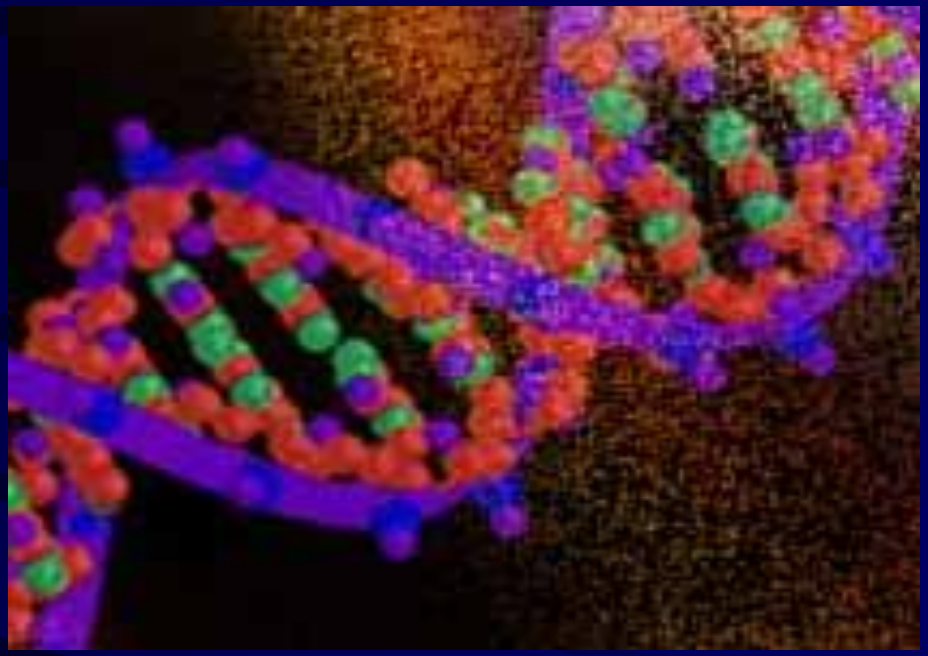
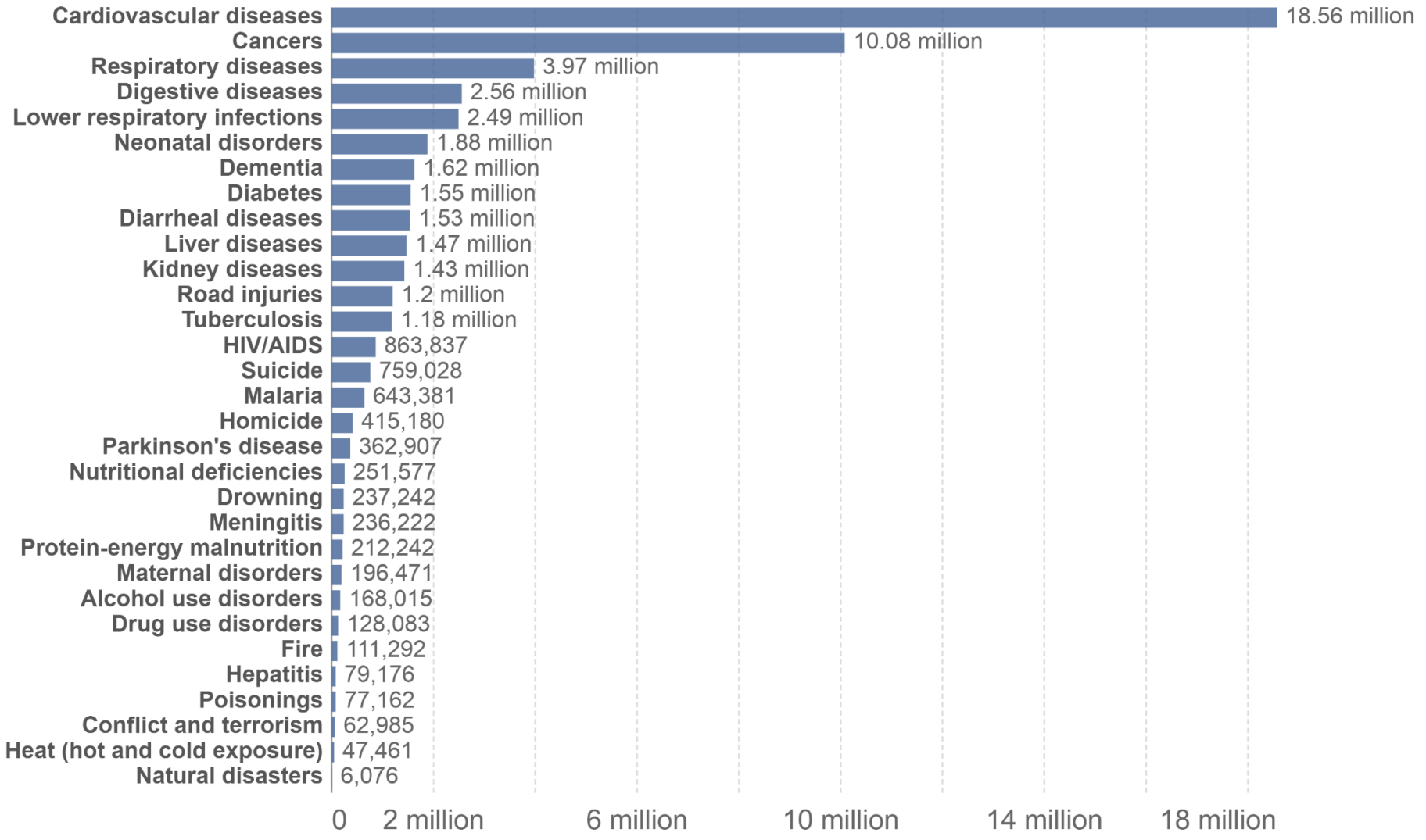
AULA DE VACINAS



Prof. Sérgio Costa Oliveira
Lab. de Imunologia de Doenças Infecciosas
Dept. de Imunologia-USP

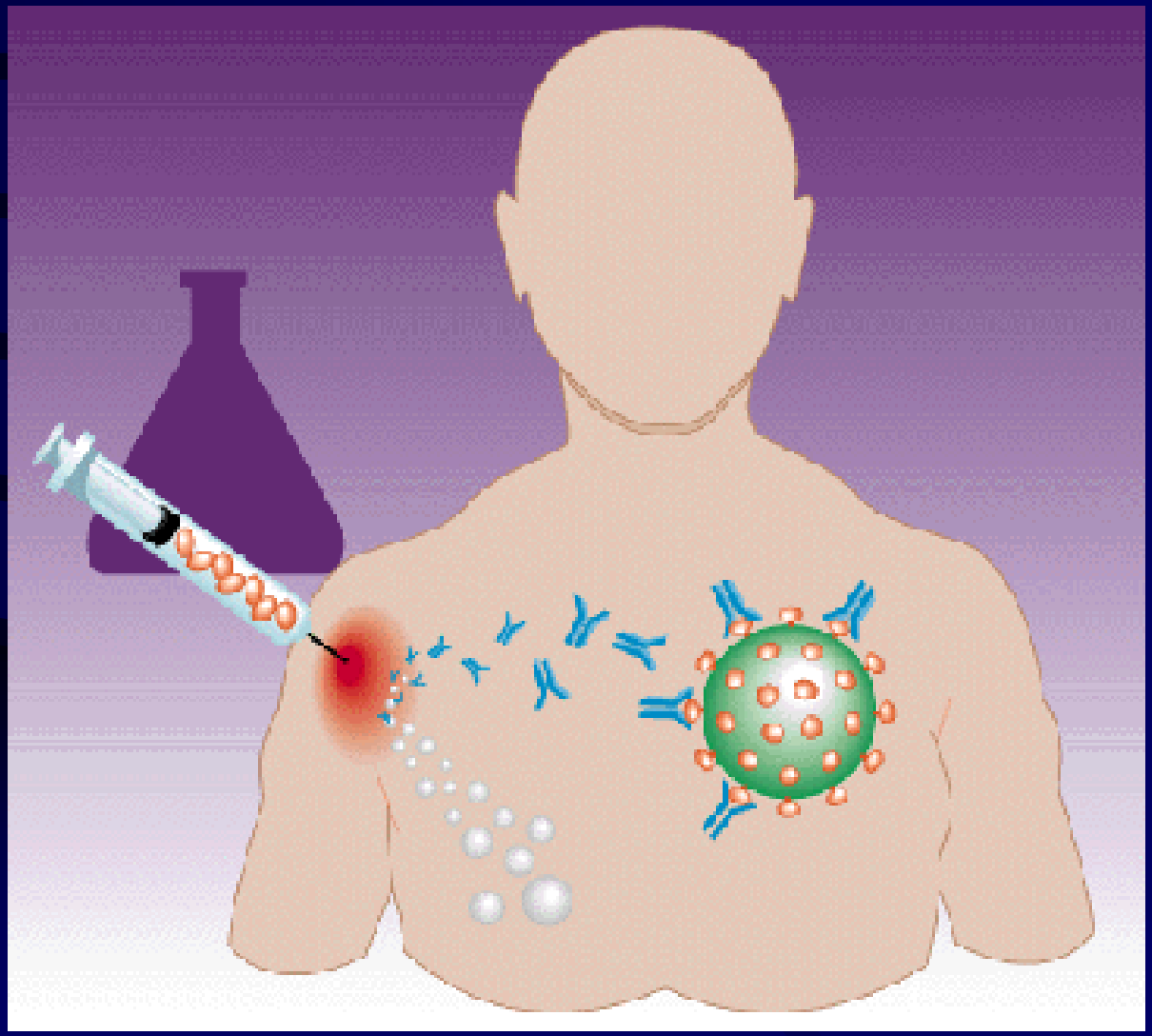
Number of deaths by cause, World, 2019



Source: IHME, Global Burden of Disease (2019)

OurWorldInData.org/causes-of-death • CC BY

Como as vacinas funcionam?



Adjuvantes

- **Substância adicionada a uma vacina para potencializar a resposta imunológica ao antígeno. O adjuvante mais frequentemente utilizado é o hidróxido de alumínio, porém, outras substâncias têm sido usadas à base de esqualeno**.

https://www.youtube.com/watch?v=_EZoKhhbrYs

Vacinas mais utilizadas na Medicina Vet

Brucelose
Febre Aftosa
Raiva
Cinomose
Parvovirose
Coronavirose
Hepatite Infecciosa Canina
Adenovirose
Parainfluenza Canina
Leptospirose Canina
Leishmaniose Canina

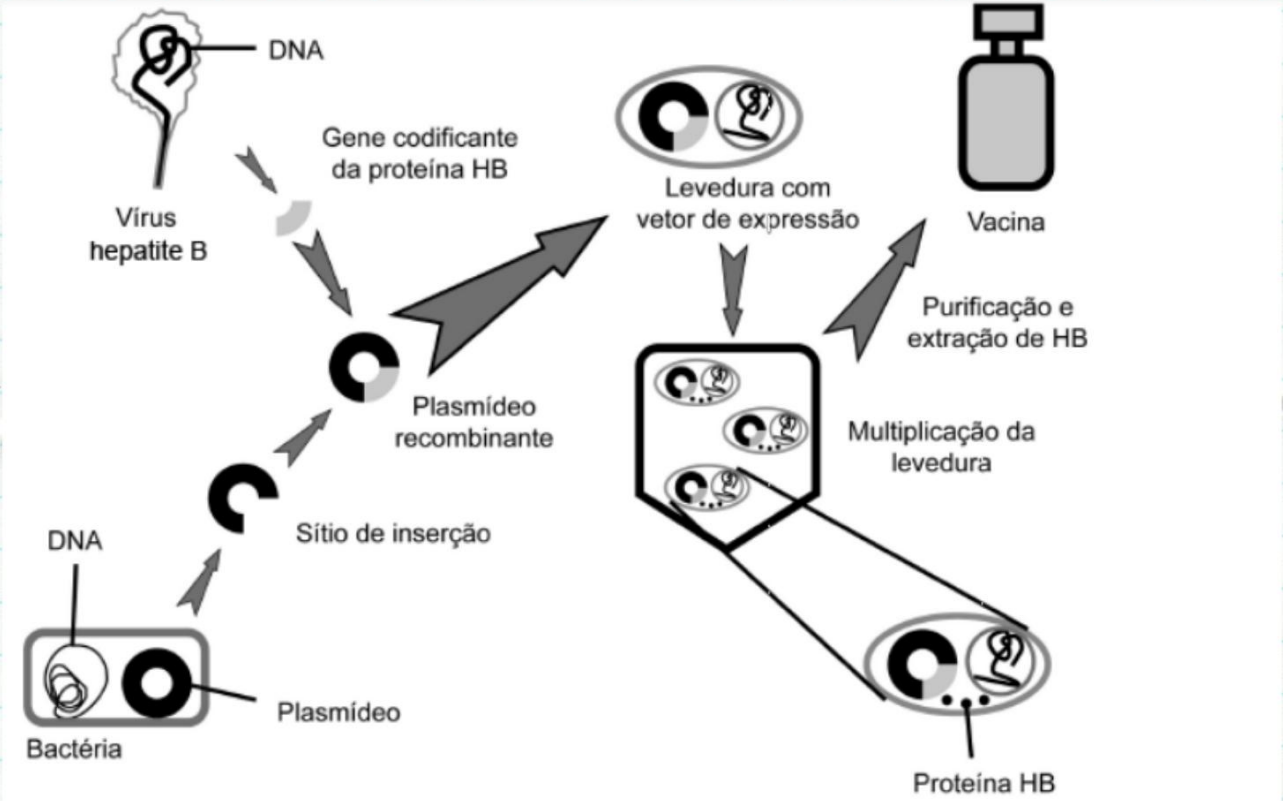
Vacinas de Primeira Geração

- Vacinas compostas por patógenos vivos atenuados ou inativados:

  - Pólio
  - Sarampo
  - Raiva
  - Tuberculose

Vacinas de Segunda Geração

- Vacinas compostas por proteínas recombinantes :
  - Hepatite B

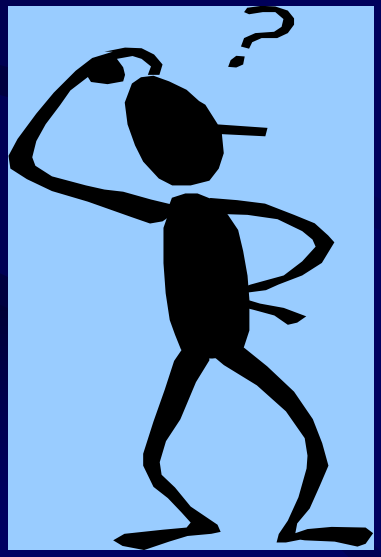


Disponível em: www.ied.edu.hk. Acesso em: 15 out. 2015 (adaptado).

