Johns Hopkins Engineering

Immunoengineering

Immunoengineering—Pathogens

Cell Engineering Design

JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

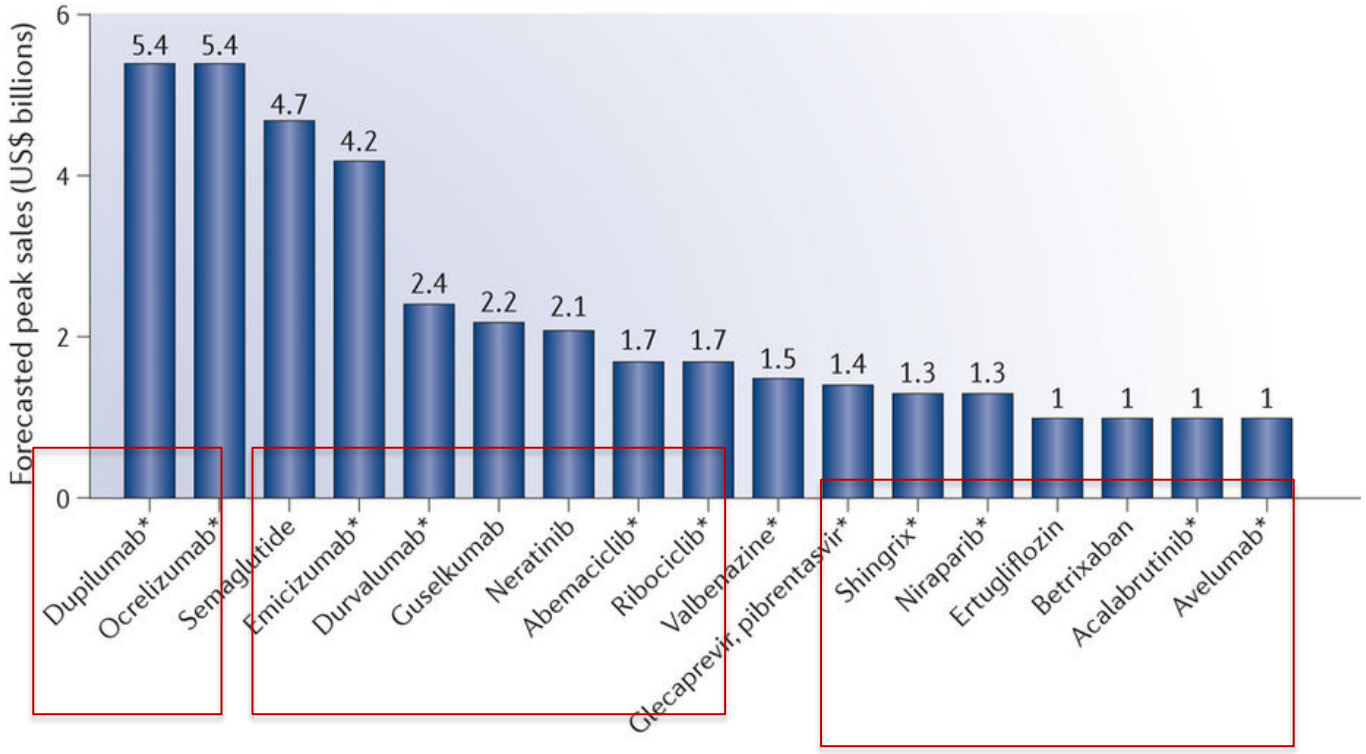
Outline: How to Develop New Biologic Therapies

- **General Design Considerations**
- **Engineered Cells**
- **Engineered Microbes & Viruses**
- Engineered Proteins – Cytokines & Antibodies
- Engineered Genetic Material

General Design Considerations

- Pharmacodynamics and Pharmacokinetics
- Route of administration
- Stability/solubility
- Biodistribution
- Metabolism/elimination
- Off-target effects
- Manufacturability
- Patent/Market
- Safety
- Cost

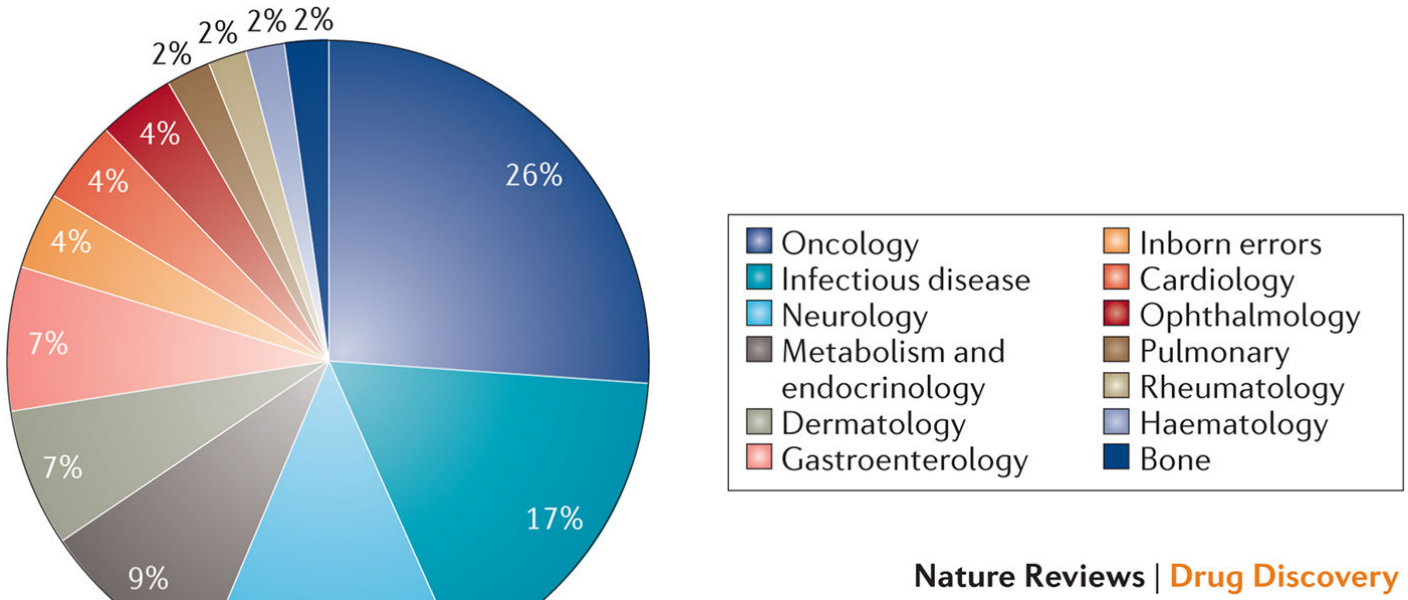
2017 FDA Drug Approvals



Nature Reviews | Drug Discovery

Mullard, Asher. "2017 FDA drug approvals." (2018): 81.

