Disciplina: BMM0586 - Virologia

Vírus como ferramentas: os vetores virais

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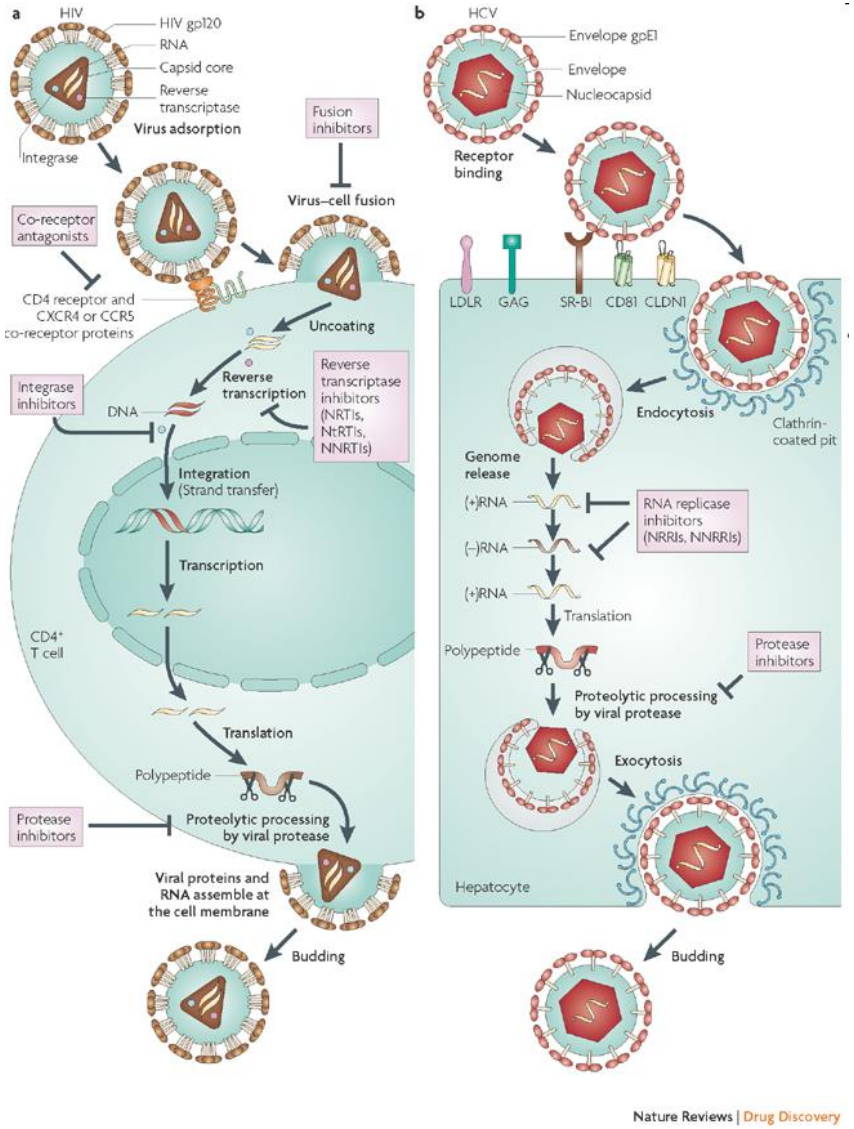

- ✓ **Os vetores virais são uma ferramenta promissora para a entrega eficaz de material genético em células.**
- ✓ **Consideram a capacidade natural de um vírus de transmitir a carga genética para as células**

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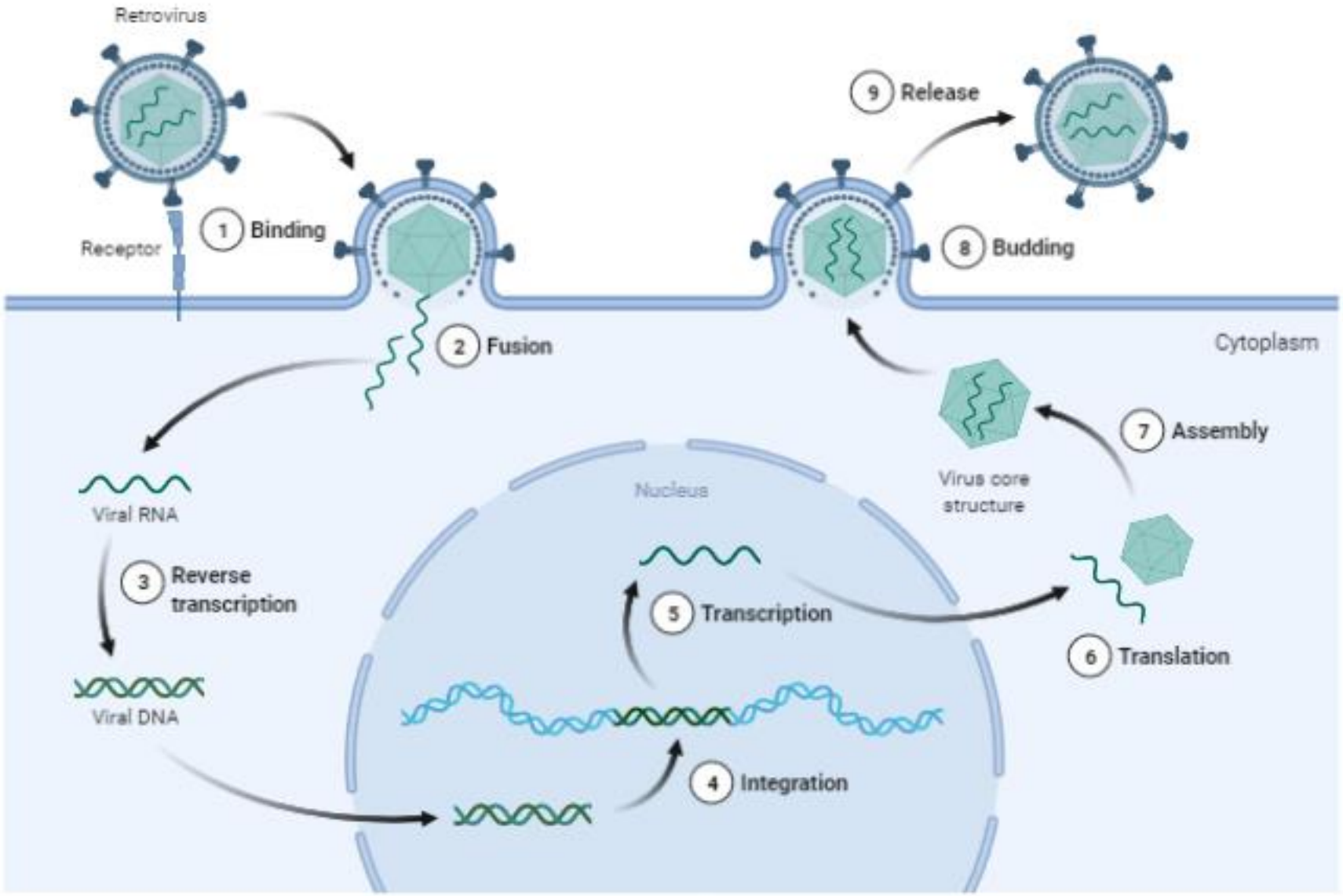
HIV



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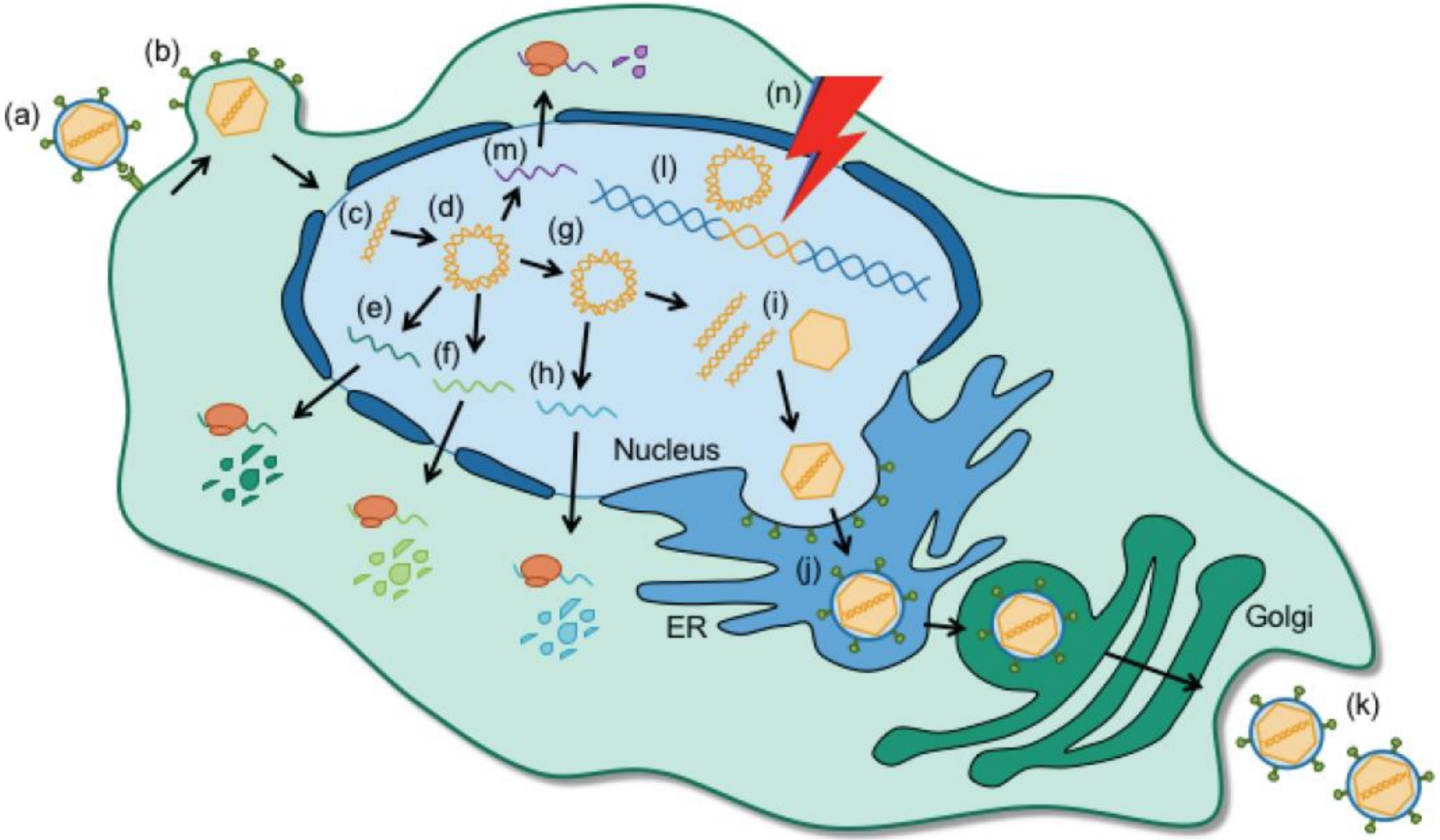
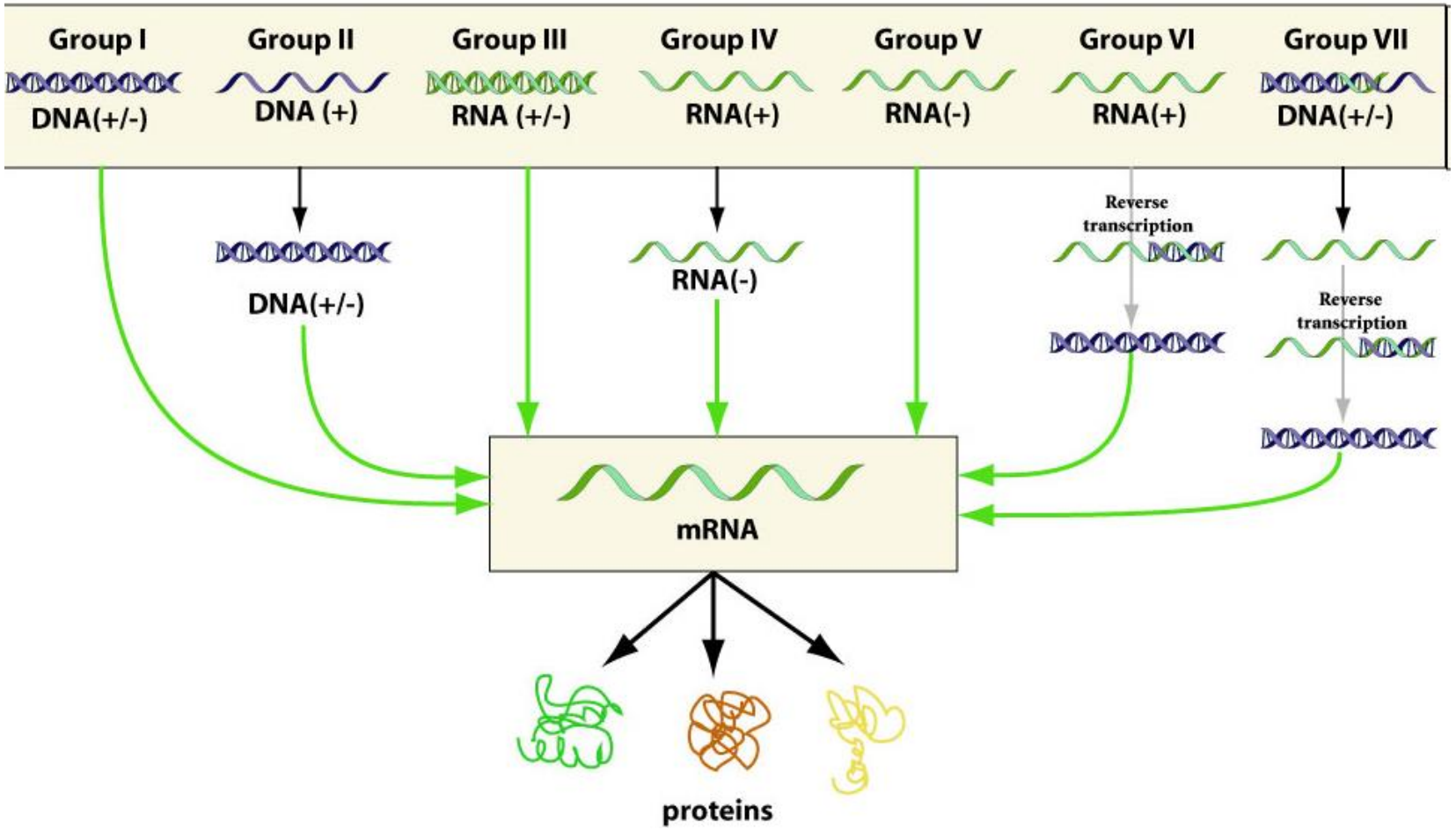
Herpes vírus (HSV)



Classificação pelo Sistema de Replicação viral

A classificação de Baltimore

- A estratégia de replicação do genoma viral depende da natureza do mesmo.



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	Adenoviridae	*Parvoviridae*	*Retroviridae*	*Retroviridae*
				
	ADENOVIRUS	AAV	γ-RETROVIRUS	LENTIVIRUS
SIZE	~90-100 nm	~25 nm	~80-100 nm	~80-100 nm
GENOME	dsDNA	ssDNA	ssRNA	ssRNA
PACKAGING CAPACITY	~8 kb – 36 kb	~4.7 kb	10 kb	8 kb
TRANSDUCTION	Dividing and non-dividing cells	Dividing and non-dividing cells	Dividing cells	Dividing and non-dividing cells
TRANSDUCTION EFFICIENCY	High	Moderate	Moderate	Moderate
INTEGRATION	Non-integrating	Non-integrating	Integrating	Integrating
EXPRESSION	Transient	Transient or stable	Stable	Stable
BIOSAFETY LEVEL	BSL-2	BSL-1	BSL-2	BSL-2
IMMUNOGENICITY	High	Low	Moderate-High	Moderate-High
GENE THERAPY STRATEGY	*In vivo*	*In vivo*	*Ex vivo*	*Ex vivo*

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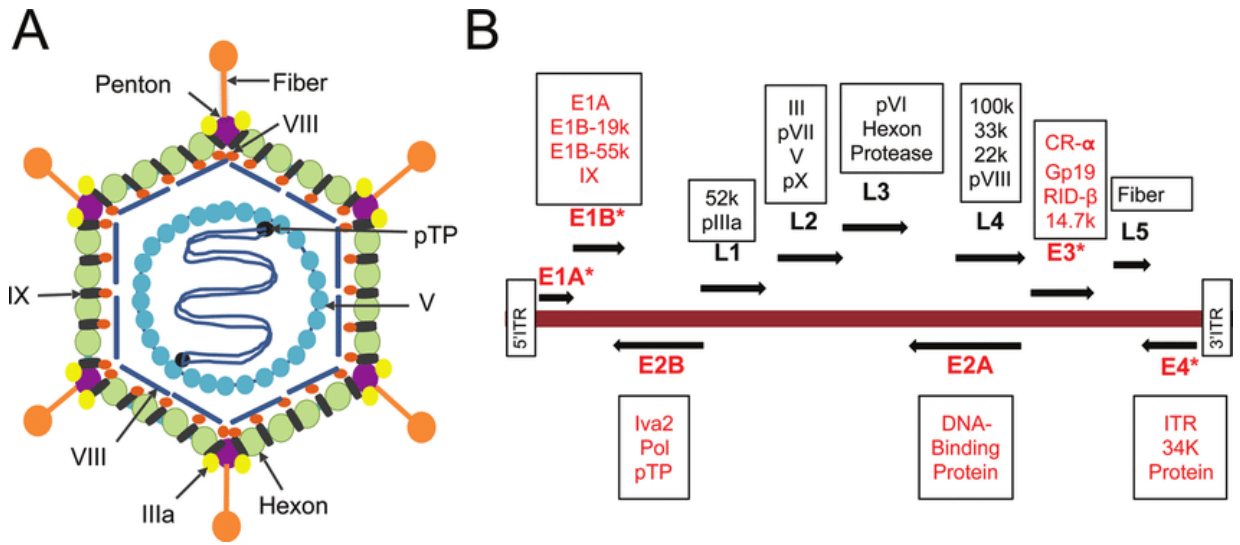
ADENOVIRUS

- ✓ DNA, 26 a 45 kb, flanqueado por ITR (inverted terminal repeats)
- ✓ DNA encapsulado em um icosaédrico não envelopado
- ✓ Capsídeo (tamanho de 90 nm de diâmetro).
- ✓ Mais usado é o Ad5
- ✓ Permanece episssomal



	ADENOVIRUS
SIZE	~90-100 nm
GENOME	dsDNA
PACKAGING CAPACITY	~8 kb – 36 kb
TRANSDUCTION	Dividing and non-dividing cells
TRANSDUCTION EFFICIENCY	High
INTEGRATION	Non-integrating
EXPRESSION	Transient
BIOSAFETY LEVEL	BSL-2
IMMUNOGENICITY	High
GENE THERAPY STRATEGY	*In vivo*

