Pros and cons of DNA vaccines against chronic viral infections and the advantages of combining different vaccination approaches

Maria Isaguliants

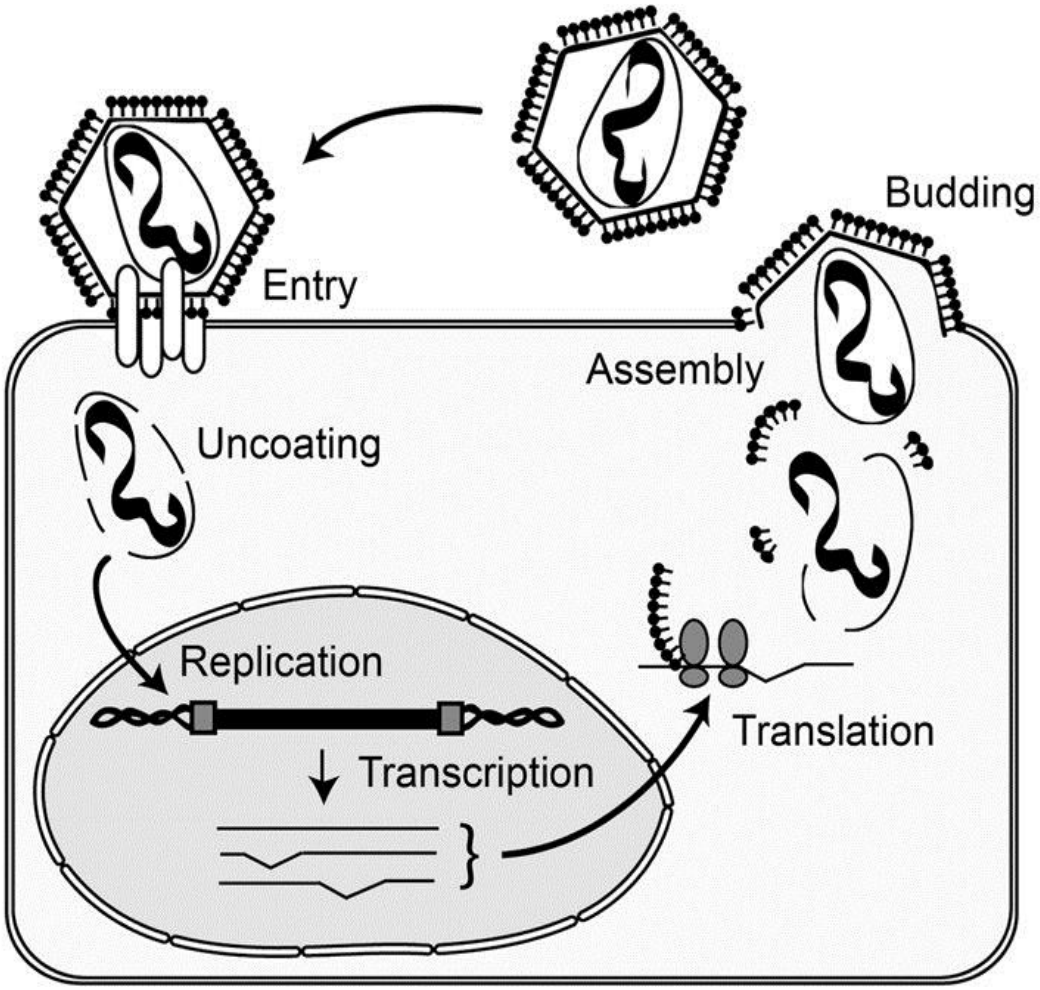
Eukaryotic cell barriers to the viral life cycle and types of subversion mechanisms that are used by the virus

Generic Viral Life Cycle

Initiation Phase
- Attachment
- Cross plasma membrane
- Uncoating
- Cross nuclear membrane

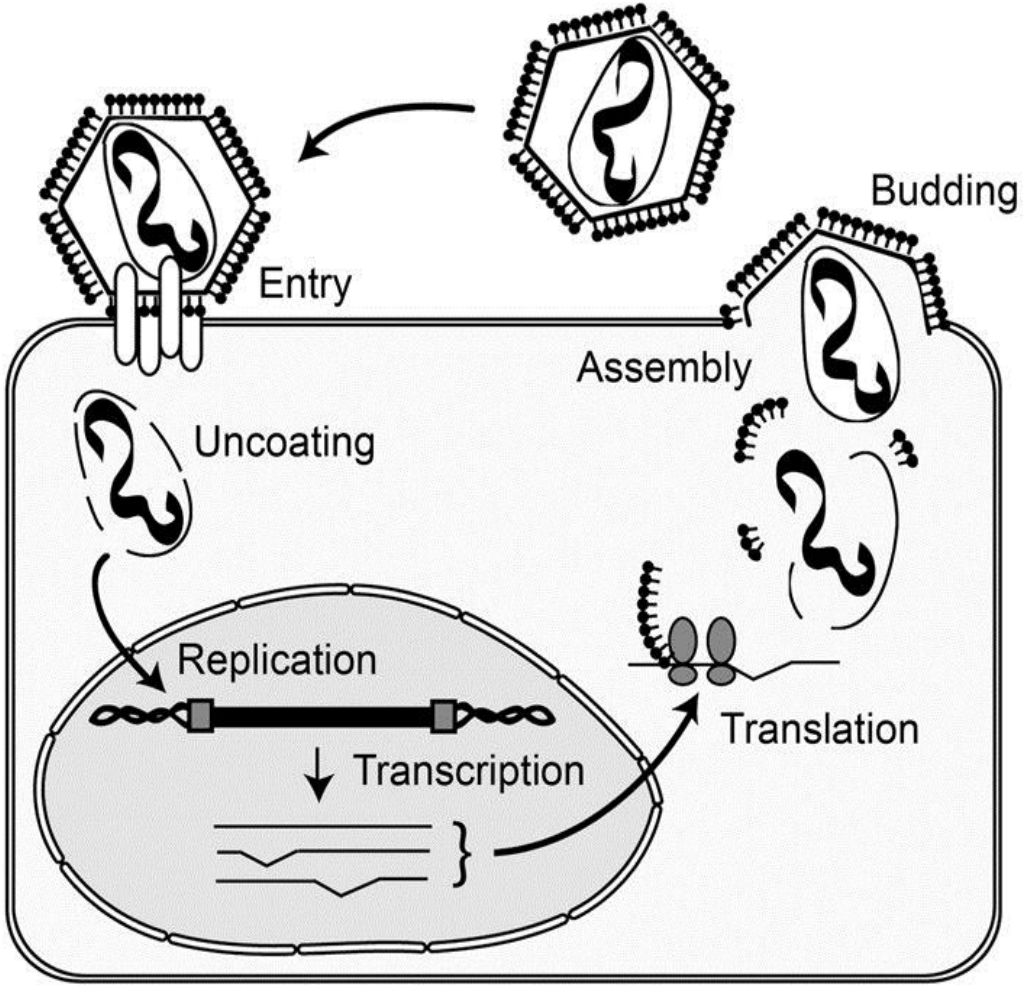
Replication Phase
- DNA synthesis
- RNA synthesis
- Protein synthesis

Release Phase
- Assembly
- Maturation
- Exit from cell



Bruggeman, L. A. Clin J Am Soc Nephrol 2007;2:S13-S19

CJASN



Common Viral Subversion Mechanisms

Molecular mimicry
- Cell surface receptors
- Immune evasion
- Cell survival

Hijacking
- Signal transduction pathways
- Transcription factors

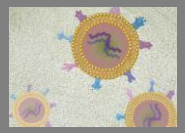
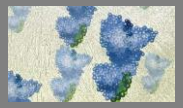
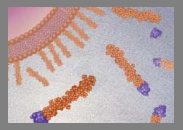
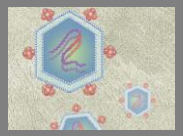
Oncogenes/Transformers
- Cell cycle control
- Access to the nucleus

Bruggeman, L. A. Clin J Am Soc Nephrol 2007;2:S13-S19

Examples of the viruses causing chronic infections:

- HIV-1
- HPV family
- HSV-1/HSV-2
- CMV
- HCV

Examples of the approaches to vaccine design

	Vaccine Type	Disease
	Live, attenuated vaccine	Measles, mumps, rubella, polio (Sabin vaccine), yellow fever
	Inactivated or “killed” vaccine	Cholera, flu, hepatitis A, Japanese encephalitis, plague, polio (Salk vaccine), rabies
	Toxoid vaccine	Diphtheria, tetanus
	Subunit vaccines	Hepatitis B, pertussis, pneumonia caused by *Streptococcus pneumoniae*
	Conjugate vaccines	*Haemophilus Influenza* type B, pneumonia caused by *Streptococcus pneumoniae*
	**DNA vaccines**	**In clinical testing**
	**Recombinant vector vaccines**	**In clinical testing**

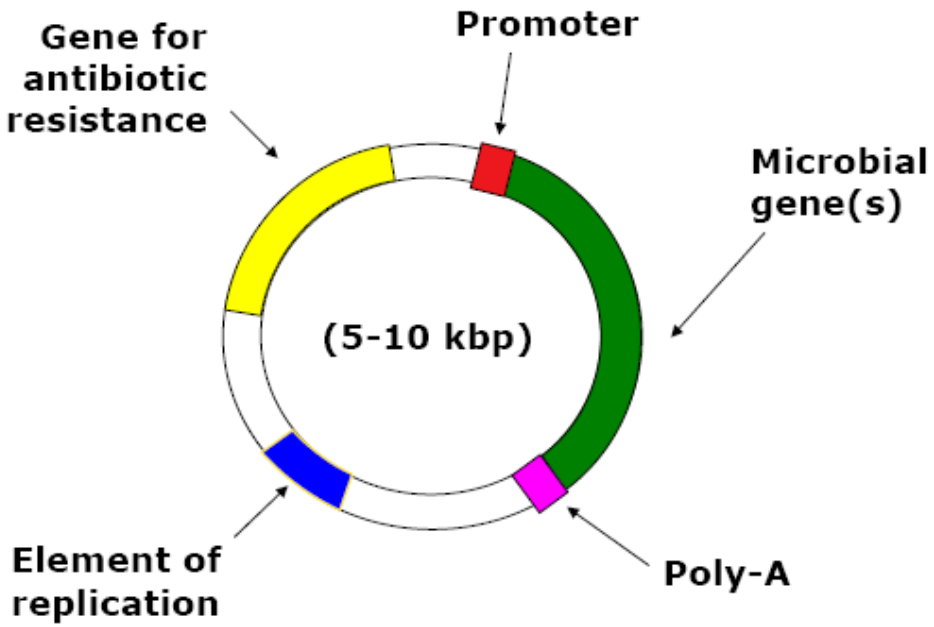
Needles versus No needles

- Oral vaccines: polio, typhoid, cholera, rotovirus, nasal influenza
- Cutaneous vaccination: liquid-jet injection deliver antigen id, sc or im