2017 FDA Approvals



Mullard, Asher. "2017 FDA drug approvals." (2018): 81.

What Does Biologic Therapy Include?

Table 2: Selected Center for Biologics Evaluation and Research approvals in 2017

From: 2017 FDA drug approvals

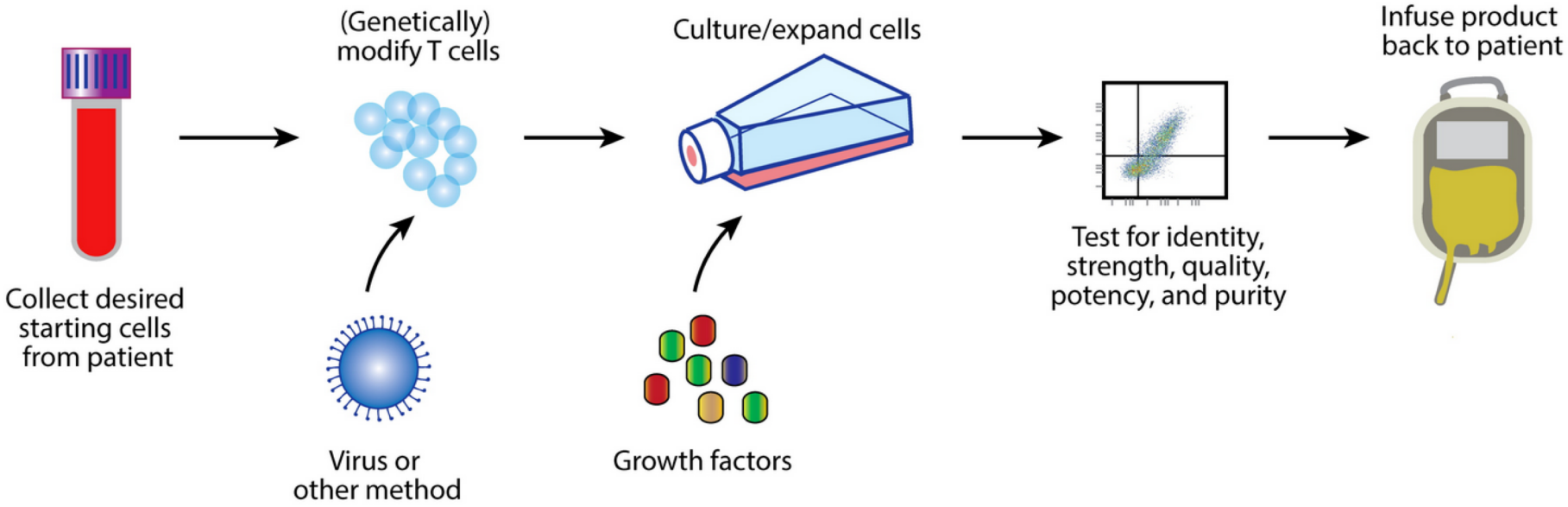
Biologic name	Sponsor	Properties	Indication
Nonacog beta pegol (Rebinyn)	Novo Nordisk	GlycoPEGylated coagulation factor IX	Haemophilia B
Tisagenlecleucel (Kymriah)	Novartis	CD19-directed CAR T therapy	B cell precursor ALL
Axicabtagene ciloleucel (Yescarta)	Kite Pharma/Gilead Sciences	CD19-directed CAR T therapy	Large B cell lymphoma
Zoster vaccine (Shingrix)	GlaxoSmithKline	Recombinant, adjuvanted herpes zoster vaccine	Prevention of herpes zoster (shingles)
Hepatitis B vaccine (Heplisav-B)	Dynavax Technologies	Adjuvanted hepatitis B vaccine	Hepatitis B prophylaxis
Voretigene neparvovec (Luxturna)	Spark Therapeutics	*RPE65* gene therapy	*RPE65*-associated inherited retinal dystrophy

Data from the FDA. ALL, acute lymphoblastic leukaemia; CAR T, chimeric antigen receptor T cell.

Mullard, Asher. "2017 FDA drug approvals." (2018): 81.

Cell Engineering - Introduction

Overview of T cell engineering

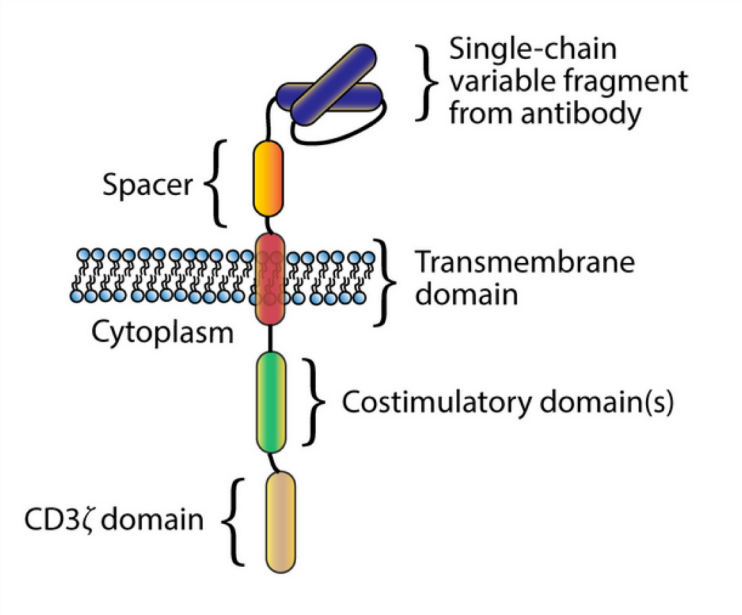


Esensten, Jonathan H., Jeffrey A. Bluestone, and Wendell A. Lim. "Engineering therapeutic T cells: from synthetic biology to clinical trials." *Annual Review of Pathology: Mechanisms of Disease* 12 (2017): 305-330