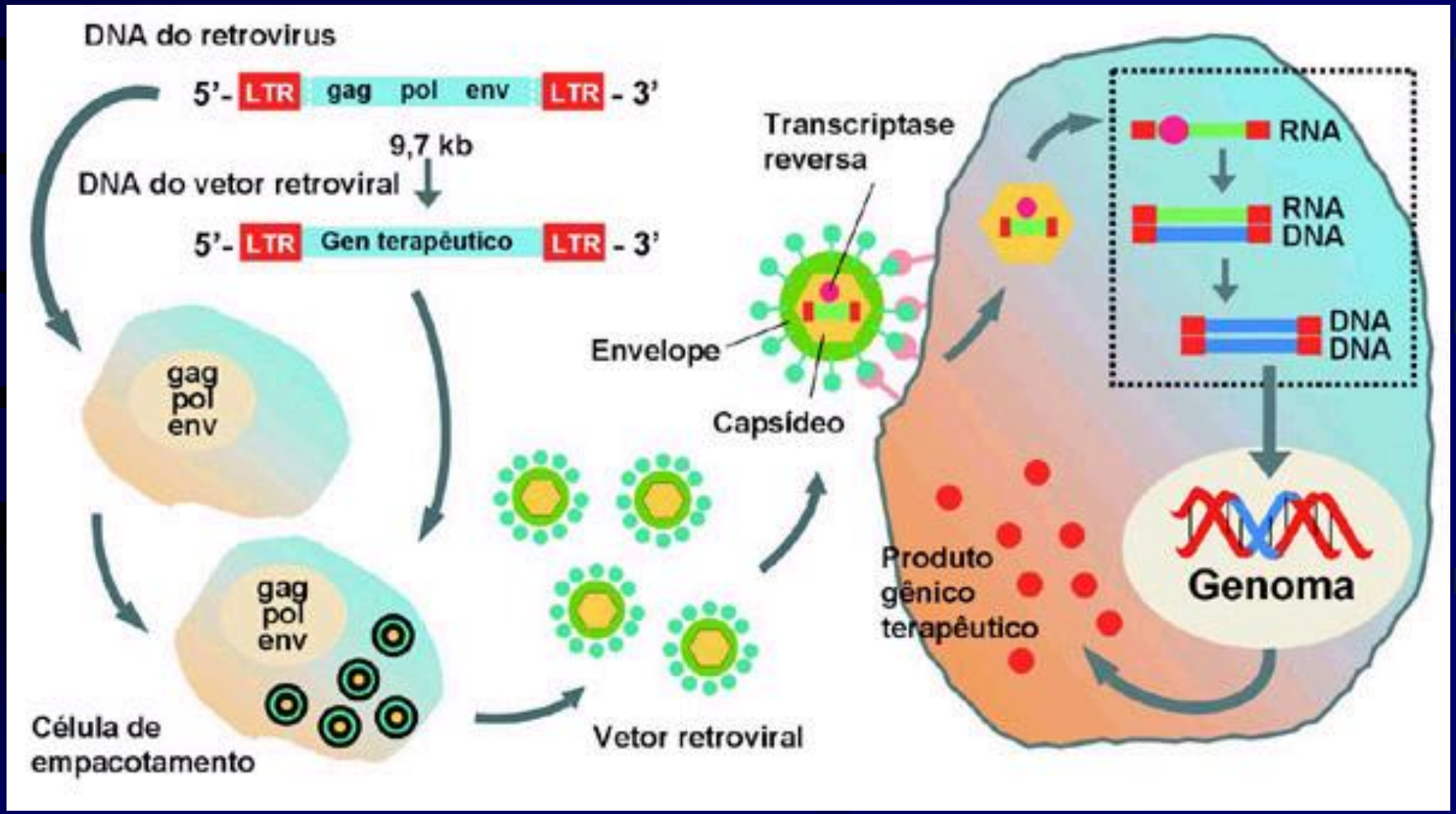
Vacinas de Terceira Geração

- Vacinas compostas por genes que codificam importantes imunógenos.

- Podemos fazer de genes importantes vacinas ?



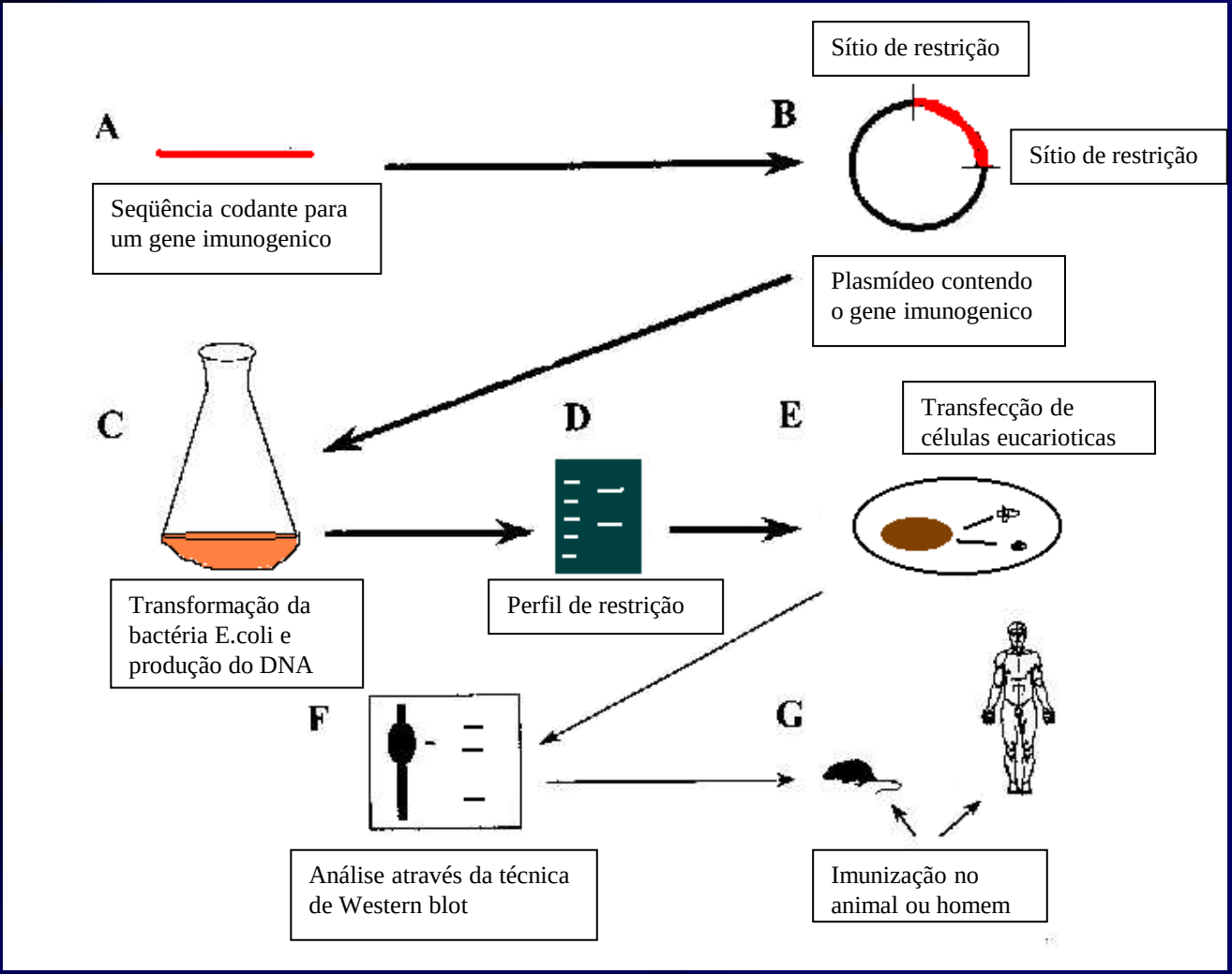
As Vacinas de DNA nasceram da Terapia Gênica



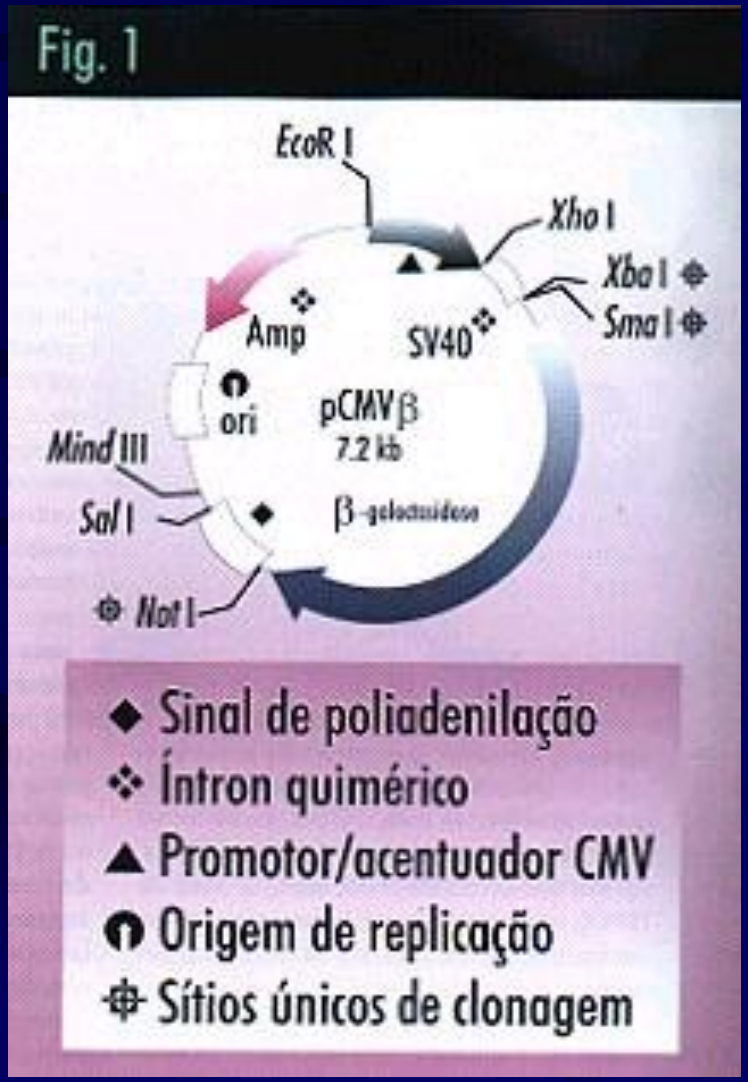
Comparação entre as vacinas tradicionais e de DNA

Características	Organismo vivo, atenuado	organismo morto ou subunidade	Vacina de DNA
Indução de $CD8^+$ CTL	Sim	Pouco comum	Sim
Indução de $CD4^+$ T helper	Sim	Sim	Sim
Fácil de preparar e purificar	Não	Não	Sim
Estabilidade ao calor	Pouco comum	Pouco comum	Sim
Barato	Não	Não	Sim