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ADENOVIRUS

Vantagens

- ✓ Empacota cerca de 30 kb
- ✓ Episssomal.
- ✓ *Dividing and non dividing cells*

Uso

- ✓ Vacinas

Desvantagens

- ✓ Alta resposta imune do hospedeiro contra o capsídeo, resultando em toxicidade e morte das células transduzidas
- ✓ Estratégia para diminuir resposta imune: uso com PEG, polietileno glicol e imunossupressores

Molecular Therapy
Oncolytics
Review

Current development in adenoviral vectors for cancer immunotherapy

Greyson Willis Grossman Biegert,[1,2,3] Amanda Rosewell Shaw,[1,2,3] and Masataka Suzuki[1,2]

[1]Department of Medicine, Section of Hematology/Oncology, Baylor College of Medicine, Houston, TX, USA; [2]Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital, Houston Methodist Hospital, Houston, TX, USA

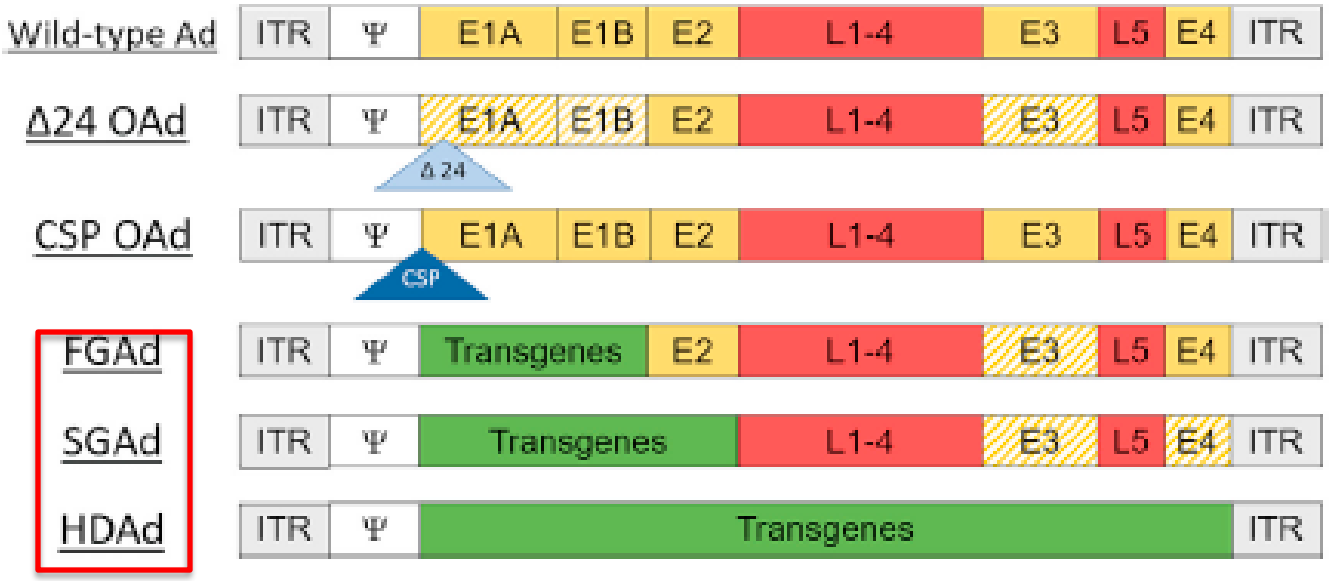

Figure 1. Genomic structure of adenoviral vectors
Wild-type adenoviral genomes are composed of inverted terminal repeats (ITRs) positioned at the termini denoted in gray; early genes 1A (E1A), E1B, E2, E3, and E4 represented in orange; late genes 1–5 (L1–5) in red; and a packaging signal (Ψ) that is responsible for packaging genomes into virion capsids represented in white. The transgene capacity is represented in green. The 24-bp deletion present in some oncolytic adenovirus virus (OAd) constructs is represented by a light-blue triangle, and the dark-blue triangle shows an example of a cancer-specific promoter insert. Striped sections represent additional deletion sites present in some constructs. The typical transgene capacity for OAds is 2–3 kb, while first-generation and second-generation Ads can accommodate up to 8 and 12 kb. Helper-dependent (HDAd) vectors have the largest transgene capacity, able to encode approximately 34 kb.

Molecular Therapy
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Current development in adenoviral vectors for cancer immunotherapy

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[1]Department of Medicine, Section of Hematology/Oncology, Baylor College of Medicine, Houston, TX, USA; [2]Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital, Houston Methodist Hospital, Houston, TX, USA

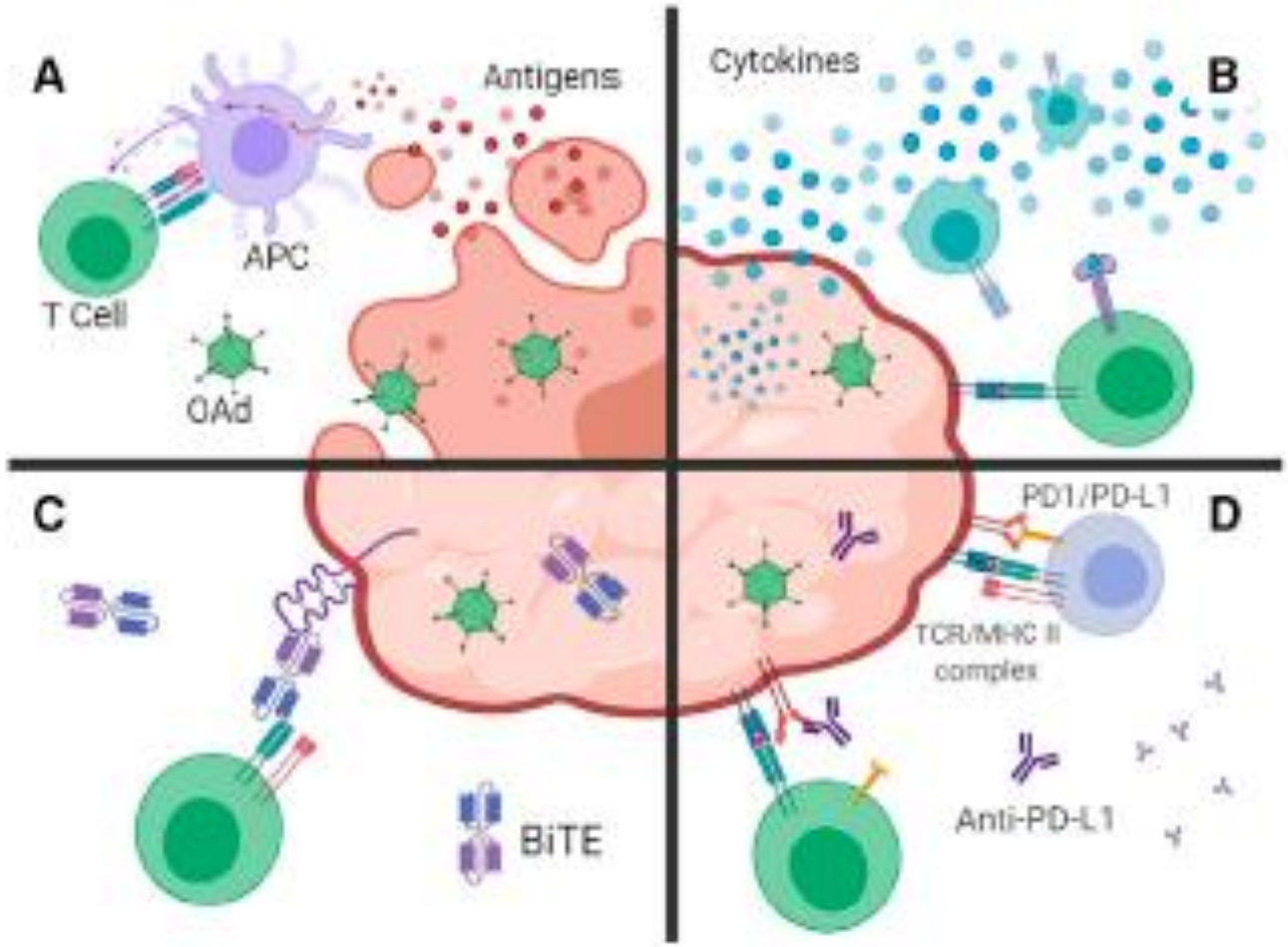
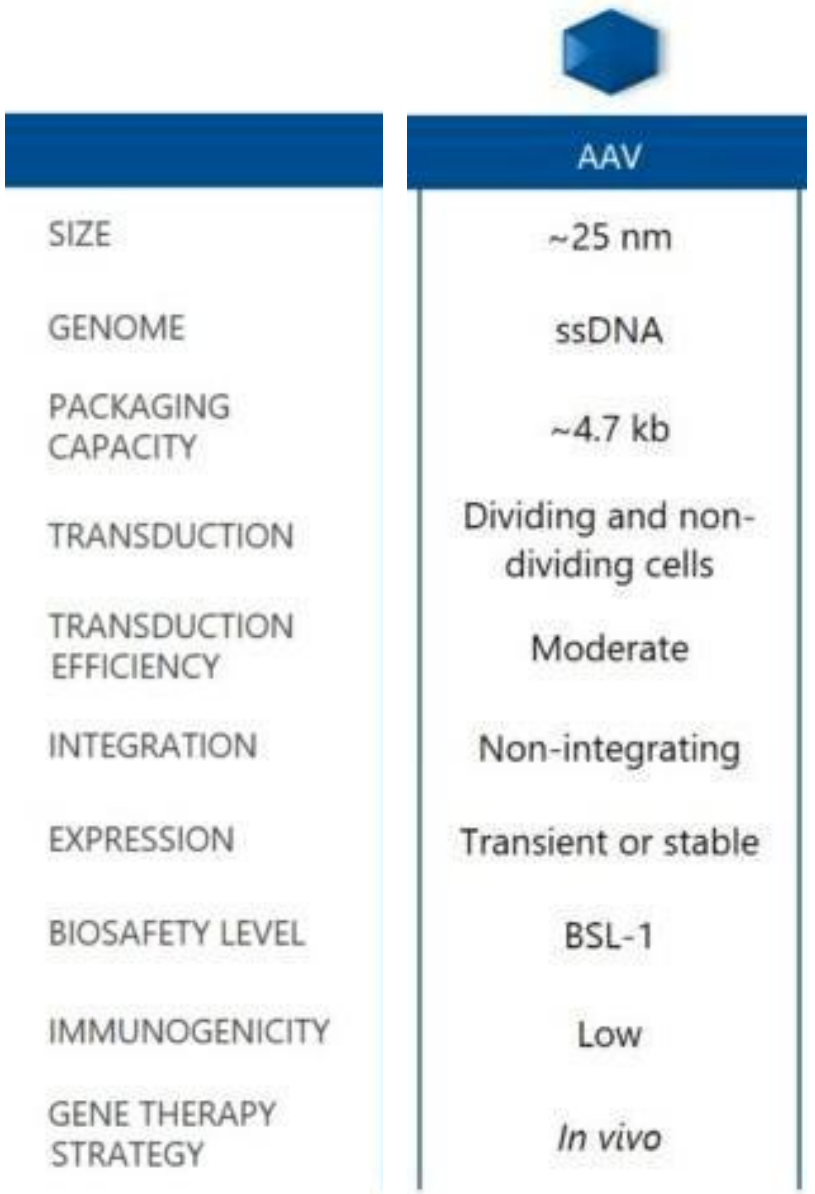


Figure 2. Current OAd mechanisms of immunostimulation

(A) Oncolysis is the primary method of action of OAds. When the tumor cell is lysed, via apoptosis or immunogenic cell death, cellular contents including tumor-associated antigens (TAAs) and viral particles are released in the intracellular space, which are then taken up by antigen-presenting cells (APCs). APCs then present these antigens (DAMPs, PAMPs, and TAAs) to effector cells, which are then directed to act against the remaining tumor cells. (B) OAds that encode cytokine transgenes enhance effector cell function, promote an immunogenic tumor microenvironment, and improve effector cell persistence. (C) OAds encoding bispecific T cell engager (BiTE) molecules enhance tumor targeting by endogenous and adoptively transferred T cells when tumor cells lack the specific antigen required to activate T cell function via the T cell receptor (TCR). (D) Immune checkpoint blockade antibodies generated by armed OAds counteract T cell hypofunction and exhaustion (gray T cell) by sterically hindering the binding of immune checkpoint receptors and their ligands such as PD-1/PD-L1.

AAV

- ✓ Parvovírus não patogênico/ *helper-Ad dependent virus*
- ✓ Composto de uma fita simples de 4,7 kb
- ✓ DNA encapsulado em um icosaédrico não envelopado
- ✓ Capsídeo (tamanho de 20 a 25 nm de diâmetro).
- ✓ Adsorção em ácido siálico, galactose, ou heparan sulfato e proteínas (AAV receptor- AAVR)
- ✓ AAV8 (fígado); AAV1 e AAV5 em SNC (Asokan, Schaffer, & Samulski, 2012



	AAV
SIZE	~25 nm
GENOME	ssDNA
PACKAGING CAPACITY	~4.7 kb
TRANSDUCTION	Dividing and non-dividing cells
TRANSDUCTION EFFICIENCY	Moderate
INTEGRATION	Non-integrating
EXPRESSION	Transient or stable
BIOSAFETY LEVEL	BSL-1
IMMUNOGENICITY	Low
GENE THERAPY STRATEGY	*In vivo*

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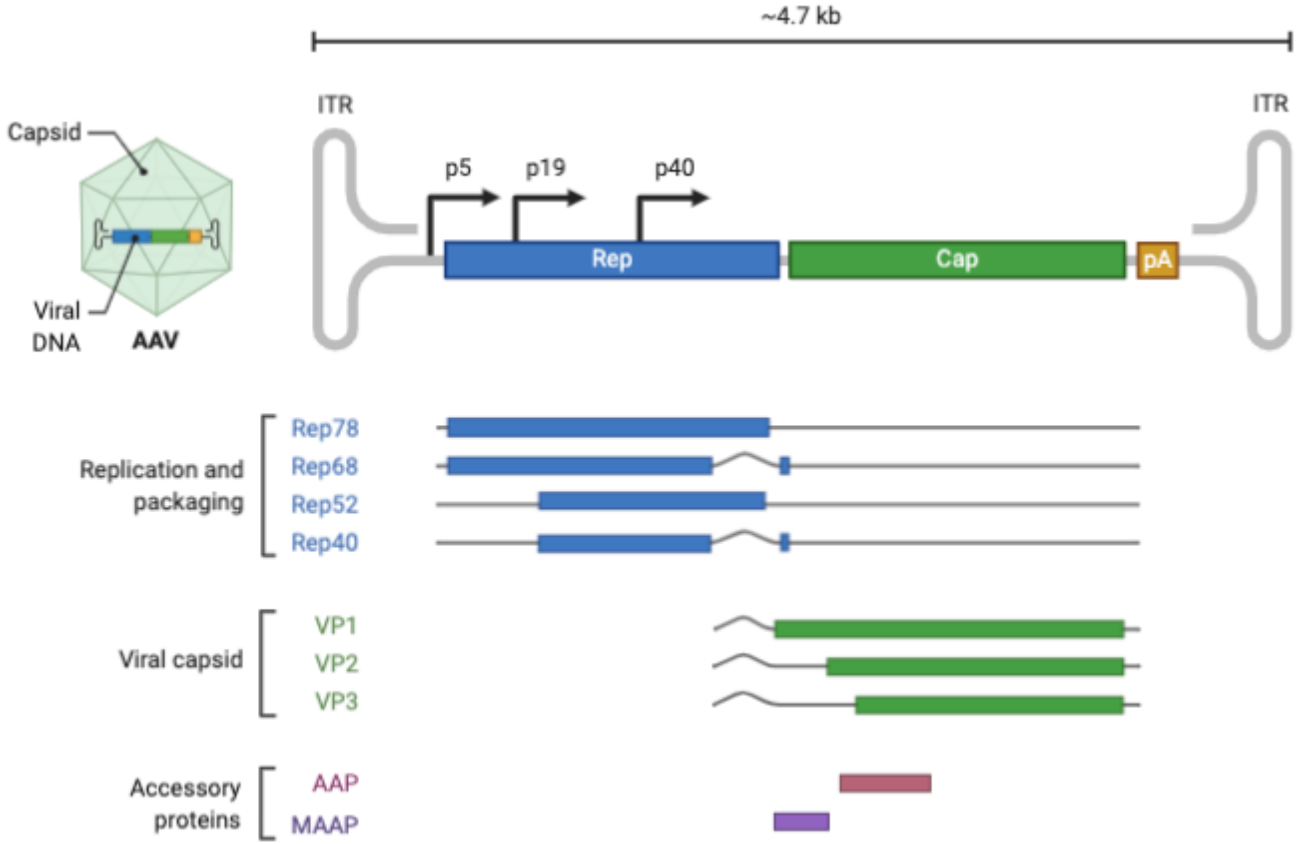
AAV

- ✓ O genoma viral é composto por três genes: Rep, Cap e AAP, flanqueado por terminal invertido repetições (ITRs) que funcionam como o vírus origem de replicação e o sinal de empacotamento.

- *REP: genes que codificam proteínas não estruturais, relacionados a replicação*
- *Cap: genes que codificam proteínas estruturais*
- *AAP: genes associados a montagem (assembly activating protein)*



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AAV

Vantagens

- ✓ Não patogênico ou citotóxico
- ✓ In vivo é pouco imunogênico
- ✓ Episssomal persite por longos períodos em células que não se dividem
- ✓ *Low integration rate*: baixa tumorigenicidade (mutagênese)

Desvantagens

- ✓ Empacota < 5 kb

Uso

- ✓ Gene replacement in vivo (gene theraphy)
- ✓ Knock in ou knoch down
- ✓ Hemofilia, cegueira, neurobiologia e anatomia do SNC (Sizemore, Seeger-Armbruster, Hughes, & Parr-Brownlie, 2016)

viruses

MDPI

Review

Adeno-Associated Virus (AAV) Gene Delivery: Dissecting Molecular Interactions upon Cell Entry

Edward E. Large, Mark A. Silveria, Grant M. Zane, Onellah Weerakoon and Michael S. Chapman *

Table 1. Native AAV structures.