Cell Engineering – CAR T cells

- Replace TCR with antibody
- Include co-stimulatory domains in intracellular signaling for all-in-one signaling
- Redirect T cell response

Chimeric antigen receptor



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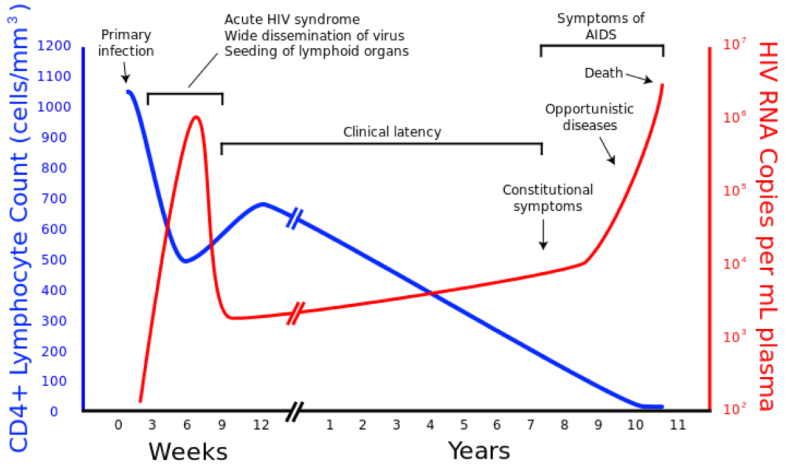
Cell Engineering – HIV Example

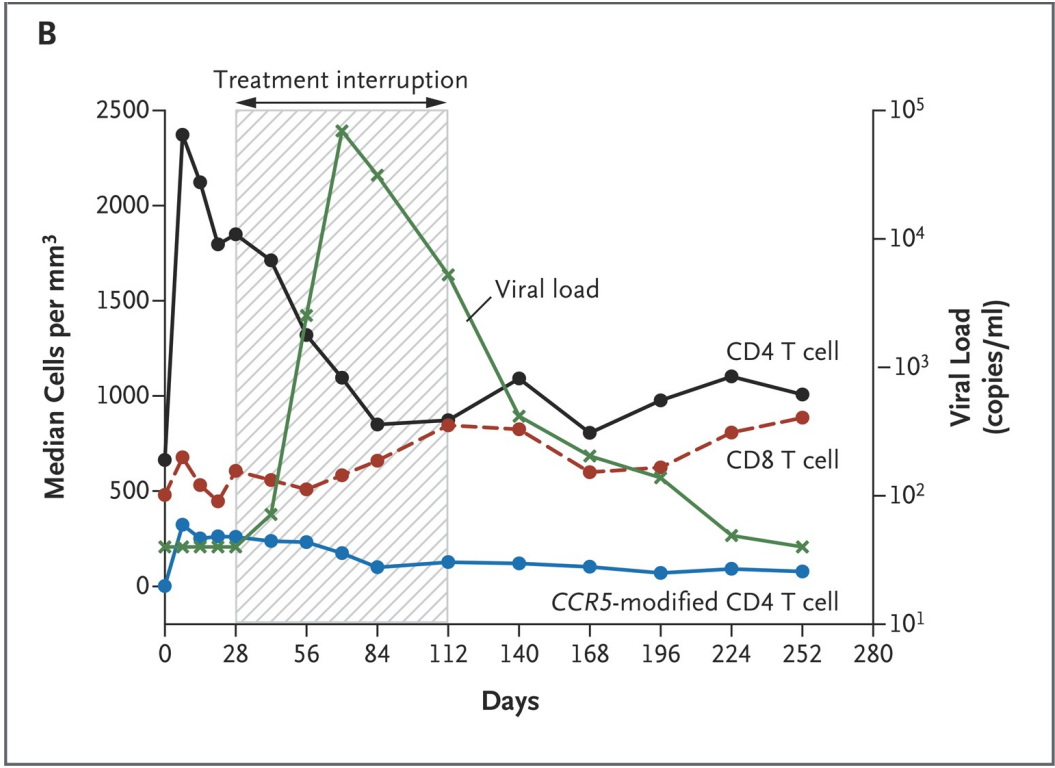
- HIV targets CD4+ T cells
- Causes Immunocompromised patients
- Opportunistic infections