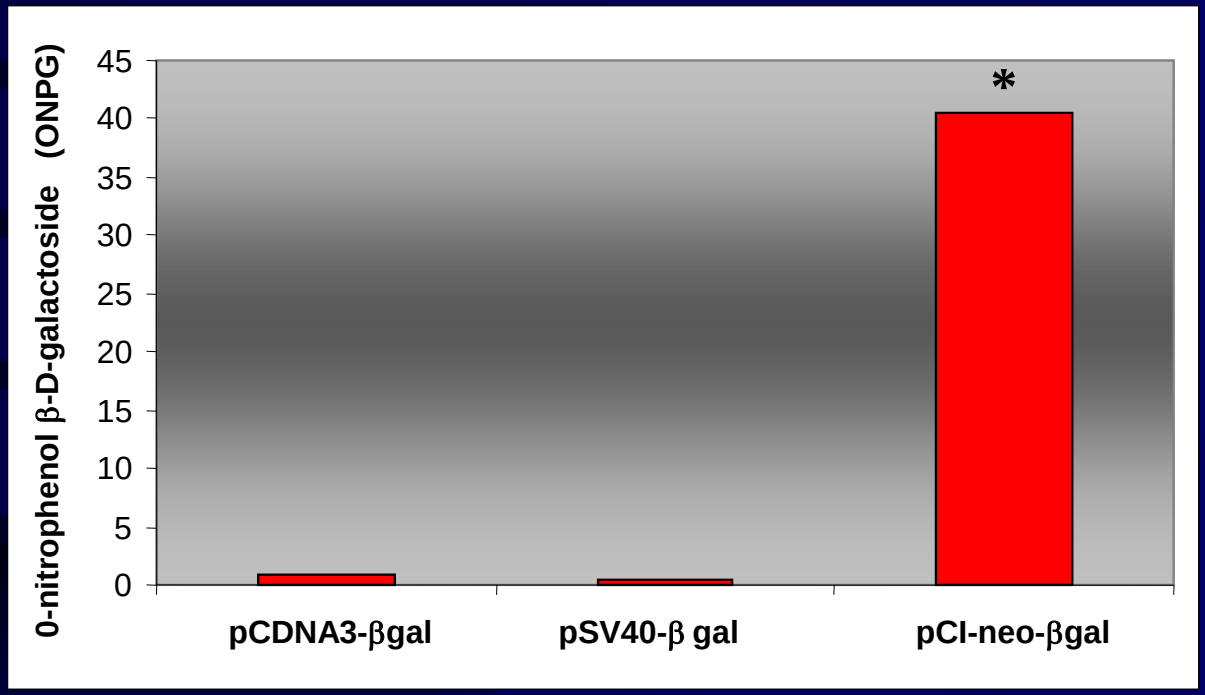
Esquema de produção de uma vacina de DNA



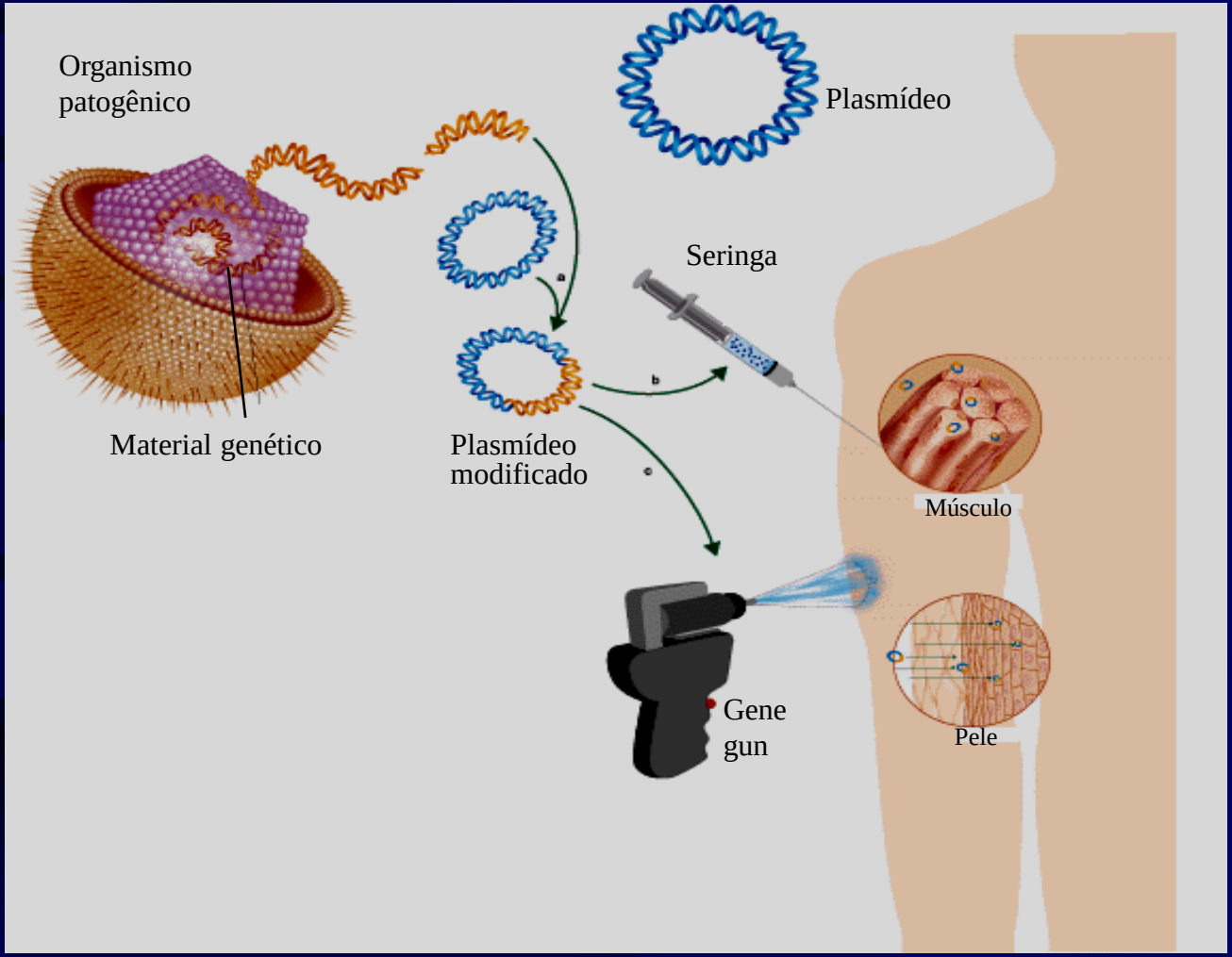
Elementos importantes em um plasmídeo utilizado em uma vacina de DNA



Comparação do nível de expressão de β-galactosidase plasmideos utilizados em vacinas de DNA

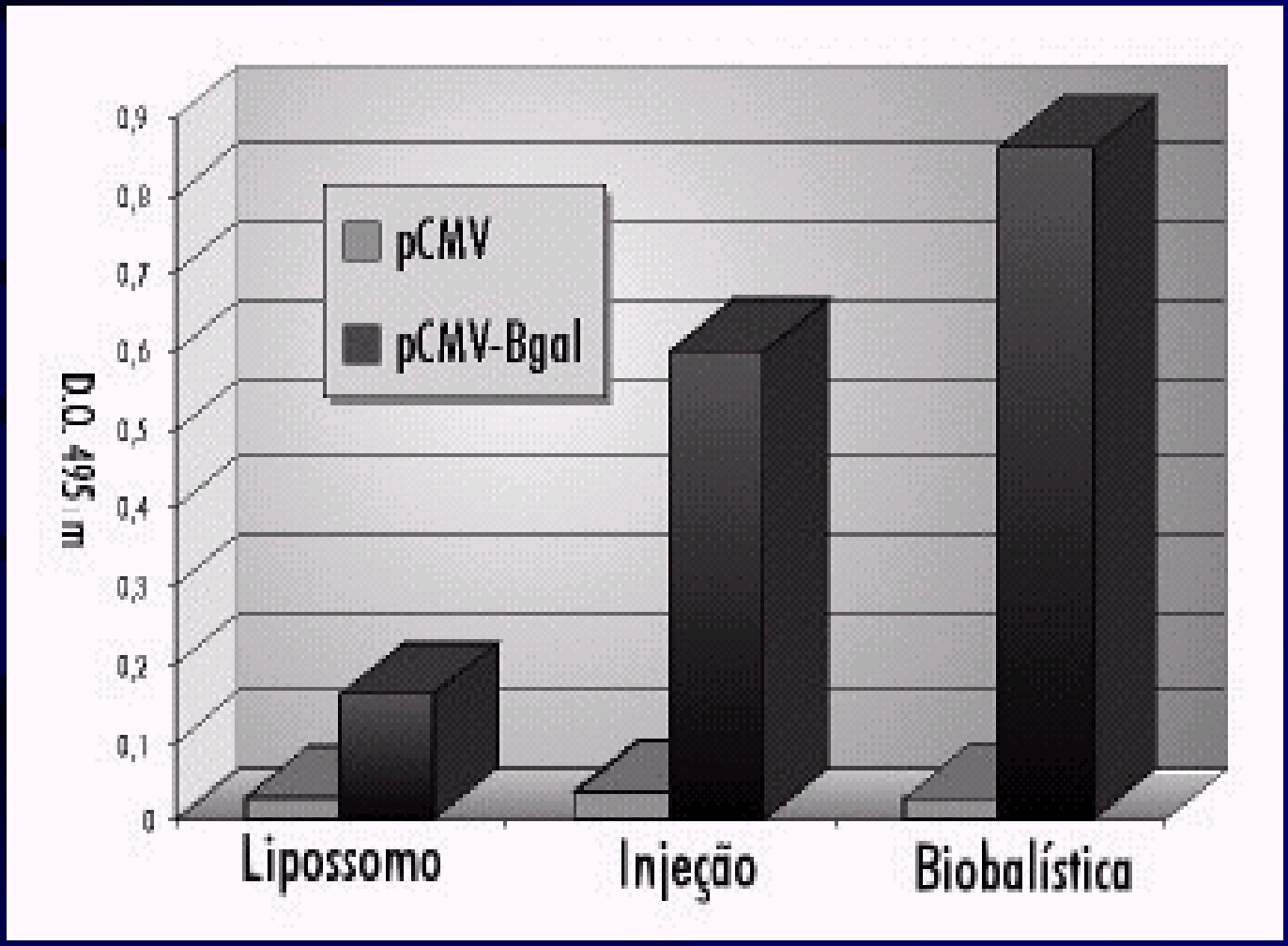


Rotas de Imunização



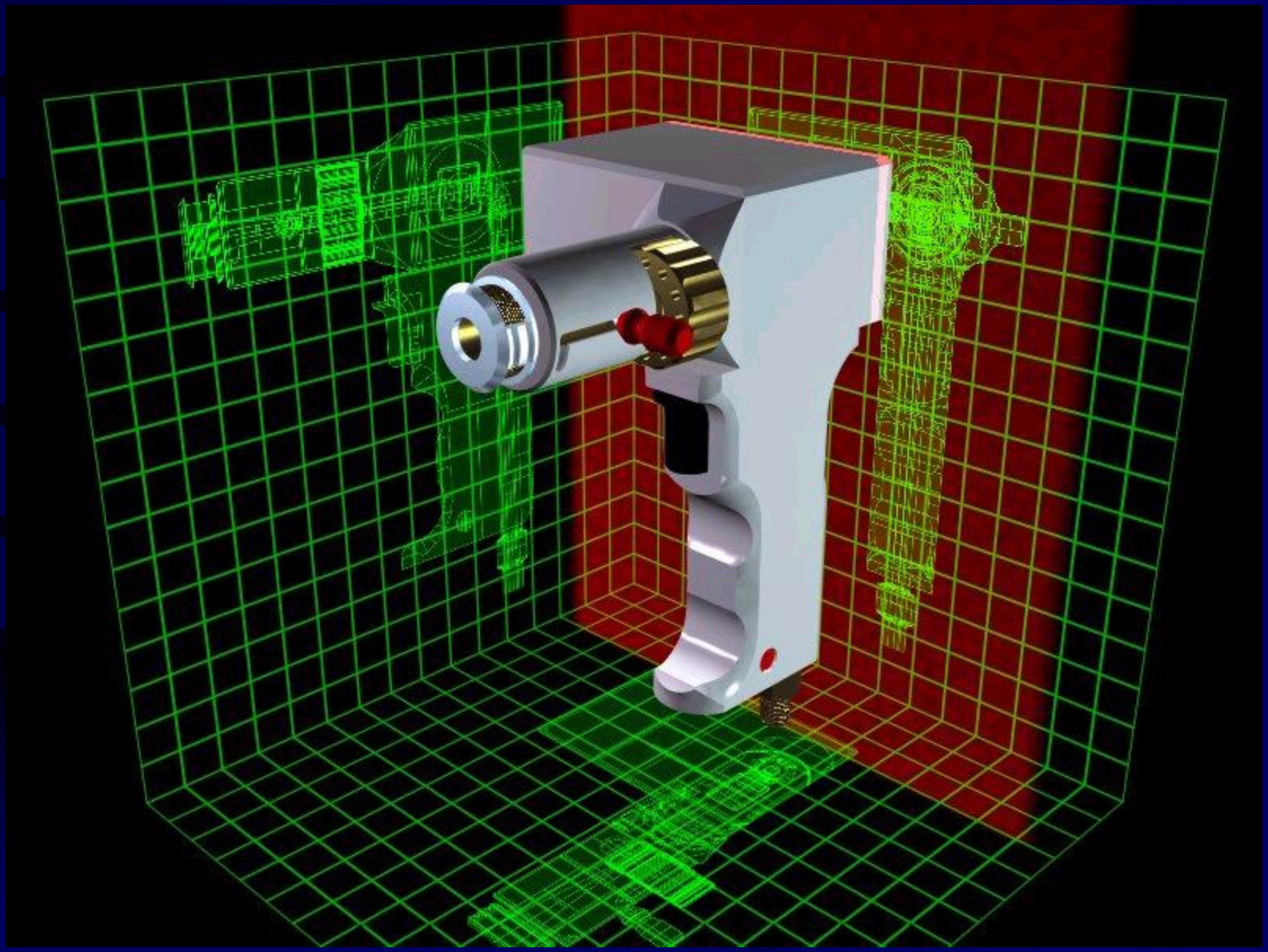
Oliveira SC, Rosinha GM, Brito CFA, et al. (1999). Gene Vaccines: Immunological properties of different delivery systems. *Braz. J. Med Biol Res*. 32(2):207-214.