Serotype	Clade	Resolution	Year	Tropism [1]	Tropism [2]	Tropism [3]	Reference	PDBid
AAV1	A	X-ray 2.5 Å	2010	Muscle, CNS, heart	Skin, lung, kidney, cervix, bone	Kidney, skin	Ng et al. [21]	3NG9
AAV2	B	X-ray 3.0 Å	2002	Liver, CNS, muscle	Skin, lung, kidney, cervix, liver, bone	Liver, kidney, cervix, retina, skin	Xie et al. [22]	1LP3
AAV3	C	X-ray 2.6 Å	2010	Muscle, stem cells	Skin, lung, kidney, cervix, liver, bone	Skin	Lerch et al. [23]	3KIC
AAV4	Unique	X-ray 3.2 Å	2006	Eye, CNS	Bone	Not detected	Govindasamy et al. [24]	2G8G
AAV5	Unique	X-ray 3.5 Å	2013	CNS, lung, eye	Not detected	Not detected	Govindasamy et al. [25]	3NTT
AAV6	A	X-ray 3.0 Å	2011	Muscle, CNS, heart, lung	Skin, lung, kidney, cervix, bone	Skin	Xie et al. [26]	4V86
AAV7	D	Cryo-EM 3.0 Å	2021	Muscle, CNS	Not detected	Not detected	Mietzsch et al. [20]	7L5Q
AAV8	E	X-ray 2.6 Å	2007	Liver, muscle, pancreas, CNS	Not detected	Not detected	Nam et al. [27]	2QA0
AAV9	F	X-ray 2.8 Å	2012	Broad distribution	Not detected	Not detected	Dimattia et al. [28]	3UX1
AAVrh.39 (AAV10-like)		Cryo-EM 3.4 Å	2020	Muscle (AAV10)	Not tested	Not tested	Mietzsch et al. [29]	6V1T
AAV11		Cryo-EM 2.9 Å	2021	Unknown	Not tested	Not tested	Mietzsch et al. [20]	7L6F
AAV12		Cryo-EM 2.5 Å	2021	Nasal	Not tested	Not tested	Mietzsch et al. [20]	7L6B
AAV13		Cryo-EM 3.0 Å	2021	Not shown	Not tested	Not tested	Mietzsch et al. [20]	7L6I
AAVDJ		Cryo-EM 4.5 Å	2012	Not shown	Not tested	Liver, kidney, cervix, retina, skin, lung	Lerch et al. [30]	3J1Q
AAVDJ		Cryo-EM 1.6 Å	2020	Not shown	Not tested	Liver, kidney, cervix, retina, skin, lung	Xie et al. [31]	7KFR

[1] Li and Samulski 2020 [32], Review; [2] Ellis et al. 2013 [33], Supplementary Table S2; [3] Grimm et al. 2008 [34], Supplementary Table S2.

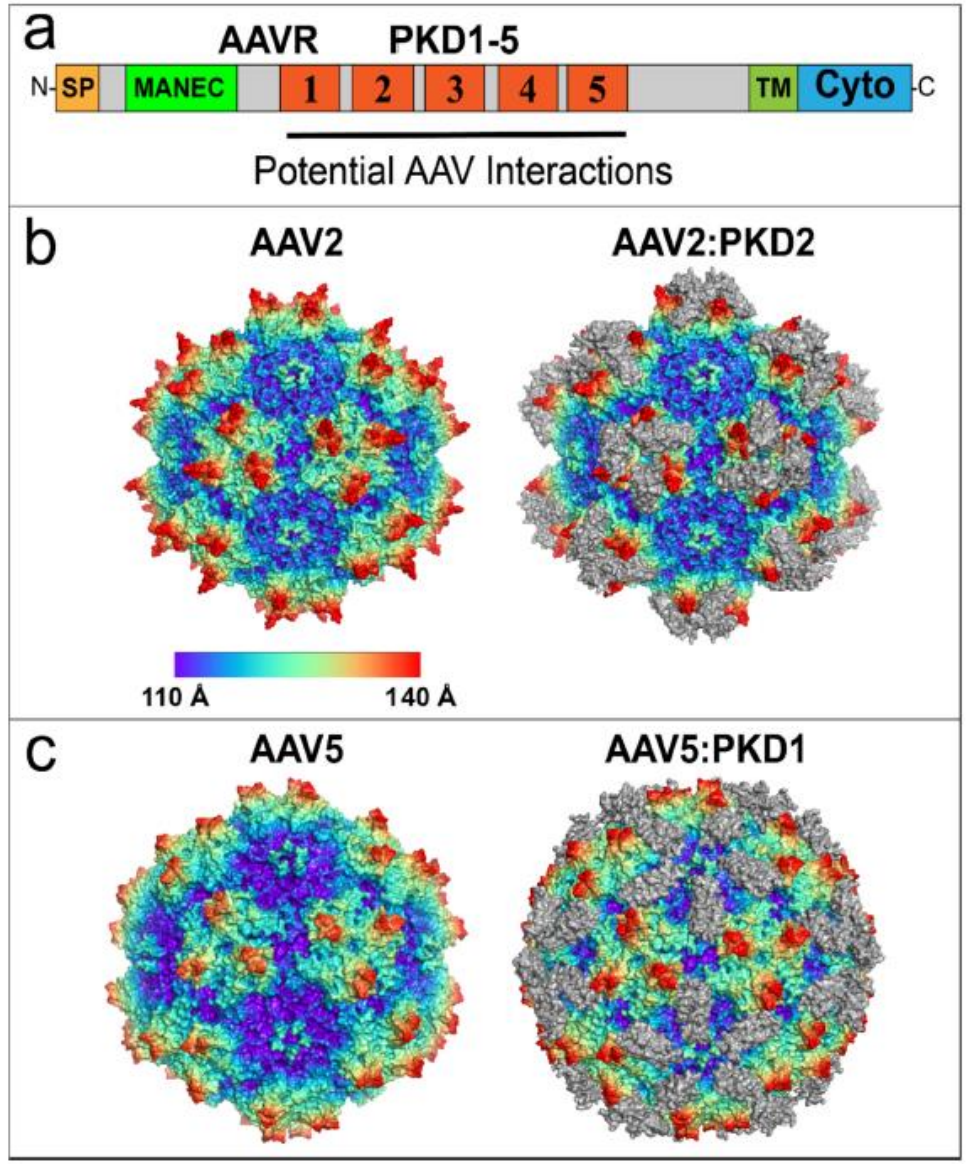
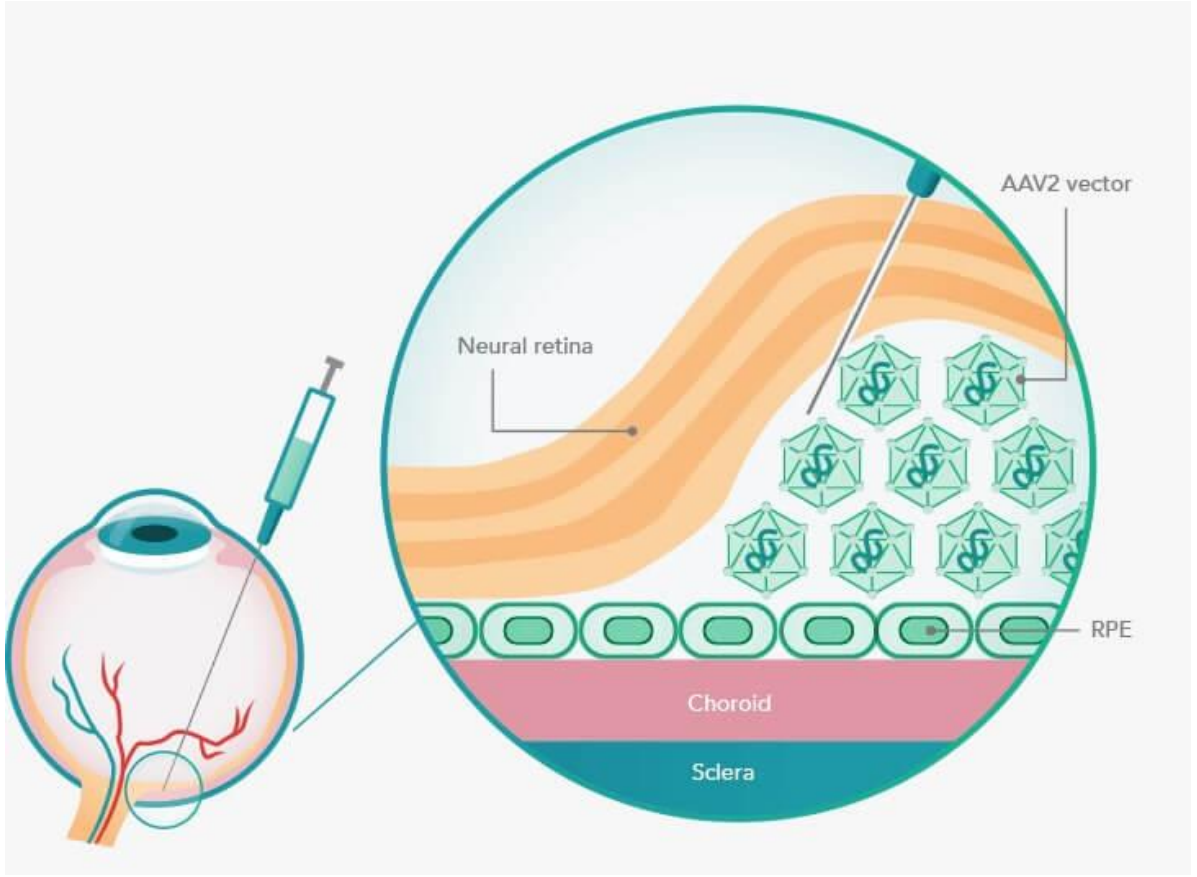


Figure 4. AAV2 and AAV5 bind to distinct AAVR PKD domains. (**a**) AAVR domain structure from N-terminus (N) to C-terminus (C): Signal Peptide (SP), Motif At the N-terminus with Eight Cysteines (MANEC), immunoglobulin-like Polycystic Kidney Disease (PKD) domains 1–5, TransMembrane (TM) helix and Cytoplasmic domain (Cyto). The region containing potential AAV interactions is composed of PKD1-5. (**b**) Native AAV2 60-mer (left) and the AAV2:PKD2 complex (right). Virus models are colored by radial distance from the center of the virion. The PKD2 domain of AAVR is colored in gray. (**c**) Virus model of native AAV5 virion (left) and the AAV5:PKD1 complex (right). Models are colored by radial distance from the center of the virion. The PKD1 domain of AAVR is colored in gray. Structures in (**b**,**c**) were prepared using PyMOL [66].

- O AAV é a base de uma indústria multibilionária e centenas de ensaios clínicos utilizaram sistemas de entrega de AAV
- A biotecnologia de vectores virais é a principal escolha para plataformas de terapia génica
- Vectores do vírus adeno-associado recombinante (rAAV) são normalmente preferidos devido à sua baixa toxicidade, à sua dependência de outros vírus para a replicação, ao seu amplo tropismo e a capacidade de infectar tanto células em divisão como células que não estão em divisão.
- Existem atualmente dois tratamentos de substituição genética por AAV disponíveis para doenças genéticas autossômicas recessivas: Luxturna (cegueira noturna) e Zolgensma (atrofia muscular espinhal, AME).

- O Luxturna rAAV baseia-se no serotipo AAV2 (rAAV2) e fornece uma cópia funcional do gene RPE65 (proteína 65 kDa específica do epitélio pigmentar da retina) no epitélio pigmentar da retina nas células epiteliais do pigmento da retina de doentes com distrofia da retina R$ 1.930.768,81



- Zolgensma utiliza o rAAV9 para fornecer uma cópia funcional do gene humano SMN1 (survival of motor neuron 1) em doentes com atrofia muscular espinal (AME) (causada por uma mutação autossômica recessiva no gene de sobrevivência sobrevivência do neurónio motor 1 (SMN1).

22 apresentações com preço máximo de R$ 2.878.906,14 para cada uma das apresentações

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RETROVIRUS

- ✓ ssRNA, 2 cópias de RNA +
- ✓ Composto de uma fita simples de 4,7 kb
- ✓ DNA encapsulado em um icosaédrico envelopado
- ✓ Capsídeo e envolope (tamanho de 80 a 120 nm de diâmetro).
- ✓ Lentivirus (non-dividing cells) HIV, gamma retrovírus (dividing cells) Moloney Murine Leukenia Virus (MoMLV). Feline immunodeficiency virus (FIV), and equine infectious anaemia virus (EIAV)

RETROVIRUS

	γ-RETROVIRUS	LENTIVIRUS
SIZE	~80-100 nm	~80-100 nm
GENOME	ssRNA	ssRNA
PACKAGING CAPACITY	10 kb	8 kb
TRANSDUCTION	Dividing cells	Dividing and non-dividing cells
TRANSDUCTION EFFICIENCY	Moderate	Moderate
INTEGRATION	Integrating	Integrating
EXPRESSION	Stable	Stable
BIOSAFETY LEVEL	BSL-2	BSL-2
IMMUNOGENICITY	Moderate-High	Moderate-High
GENE THERAPY STRATEGY	*Ex vivo*	*Ex vivo*

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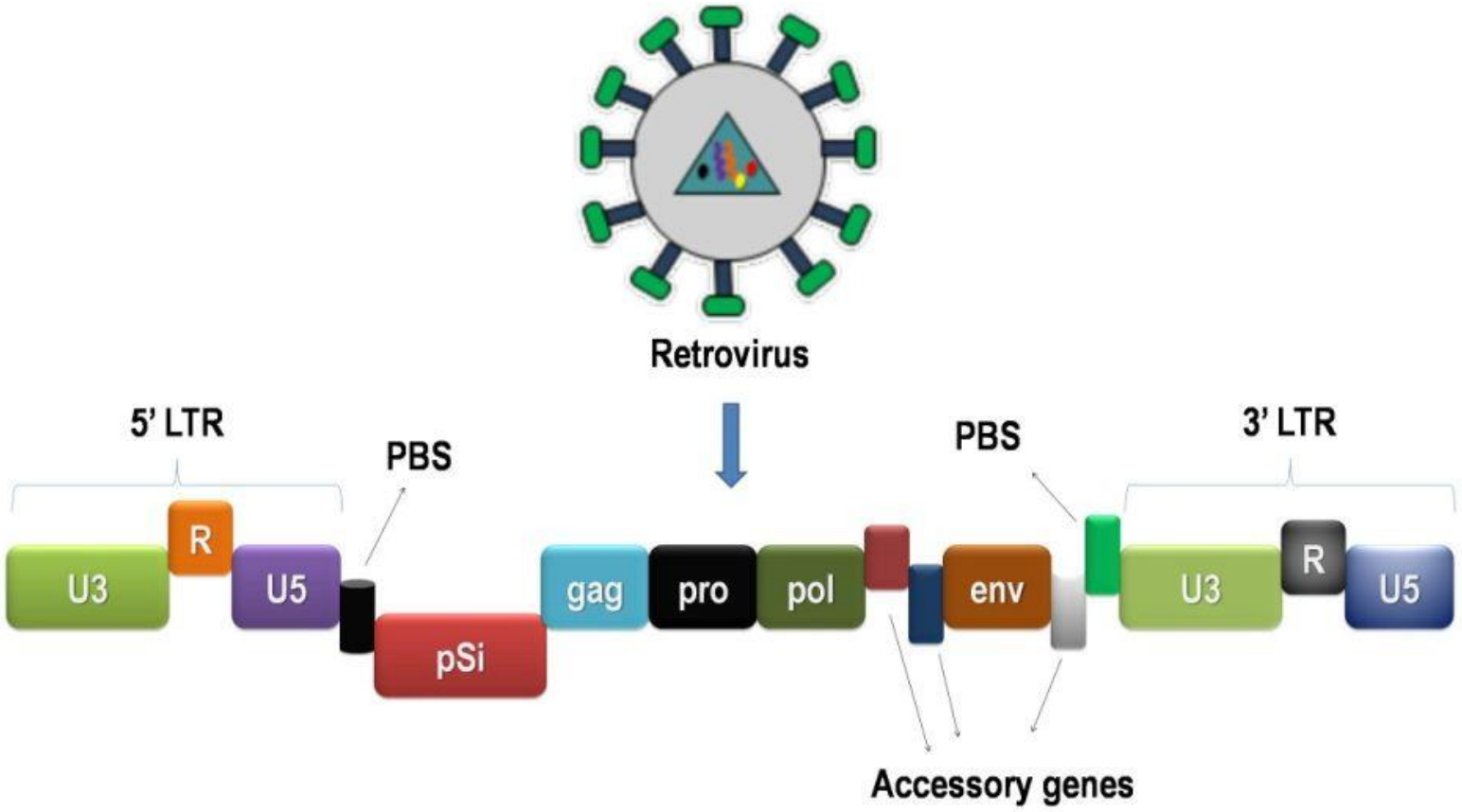
RETROVIRUS

- ✓ O genoma viral é composto por três genes: *gag, pol e env* e genes acessórios (*tat, rev, vpr, vpu, nef, and vif*) flanqueados por terminal longo invertido repetições (LTRs)
- ✓ Transcriptase reversa faz cDNA