https://en.wikipedia.org/wiki/HIV#/media/File:Hiv-timecourse_copy.svg

Cell Engineering – HIV Example

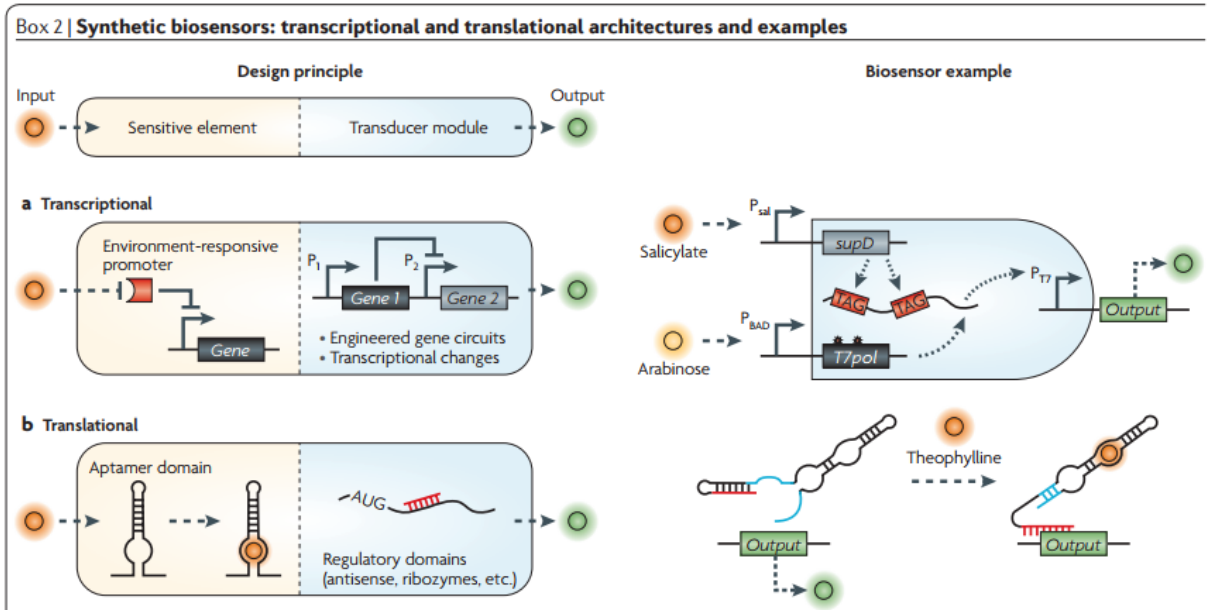
- CCR5 is used by HIV to enter CD4+ T cells
- Genetically delete CCR5 from patient CD4+ T cells
- Extended survival of modified CD4+ T cells



Tebas, Pablo, et al. "Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV." *New England Journal of Medicine* 370.10 (2014): 901-910.

Microbe Engineering - Introduction

- Detection devices
- Disease mechanism
- Drug discovery
- Drug production
- Therapy

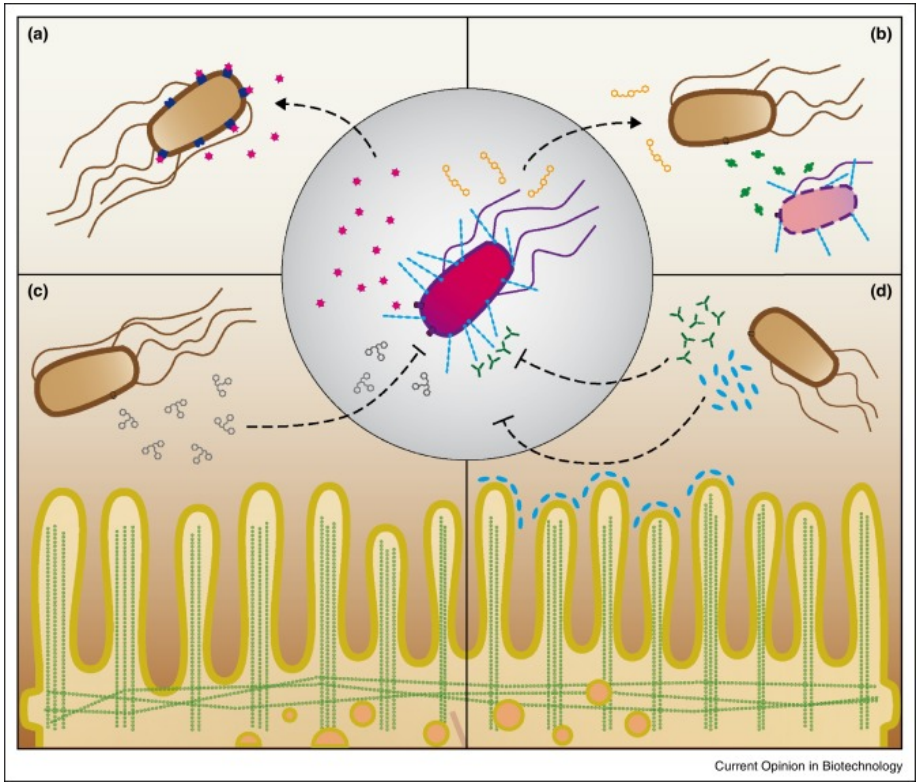


Khalil, Ahmad S., and James J. Collins. "Synthetic biology: applications come of age." *Nature Reviews Genetics* 11.5 (2010): 367.

Microbe Engineering - Introduction

(A) Toxin neutralization using modified surface components.

(C) Triggering repression of virulence genes by the release of alternative quorum sensing signals (gray molecules)

