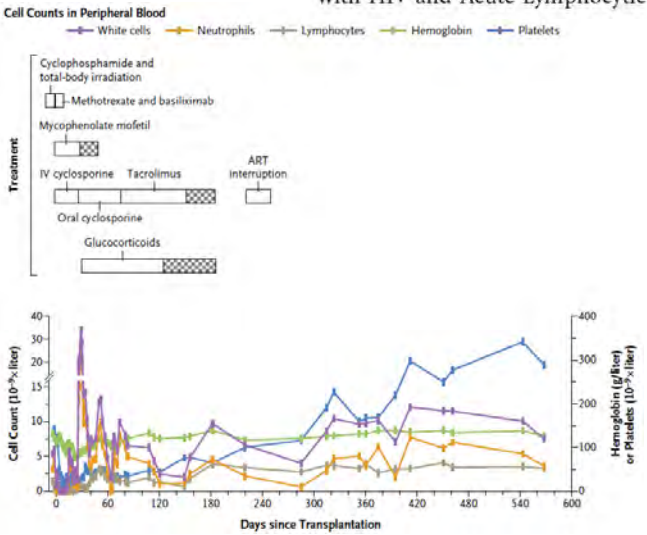

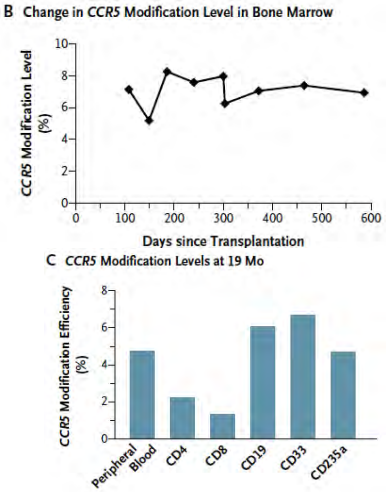

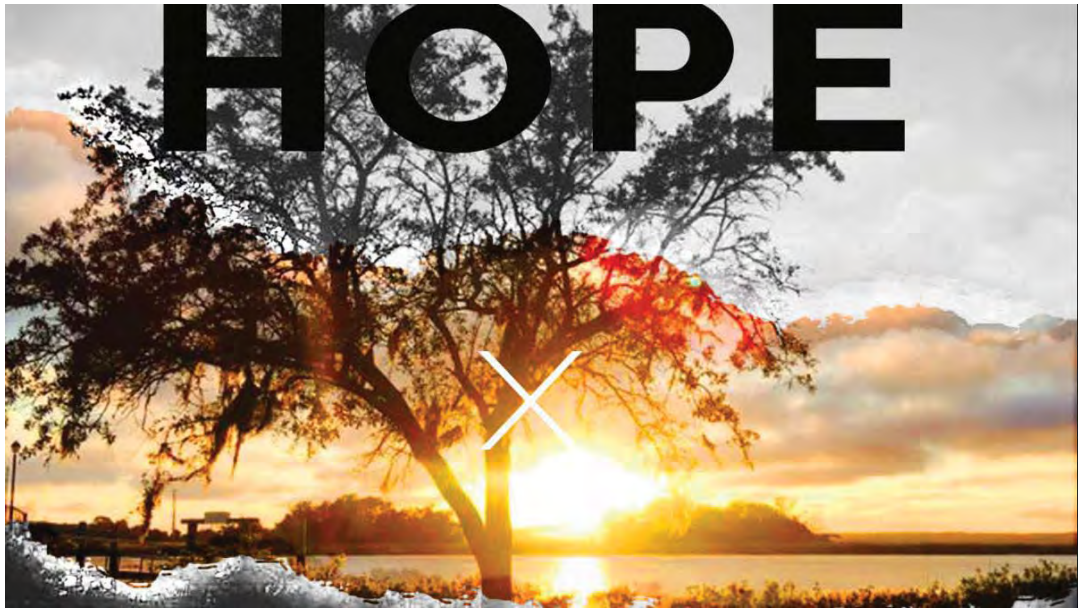
HOPE