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RETROVIRUS

Vantagens

- ✓ Empacota cerca de 9kb
- ✓ Integra no genoma, *long-term gene expression.*

Desvantagens

- ✓ Pode ser silenciado
- ✓ Integra no genoma, mutagênese

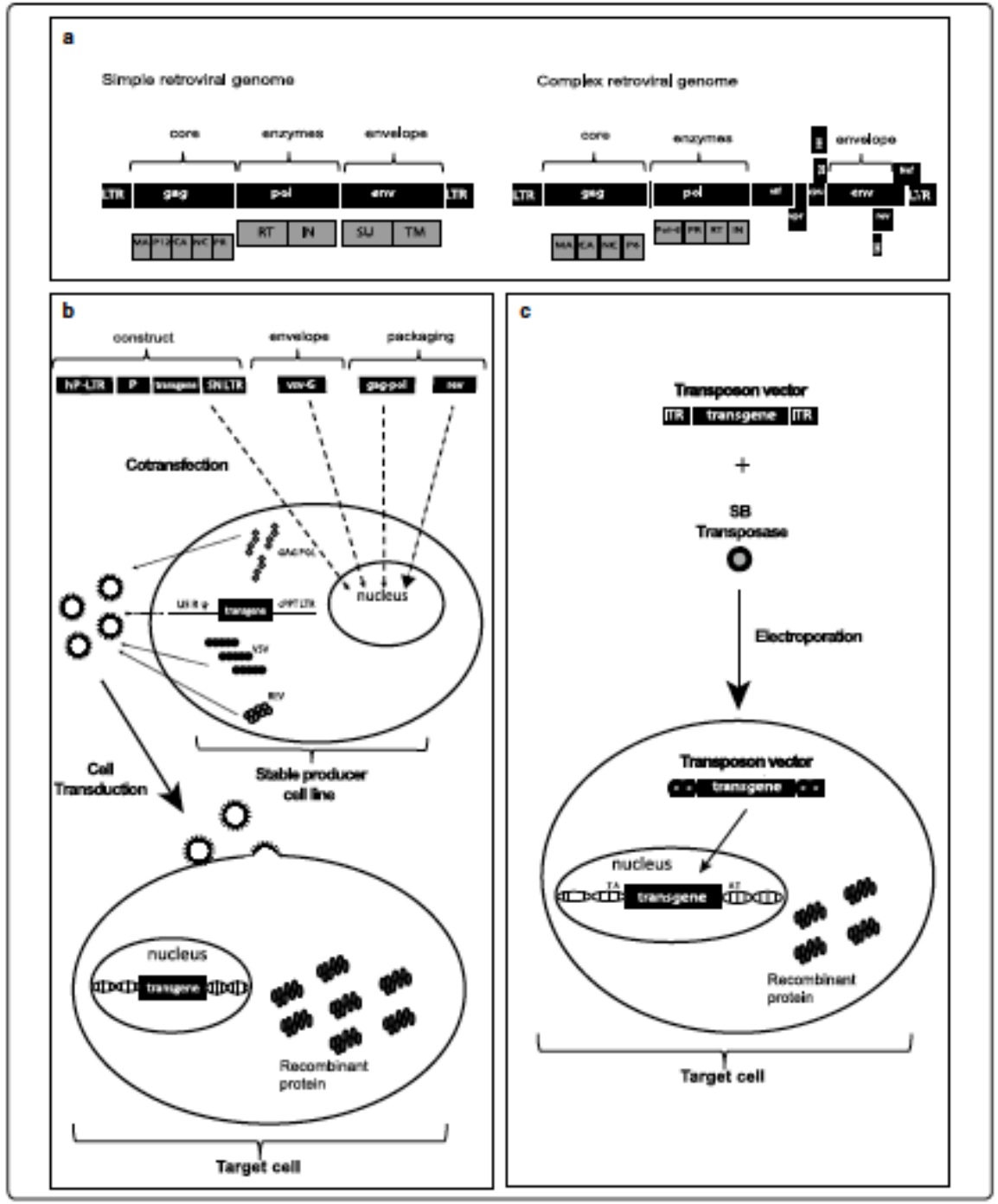
Uso

- ✓ Ex vivo delivery (gene theraphy)
- ✓ Doenças do sangue, SNC, metabólicas
- ✓ CARs terapia anti câncer (leucemia)

Retroviral vectors and transposons for stable gene therapy: advances, current challenges and perspectives

José Eduardo Vargas[1†], Leonardo Chicaybam[2,3†], Renato Tetelbom Stein[1], Amilcar Tanuri[4], Andrés Delgado-Cañedo[5] and Martin H. Bonamino[2,3*]

Usando uma célula empacotadora



Transfecção

	Adenoviridae	*Parvoviridae*	*Retroviridae*	*Retroviridae*
				
	ADENOVIRUS	AAV	γ-RETROVIRUS	LENTIVIRUS
SIZE	~90-100 nm	~25 nm	~80-100 nm	~80-100 nm
GENOME	dsDNA	ssDNA	ssRNA	ssRNA
PACKAGING CAPACITY	~8 kb – 36 kb	~4.7 kb	10 kb	8 kb
TRANSDUCTION	Dividing and non-dividing cells	Dividing and non-dividing cells	Dividing cells	Dividing and non-dividing cells
TRANSDUCTION EFFICIENCY	High	Moderate	Moderate	Moderate
INTEGRATION	Non-integrating	Non-integrating	Integrating	Integrating
EXPRESSION	Transient	Transient or stable	Stable	Stable
BIOSAFETY LEVEL	BSL-2	BSL-1	BSL-2	BSL-2
IMMUNOGENICITY	High	Low	Moderate-High	Moderate-High
GENE THERAPY STRATEGY	*In vivo*	*In vivo*	*Ex vivo*	*Ex vivo*

Vector	MoMLV retroviral	Lentiviral	Adenoviral	Helper-dependent adenoviral	Recombinant HSV	HSV amplicon	AAV
Family	Retroviridae	Retroviridae	Adenoviridae	Adenoviridae	Herpesviridae	Herpesviridae	Parvoviridae
Particle size (nm)	100	100	70-120	70-120	120-300	120-300	20-25
Cargo	RNA	RNA	dsDNA	dsDNA	dsDNA	dsDNA	ssDNA
Packaging capacity (kB)	7-8	7-9	8-10	Up to 36	30-50	Up to 150	4.8
Vector yield (transducing units ml-1)	1.00E+09	1.00E+09	1.00E+12	1.00E+12	1.00E+11	1.00E+08	1.00E+13
Chromosomal integration?	Yes	Yes	No	No	No	No	No
Oncolytic?	No	No	Yes/no	No	Yes/no	No	No
Infects post-mitotic cells?	No	Yes	Yes	Yes	Yes	Yes	Yes
Risk of oncogene activation?	Yes	Yes	No	No	No	No	No

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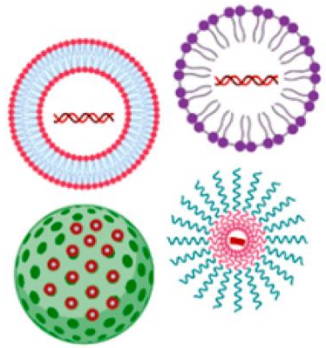
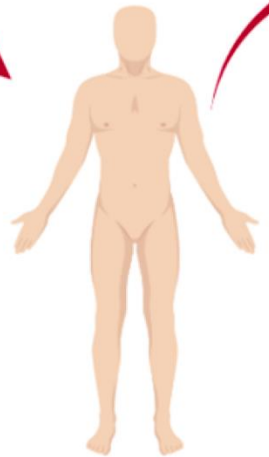
In vivo gene therapy

Ex vivo gene therapy

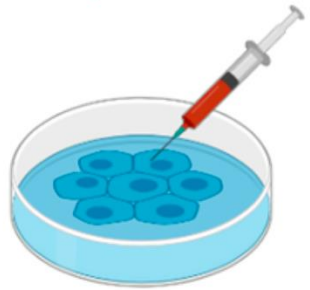
Non-viral delivery system



Nanoparticles that can potentially be used for HCC gene therapy



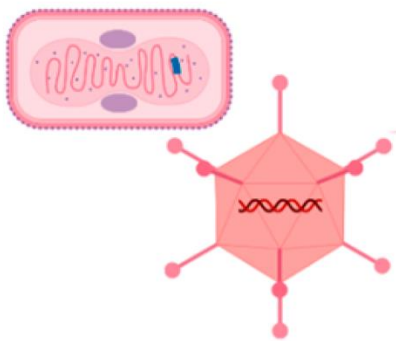
cells isolated from patient



cells modified *in vitro*

Modified cells injected back into the patient

Viral delivery systems



Viruses currently being used for HCC gene therapy

Hepato Carcinoma Celular

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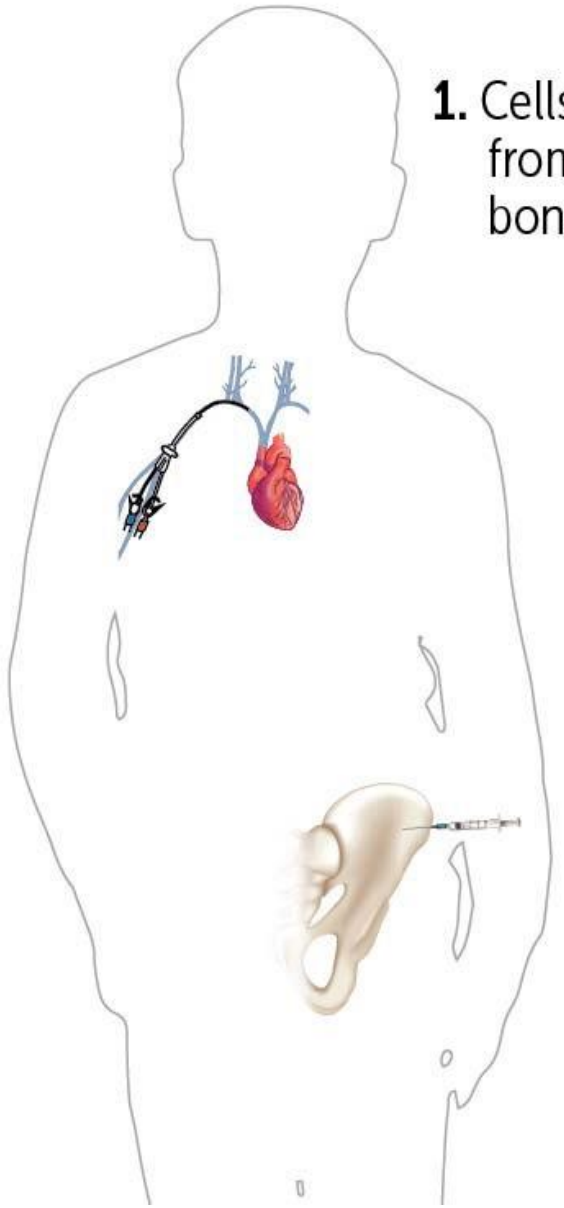
Ex-Vivo Gene Therapy - Cell-Based Delivery



1. Cells are collected from blood or bone marrow

2. Patient's target cells (e.g. stem cells or T cells) are isolated

3. A theraputic gene is packaged into a delivery vehicle (vector), such as an engineered virus

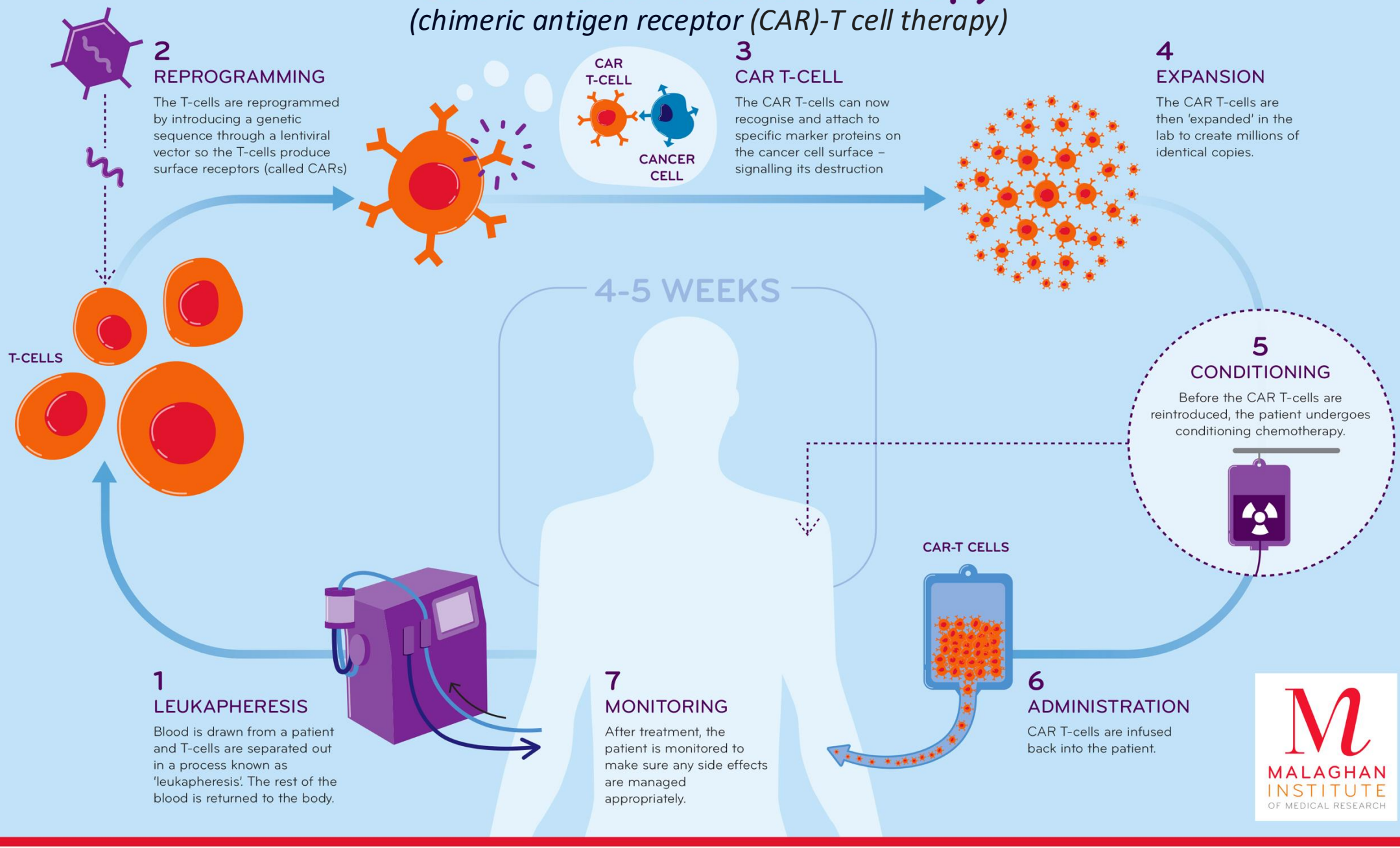
4. Vector is introduced to the patient's stem cells

5. Genetically modified cells are returned to the patient who may require specific conditioning

Boston Children's Hospital

CAR T-Cell Cancer Therapy

(chimeric antigen receptor (CAR)-T cell therapy)



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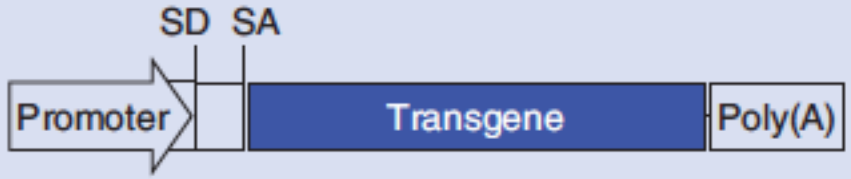
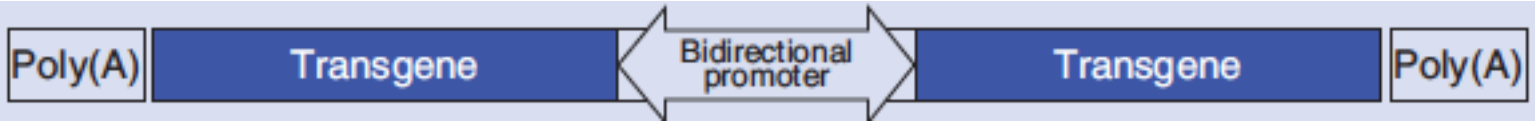
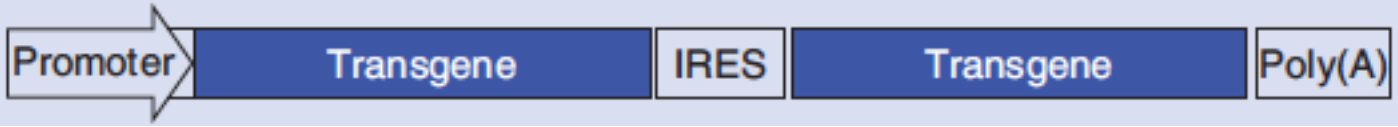
Como construir um vetor viral?