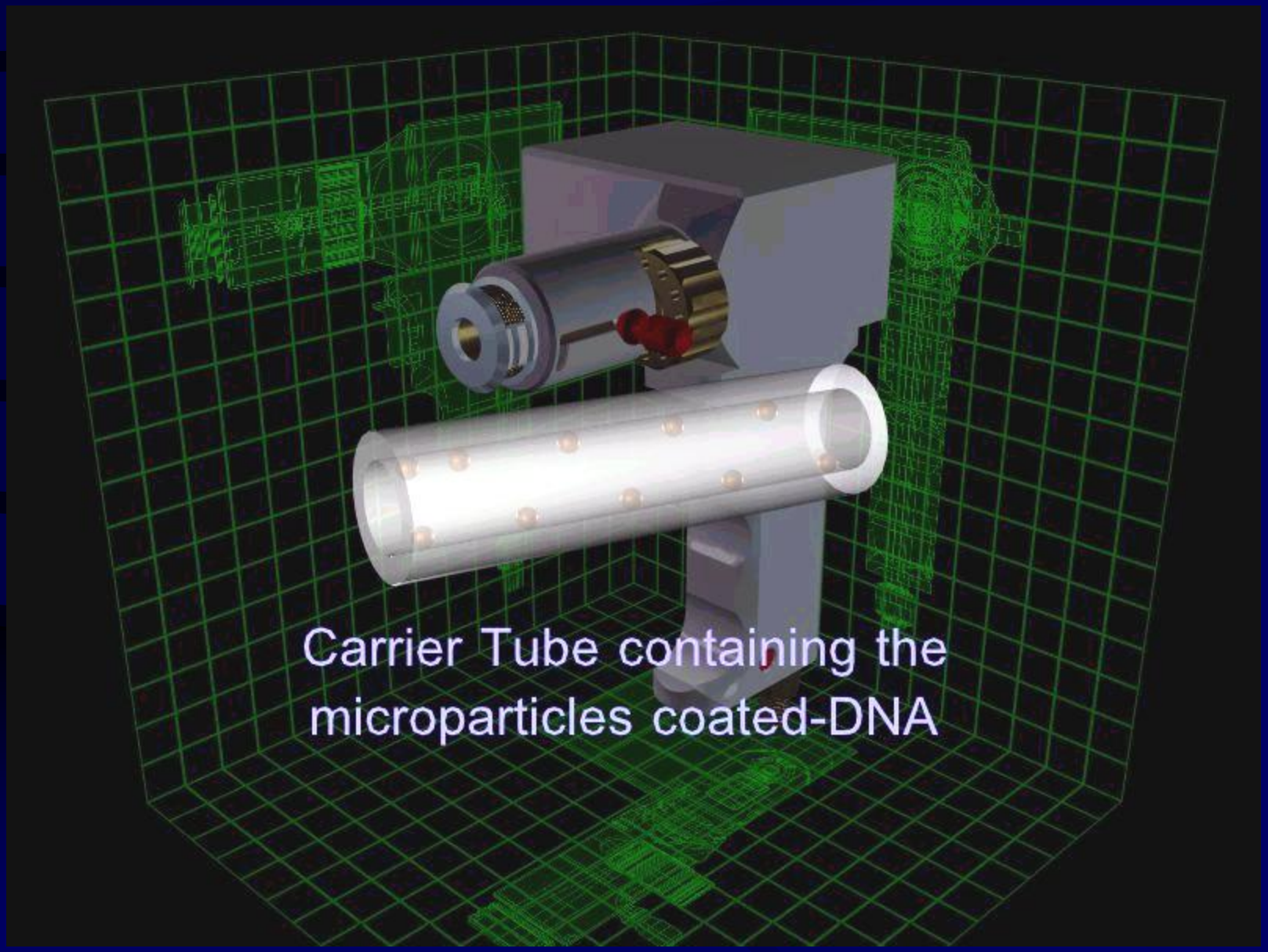
Níveis de IgG anti-βgal utilizando diferentes rotas de imunização

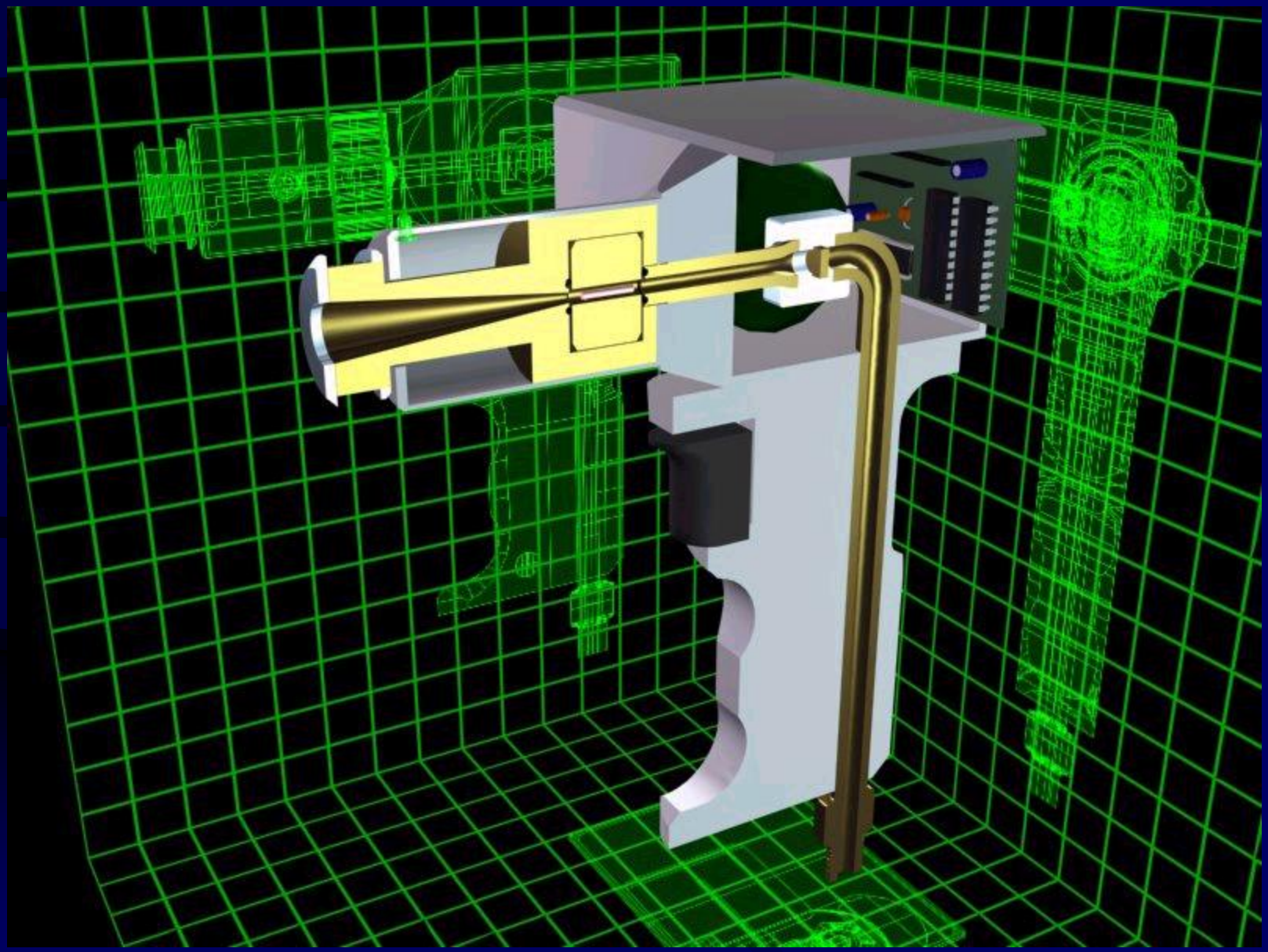


Azevedo V, Oliveira S.C. 1998. Vacinas de DNA: O Paradigma das Vacinas gênicas. Biotecnolgia Ciência e Desenvolvimento 5:40-43.