  (Advantage-Langerhans cells in skin-requires lower dose of antigen. Disadvantage-blood transfer)
- Particle bombardment of the skin - epidermal powder administration
- Topical application to skin using adjuvants and or permeabilizing agents

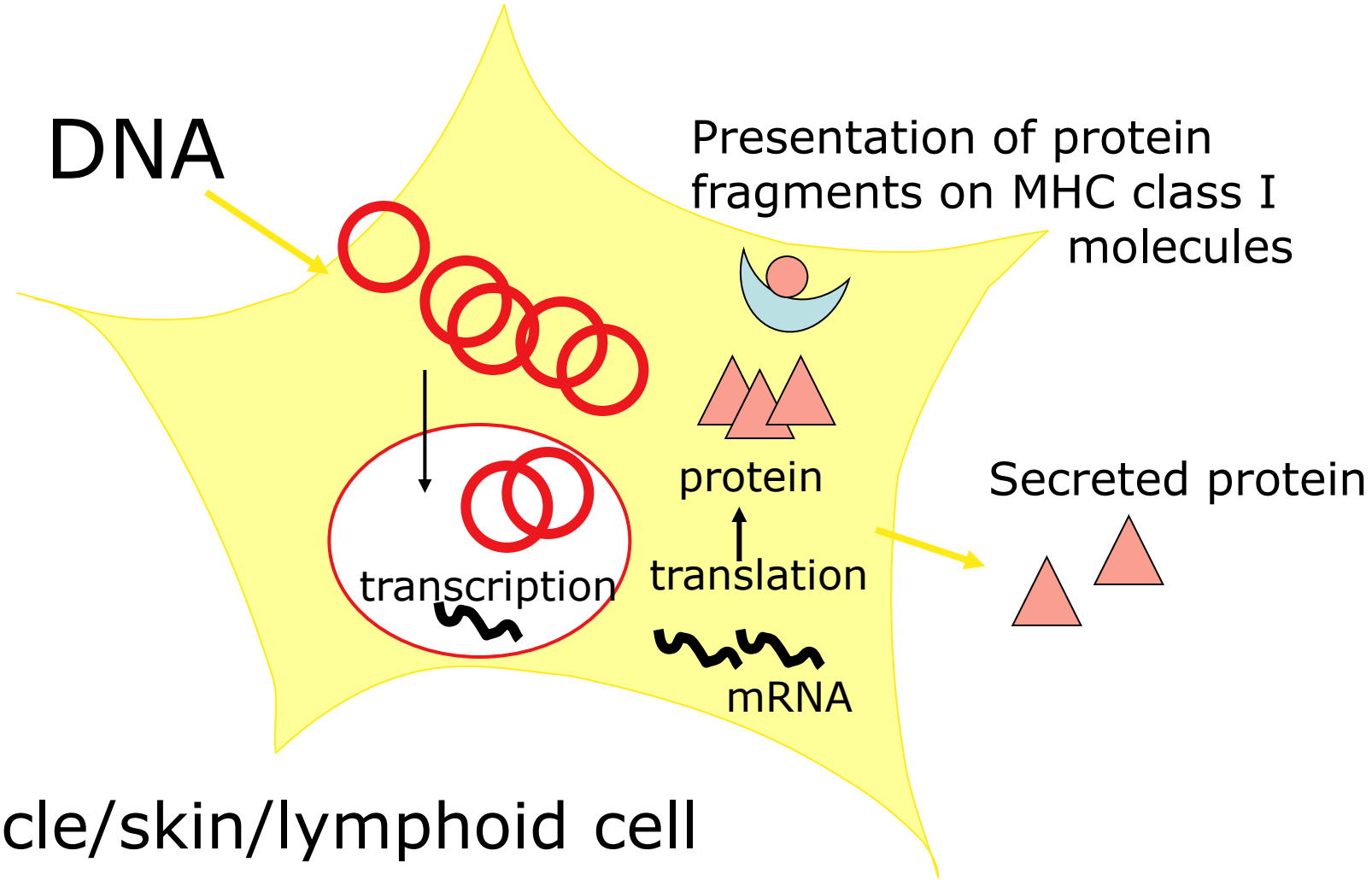
Genetic vaccines – new technique, new hope

- Genetic vaccines are based on DNA or RNA
- Encodes the vaccine antigen
- Stimulates both B and T cell responses
- Induces an immune response resembling that during a viral infection



G. Karlsson-Hedestam

The cells of the body act as vaccine factories



G. Karlsson-Hedestam, SMI 2006

DNA vaccines

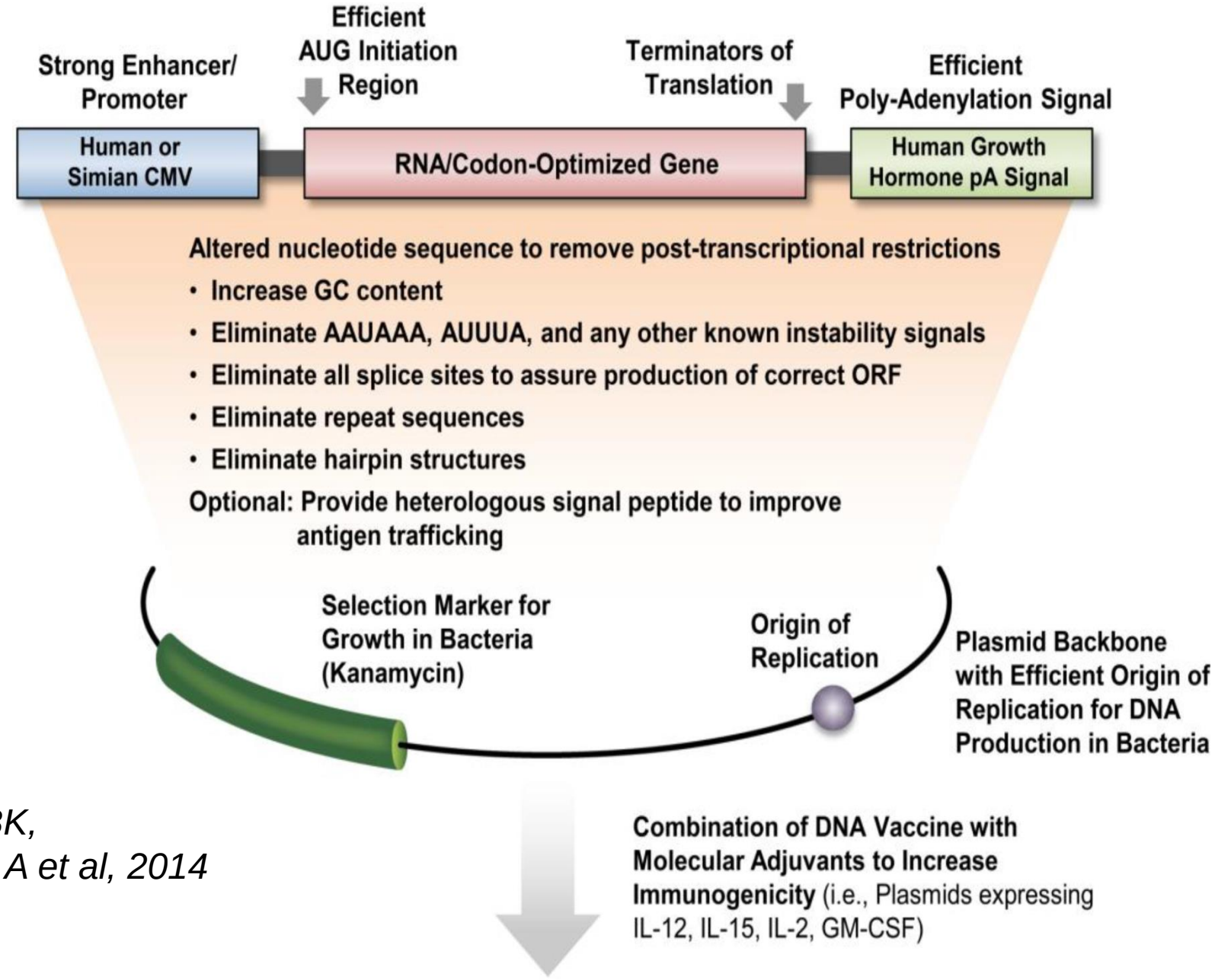
PROS

- Efficacy
  - Immunity similar to a modified live
- Stability
  - Highly stable over long periods of time