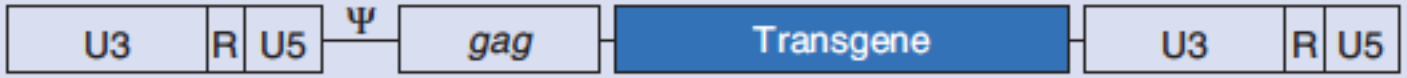
BOX 2. TRANSGENE EXPRESSION CASSETTES



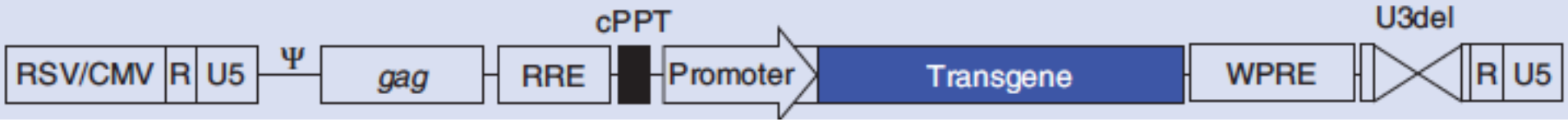




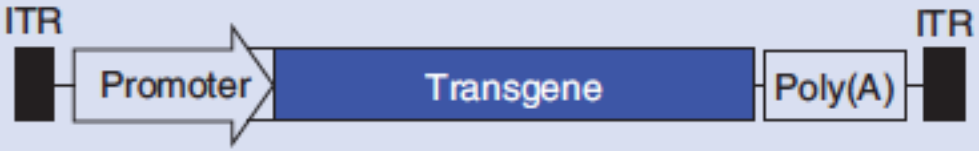
Retrovirus Vectors



Lentivirus Vectors

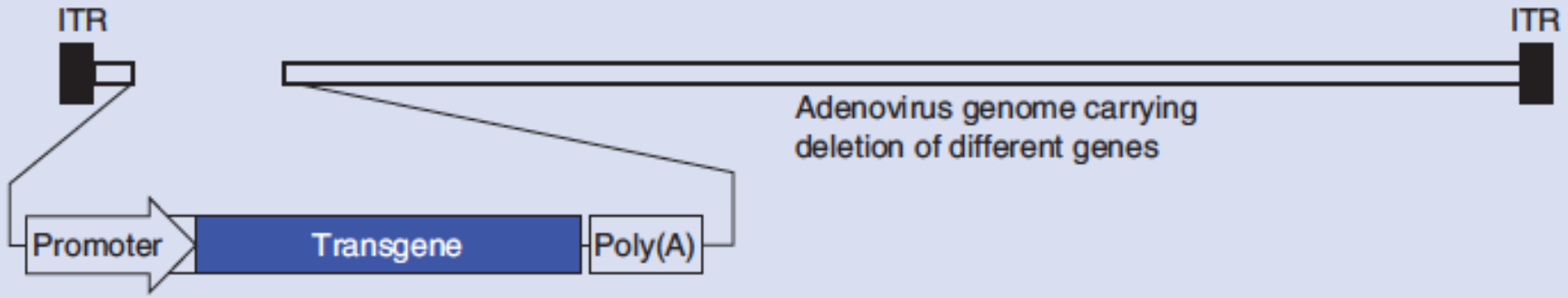


AAV Vectors



Adenovirus Vectors

Standard Ad vector



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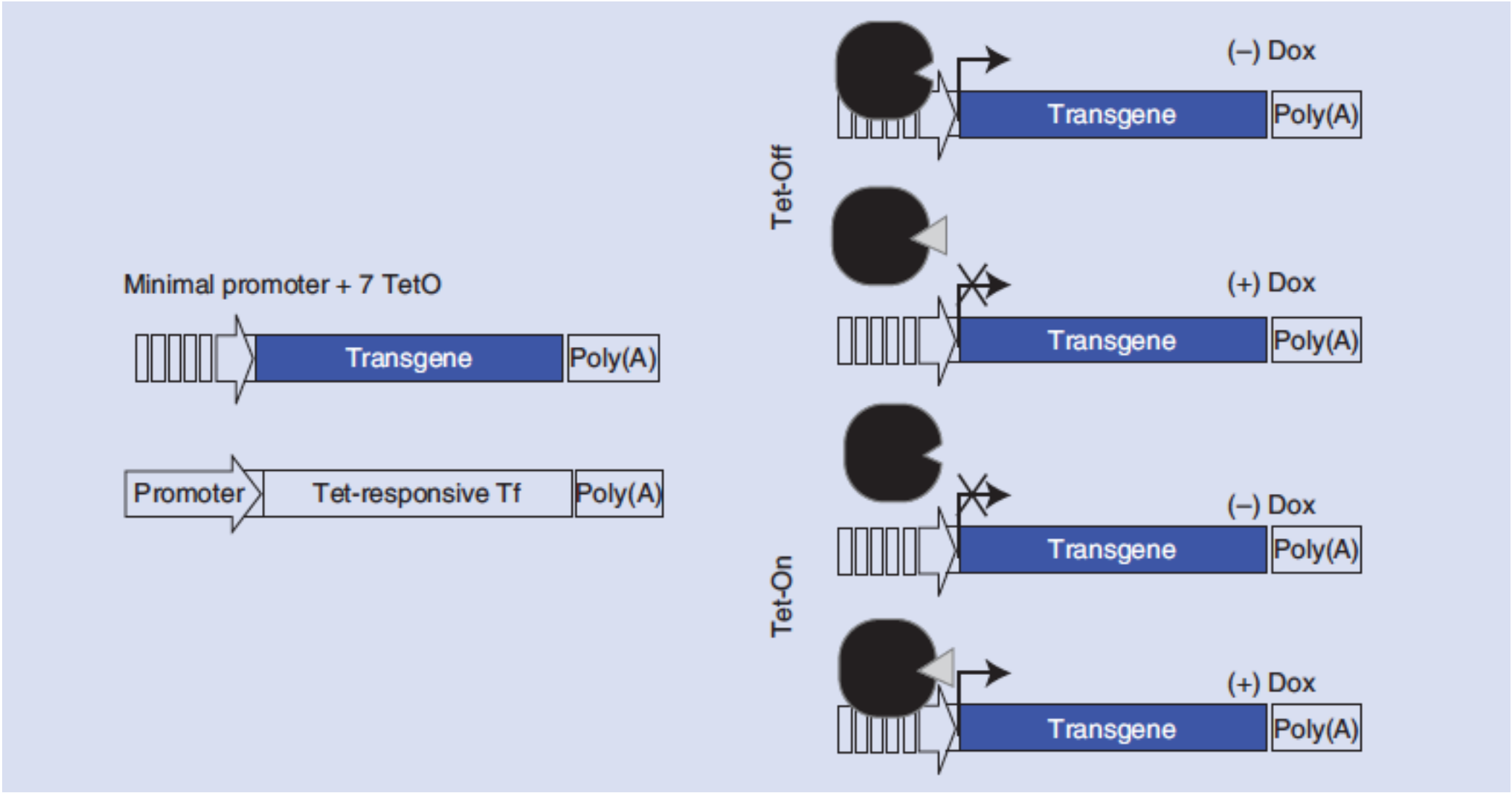
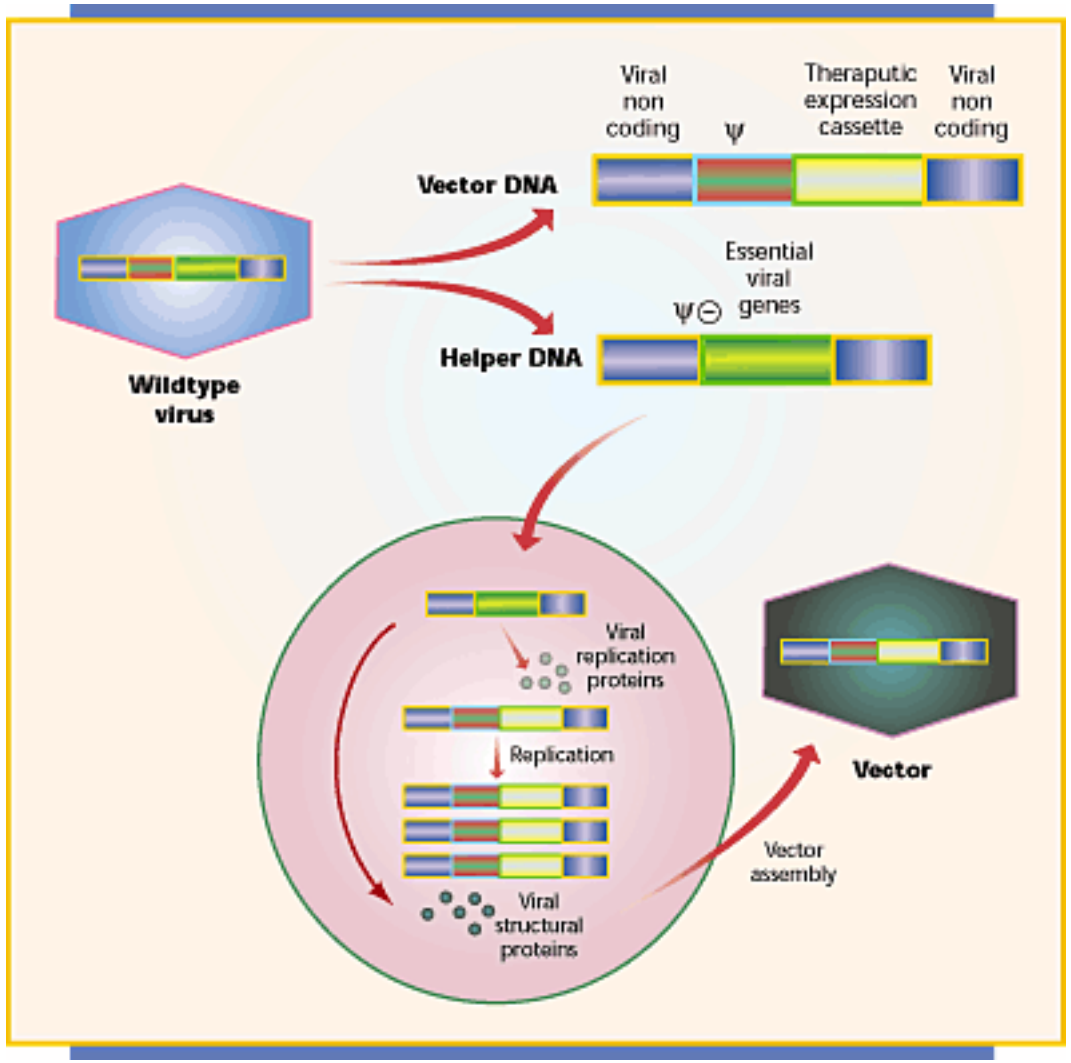
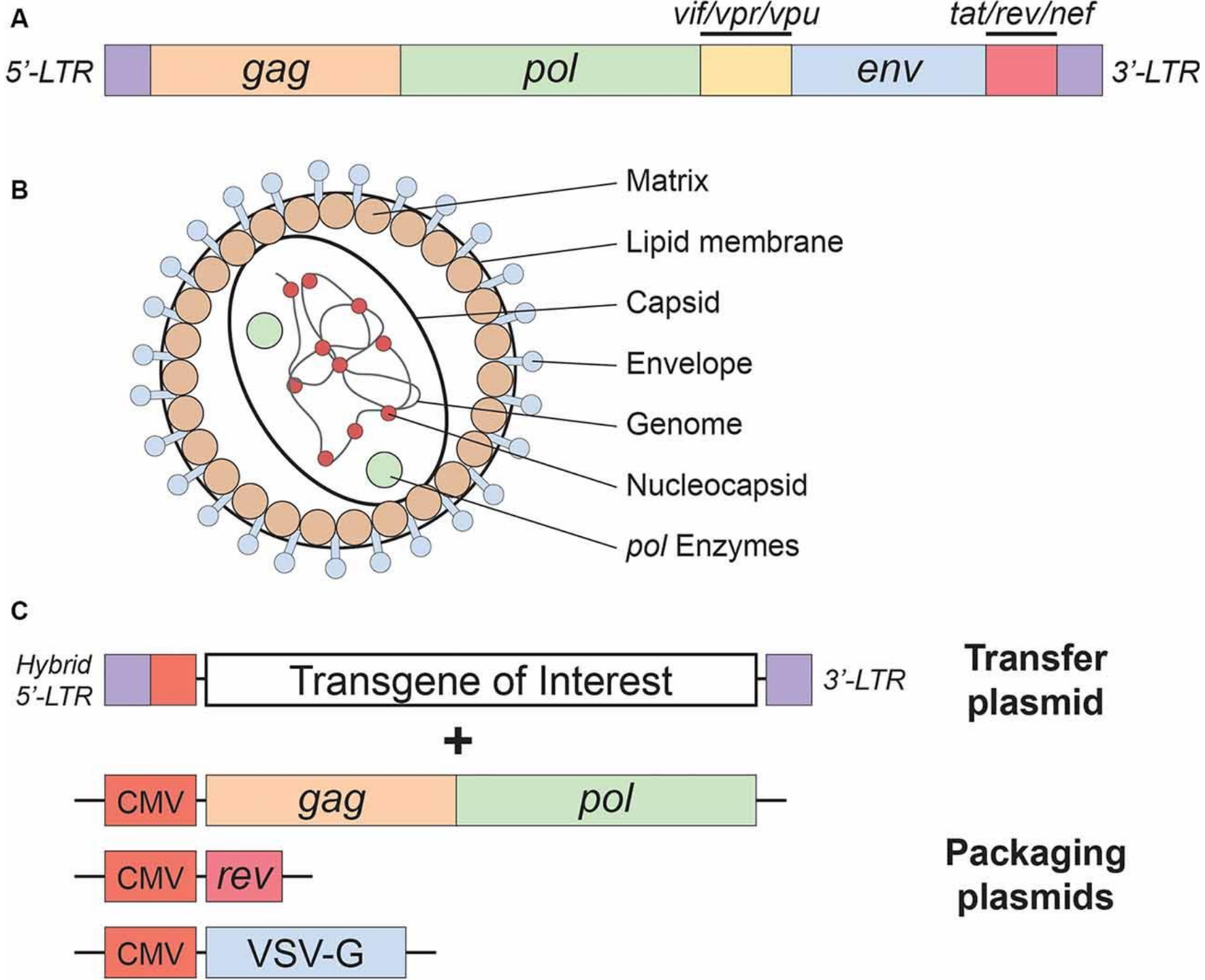
Vetores com a expressão controlada



- ✓ **Os vetores virais são geneticamente modificados de modo que seu capacidade de replicação é prejudicada ou removida**




A
vif/vpr/vpu
tat/rev/nef
5'-LTR
gag
pol
env
3'-LTR
B
Matrix
Lipid membrane
Capsid
Envelope
Genome
Nucleocapsid
pol Enzymes
C
Hybrid 5'-LTR
Transgene of Interest
3'-LTR
Transfer plasmid
+
CMV
gag
pol
CMV
rev
CMV
VSV-G
Packaging plasmids

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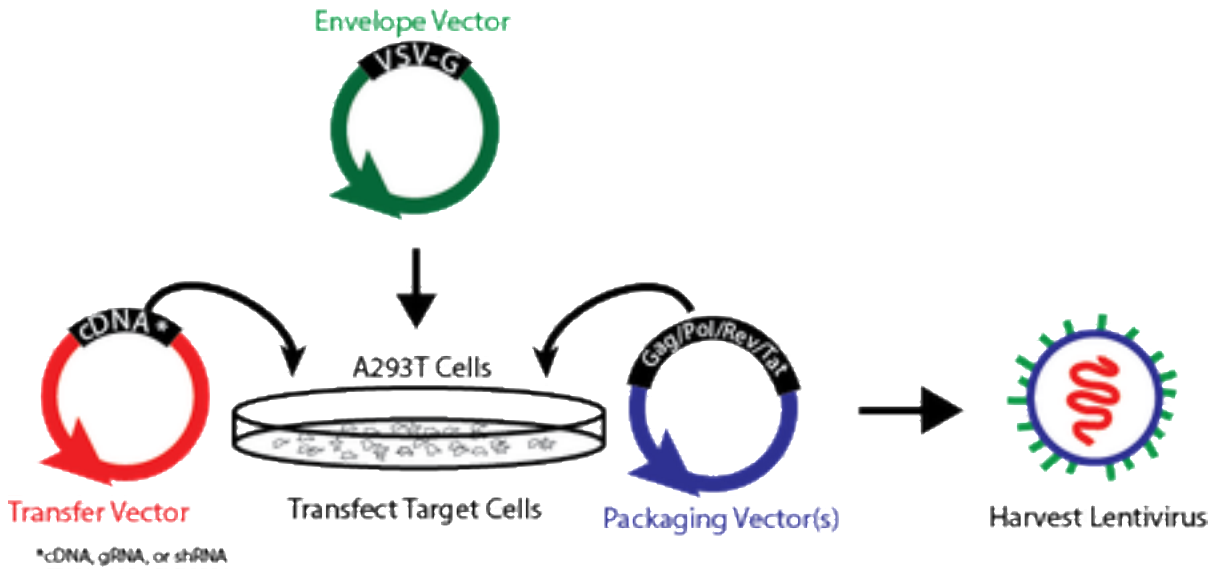
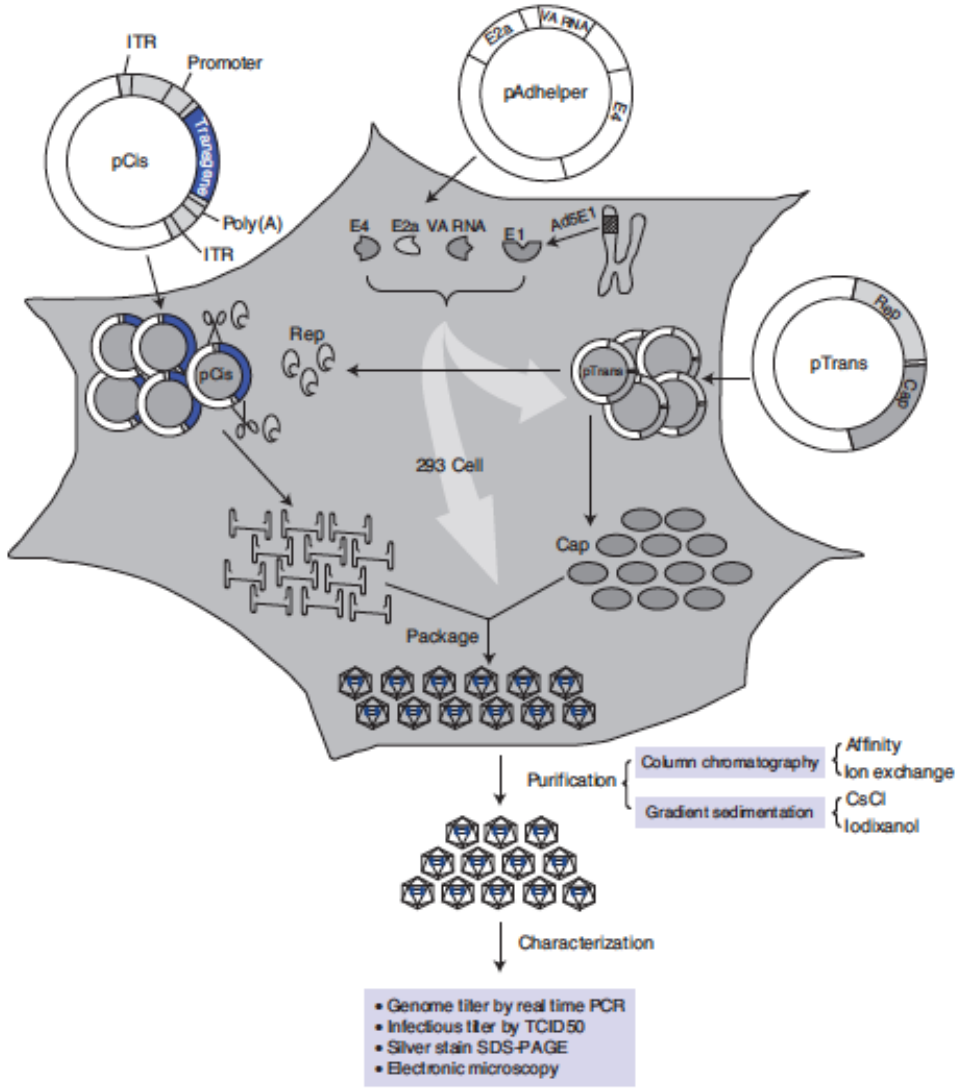
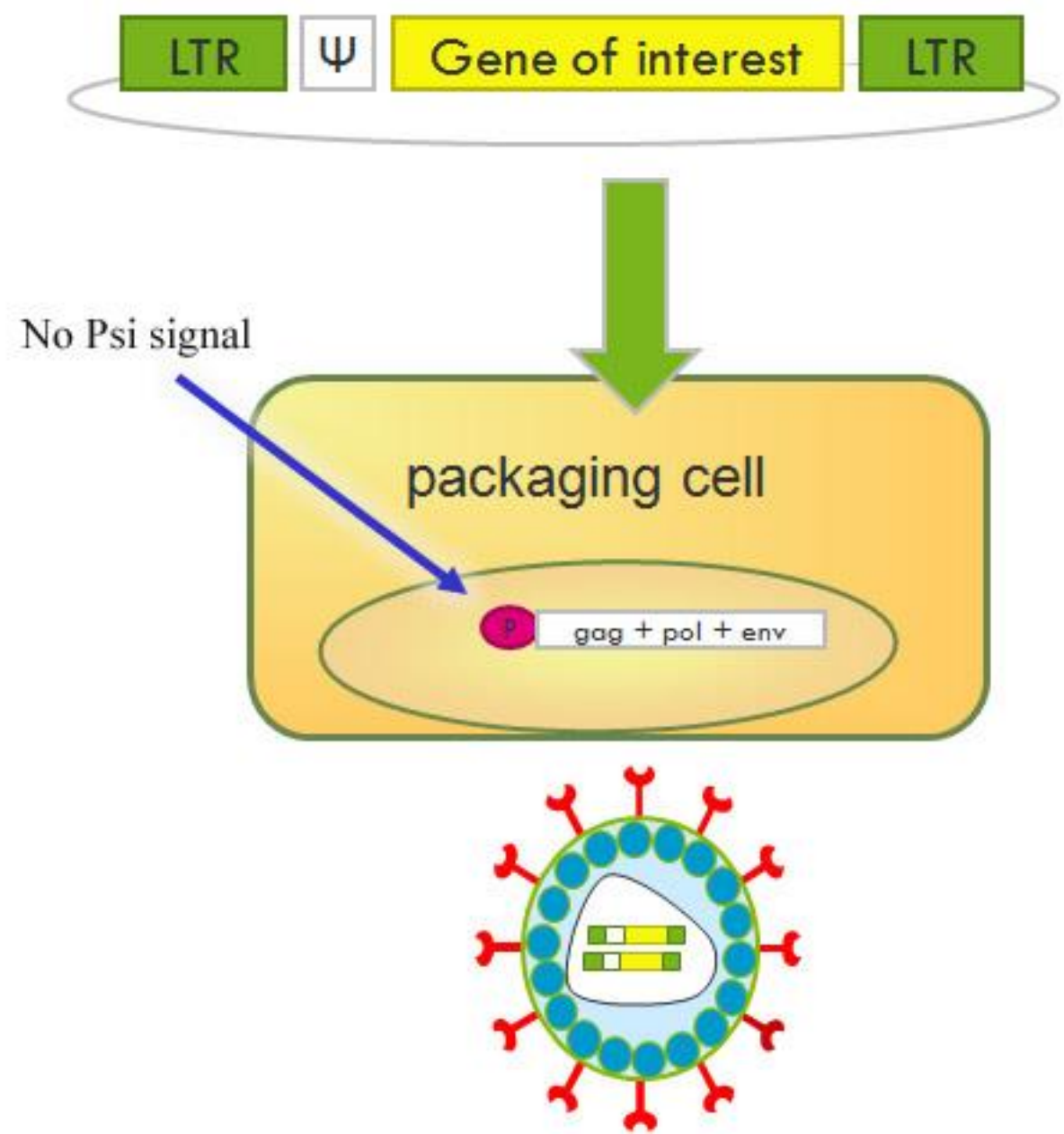
Tripla transfecção