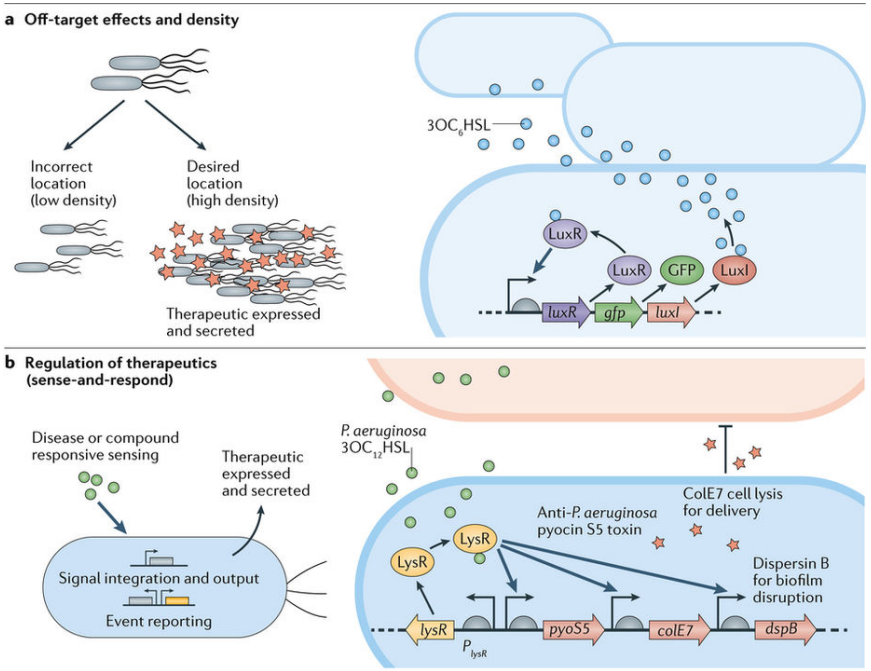


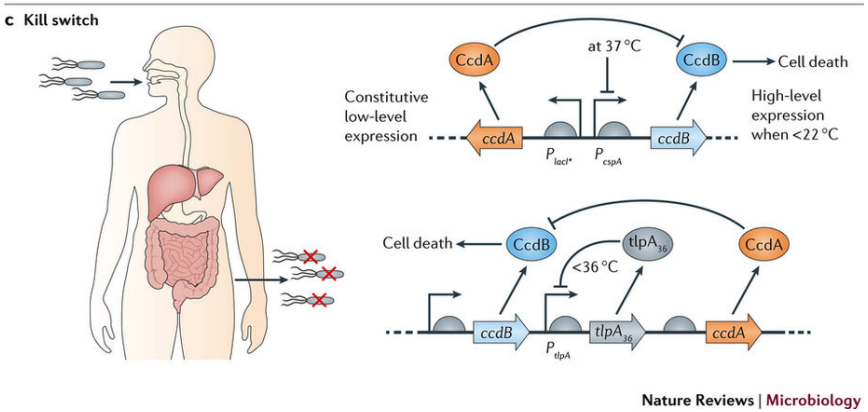
(B) Production of antimicrobial factors (green molecules) upon sensing signals from the pathogen mediating killing

(D) Bacteria can be engineered to secrete antibodies and adhesin subunits that competitively inhibit pathogen adhesion.

Piñero-Lambea, Carlos, David Ruano-Gallego, and Luis Ángel Fernández. "Engineered bacteria as therapeutic agents." *Current opinion in biotechnology* 35 (2015): 94-102.

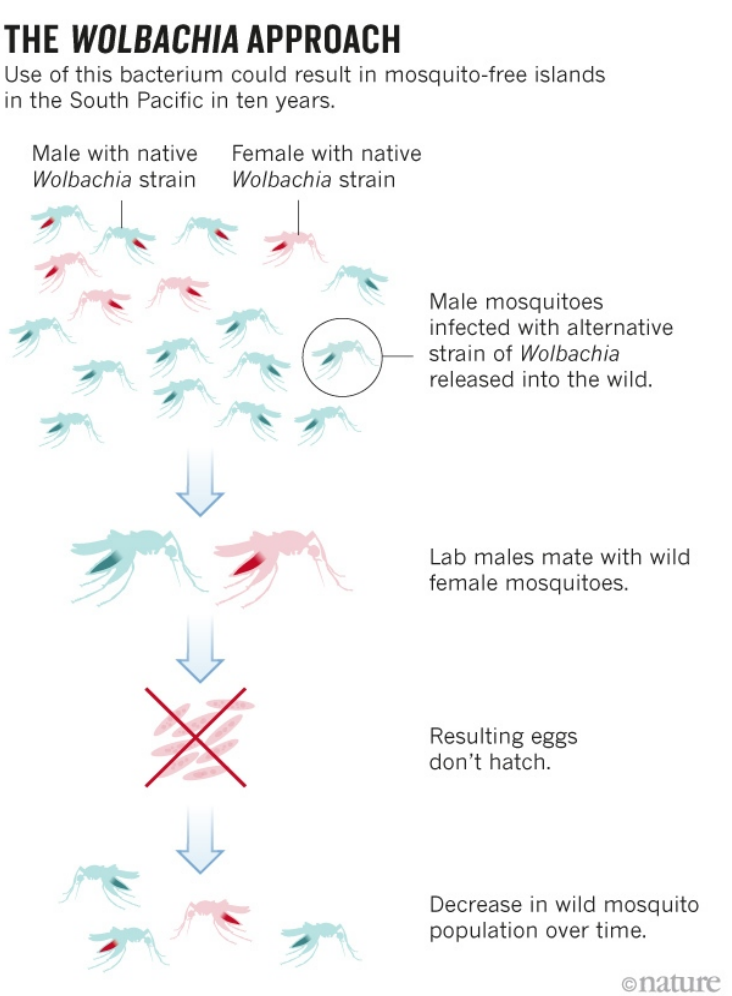
Microbe Engineering - Introduction





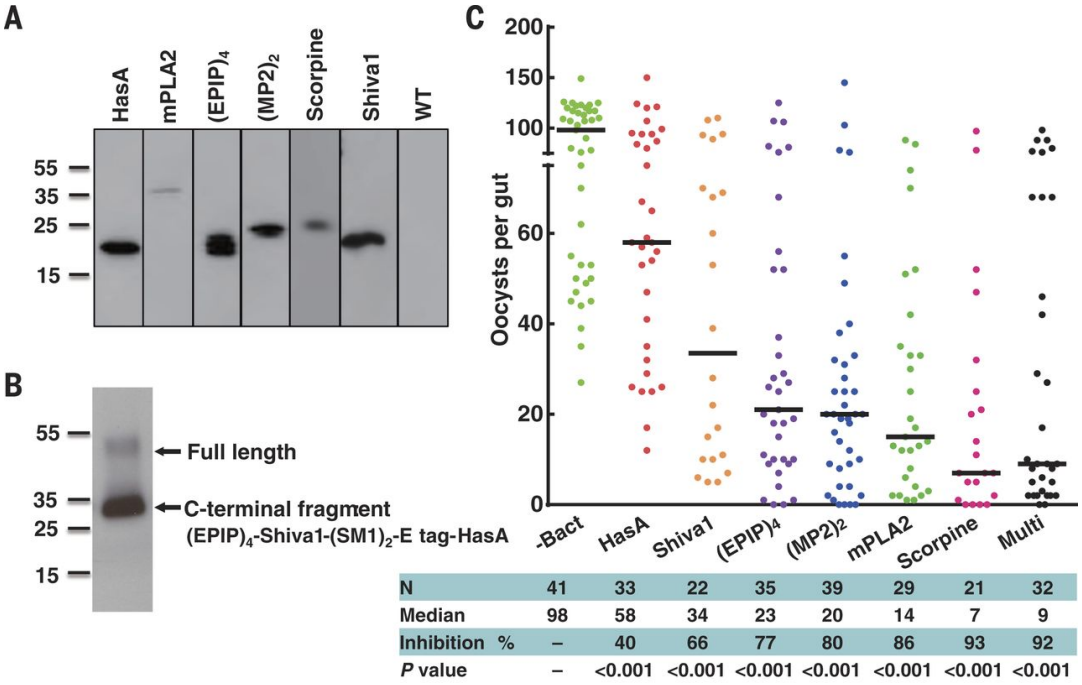
Riglar, David T., and Pamela A. Silver. "Engineering bacteria for diagnostic and therapeutic applications." *Nature Reviews Microbiology* (2018).