CONS

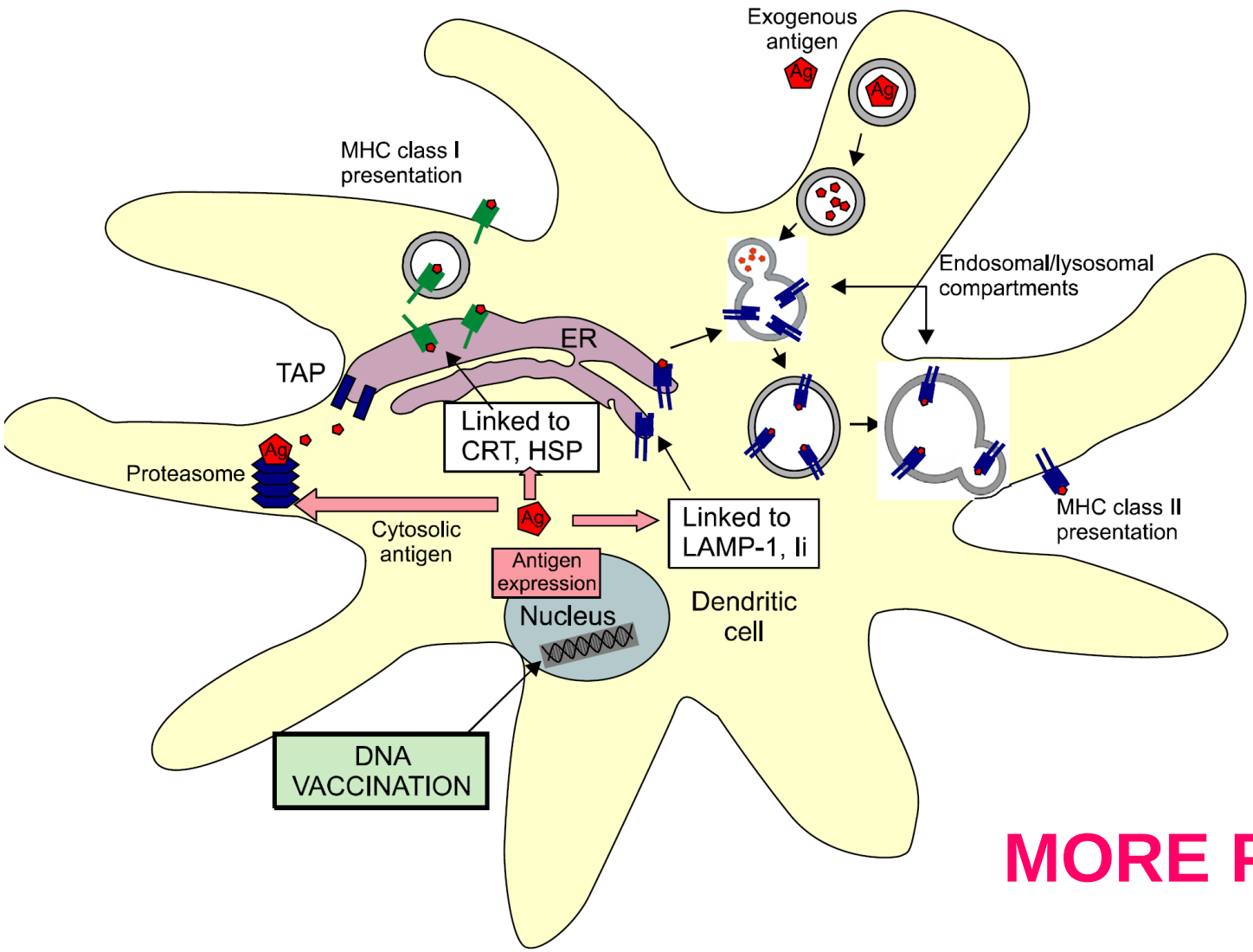
- Efficacy
  - Protective antigen must be known
  - Possible need for multiple injections
  - Prime/boost may require different technology
  - Limited data on in field conditions
- Safety
  - Unknown risk associated with incorporation into genome – food producing animals

Efficacy: stepwise changes make a difference



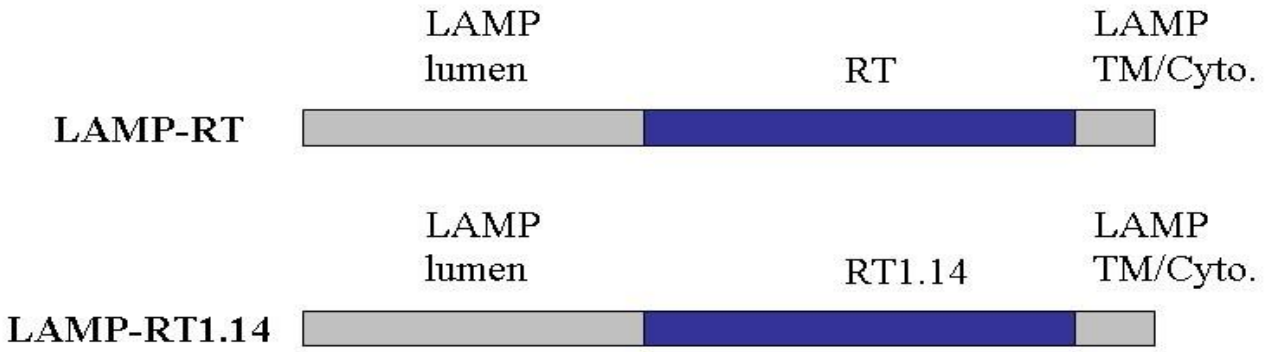
Felber BK,
Velantin A et al, 2014

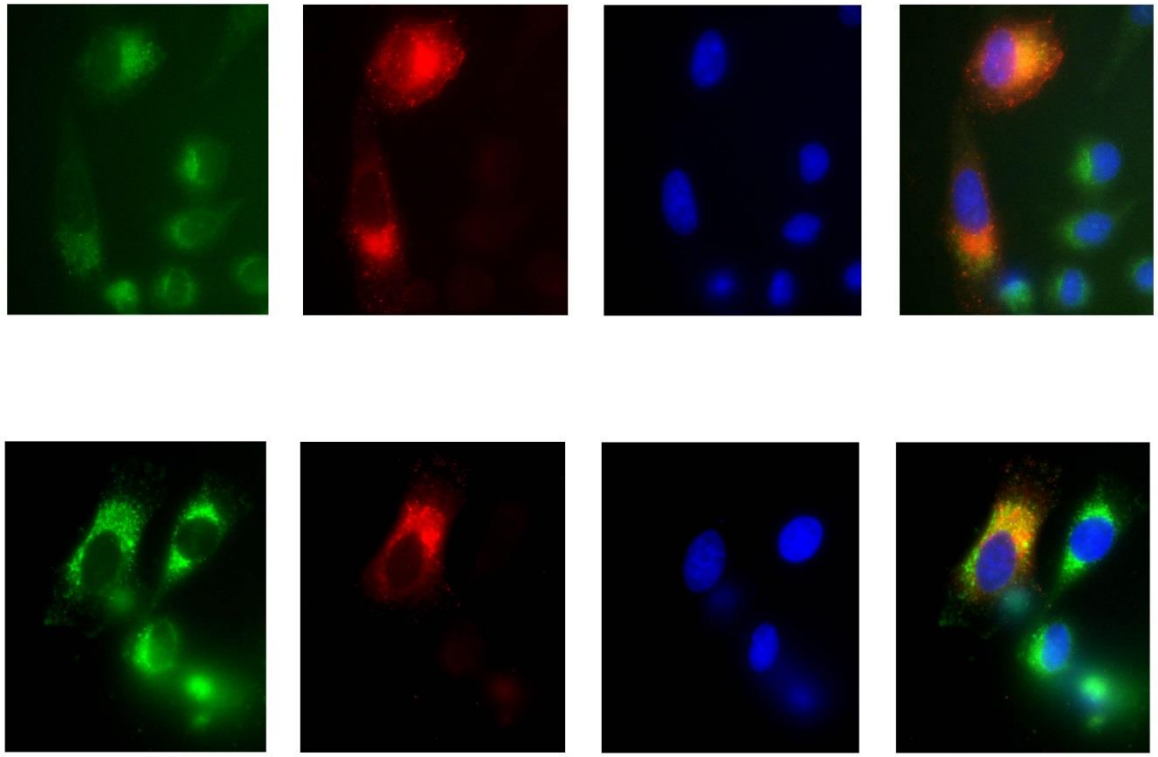
Manipulations with encoded antigens allowing retargeting of processing and presentation



MORE PROS

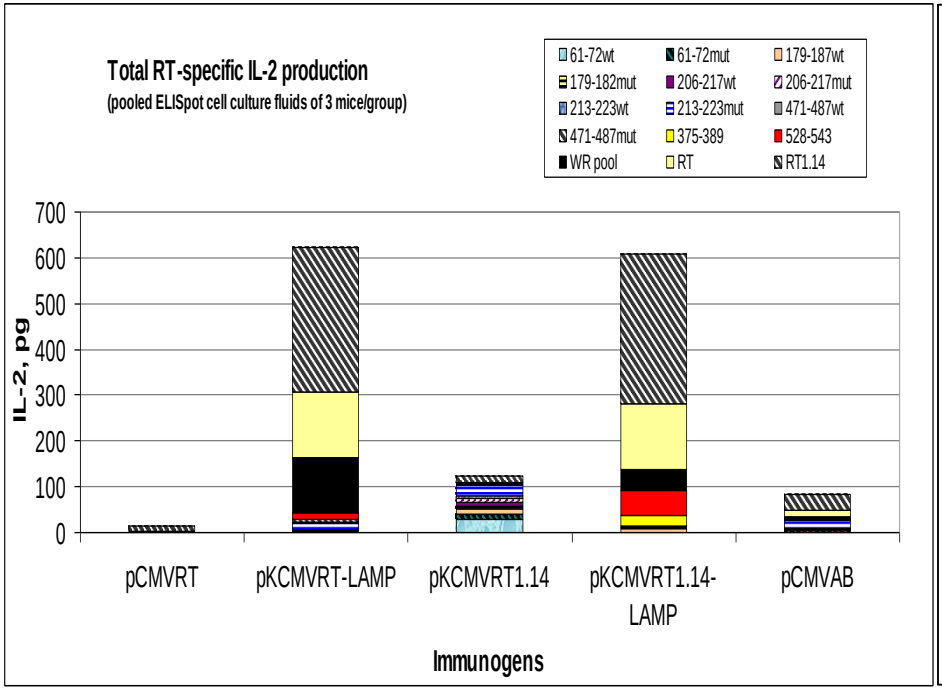
Targeting of drug resistant HIV-1 reverse transcriptase (RT) to the lysosomal degradation pathway