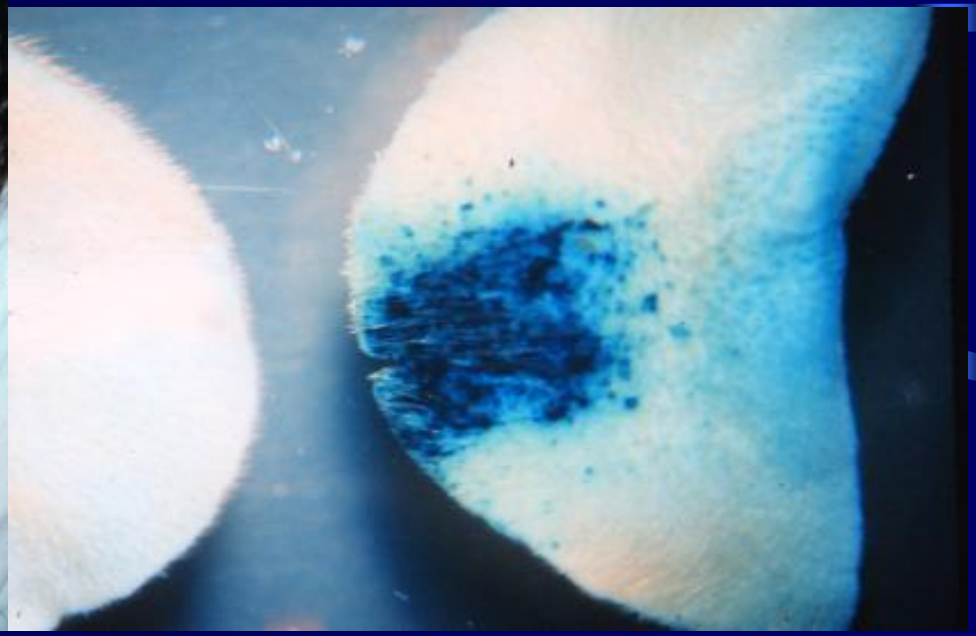
Gene gun ou Biobalísitca




Carrier Tube containing the
microparticles coated-DNA

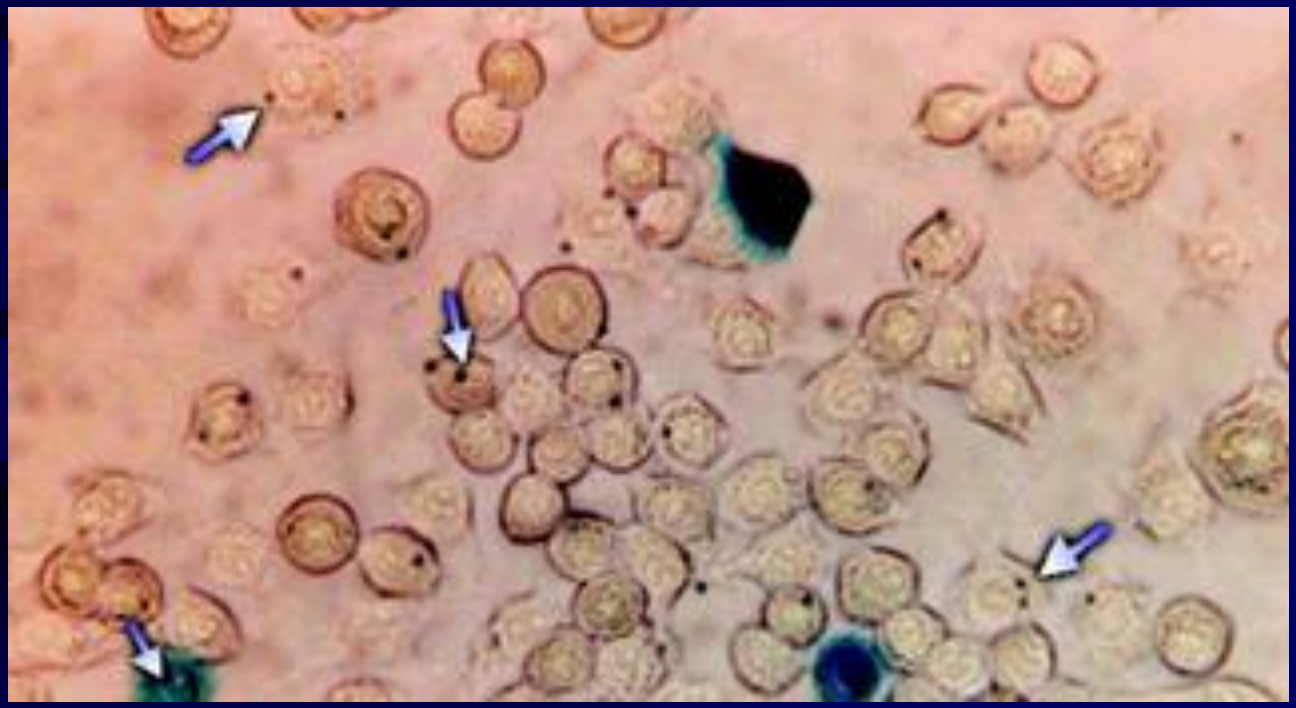


Gene Gun

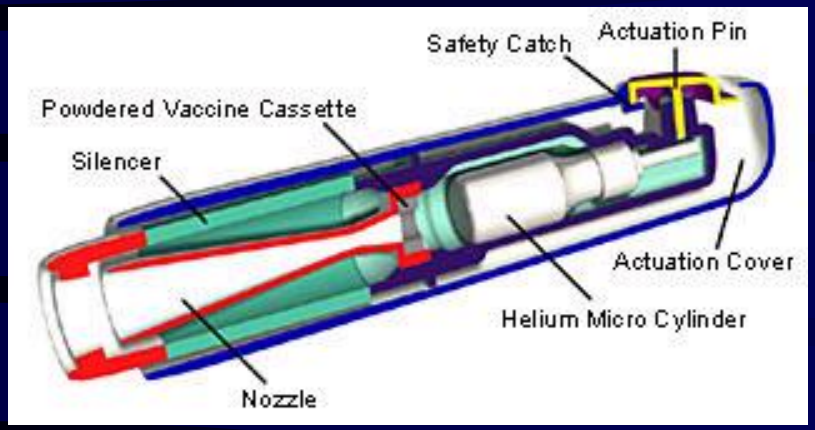


Oliveira SC, Harms JS, Rosinha GMS, Rodarte RS, Rech EL, Splitter GA (2000). Biolistic-mediated gene transfer of bovine herpesvirus-1 glycoprotein D induces neutralizing antibody in its natural host. Journal of Immunological Methods 245:109-118.

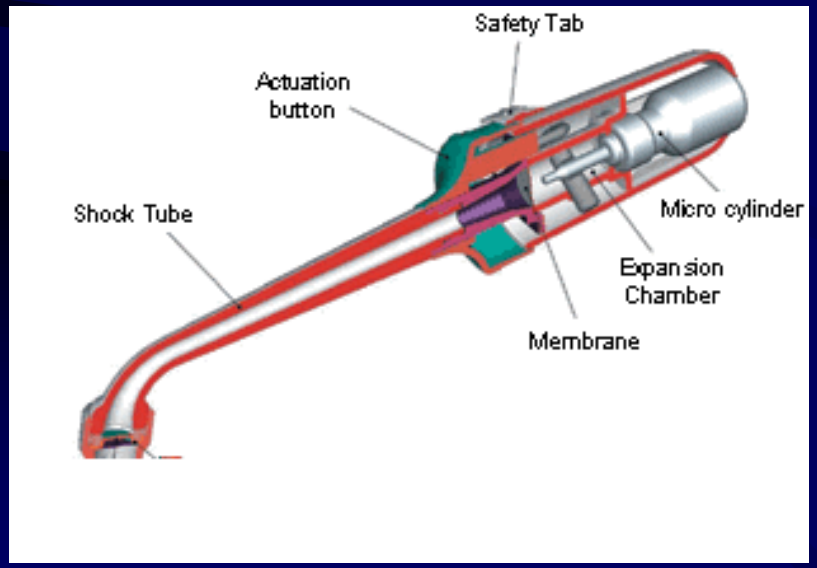
Expressão de β-galactosidase in vitro



Novos equipamentos descartáveis



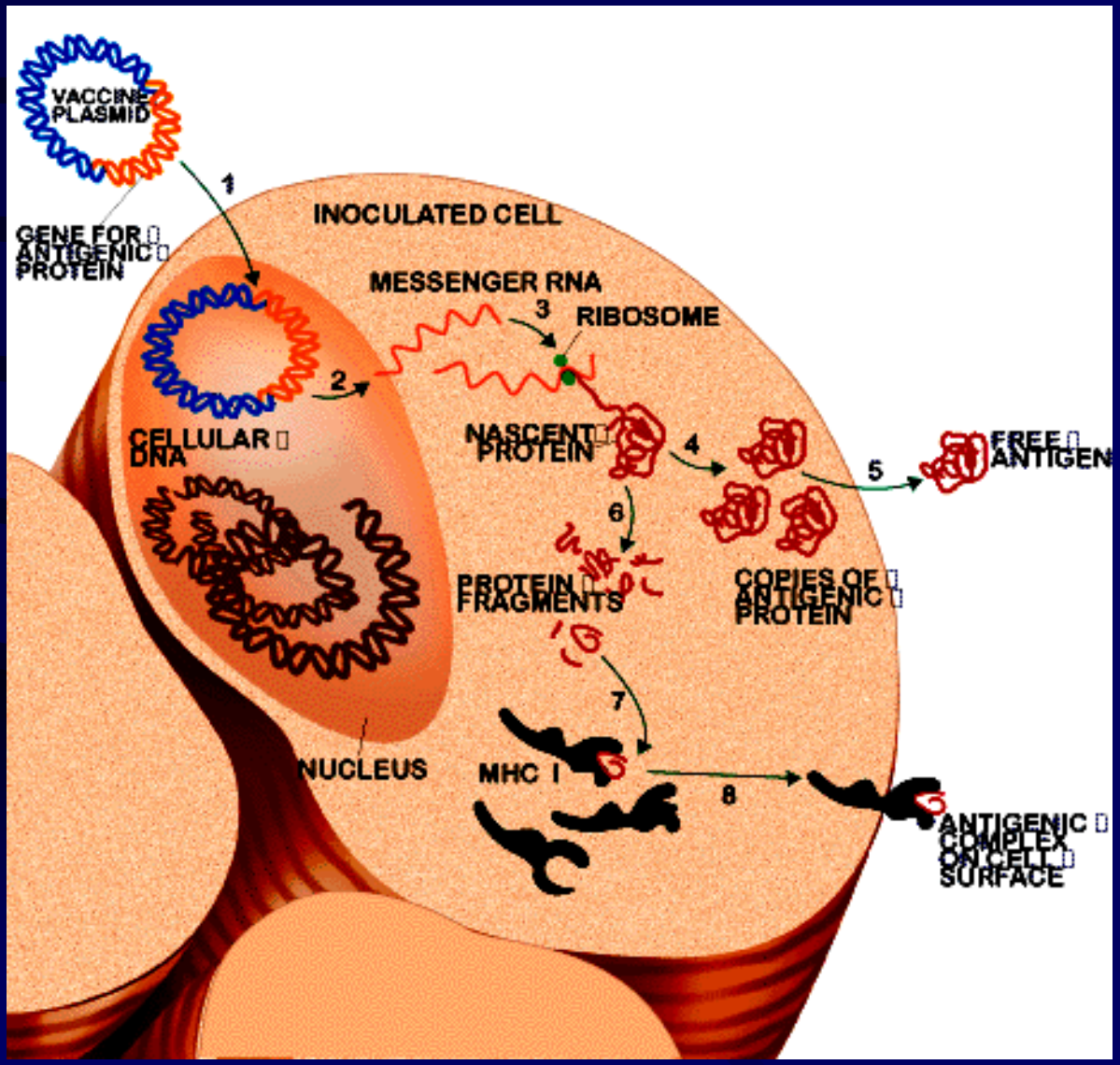
epidermal



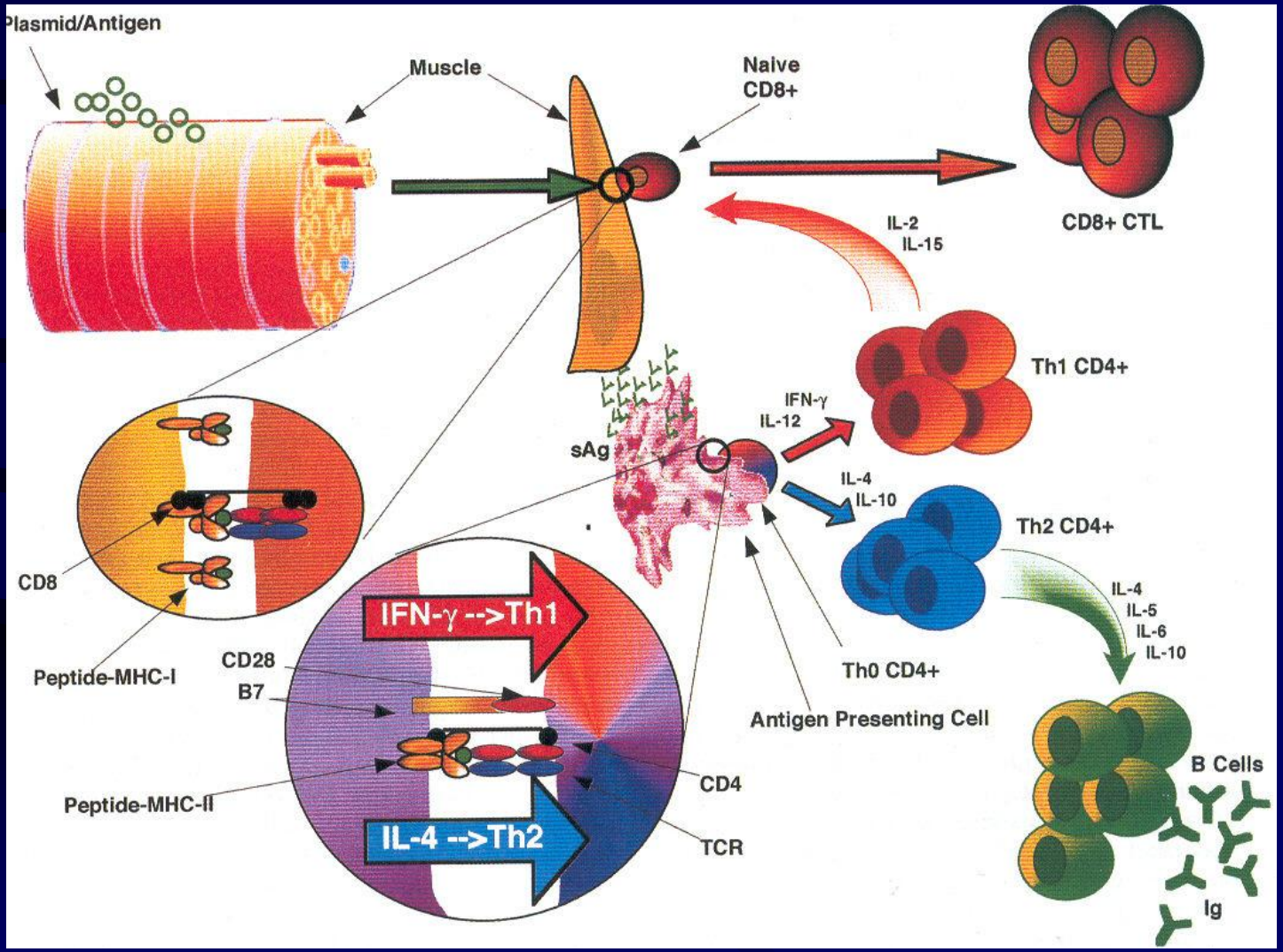
oral

Injeção Intramuscular

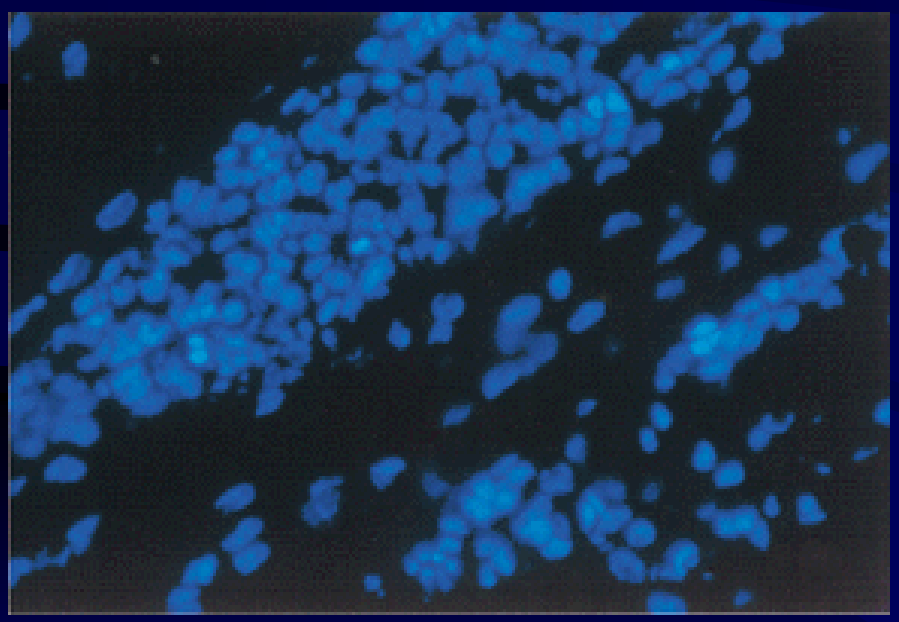
Apresentação de antígeno na imunização gênica