Example - Microbial Engineered Therapies for Malaria

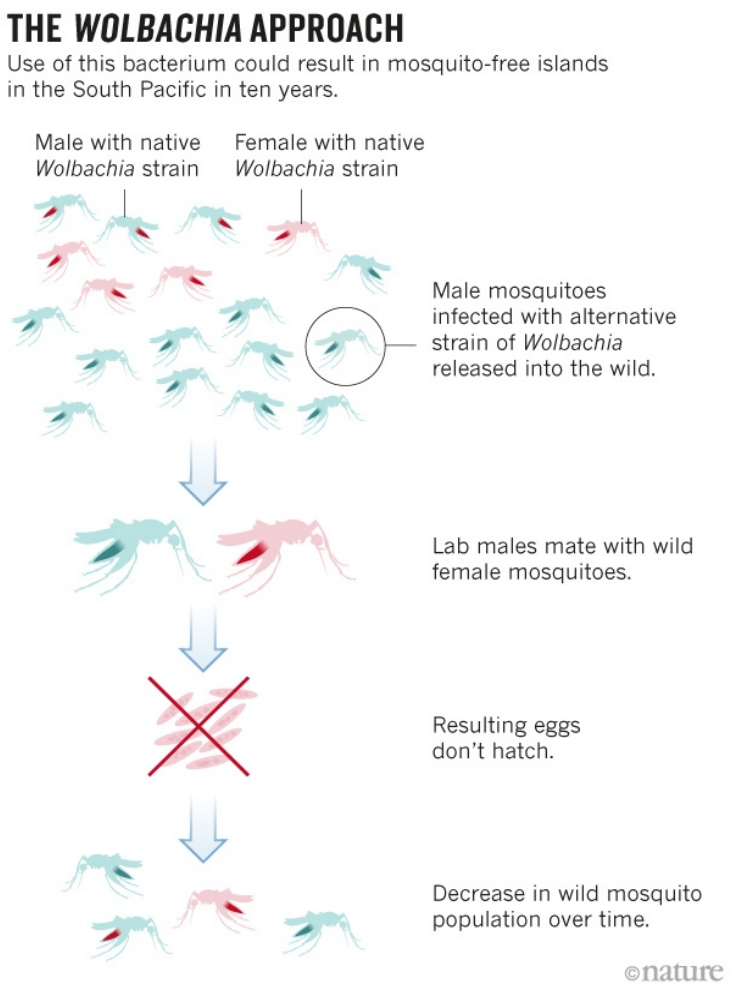


Marris, Emma. "Bacteria could be key to freeing South Pacific of mosquitoes." *Nature* 548.7665 (2017): 17-18.
Wang, Sibao, et al. "Driving mosquito refractoriness to Plasmodium falciparum with engineered symbiotic bacteria." *Science* 357.6358 (2017): 1399-1402..

Example - Microbial Engineered Therapies for Malaria



N	41	33	22	35	39	29	21	32
Median	98	58	34	23	20	14	7	9
Inhibition %	–	40	66	77	80	86	93	92
P value	–	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001