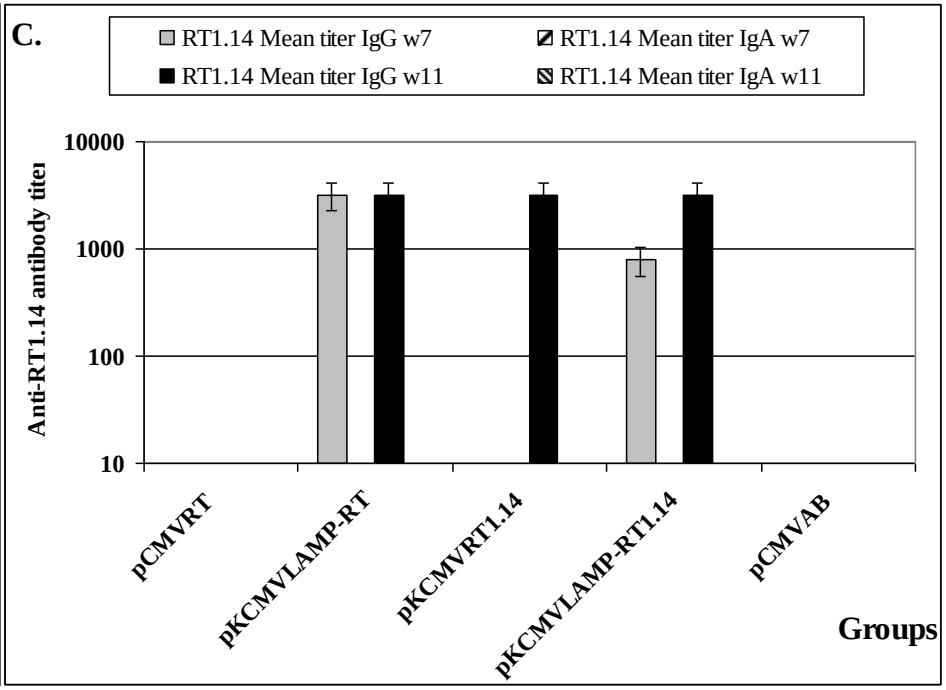




Isaguliants M & Starodubova E, 2009

Potentiating immune response against drug resistant HIV-1 RT after its targeting to the lysosomal degradation pathway



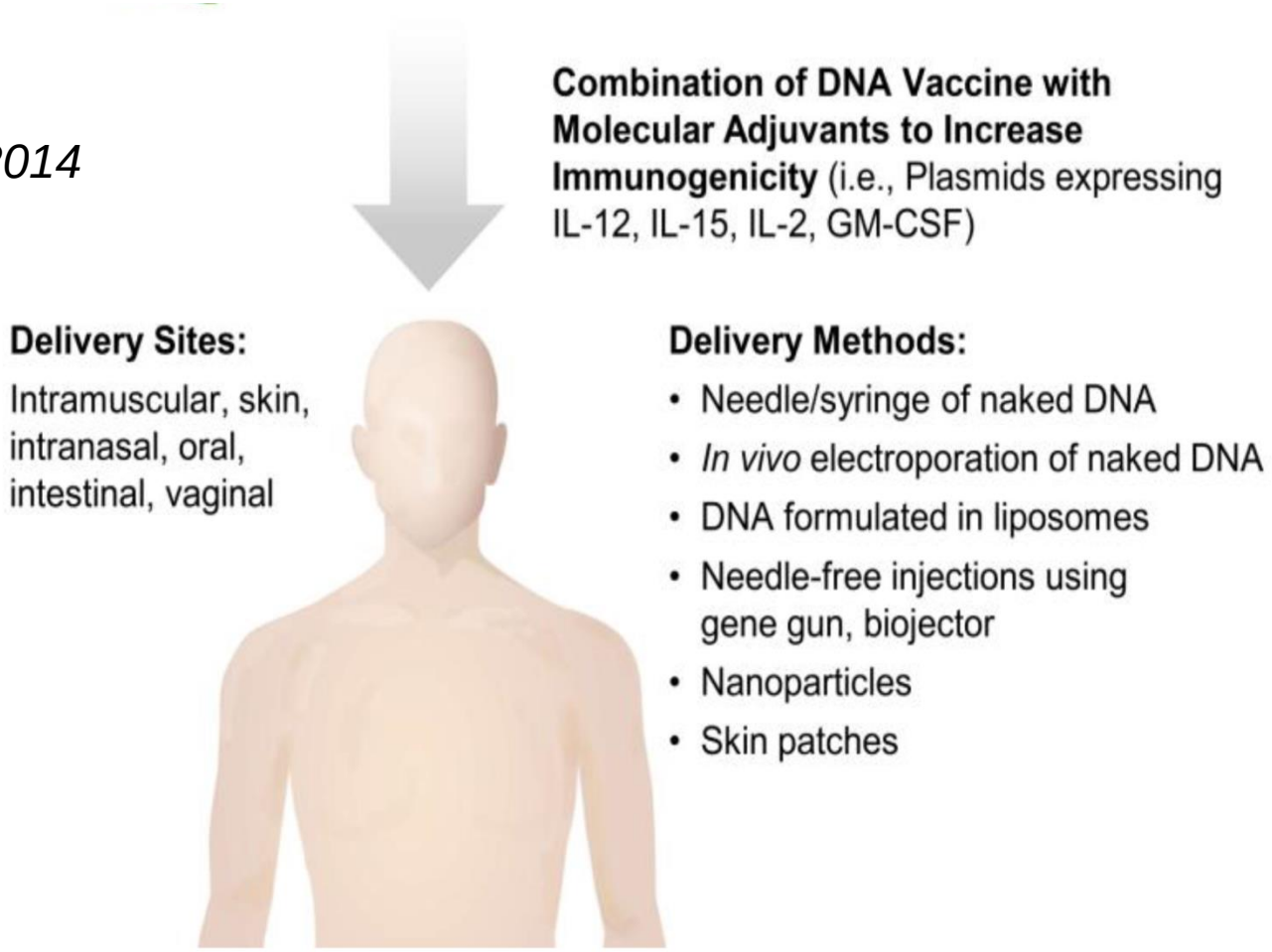


Specific IL-2 production

Specific IgG response

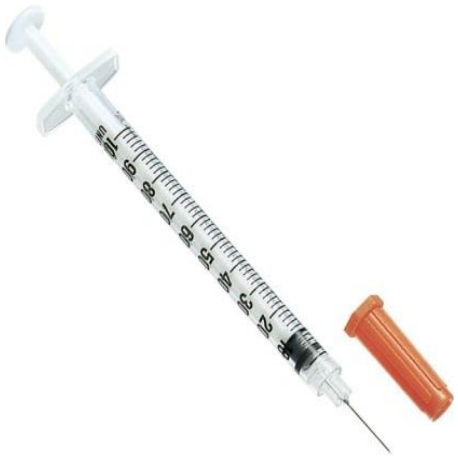
Efficacy: stepwise changes make a difference

Felber BK,
Velantin A et al, 2014



Ways of gene delivery

29G needles
OR



Microneedles

Micronjet (Nanopass Technologies)

Biojector (delivery by gas pressure)

Dermavax, Cellectis

Electroporation

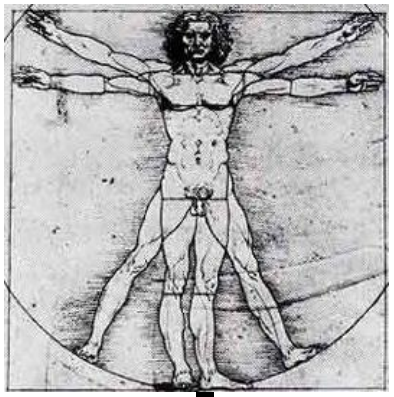
CUY21EDIT, BEX

OLD CONS

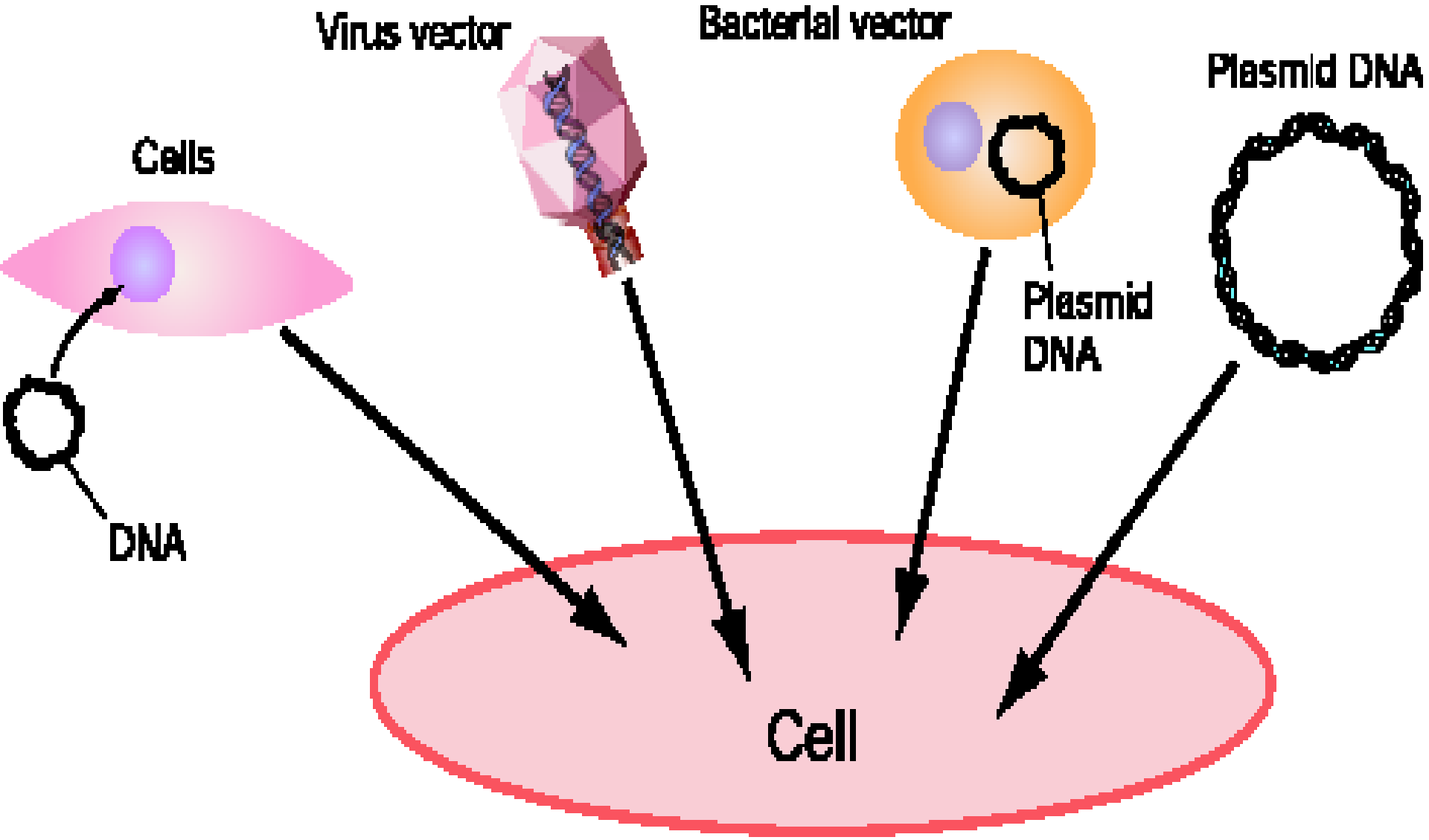
-Very efficient in (small) animal models, cheap, easy to produce, handle and store;
- Safe in small animals, larger species, humans