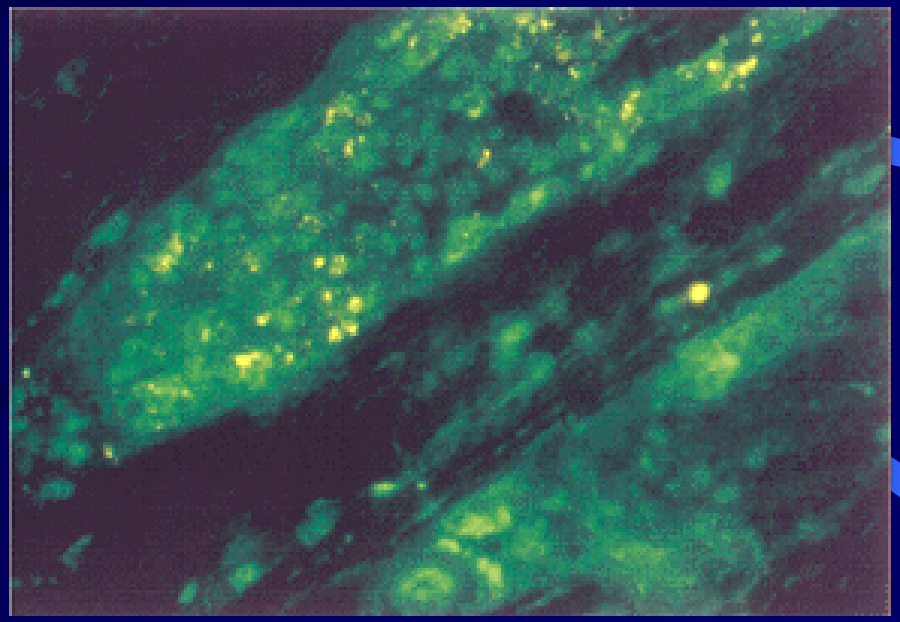


Resposta imune gerada

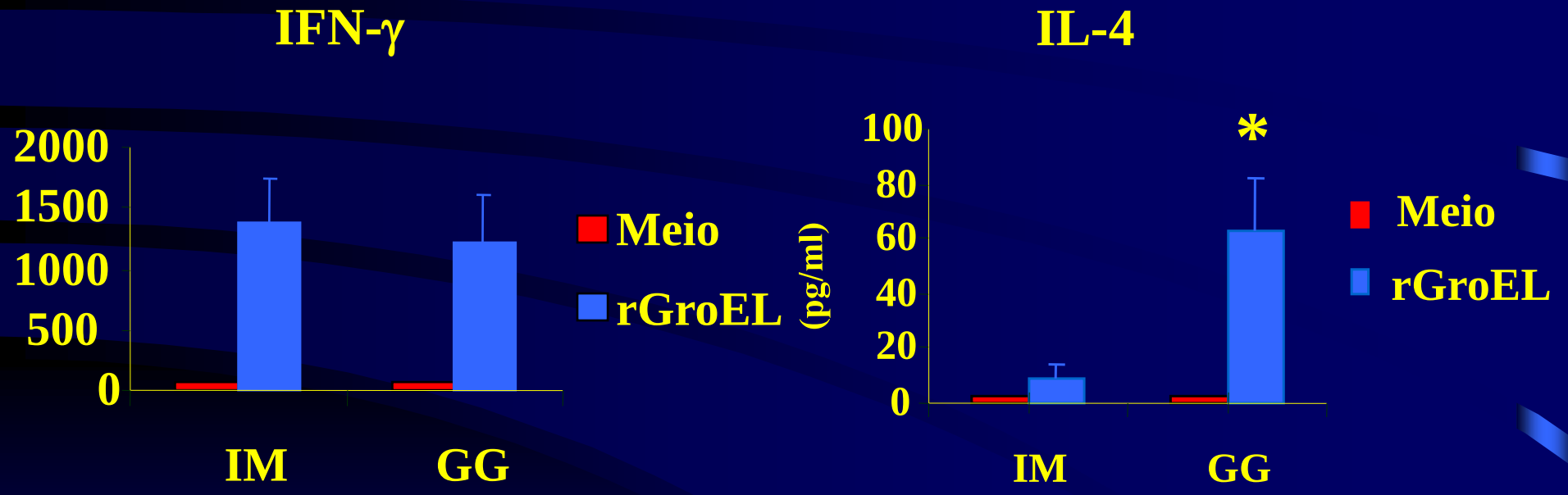


Células Musculares expressando o antígeno de interesse



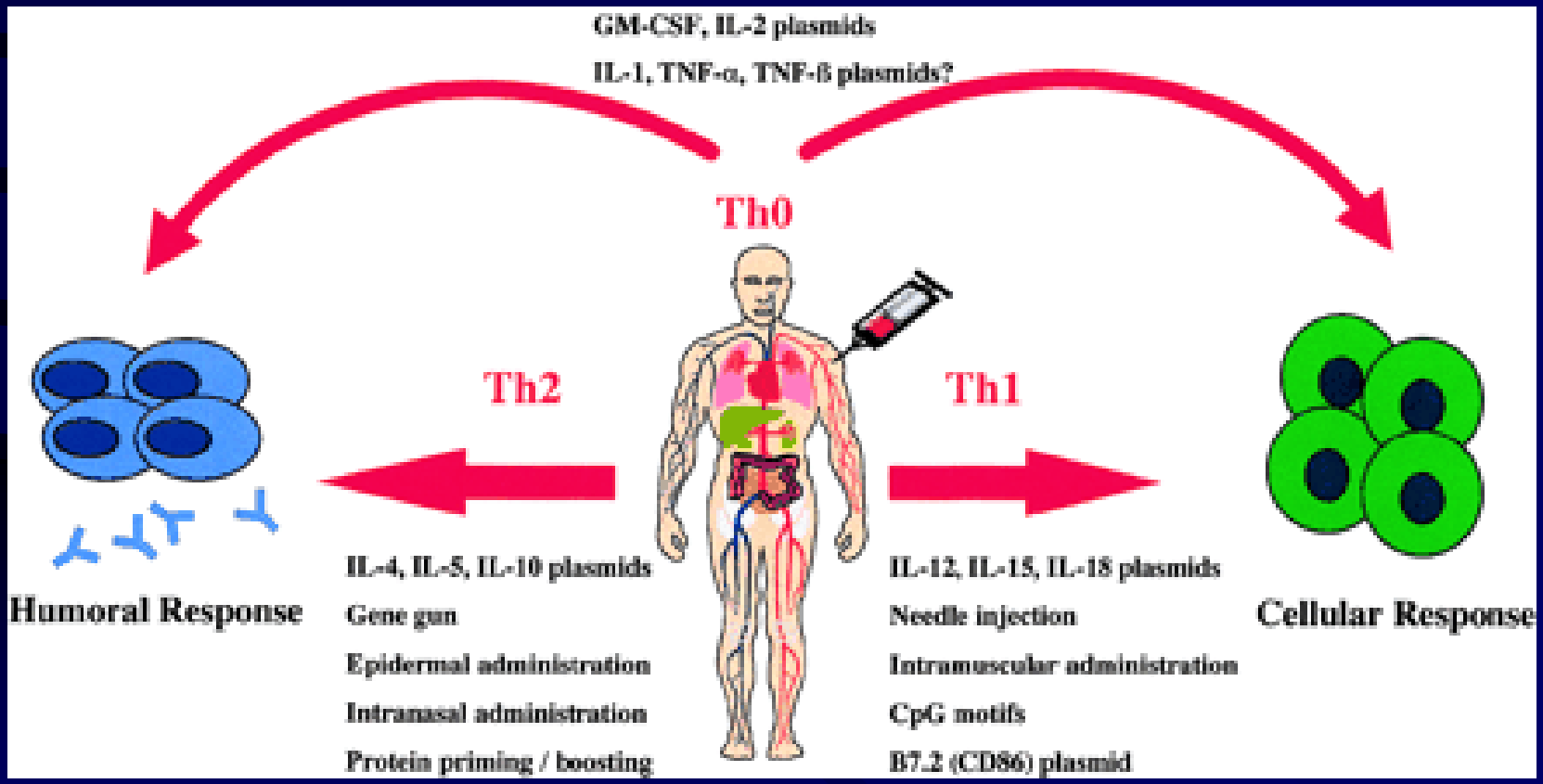


Análise da produção de citocinas após imunização com o gene que codifica a proteína de choque térmico GroEL