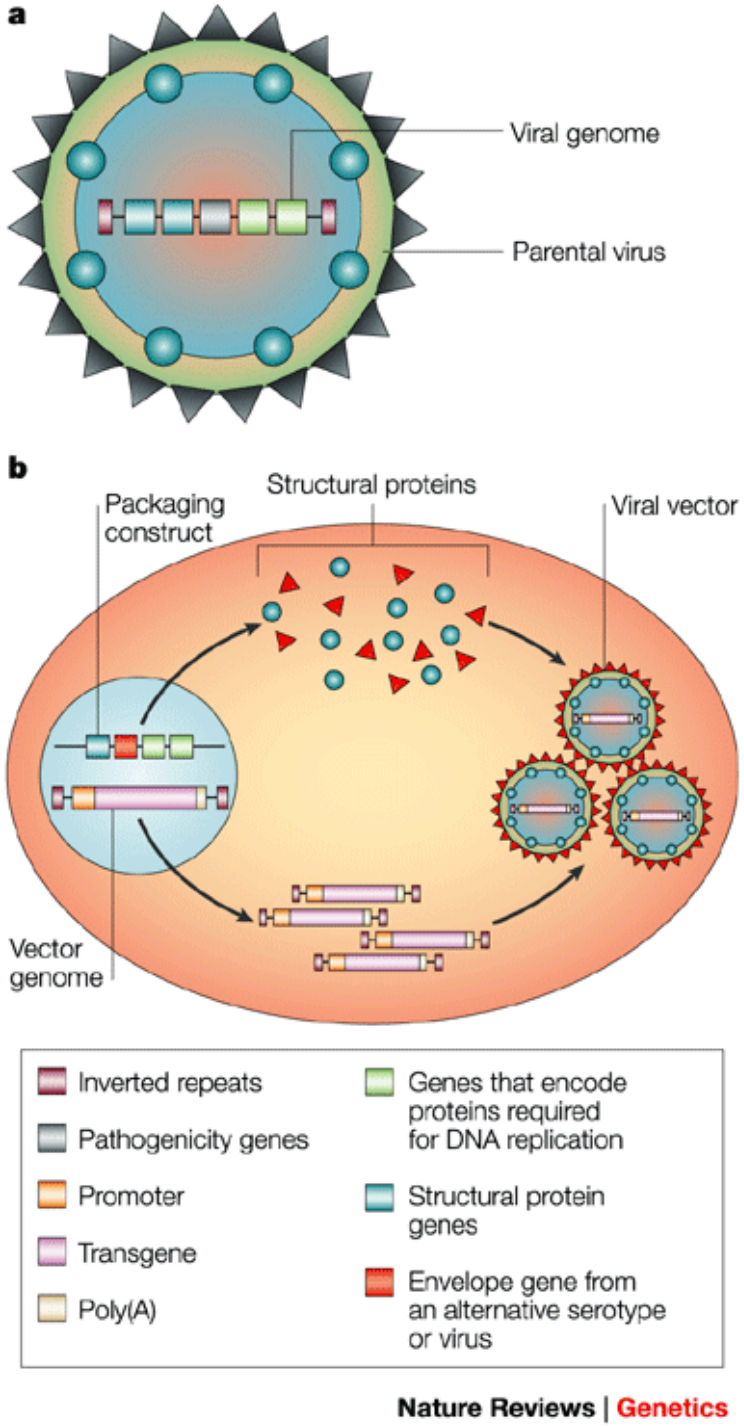


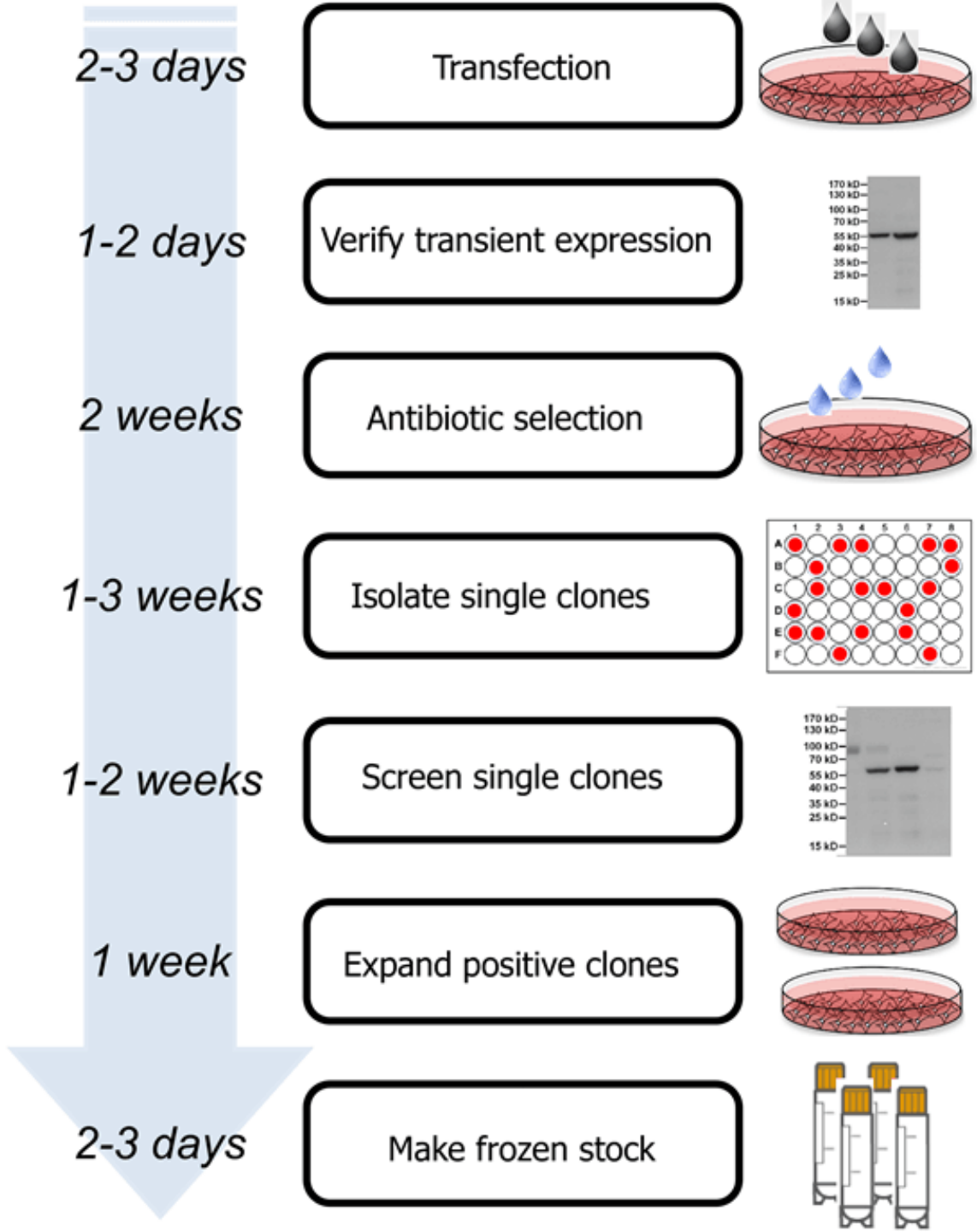


Células empacotadoras

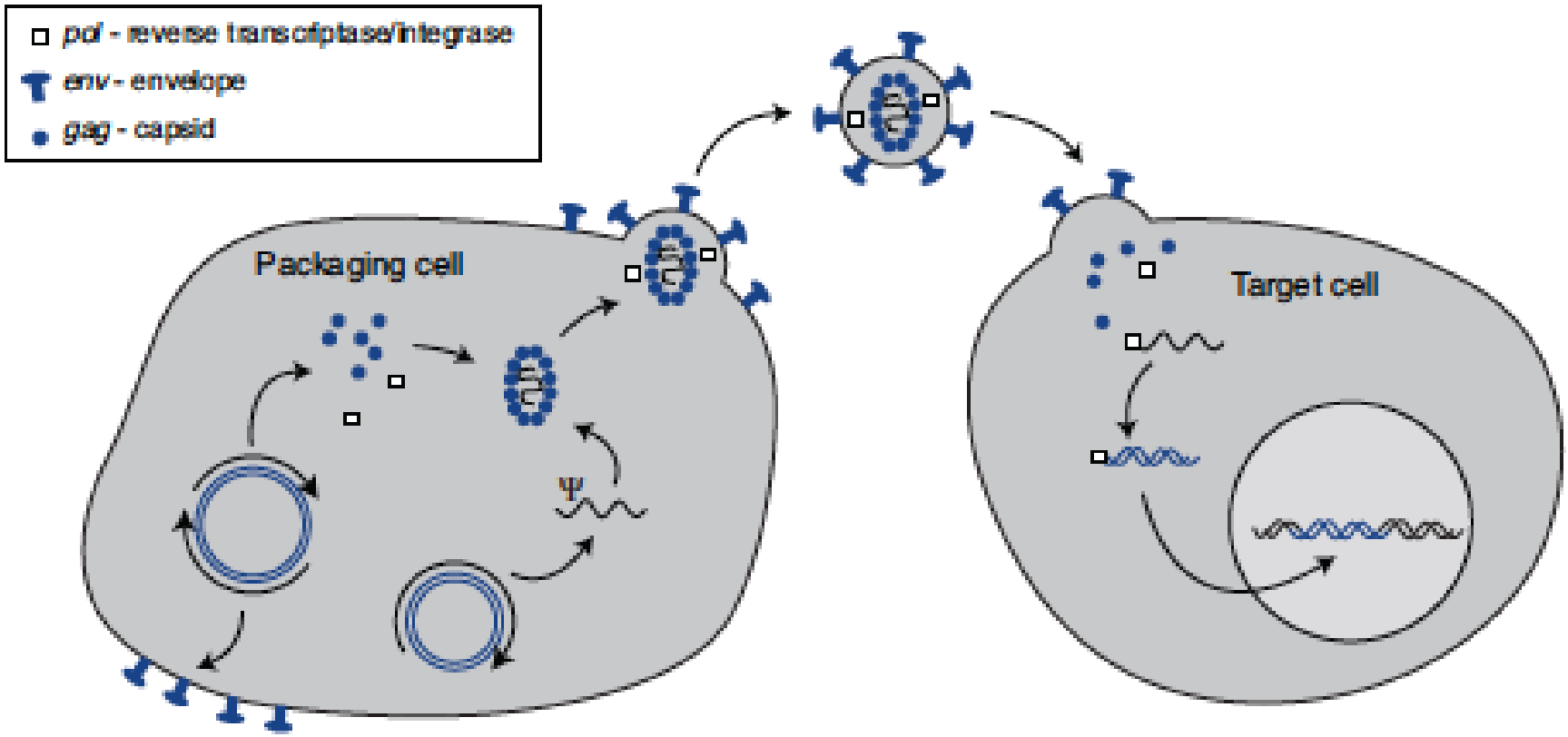


Células empacotadoras




2-3 days
Transfection
1-2 days
Verify transient expression
2 weeks
Antibiotic selection
1-3 weeks
Isolate single clones
1-2 weeks
Screen single clones
1 week
Expand positive clones
2-3 days
Make frozen stock

Produção de vírus recombinantes em células empacotadoras e posterior infecção na célula-alvo



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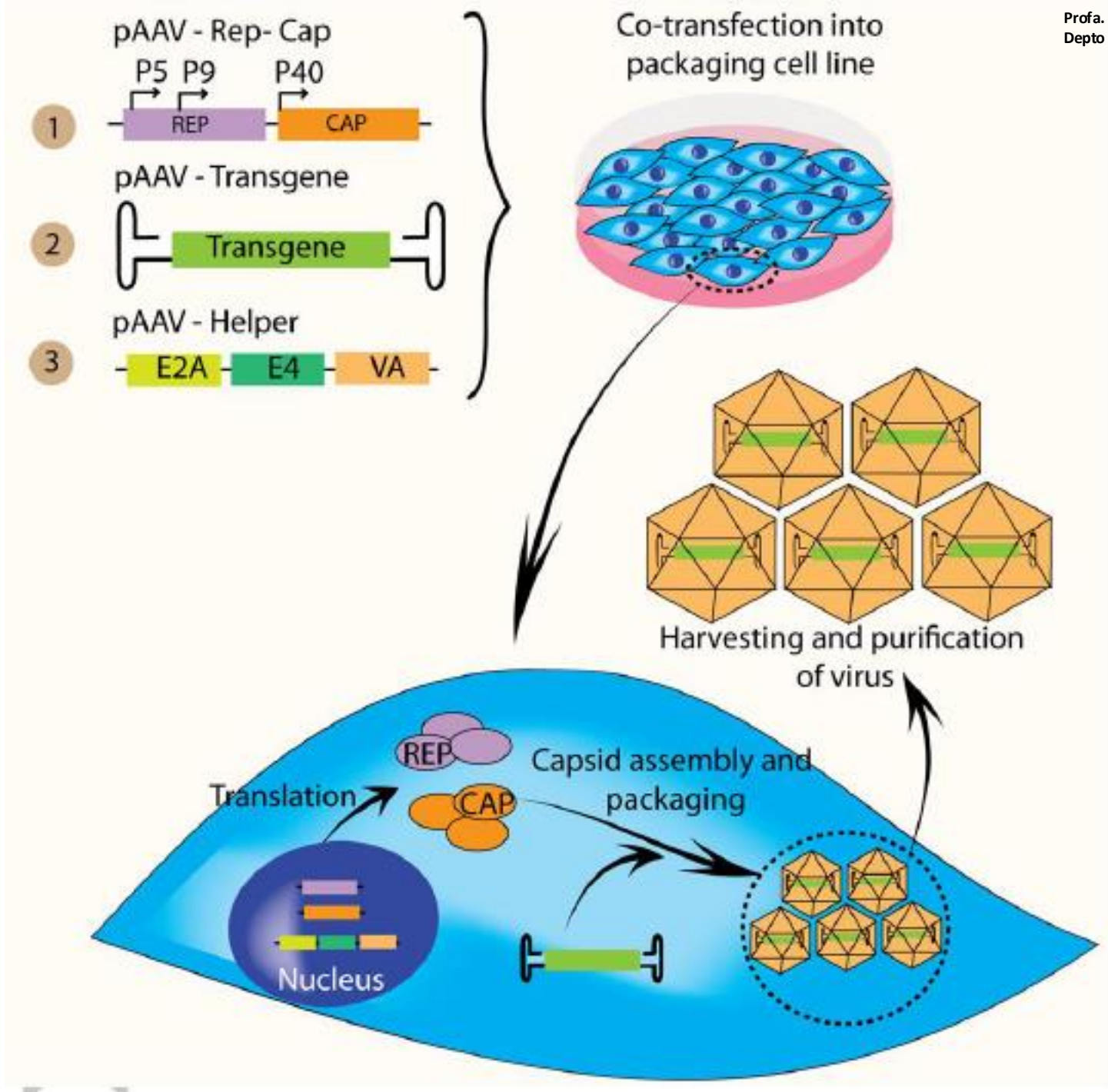
***Harvesting* de vírus recombinantes**