NEW CONS

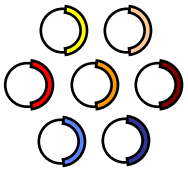
- Low immunogenicy in larger species;
- Difficult to enhance the efficacy in large species;
- Multiple clinical trials with little success.

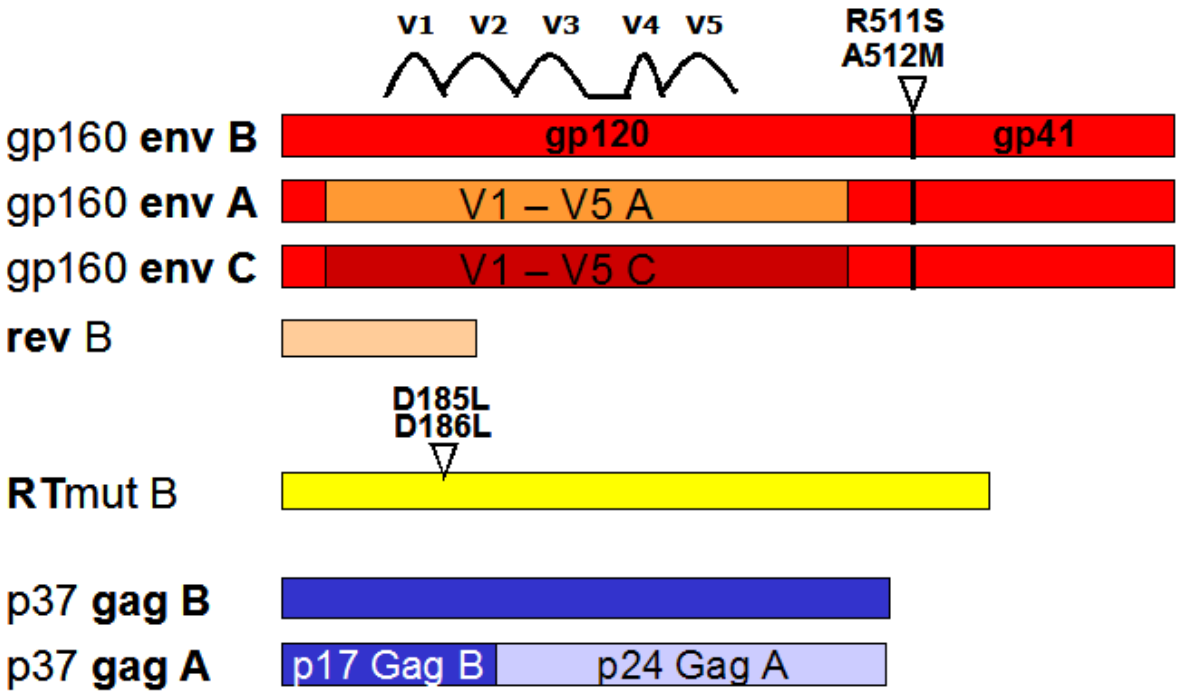


Methods for gene delivery

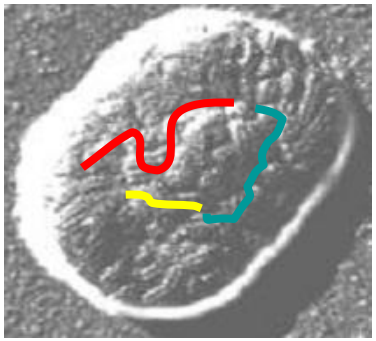


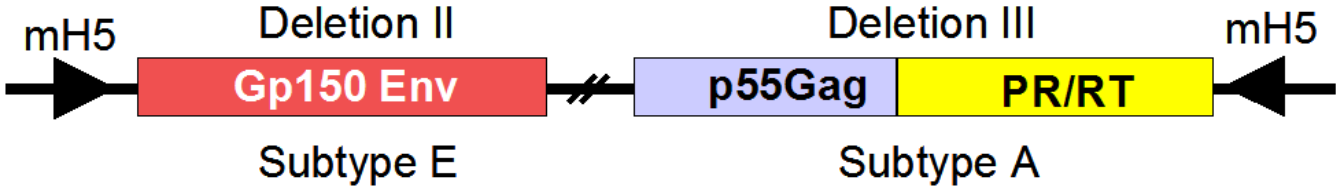
The plasmids





The recombinant Modified Vaccinia virus Ankara

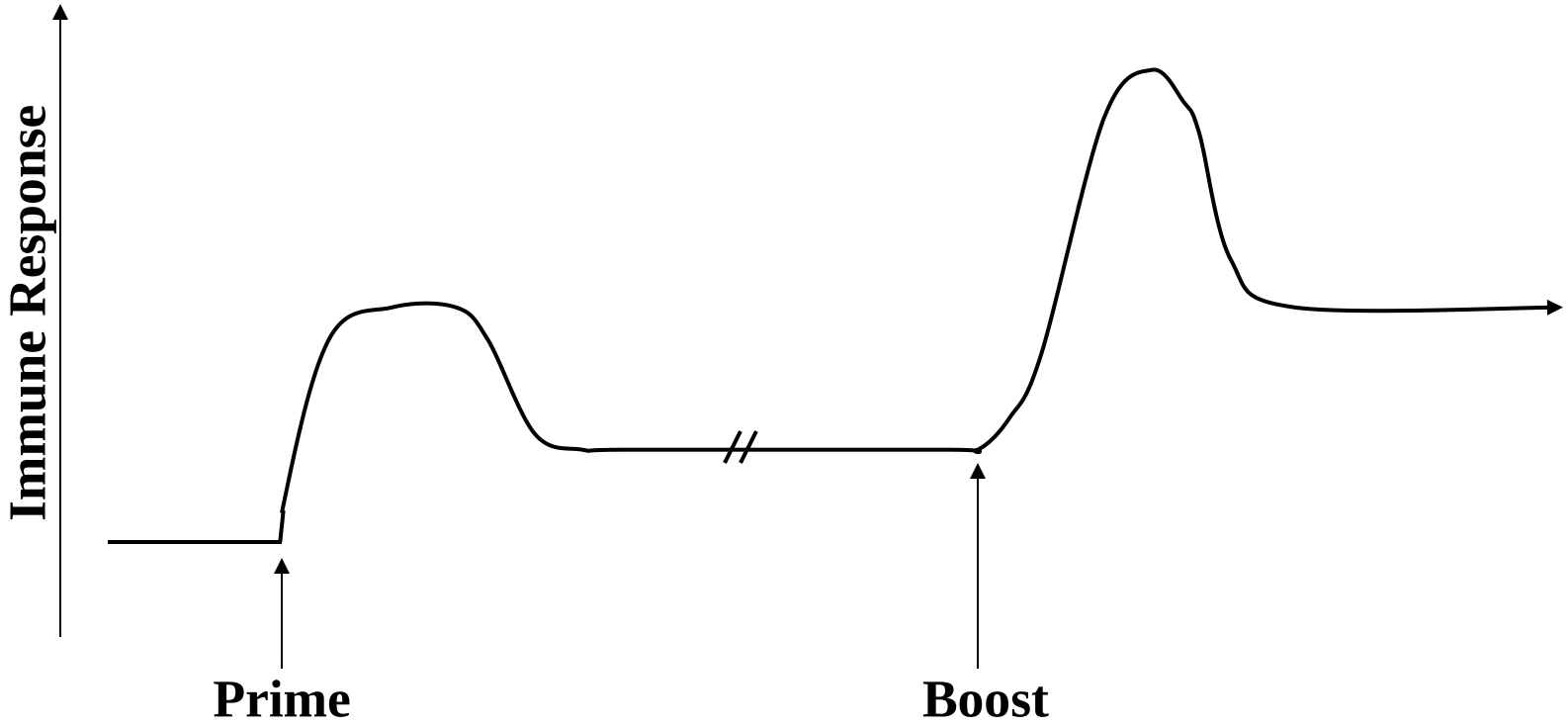




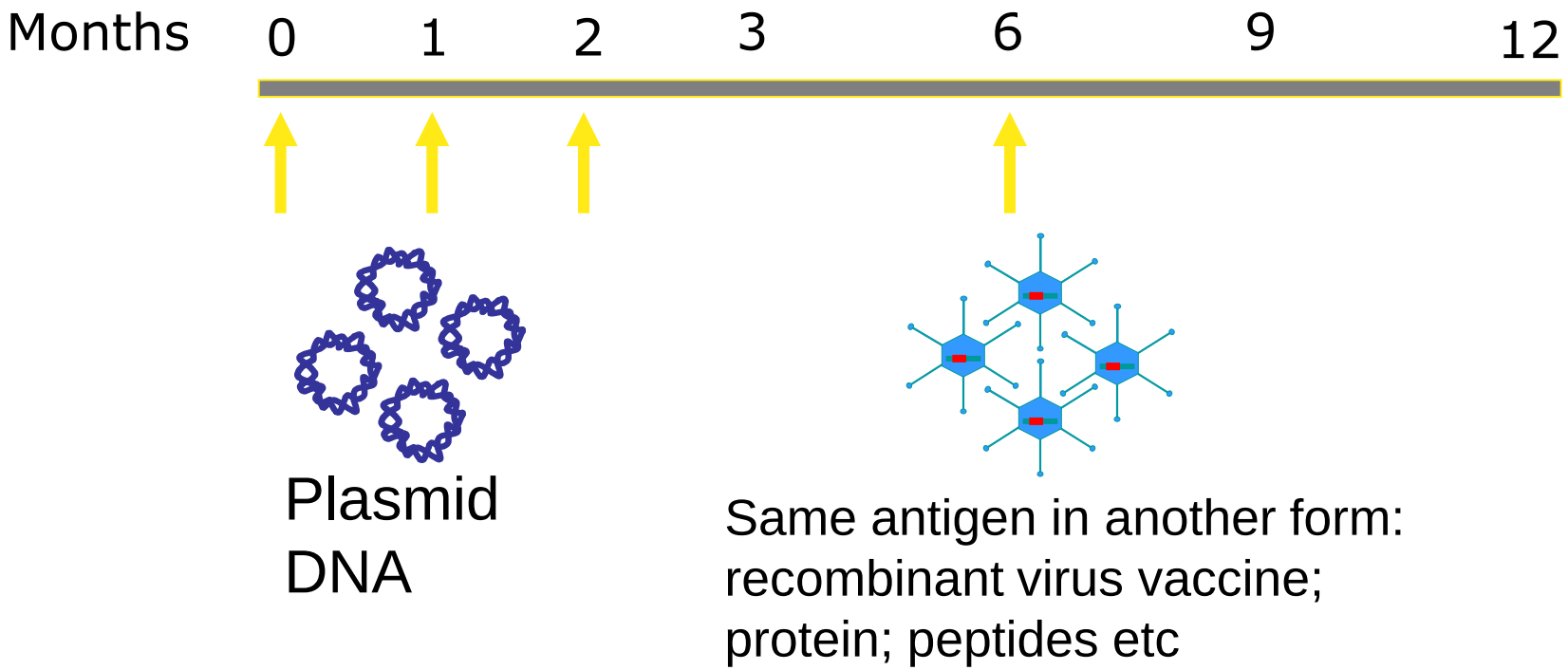
HIVIS:

Example of therapeutic HIV vaccine

Prime Boost Strategy



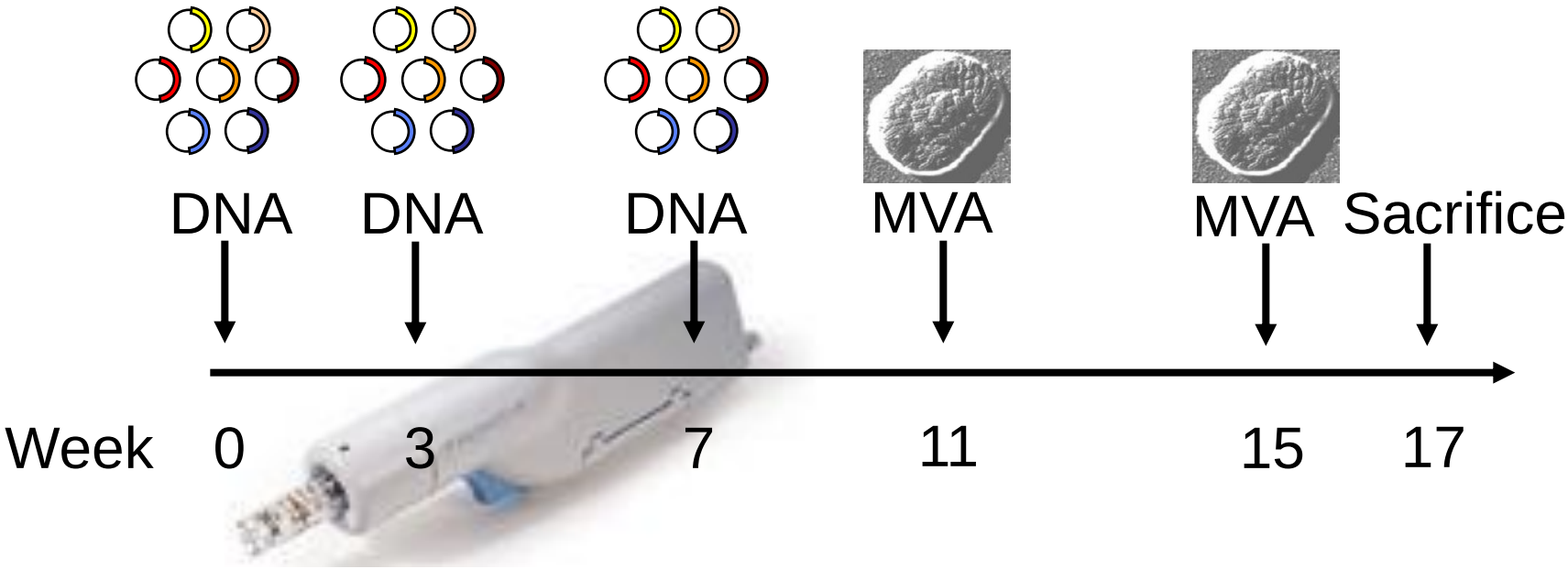
Heterologous Prime-Boost



- Same antigen encoded by the plasmid and presented by the heterologous boost;
- Focuses the immune system on the vaccine antigen

G. Karlsson-Hedestam

Wahren B et al, 2007



25ug/plasmid injected intradermally using the Biojector. Plasmid injected as 2 entities (env/rev and gag/RT)

1ug rGM-CSF injected at the site of injection of envelope-encoding DNA

Animals boosted with 10^7 pfu i.m. rMVA

HIV VACCINE TRIALS NETWORK

MVA and Ad5 as Vectors in HVTN Trials

- HVTN 055 Therion MVA or MVA+FPV
- HVTN 065 GeoVax MVA or DNA+MVA
- HVTN 050 Merck Ad5 gag
- HVTN 502/503 Merck Ad5 gag/pol/nef