* p< 0,05

Mecanismos utilizados para modular a resposta imune na imunização gênica



O uso de DNA encapsulado em microesferas para potenciar a resposta imune

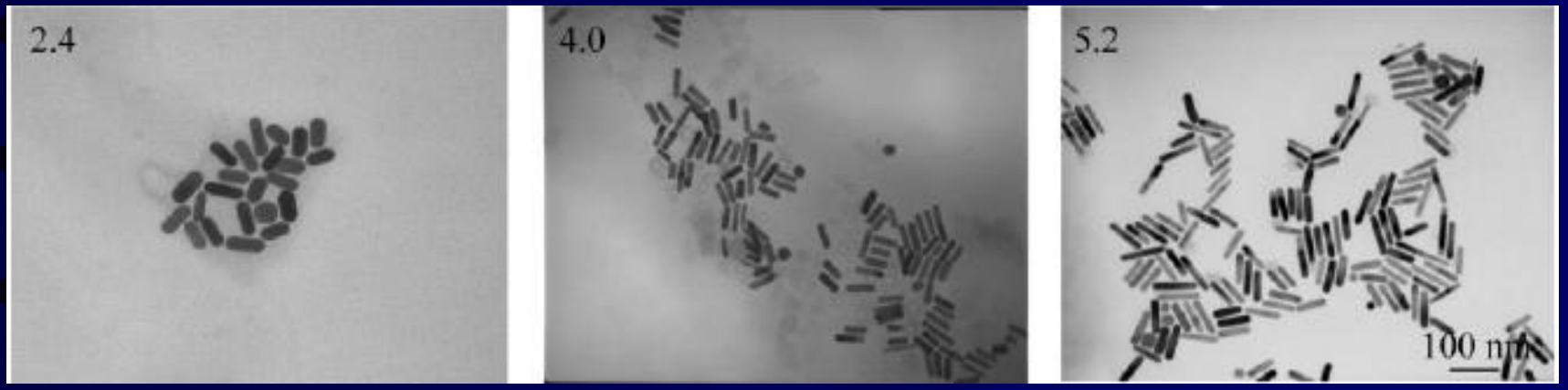
INTRODUÇÃO

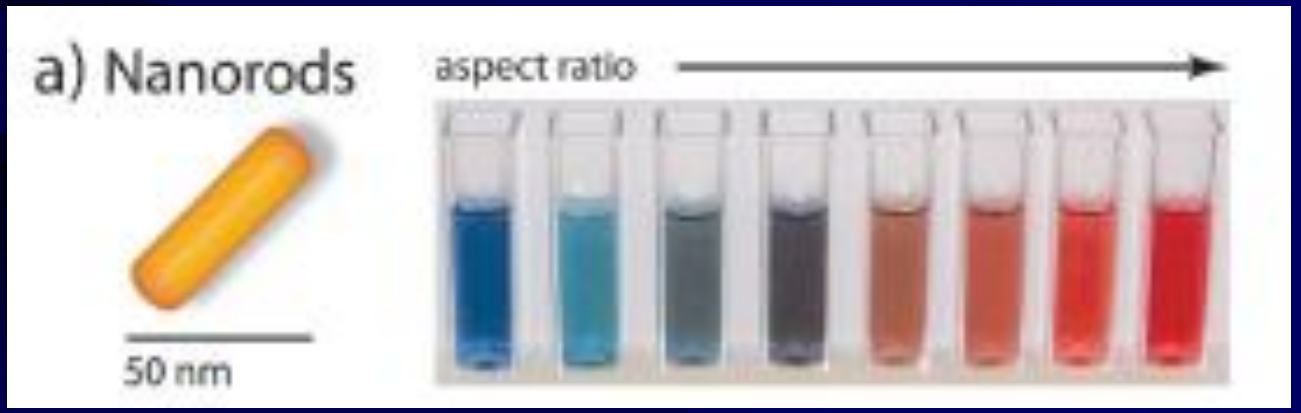
Nanotecnologia

Nanos = Anão

1nm ——— 100nm

Nanobastões de Ouro

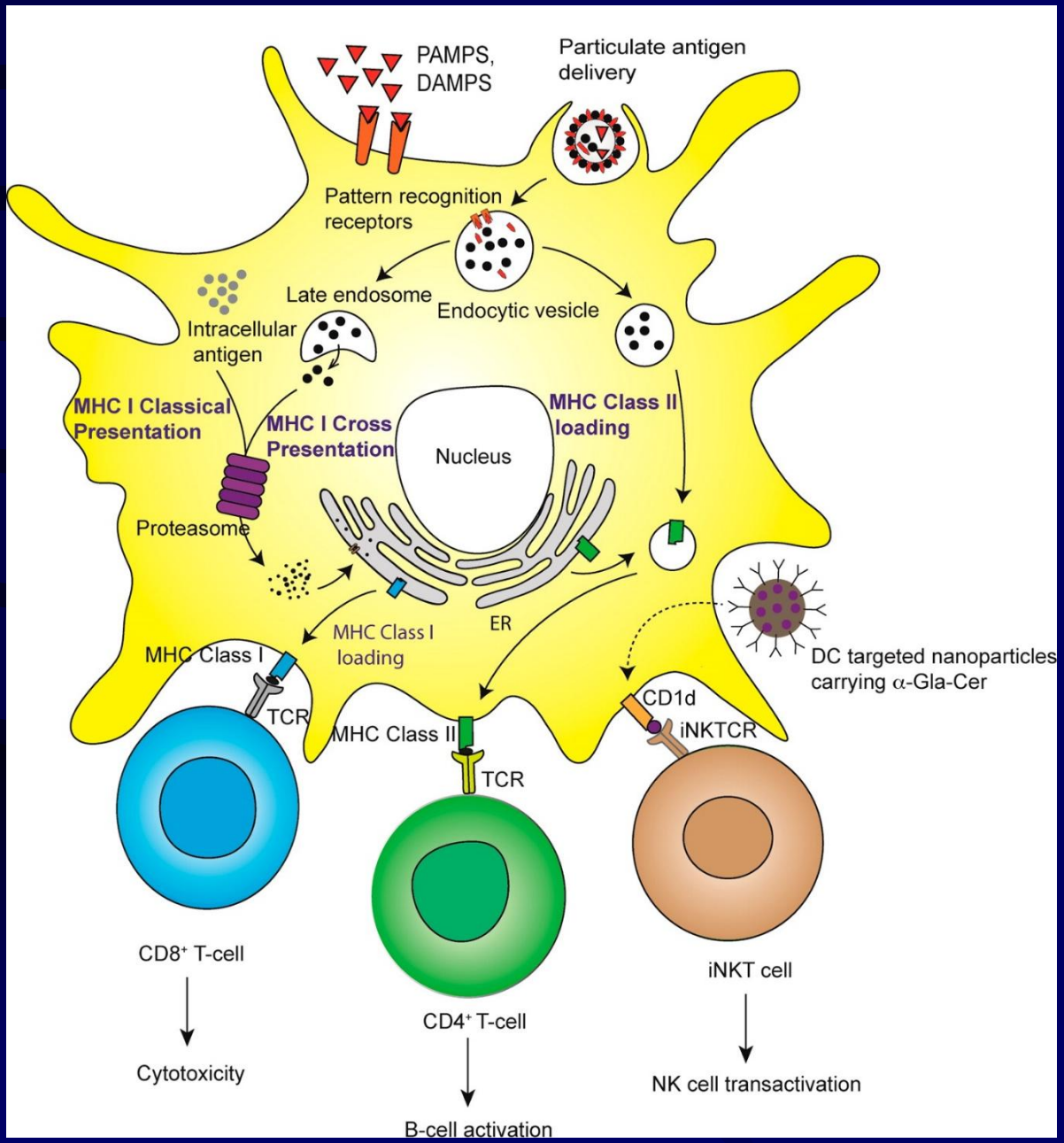




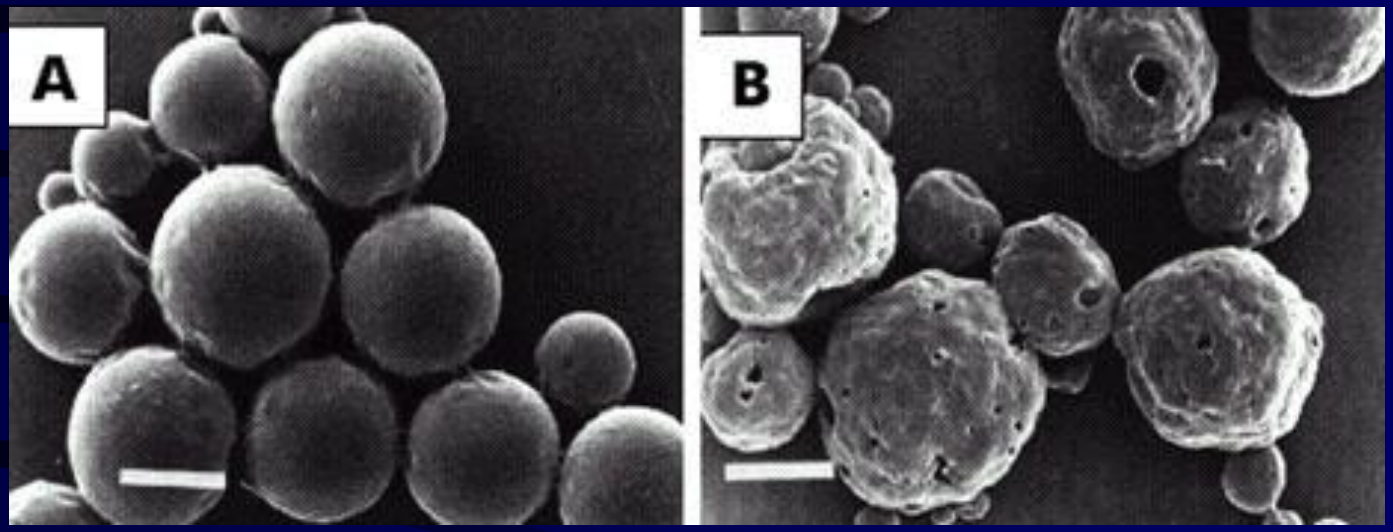
Dreaden *et al.*, 2012

Nanobastões de Ouro

Apresentação de antígeno encapsulado nas microesferas



Microesferas de PLGA



Microesferas após o preparo

Microesferas após a liberação do antígeno

BIOSSEGURANÇA DAS VACINAS GÊNICAS

Preocupações com a Biossegurança na utilização das vacinas de DNA

1. Integração do plsmídeo no genoma da célula do hospedeiro

1.1. Ativação de oncogenes
1.2. Inativação de genes supressores de tumor

2. Risco potencial de uma reação autoimune

Cálculo da frequência de mutação induzida

-A probabilidade de um plasmideo integrar no genoma
-de qualquer célula é de 1/150,000 (6.7×10^{-6}).

-A probabilidade de um plasmideo produzir uma mutação em um gene específico é de 1/5,000 (2×10^{-4}).

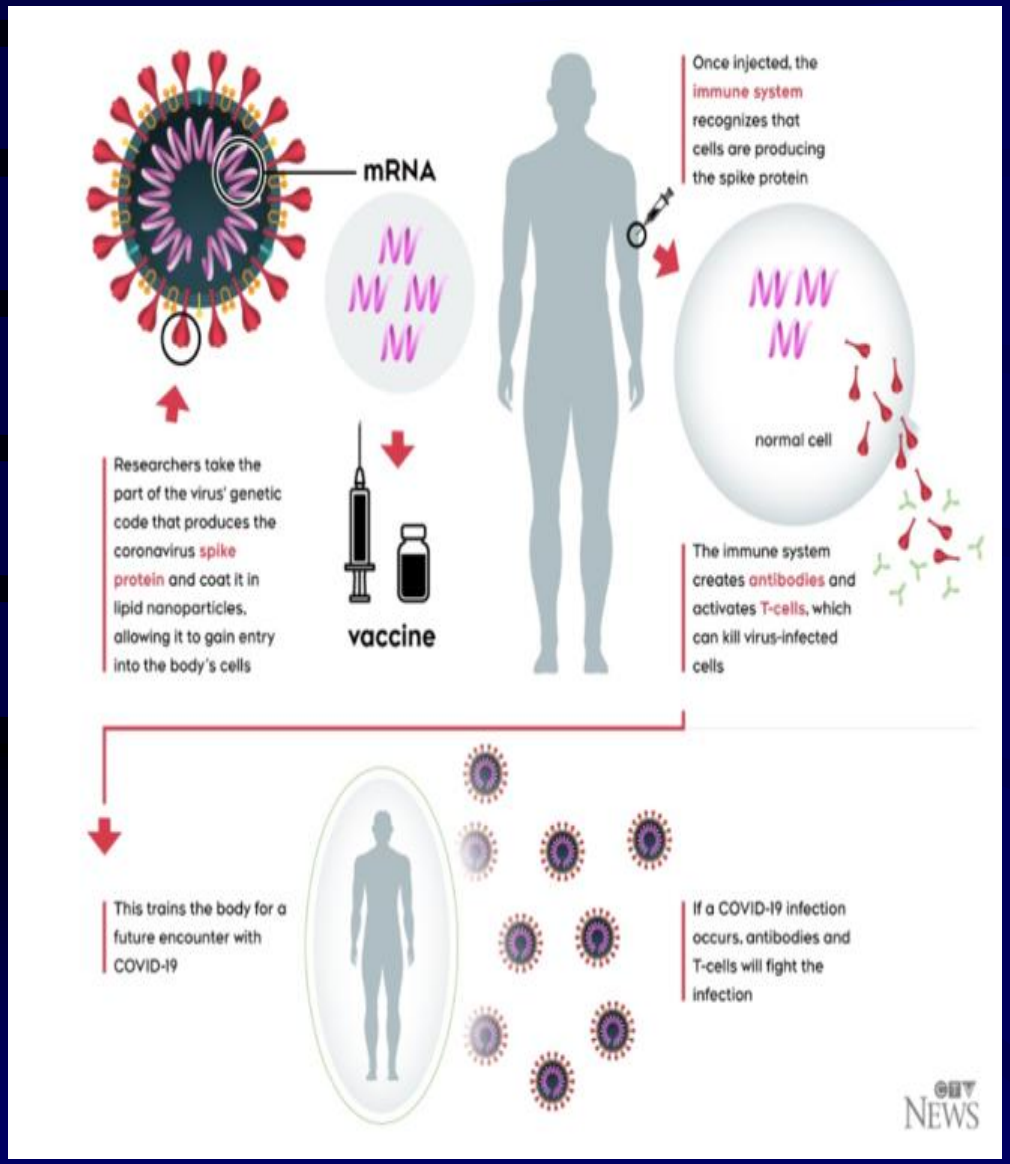
-A probabilidade de uma mutação induzida por uma integração do plasmideo é de:
$(2 \times 10^{-4}) \times (6.7 \times 10^{-6}) = 1.3 \times 10^{-9}$

- Sabendo que a frequência de mutação espontânea de uma célula é de 2×10^{-6}, a probabilidade de uma mutação causada pela vacina de DNA ocorrer é 1.000 vezes menor do que a frequência de mutação espontânea que ocorre em uma dada célula (Nichols et al. 1995).