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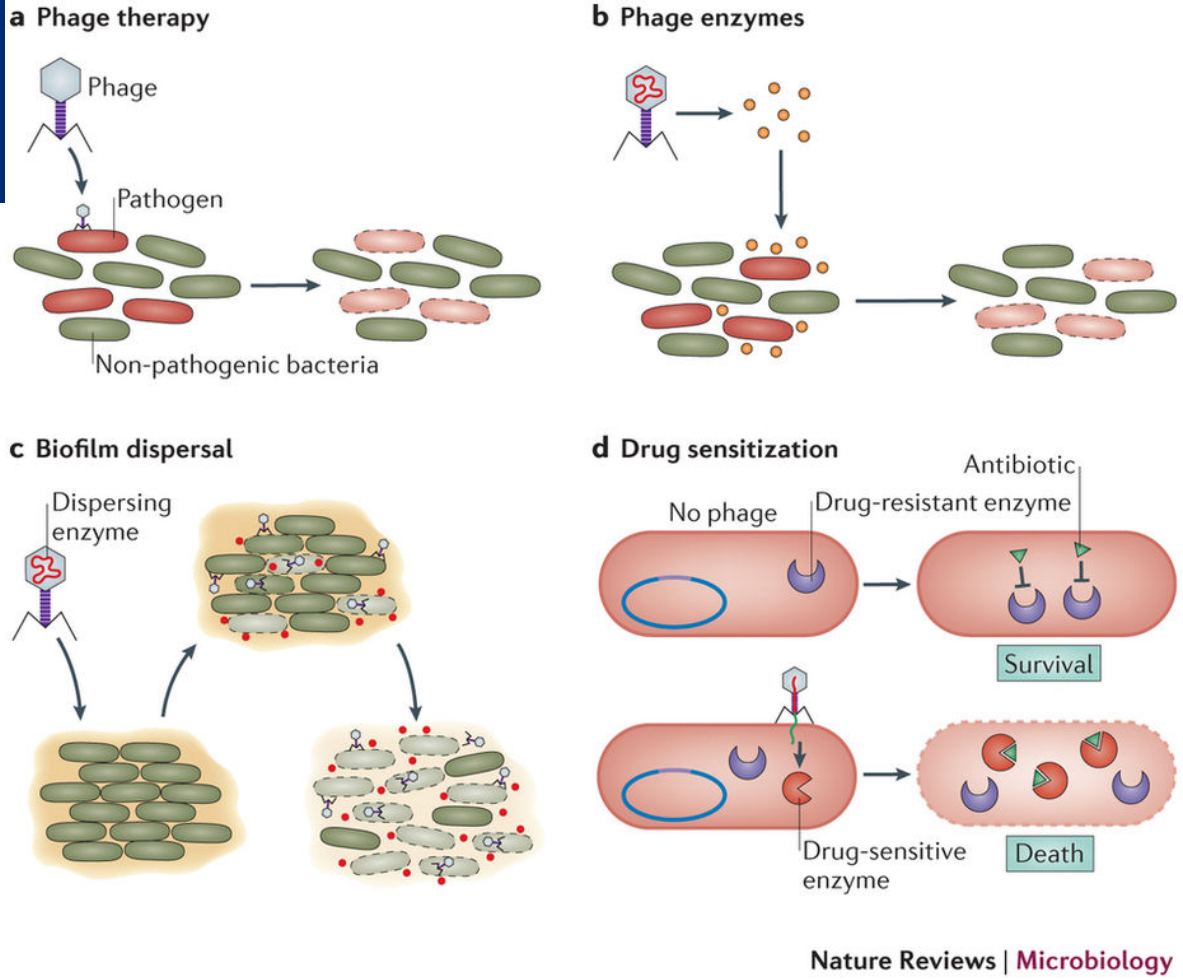
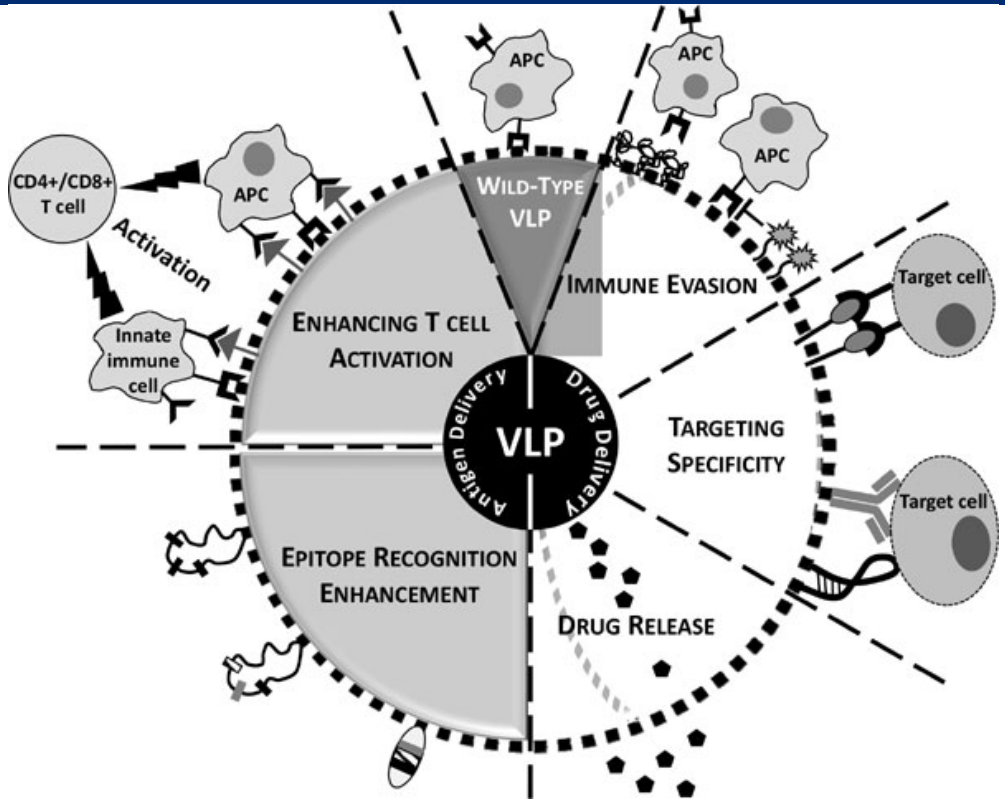
Viral Engineering



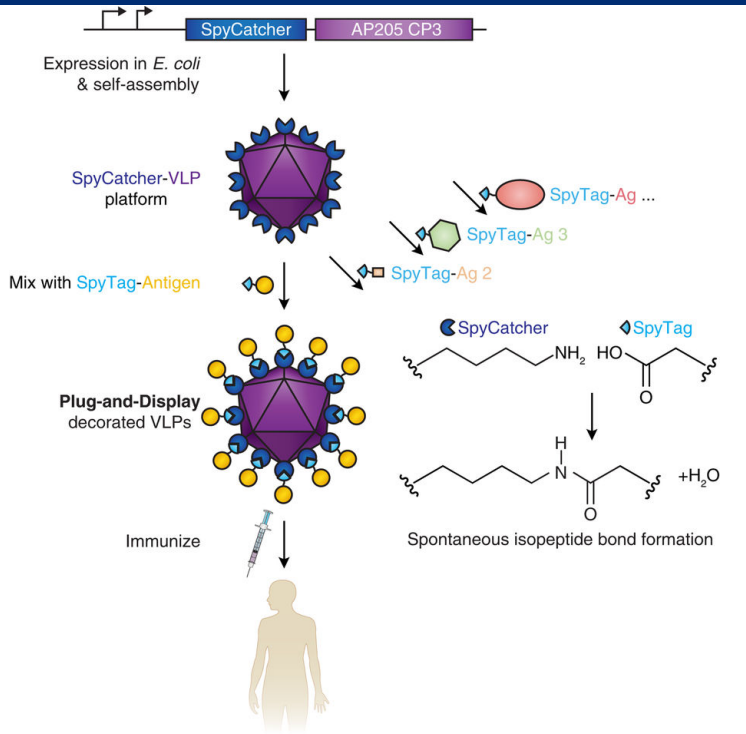
Salmond, George PC, and Peter C. Fineran. "A century of the phage: past, present and future." *Nature Reviews Microbiology* 13.12 (2015): 777.