- HVTN 054 VRC Ad5 env/gag/pol
- HVTN 057 VRC DNA + Ad5 env/gag/pol
- HVTN 069 VRC DNA + Ad5 env/gag/pol
- HVTN 204 VRC DNA + Ad5 env/gag/pol

Thai HIV Vaccine Study

- Community-based study
  - Not specifically high-risk
  - Moderate risk more likely predominated
- 31.2% Efficacy to prevent infection
  - Two-tailed p value 0.039 with 95% CI 1.1%-52.1%
  - 51/8197 infected vaccinees
  - 74/8198 infected placebo

Rerks-Ngarm S, Pitisuttithum P et al, 2009

From HIVIS to TaMoVac

Designation	N (+placebo)	2004	2006	2007	2009	2010	2011	2012 2013	2014
HIVIS01/02/05 Stockholm	40 phase 1	3xDNA	1st MVA	Published	2nd MVA				Published
HIVIS03/06 Dar es Salaam	40 (+20) phase 1/2			3xDNA + 1st MVA	2nd MVA		Published	3rd MVA	Analysis ongoing
TaMoVac I (Tz) Dar + Mbeya	108 (+12) phase 2					3xDNA 2xMVA		rgp140/ GLA	I: In press II: Manuscript
TaMoVac I (Moz) Maputo	20 (+4) phase 1						3xDNA 2xMVA		Analysis ongoing
HIVIS07 Stockholm	22 (+5) phase 1						3xDNA +/- EP 2xMVA +/- rgp140		Manuscript submitted
TaMoVac II Dar+ Mbeya+ Maputo	180 +(18) phase 2							3xDNA +/- EP	Addition of rgp140/GLA to MVA boost

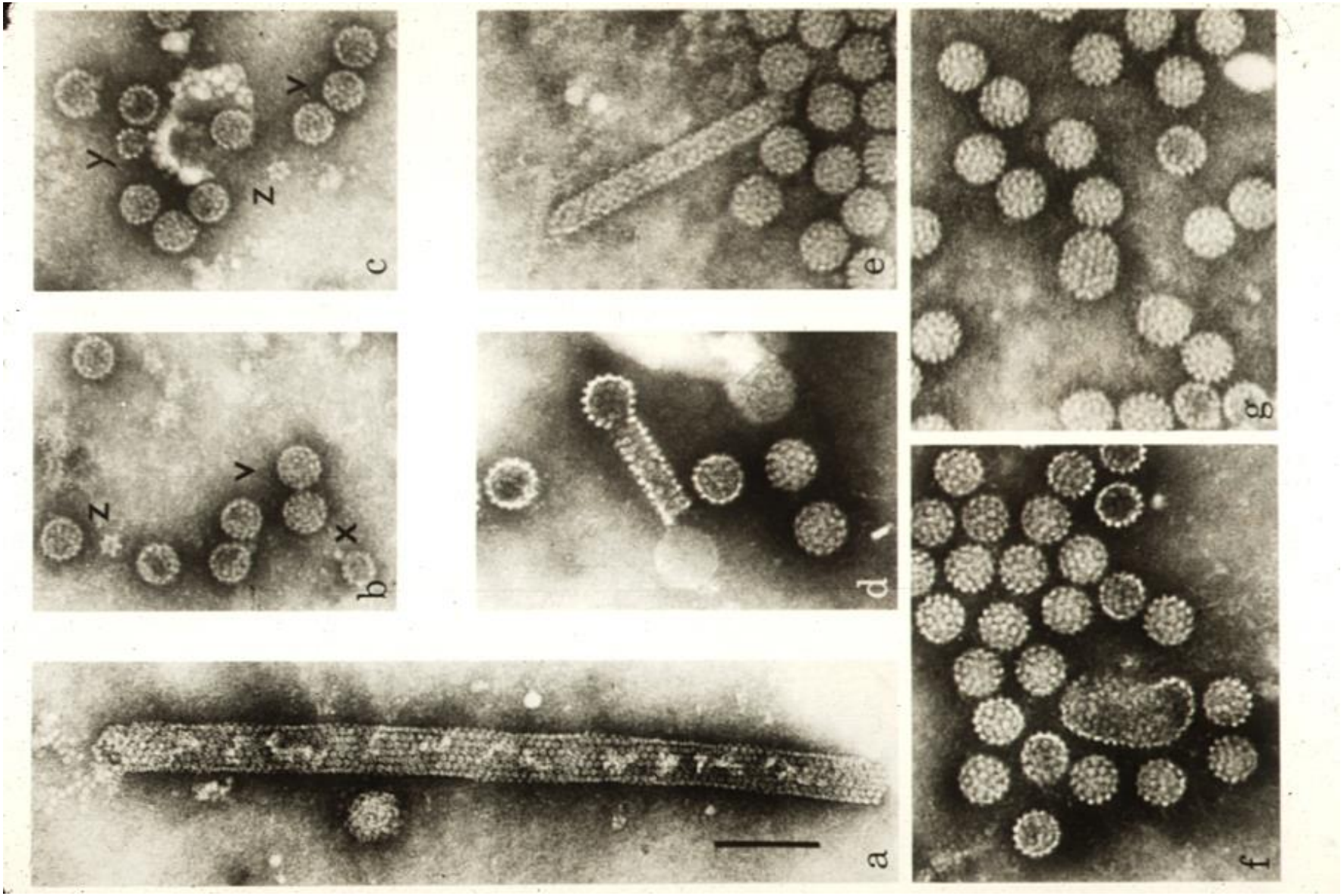
MVA: MVA-CMDR DNA: 7 plasmid DNA multigene/multisubtype vaccine EP: Electroporation