Vacinas contra COVID-19

https://youtu.be/KZ9EM2NBhQI

mRNA Vaccine



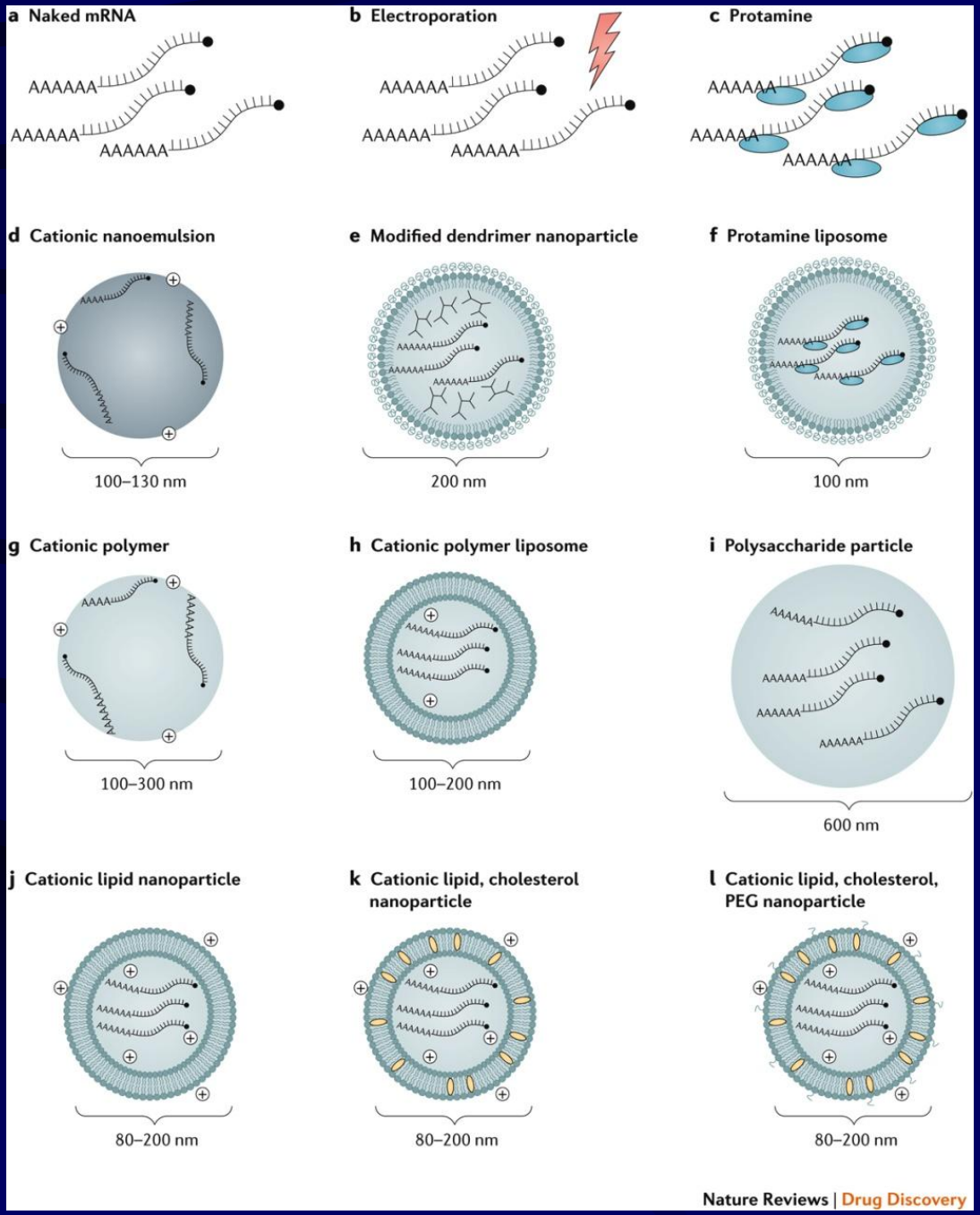
mRNA vaccines are a new type of vaccine to protect against infectious diseases. To trigger an immune response, many vaccines put a weakened or inactivated germ into our bodies. Not mRNA vaccines.

Instead, they teach our cells how to make a protein—or even just a piece of a protein—that triggers an immune response inside our bodies. That immune response, which produces antibodies, is what protects us from getting infected if the real virus enters our bodies.

Table 1 | **mRNA vaccine complexing strategies for *in vivo* use**

Delivery system type	Route of delivery	Species	Target
Commercial transfection reagent	i.n.	Mouse	OVA[145]
Protamine	i.d.	Mouse, ferret, pig and human	Influenza virus[18,52], melanoma[150], non-small-cell lung cancer[200], prostate cancer[36,52,151], rabies virus[56], OVA[36,52,155] and Lewis lung cancer[155]
Protamine liposome	i.v.	Mouse	Lung cancer[201]
Polysaccharide particle	s.c.	Mouse and rabbit	Influenza virus[98]
Cationic nanoemulsion	i.m.	Mouse, rabbit, ferret and rhesus macaque	Influenza virus[96], RSV[50], HIV-1 (REFS 50,97), HCMV[50], *Streptococcus* spp.[100], HCV and rabies virus[87]
Cationic polymer	s.c. and i.n.	Mouse	Influenza virus[99], andHIV-1 (REFS 110,111)
Cationic polymer liposome	i.v.	Mouse	Melanoma[202,203], pancreatic cancer[204]
Cationic lipid nanoparticle	i.d., i.v. and s.c.	Mouse	HIV-1 (REF. 109) and OVA[152]
Cationic lipid, cholesterol nanoparticle	i.v., s.c. and i.s.	Mouse	Influenza virus[59,108], melanoma[59,141], Moloney murine leukaemia virus, OVA, HPV andc olon cancer[59]
Cationic lipid, cholesterol, PEG nanoparticle	i.d., i.m. and s.c.	Mouse, cotton rat and rhesus macaque	Zika virus[20,85,112], influenza virus[22,94,95,205], RSV[19], HCMV, rabies virus[87] and melanoma[153]
Dendrimer nanoparticle	i.m.	Mouse	Influenza virus, Ebola virus, *Toxoplasma gondii*[89] and Zika virus[88]

HCMV, human cytomegalovirus; HCV, hepatitis C virus; HPV, human papillomavirus; i.d., intradermal; i.m., intramuscular; i.n., intranasal; i.s., intrasplenic; i.v., intravenous; OVA, ovalbumin-expressing cancer models; PEG, polyethylene glycol; RSV, respiratory syncytial virus; s.c., subcutaneous.


a Naked mRNA
AAAAAA
AAAAAA
AAAAAA
b Electroporation
AAAAAA
AAAAAA
AAAAAA
c Protamine
AAAAAA
AAAAAA
AAAAAA
d Cationic nanoemulsion
100–130 nm
e Modified dendrimer nanoparticle
200 nm
f Protamine liposome
100 nm
g Cationic polymer
100–300 nm
h Cationic polymer liposome
100–200 nm
i Polysaccharide particle
AAAAAA
AAAAAA
AAAAAA
600 nm
j Cationic lipid nanoparticle
80–200 nm
k Cationic lipid, cholesterol nanoparticle
80–200 nm
l Cationic lipid, cholesterol, PEG nanoparticle
80–200 nm
Nature Reviews | Drug Discovery

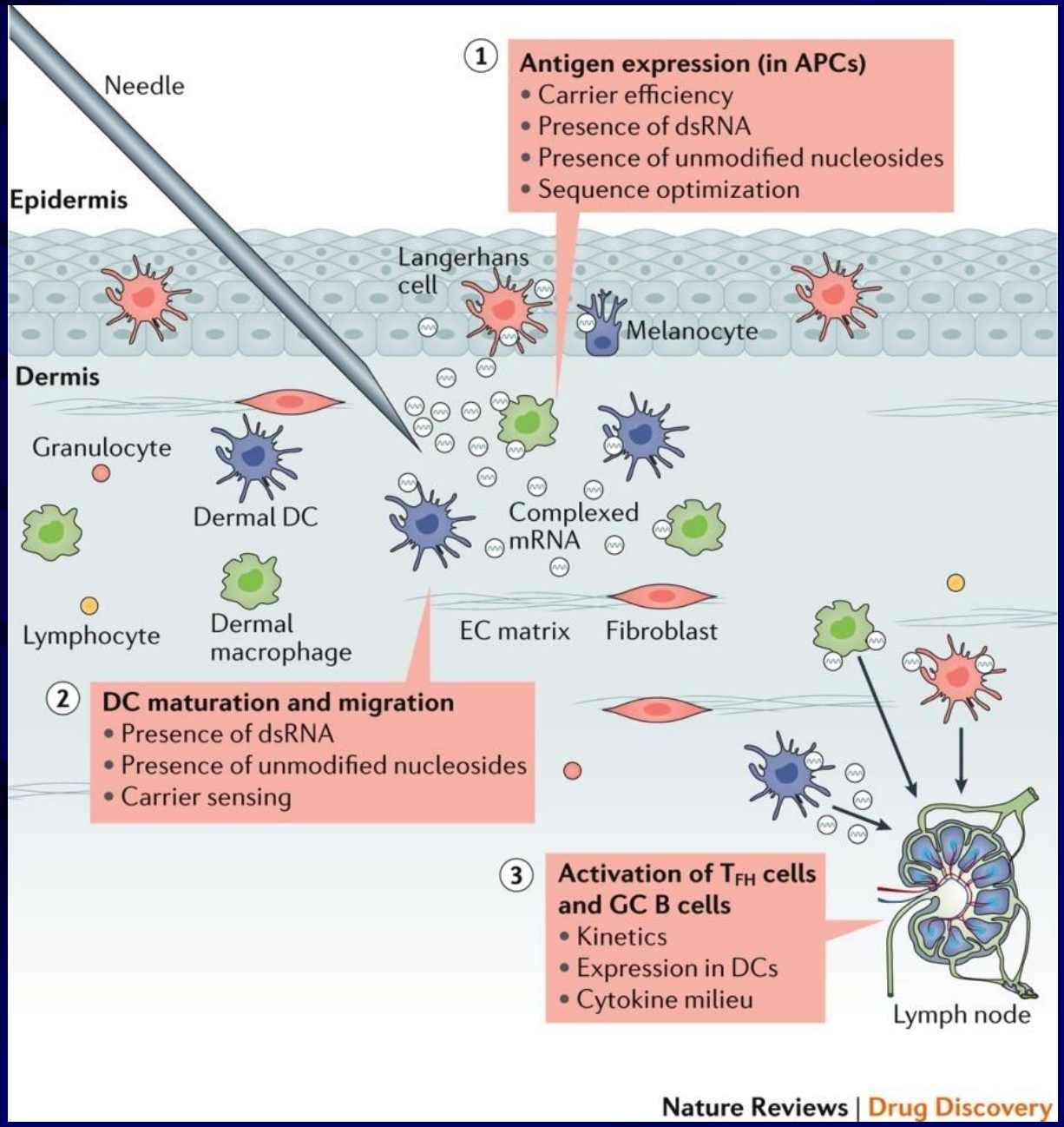

Needle
1
Antigen expression (in APCs)
• Carrier efficiency
• Presence of dsRNA
• Presence of unmodified nucleosides
• Sequence optimization
Epidermis
Langerhans cell
Melanocyte
Dermis
Granulocyte
Dermal DC
Complexed mRNA
Lymphocyte
Dermal macrophage
EC matrix
Fibroblast
2
DC maturation and migration
• Presence of dsRNA
• Presence of unmodified nucleosides
• Carrier sensing
3
Activation of T_{FH} cells and GC B cells
• Kinetics
• Expression in DCs
• Cytokine milieu
Lymph node
Nature Reviews | Drug Discovery

Table 2 | **Clinical trials with mRNA vaccines against infectious diseases**

Sponsoring institution	Vaccine type (route of administration)	Targets	Trial numbers (phase)	Status
Argos Therapeutics	DC EP with autologous viral Ag and CD40L mRNAs (i.d.)	HIV-1	• NCT00672191 (II) • NCT01069809 (II) • NCT02042248 (I)	• Completed[105] • Completed; results NA • Completed; results NA
CureVac AG	RNActive viral Ag mRNA (i.m., i.d.)	Rabies virus	NCT02241135 (I)	Active[56,91]
Erasmus Medical Center	DC loaded with viral Ag mRNA with TriMix (i.nod.)	HIV-1	NCT02888756 (II)	Recruiting
Fundació Clínic per la Recerca Biomèdica	Viral Ag mRNA with TriMix (NA)	HIV-1	NCT02413645 (I)	Active
Massachusetts General Hospital	DC loaded with viral Ag mRNA (i.d.)	HIV-1	NCT00833781 (II)	Completed[104]
McGill University Health Centre	DC EP with autologous viral Ag and CD40L mRNAs (i.d.)	HIV-1	NCT00381212 (I/II)	Completed[102]
Moderna Therapeutics	Nucleoside-modified viral Ag mRNA (i.m.)	Zika virus	NCT03014089 (I/II)	Recruiting[85]
		Influenza virus	NCT03076385 (I)	Ongoing[22]

The table summarizes the clinical trials registered at ClinicalTrials.gov as of 5 May 2017. Ag, antigen; CD40L, CD40 ligand; DC, dendritic cell; EP, electroporated; i.d., intradermal; i.m., intramuscular; i.nod., intranodal; NA, not available.

Facts about COVID-19 mRNA Vaccines

- They CANNOT give someone COVID-19.
  - mRNA vaccines do not use the live virus that causes COVID-19.

- They DO NOT affect or interact with our DNA in any way.
  - mRNA never enters the nucleus of the cell, which is where our DNA (genetic material) is kept.
  - The cell breaks down and gets rid of the mRNA soon after it is finished using the instructions.

Vaccine Side Effects (Adverse Events)

- Side effects are expected
- When the body's immune system mounts a response to a natural infection OR vaccination the result is local and/or systemic inflammation
- Local inflammation: injection site soreness, redness, swelling
- Systemic inflammation includes fever, muscle aches, headache
- The stronger the immune response, the more prominent the side effects
- The healthier the individual, the stronger the immune response

Desafios para o desenvolvimento de vacinas

HIV

Antígenos alvo?
Qual é o tipo de resposta imune eficiente ?
Taxa de mutação e diferentes cepas virais

COVID-19

Taxa de mutação e diferentes cepas variantes

Doenças Parasitárias

variação antigênica
complexidade antigênica
Qual é o tipo de resposta imune eficiente ?
custo-benefício
baixos níveis de proteção

Cancer

identificação dos antígenos tumorais
utilização de adjuvantes para potenciar a resposta imune
níveis de imunoproteção