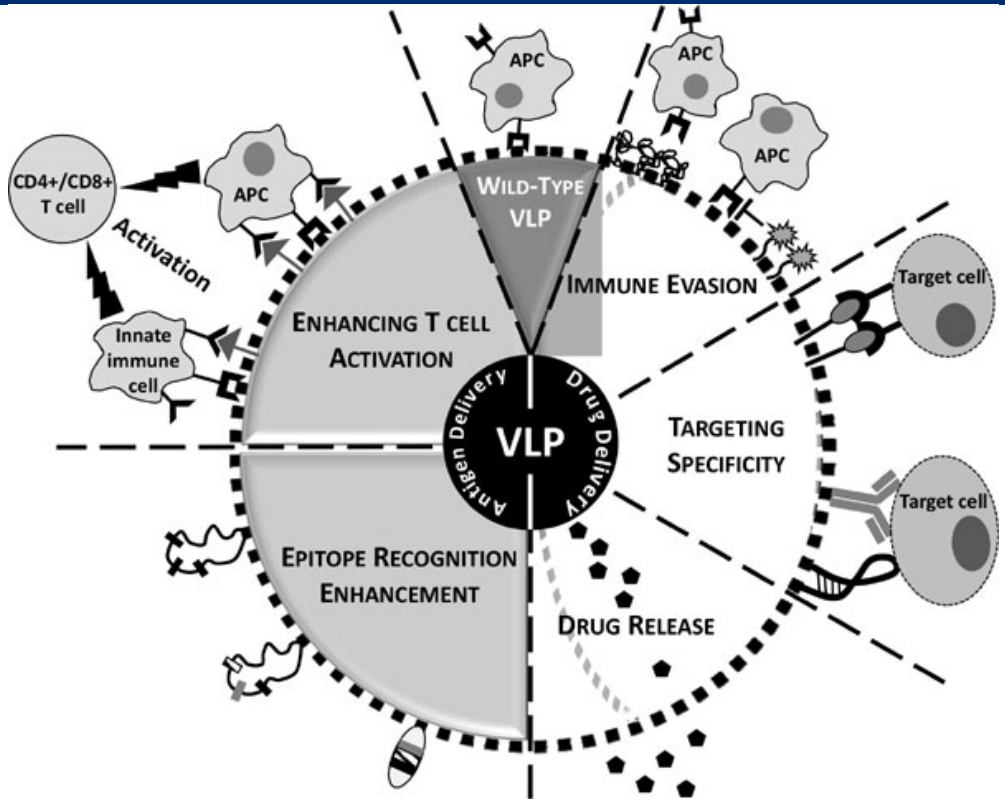
Engineering Virus-like Particles



D Hill, Brett, et al. "Engineering Virus-like Particles for Antigen and Drug Delivery." *Current Protein and Peptide Science* 19.1 (2018): 112-127.

Example - Virus-like Particles for Malaria Vaccine

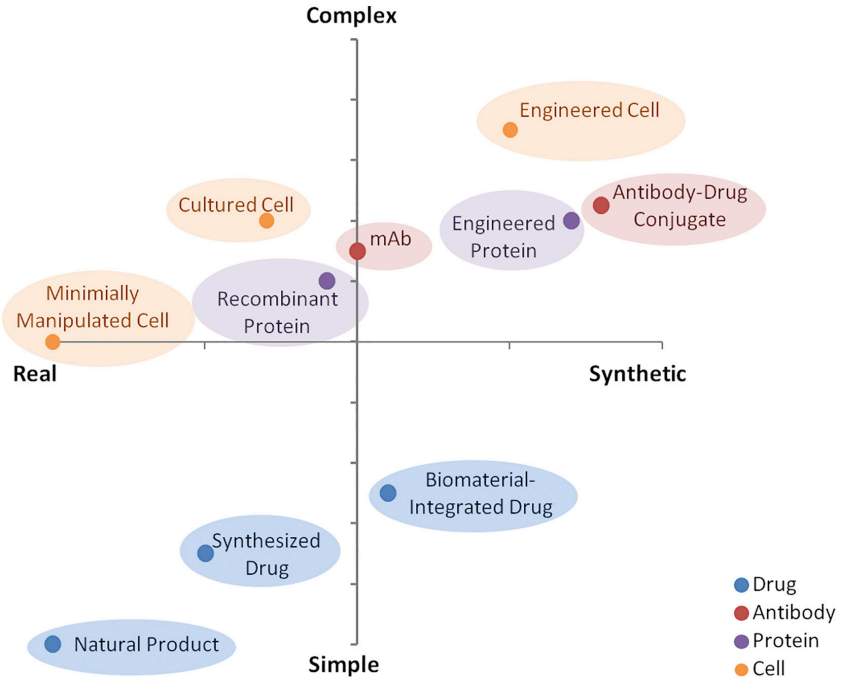




D Hill, Brett, et al. "Engineering Virus-like Particles for Antigen and Drug Delivery." *Current Protein and Peptide Science* 19.1 (2018): 112-127.
Brune, Karl D., et al. "Plug-and-Display: decoration of Virus-Like Particles via isopeptide bonds for modular immunization." *Scientific reports* 6 (2016): 19234.

Opportunities & Challenges for Cell Engineering

- Genetic engineering technique developments
  - Off-target effects
- Approval of CAR T cell therapies
  - $475,000 & $373,000
- Early stages
  - Lack of data and applications
- Lack of engineering involvement
  - Manufacturing standardization



Lipsitz, Yonatan Y., et al. "Achieving efficient manufacturing and quality assurance through synthetic cell therapy design." *Cell stem cell* 20.1 (2017): 13-17.