Thai HIV Vaccine Trial:
Surprising findings

- Protection correlated with Ab against V2 region
- Early, high protective immune response
  - First 12 months post-vaccination cumulative vaccine efficacy was est. 60.5% (95 % CI 22–80)
  - Efficacy declined quickly

Hence: Maybe additional boost or other ↑immune response can ↑ efficacy.

Papilloma Virus Vaccines



Common HPV Types Associated With Benign and Malignant Disease

	HPV Types	Manifestations
High-Risk	Types 16, 18, 31, 33, and 45	Low-grade cervical changes High-grade cervical changes Cervical cancer Anogenital and other cancers
Low-Risk	Types 6 and 11	Benign low-grade cervical changes Condylomata acuminata (Genital warts)

Cox JT, 1995; Munoz et al., 2003.

Papilloma virus gene function

ORF	Function
L1	L1 protein, major capsid protein
L2	L2 protein, minor capsid protein
E1	Initiation of viral DNA replication
E2	Transcriptional regulatory protein with an auxillary role in viral DNA replication
E3	No known function
E4	Late protein; disrupts cytokeratins
E5	Membrane-transforming protein; interacts with growth factor receptors
E6	Transforming protein of HPVs; targets degradation of p53
E7	Transforming protein of HPVs; binds to the retinoblastoma protein
E8	No known function

ORF, open reading frame; HPV, human papillomavirus.

(From Fields Virology, 4th ed, Knipe & Howley, eds, Lippincott Williams & Wilkins, 2001, Table 66-1)

Vaccination aims:

Prevention of HPV infection: generation of humoral mucosal immunity

Therapy of HPV-associated tumor: generation of cellular immunity directed against oncogene products E6/E7.

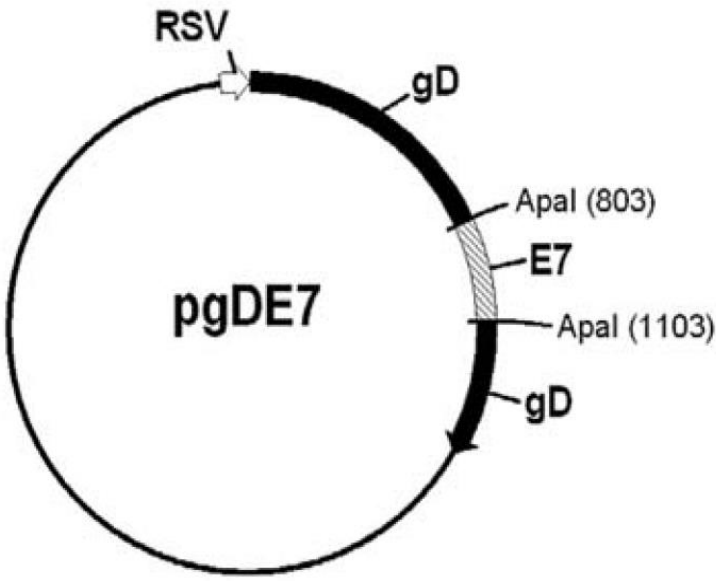
Vaccination approaches:

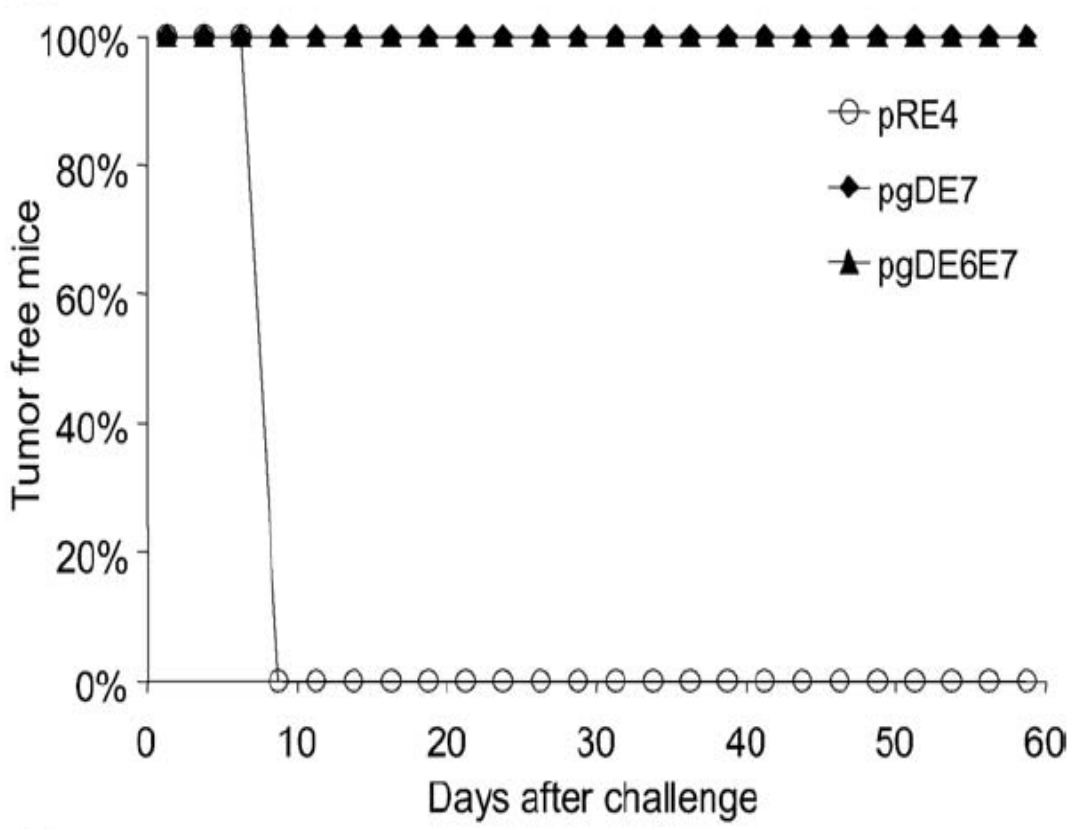
Delivery of: L1 or L1/L2 virus–like particles (VLPs).

Delivery of vaccinia vector expressing E6/E7

Delivery of chimeric VLPs (with E6/E7 peptides or L1/L2-E7 fusions)