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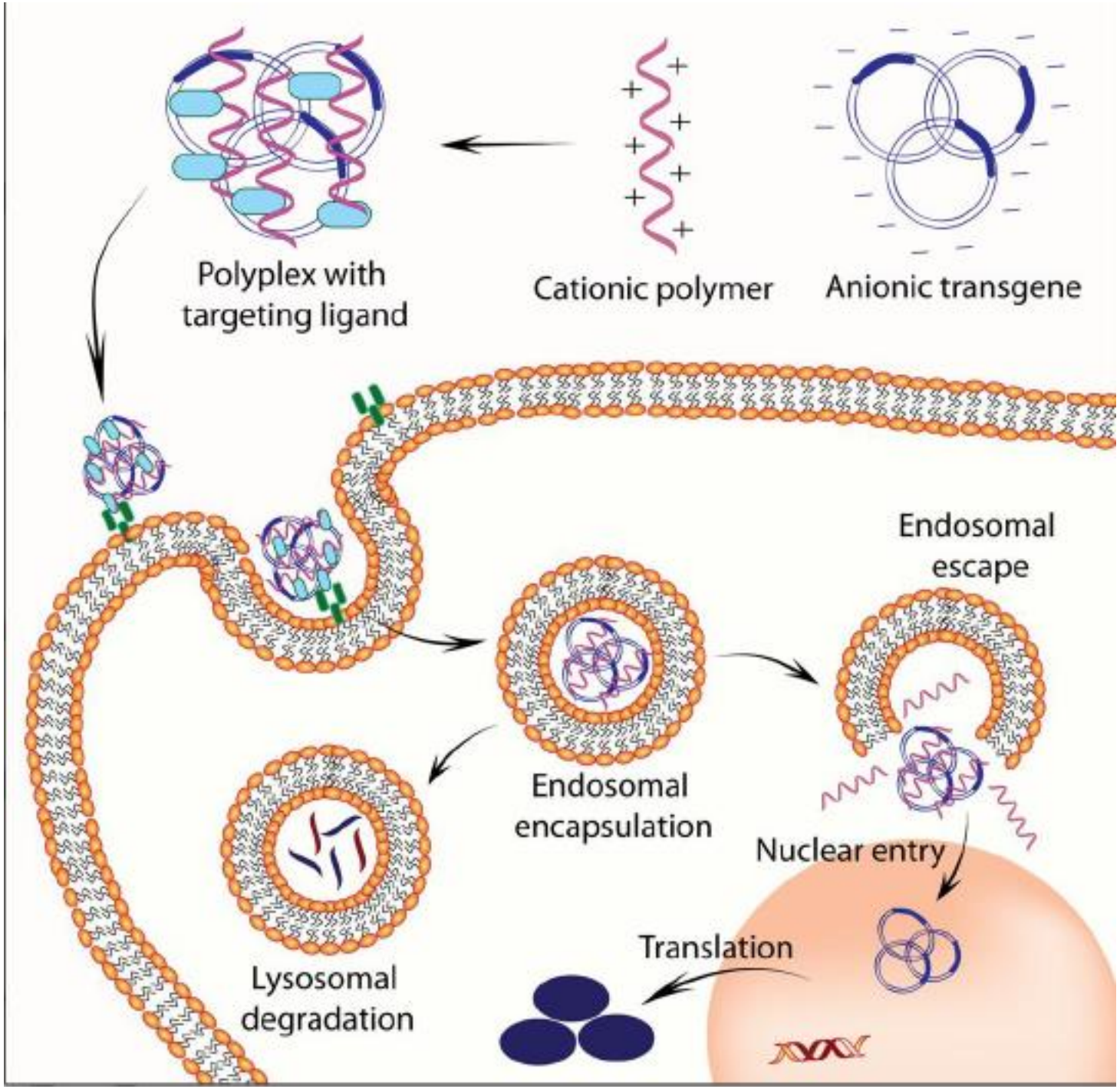
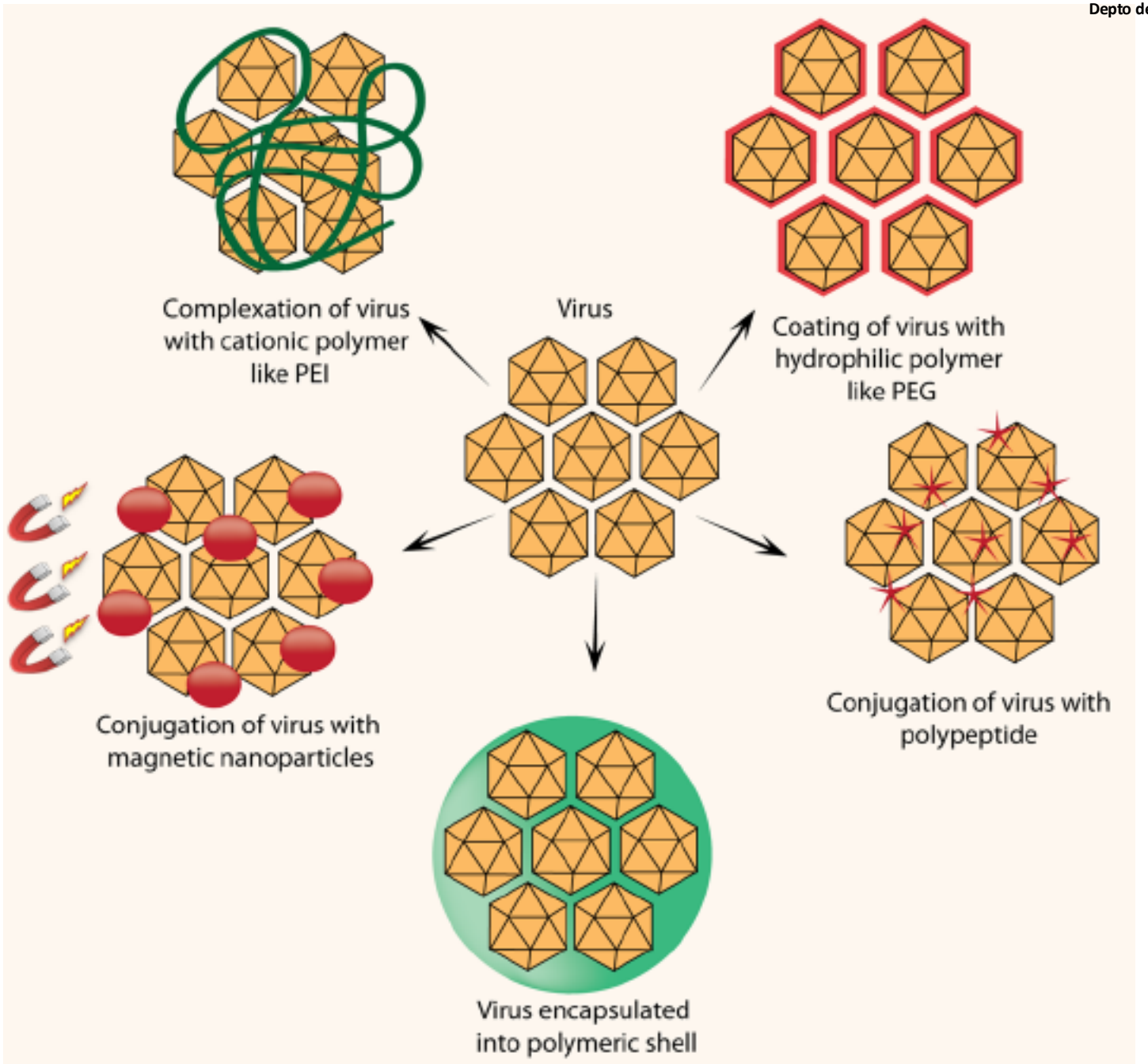
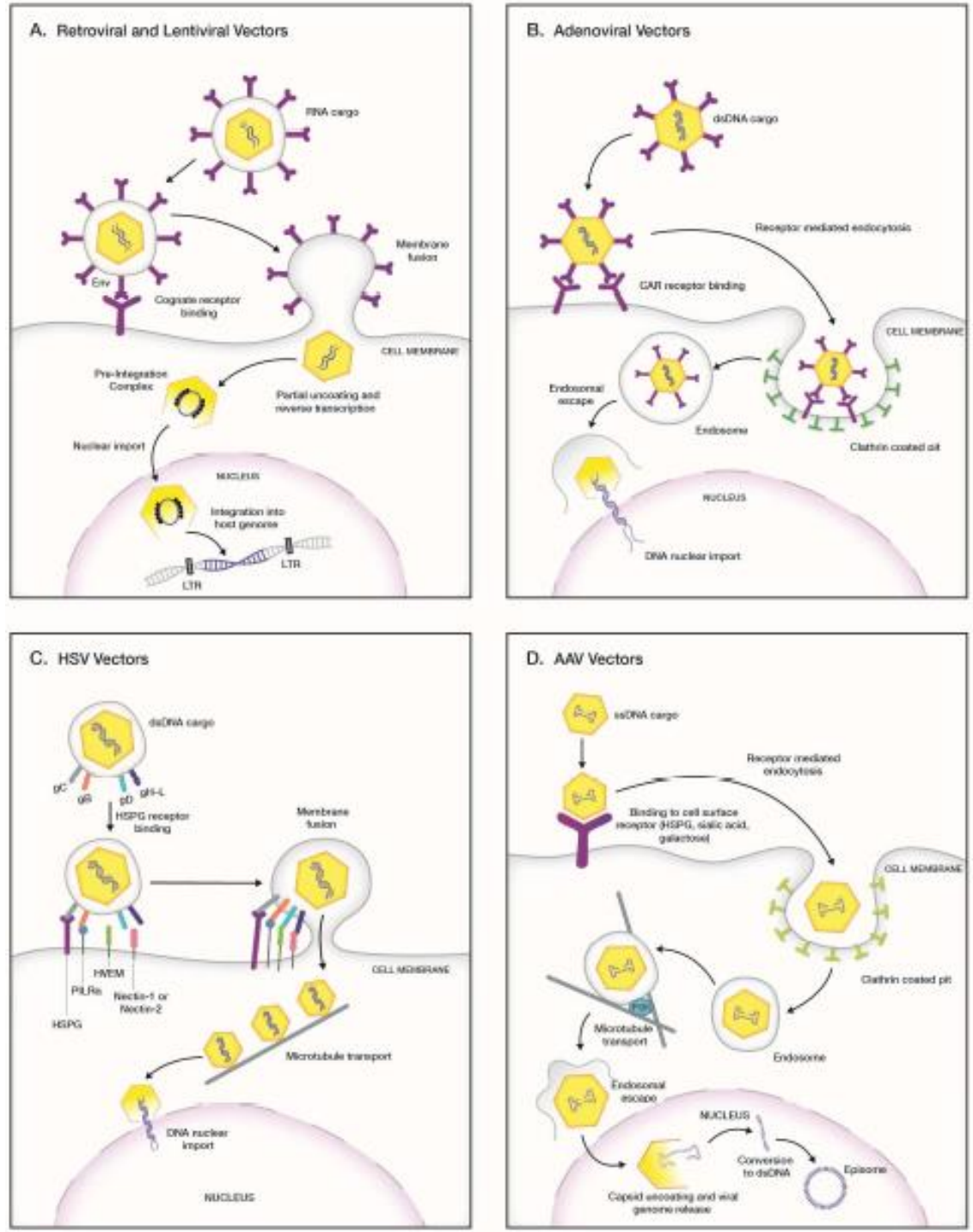
***Harvesting* de vírus recombinantes**



***Harvesting* de vírus recombinantes**




A. Retroviral and Lentiviral Vectors
RNA cargo
Membrane fusion
Env
Cognate receptor binding
CELL MEMBRANE
Pre-Integration Complex
Partial uncoating and reverse transcription
Nuclear import
NUCLEUS
Integration into host genome
LTR
LTR
B. Adenoviral Vectors
dsDNA cargo
Receptor mediated endocytosis
CAR receptor binding
CELL MEMBRANE
Endosomal escape
Endosome
Clathrin coated pit
NUCLEUS
DNA nuclear import
C. HSV Vectors
dsDNA cargo
gC
gB
gD
gH-L
HSPG receptor binding
Membrane fusion
CELL MEMBRANE
HVEM
PILRa
Nectin-1 or Nectin-2
HSPG
Microtubule transport
DNA nuclear import
NUCLEUS
D. AAV Vectors
ssDNA cargo
Receptor mediated endocytosis
Binding to cell surface receptor (HSPG, sialic acid, galactose)
CELL MEMBRANE
Clathrin coated pit
Microtubule transport
Endosome
Endosomal escape
NUCLEUS
Conversion to dsDNA
Episome
Capsid uncoating and viral genome release

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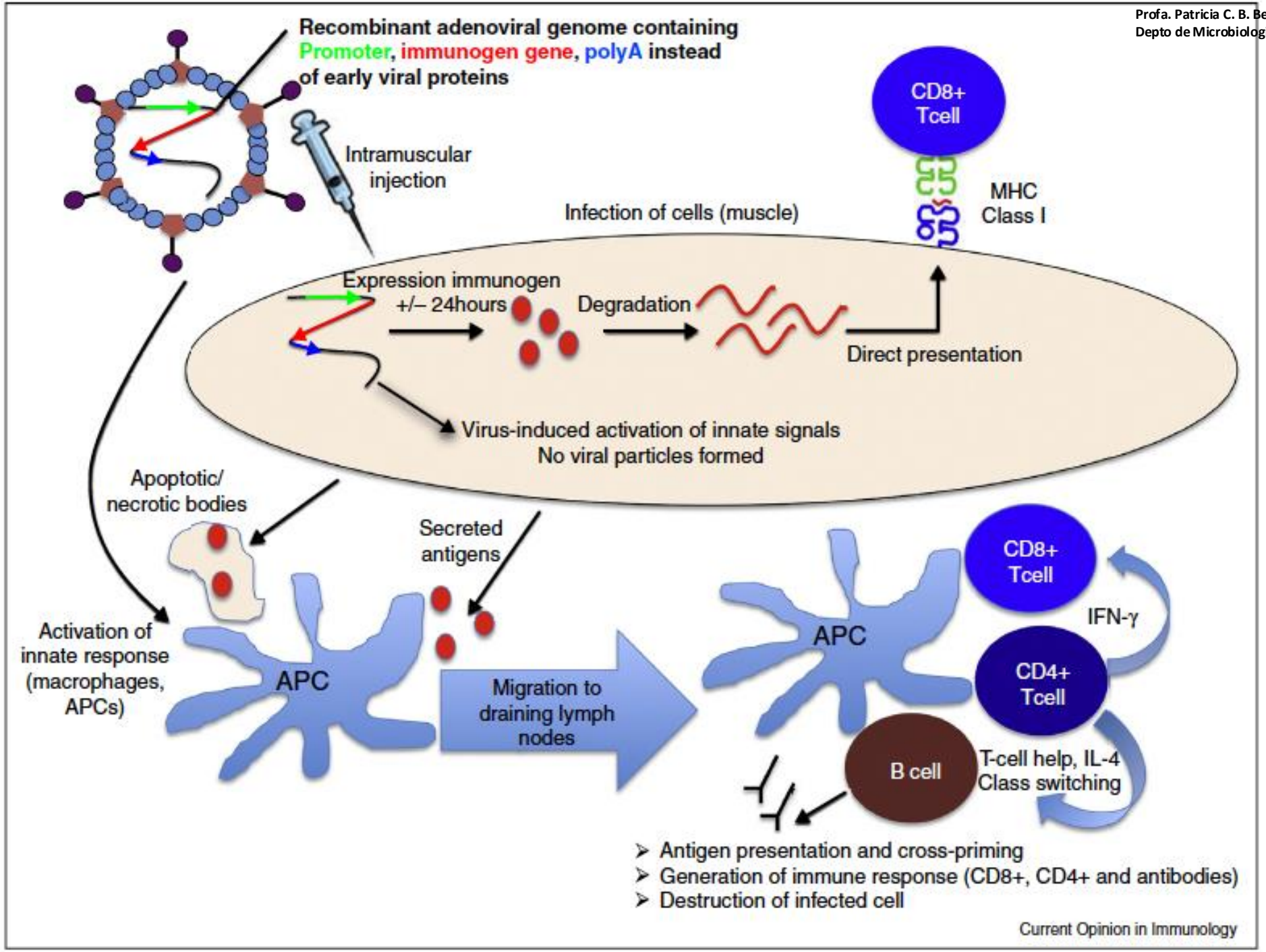
Vírus recombinantes e Vacinas



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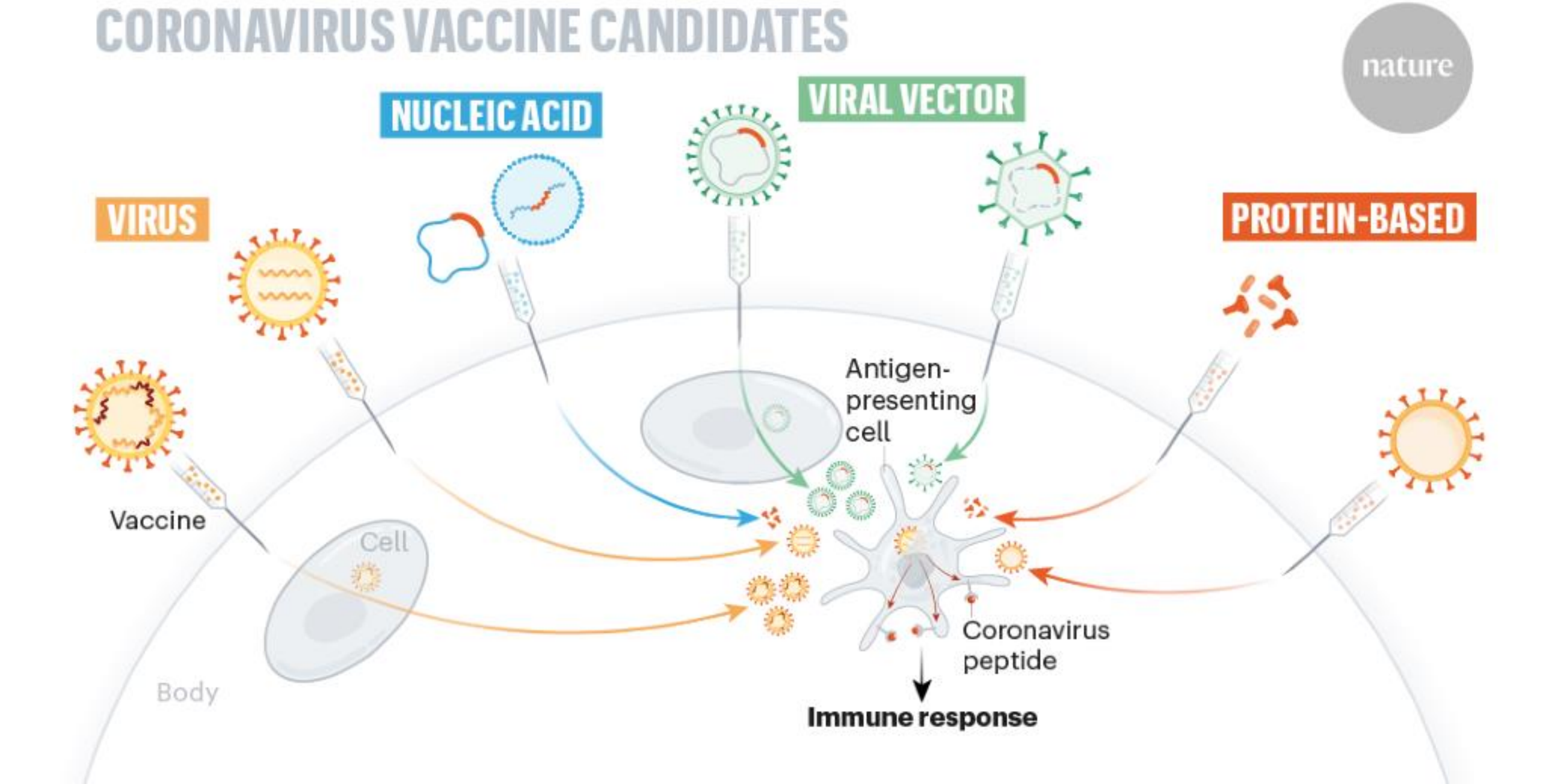