Anti-tumor DNA vaccines based on the expression of HPV16 E6/E7 oncoproteins

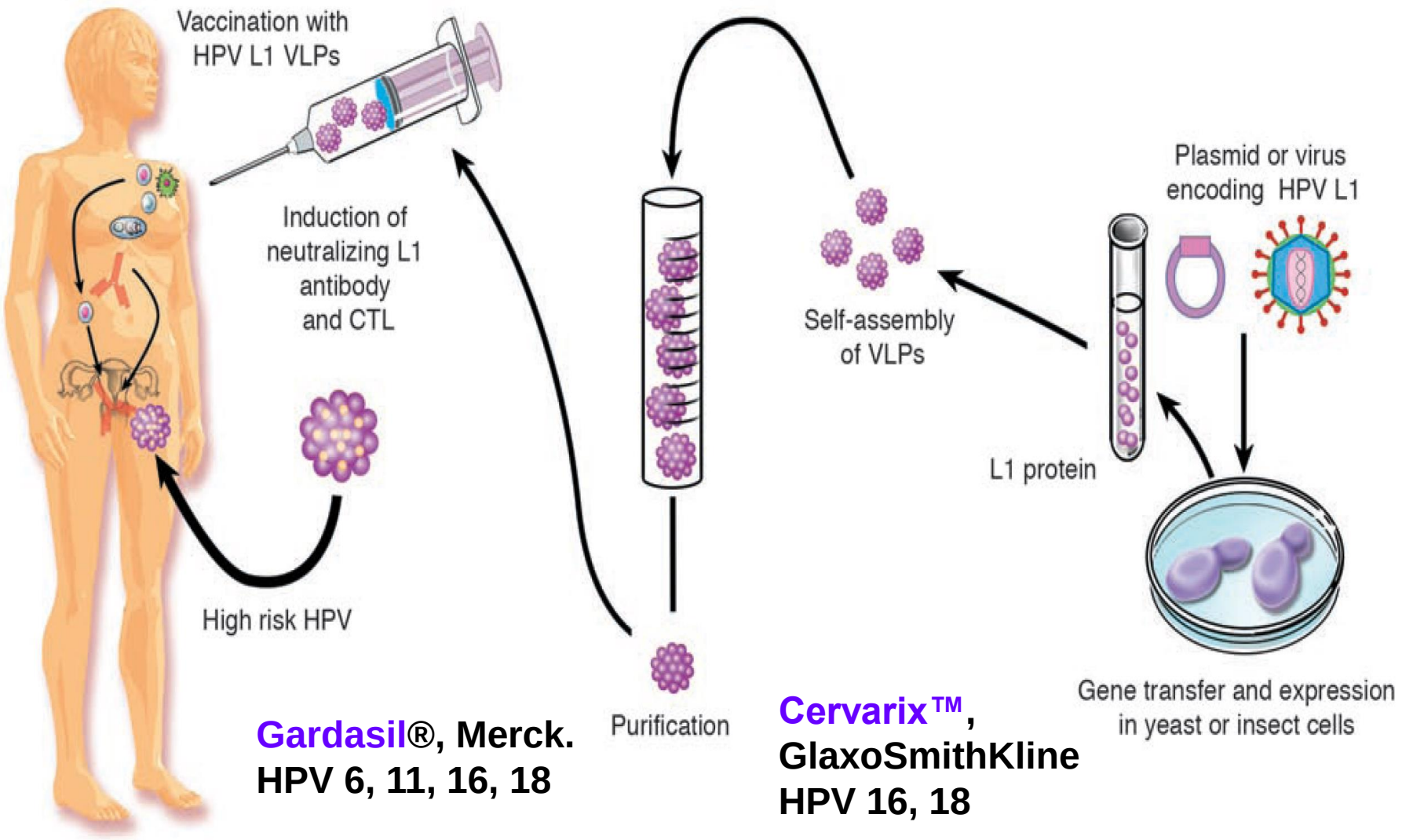




C57Bl/6 mice immunized with gD/E6/E7 DNA and challenged with neoplastic TC-1 cells expressing HPV16 E6 & E7

Lasaro MO et al, 2005

Vaccines are based on virus-like particles (VLPs) derived from the non-infectious L1 outer capsid proteins (*Fife, Wheeler et al. 2004*). The vaccines are non-infectious.



Gardasil®, Merck.
HPV 6, 11, 16, 18

Cervarix™, GlaxoSmithKline
HPV 16, 18

Harper, Franco et al. 2004; Sattler et al 2005; Skjeldestad and Committee 2005; Villa, Costa et al. 2005

Three FDA-approved vaccines—Gardasil®, Gardasil-9®, and Cervarix®—prevent HPV infection and therefore guard against the major cause of cervical and ano-genital cancers and potentially head and neck cancer.

Gardasil® protects against the HPV types 16, 18, 6, and 11.

Gardasil-9® is approved for the prevention of cervical, vulvar, vaginal, and anal cancers caused by HPV types 16, 18, 31, 33, 45, 52, and 58, and for the prevention of genital warts caused by HPV types 6 or 11

Men between the ages of 9 and 26 may receive Gardasil-9® to protect their future partners and to protect themselves against anal cancer and potentially head and neck cancer, as well as genital warts.

Cervarix is FDA approved for use in preventing the two strains of HPV that cause most cervical cancers, HPV 16 and 18.

http://www.cancerresearch.org/cancer-immunotherapy/impacting-all-cancers/cervical-cancer#sthash.qbSQJ5H1.dpuf

First HPV DNA vaccines clinical trials

- DNA vaccine encoding a signal sequence linked to E7 with abolished Rb binding site (E7detox) and **fused to heat shock protein 70 (Sig/E7detox/Hsp70)** - Phase I trials on HPV-16 positive patients with high-grade CIN lesions 2/3. Homologous DNA-prime-boost vaccination regimen of three vaccinations per patient, at three dose levels, 500, 1,000, and 3,000 ug. Regression in 3/9 pts (*Dr Trimble, Johns Hopkins University*).

- DNA vaccine encoding **calreticulin (CRT) fused to E7detox** using a PowderMed/ Pfizer proprietary gene gun device - Phase I in HPV-16 positive patients with stage 1B1 cervical cancer (*Dr Alvarez, University of Alabama at Birmingham*)

Hung CF et al, DNA vaccines for cervical cancer, 2007

Therapeutic DNA-based HPV Vaccines

A phase II clinical trial of **TVGV-1 vaccine** for patients with HPV-induced cervical pre-cancer (NCT02576561).

A phase I/II trial of **VGX-3100**, a vaccine that targets HPV types 16 and 18, and **INO-9012**, a DNA construct that induces human interleukin 12 (IL-12), are being tested in patients with cervical cancer (NCT02172911).

ADXS11-001, a vaccine against the E7 protein, which is made by HPV, is in phase I/II trials in patients with anal cancer (NCT01671488).

There are two phase I clinical trials testing **pNGVL4a/E7 (Detox)/HSP70 DNA vaccine** in patients with HPV16+ cervical intraepithelial neoplasia. The first one will determine the best dose (NCT00988559) and the second one will be a combination with **imiquimod**, an innate immune activator (NCT00788164).

http://www.cancerresearch.org/cancer-immunotherapy/impacting-all-cancers/cervical-cancer#sthash.qbSQJ5H1.dpuf

Reasons for failure of herpes candidate vaccines

- Poorly controlled studies
- Insufficient dose
- Insufficient immunogenicity (Ab)
- No induction of CTL
- No induction of mucosal antibodies
- In case of recurrent disease, difficult to eradicate reservoir

Attempts to create a vaccine against Herpes simplex

- Auto-inoculation
- Live, deletion mutants (replication limited)
- Pox virus and adenovirus vectors for glycoproteins
- Inactivated whole virus
- Inactivated infected cell extracts
- Subunit glycoproteins
- Disabled Infectious Single Cycle (DISC)
- **DNA plasmids**
- Peptides

HSV-1 glycoproteins

gB, gC, gD, gE, gG, gH, gI

DNA-immunization

Antigen	Model	Authors
gB/Sindbis	HSV-1, mouse	Hariharen 1998
gB	HSV-1, mouse	Manicken 1995
gD/bupivacaine	HSV-2, mouse.g.pig	Bernstein 1999
tgB + gD 1996/1997	HSV-2, mouse/g.pig	McClements
gD + IL12	HSV-2, mouse	Sin 1999
gD	HSV-1, mouse	Ghiasi 1995
gd/tgD	BHV-1, cattle	Van Drunnen 1998
ICP27	HSV-1, mouse	Manicken 1995

Benefits of host immune response to CMV

- Maternal immunity ameliorates effects of intrauterine infection on fetus
- Premature neonates with maternal antibody are protected from postnatal infection
- Pre-transplant immunity protects organ allograft recipients from severe disease
- Passively administered antibody protects organ allograft recipients
- Protective efficacy demonstrated in murine and guinea pig models of (homologous strain) CMV infection using attenuated strains
- Experience with Towne vaccine in humans

Experimental CMV vaccines

- Live attenuated Towne strain
- Recombinant of Towne with genes from virulent virus
- Subunit gB glycoprotein
- Subunit gH glycoprotein
- **DNA plasmids**
- gB in adenovirus vector
- Multiple genes in canarypox vector
- **Prime boost with canarypox/subunit gB**

Vaccines	Status	Characteristics and results	Ref.
gB/MF59 adjuvant	Phase II study completed	Acceptable safety for further studies Evaluated in HCMV-seronegative women within 1 year after they had given birth Vaccine efficacy of 50% on the basis of infection rates per 100 person years	[20]
gB/pp65/IE1 alphavirus replicon trivalent vaccine	Phase I study completed	Favorable safety profile Evaluated in healthy, nonpregnant adults Elicits humoral and cellular immune responses Based on replication-deficient alphavirus technology	[39]
gB/pp65 bivalent DNA vaccine	Ongoing Phase II study	Well tolerated with no serious adverse events in a Phase I study HCMV-seropositive or -seronegative healthy adults in Phase I and HCT recipients in Phase II studies Higher frequencies of HCMV-specific pp65 and gB T cells compared with placebo	[42,47]
Towne ± rhIL-12 ± priming by DNA vaccine encoding pp65, IE1 and gB	Phase I studies completed	Favorable safety profile; no evidence for viral latency or viral shedding in recipients Evaluated in HCMV-seronegative healthy adults Augmentation of immunogenicity by inclusion of rhIL-12 or DNA vaccine in Phase I studies	[52,53]

TABLE 66–1. Comparative Features of Hepatitis Viruses

Feature	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
Common name	"Infectious"	"Serum"	"Non-A, non-B–post-transfusion"	"Delta agent"	"Enteric non-A, non-B"
Virus structure	Picornavirus; capsid, RNA	Hepadnavirus; envelope, DNA	Flavivirus; envelope, RNA	Viroidlike; envelope, circular RNA	Norovirus; capsid, RNA
Transmission	Fecal-oral	Parenteral, sexual	Parenteral, sexual	Parenteral, sexual	Fecal-oral
Onset	Abrupt	Insidious	Insidious	Abrupt	Abrupt
Incubation period (days)	15–50	45–160	14–180	15–64	15–50
Severity	Mild	Occasionally severe	Usually subclinical; 70% chronicity	*Coinfection* with HBV occasionally severe; *superinfection* with HBV often severe	Normal patients, mild; pregnant women, severe
Mortality pregnant	<0.5%	1%–2%	~4%	High to very high	Normal patients, 1%–2%; women, 20%
Chronicity/ carrier state	No	Yes	Yes	Yes	No
Other disease associations	None	Primary hepatocellular carcinoma, cirrhosis	Primary hepatocellular carcinoma, cirrhosis	Cirrhosis, fulminant hepatitis	None
Laboratory diagnosis	Symptoms and anti-HAV IgM	Symptoms and serum levels of HBsAg, HBeAg, and anti-HBc IgM	Symptoms and	Anti-HDV ELISA	—

ELISA, Enzyme-linked immunosorbent assay; HAV, hepatitis A virus;

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Hepatitis Viruses

THANK YOU FOR YOUR ATTENTION!!!