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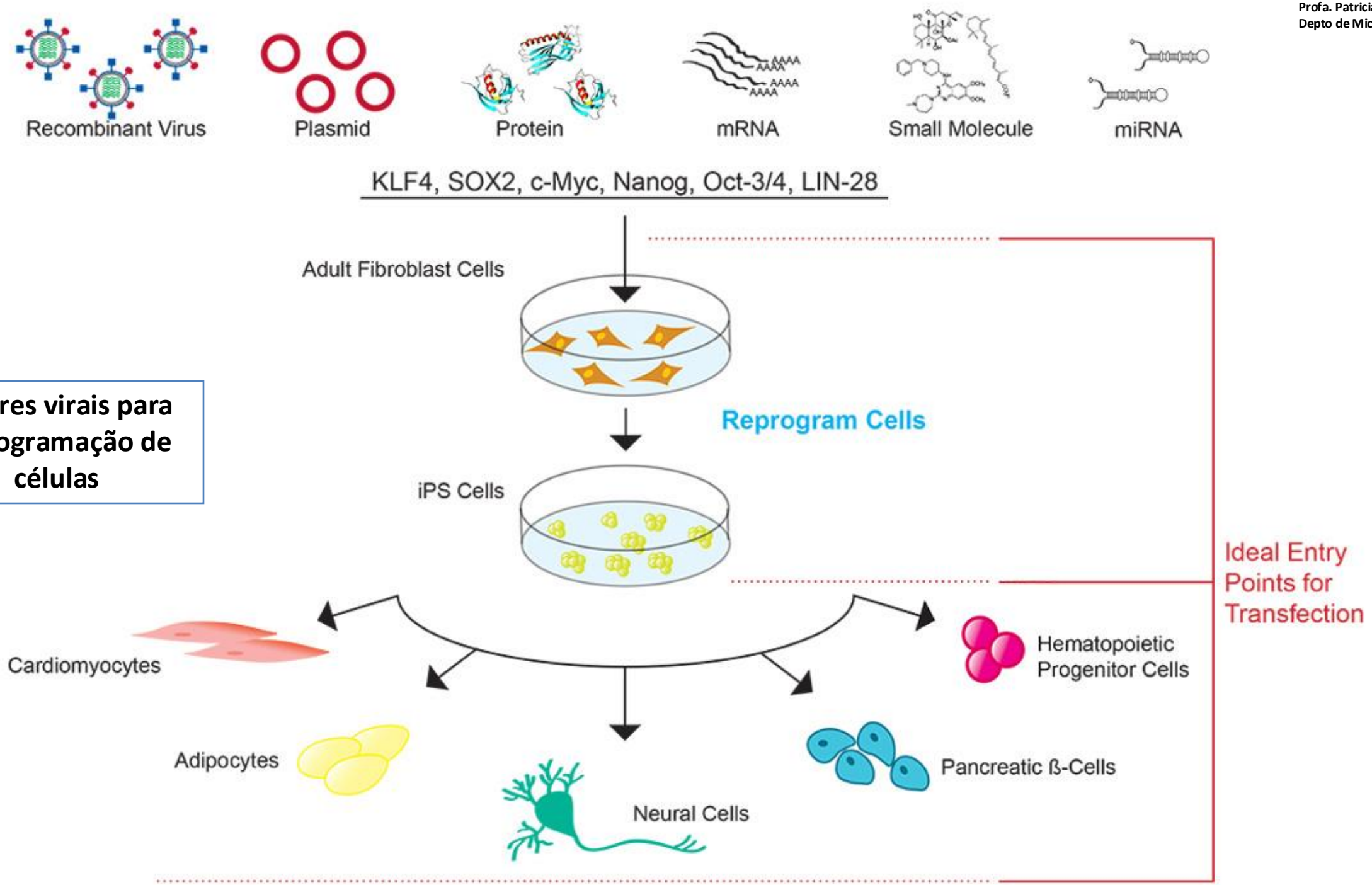
Vetores virais para reprogramação de células



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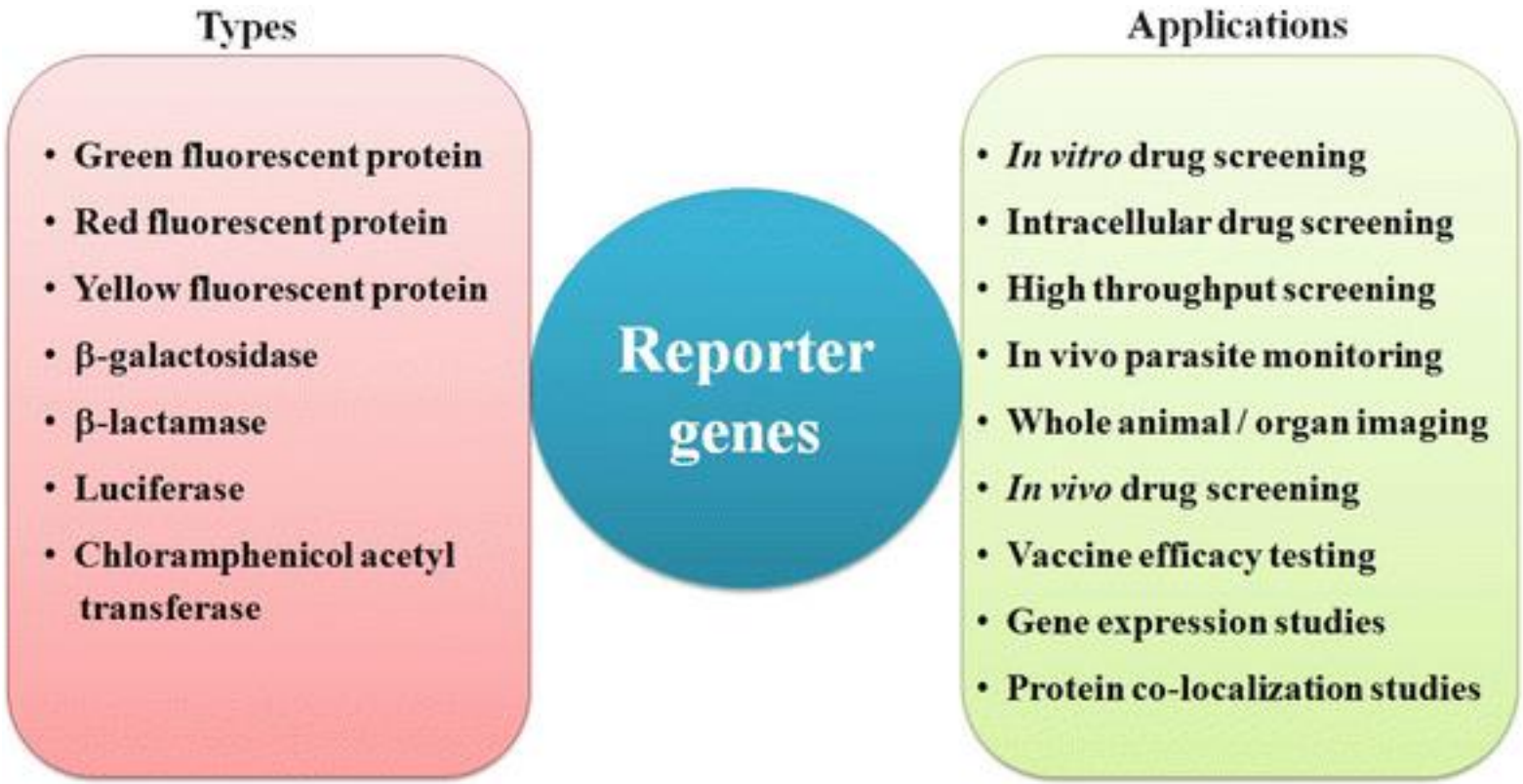
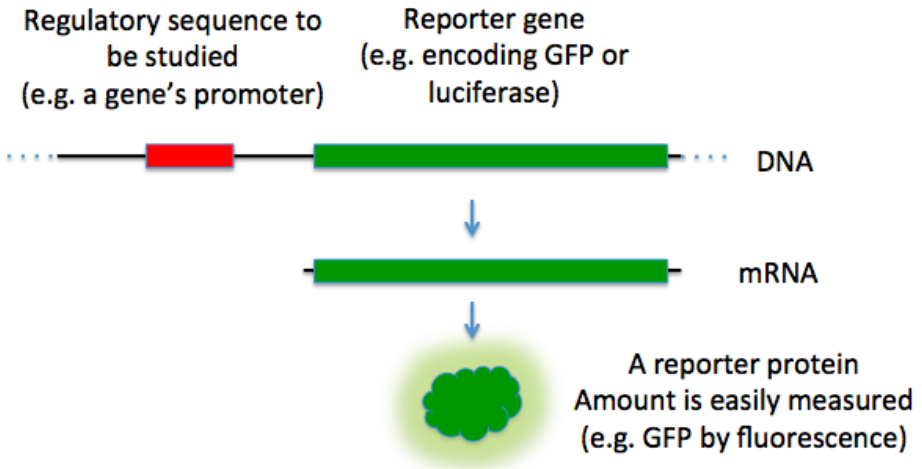
Vetores virais para marcação de células





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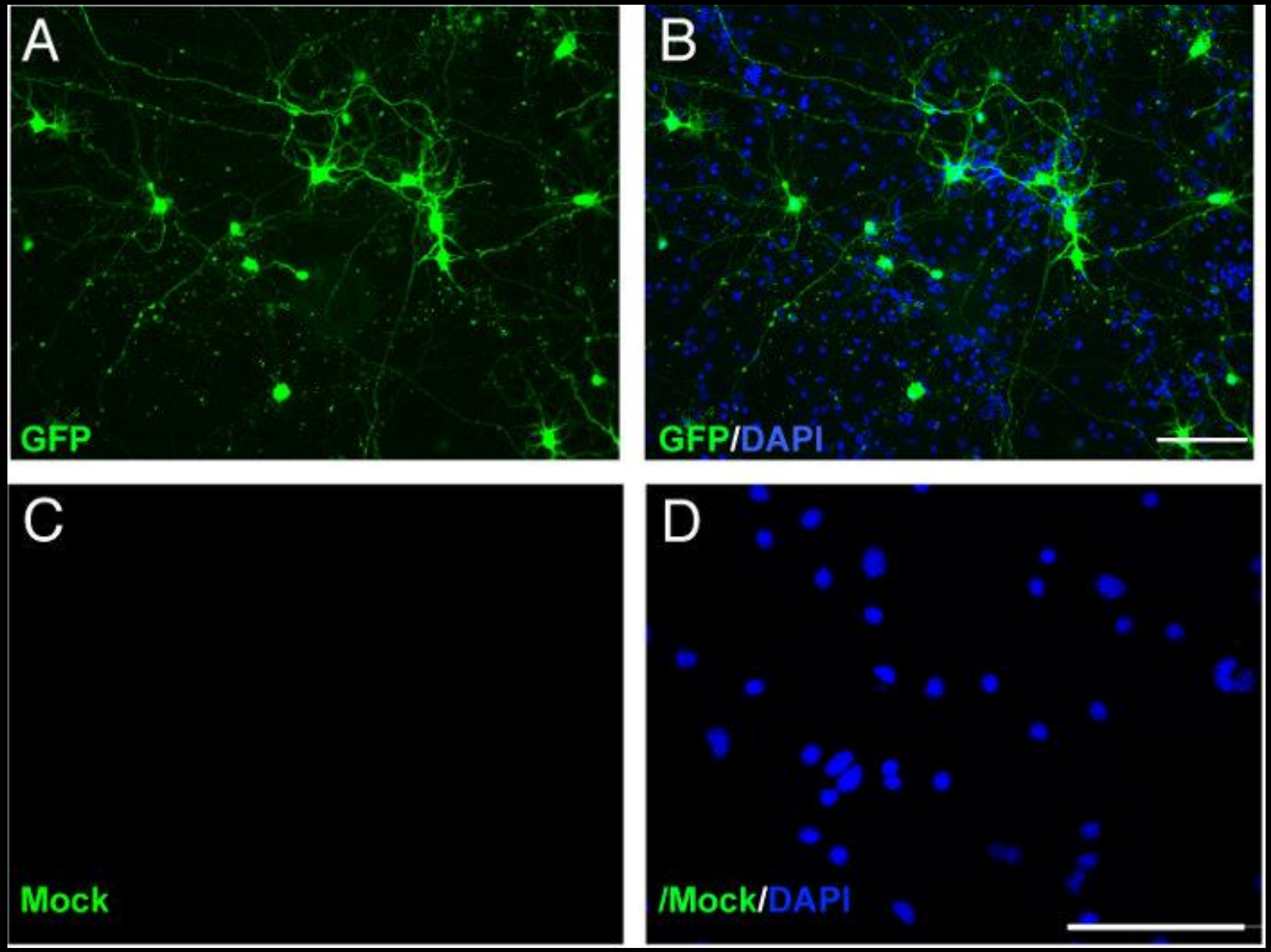

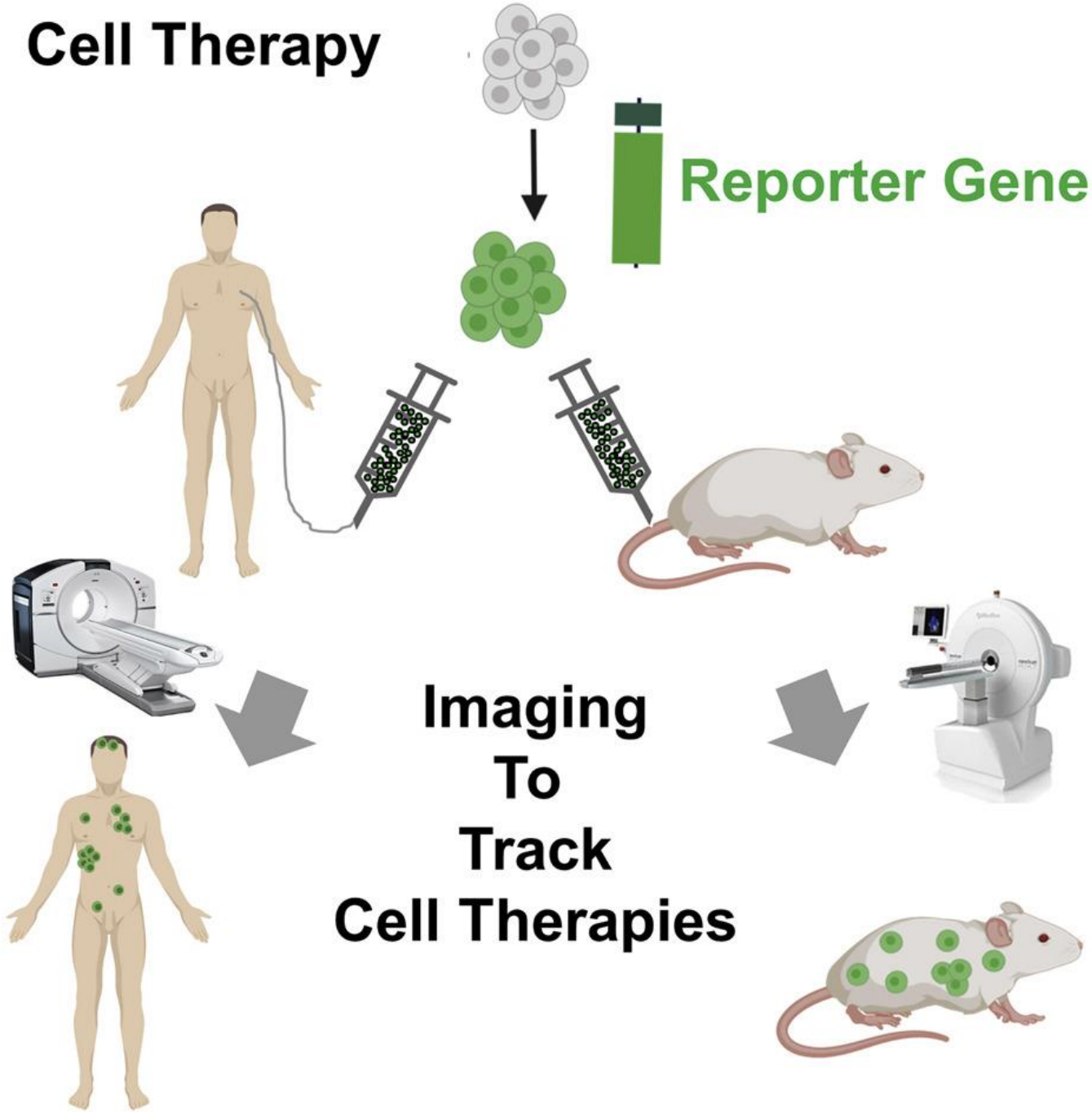
Cell Therapy
Reporter Gene
Imaging
To
Track
Cell Therapies

Disease	Vector	Transgene	Phase	Trial code
Ex vivo				
Alzheimer's disease	Retrovirus	NGF	I	US-0322
Metachromatic leukodystrophy	Lentivirus	ARSA	I, II	Biffi et al., 2013
Multiple sclerosis	Retrovirus	MBP	I, II	US-0851
Wiskott-Aldrich syndrome	Lentivirus	WASP	I, II	Aiuti et al., 2013
X-linked adrenoleukodystrophy	Lentivirus	ABCD1	I, II	Cartier et al., 2009
In vivo				
AADC deficiency	AAV	AADC	I, II	NCT01395641
Alzheimer's disease	AAV	NGF	I, II	NCT00087789, NCT00876863
Batten disease	AAV	CLN2	I	NCT00151216
Batten disease	AAV	CLN2	I, II	NCT01414985
Canavan disease	AAV	ASPA	I	Leone et.al., 2012
Giant axonal neuropathy	AAV	GAN	I	NCT02362438
Glioblastoma	Oncolytic poliovirus	-	I	NCT01491893
Glioblastoma multiforme (GBM), other gliomas	Oncolytic adenovirus	-	I	NCT00805376, NCT01956734, NCT02197169
Glioblastoma multiforme, other gliomas	Retrovirus	CD	I, II/III	NCT01470794, NCT02414165
Glioblastoma, other gliomas	Oncolytic HSV	-	I	NCT02031965
Glioblastoma, other gliomas	Oncolytic HSV	-	I	NCT00028158, NCT00157703
Leber's hereditary optic neuropathy	AAV	MT-ND4	I	NCT02161380
Metachromatic leukodystrophy	AAV	ARSA	I, II	NCT01801709
MPS IIIA (Sanfilippo Disease Type A)	AAV	SGSH, SUMF1	I, II	NCT01474343, NCT02053064
Parkinson's disease	AAV	GAD	I, II	NCT00195143, NCT00643890
Parkinson's disease	AAV	NTRN	I, II	NCT00252850, NCT00400634
Parkinson's disease	Lentivirus	TH, AADC, CH1	I, II	NCT00627588, NCT01856439
Parkinson's disease	AAV	GDNF	I	NCT01621581
Parkinson's disease	AAV	AADC	I, II	NCT02418598
Parkinson's disease	AAV	AADC	I	NCT00229736
Pompe disease	AAV	GAA	I, II	NCT00976352
Pompe disease	AAV	GAA	I	NCT02240407
Spinal muscular atrophy type 1	AAV	SMN	I	NCT02122952