Technologické aspekty vývoje genových terapií

Slabý Ondřej

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Masarykův onkologický ústav, Brno

Co je to genová terapie?

- The insertion of usually genetically altered genes into cells especially to replace defective genes in the treatment of genetic disorders or to provide a specialized diseasefighting function

- *Merriam-Webster Dictionary*

- Experimental treatment of a genetic disorder by replacing, supplementing, or manipulating the expression of abnormal genes with normally functioning genes

- *Natio*

- It is
  gene

- *American Society of Gene and Cell Therapy*

- Gene therapy is the use of DNA as a pharmaceutical agent to treat disease

- *Wikipedia*

Genová terapie je nástroj moderní terapie založený na cílené modifikaci (či interakci?) s buněčnou DNA/RNA za účelem aktivace nebo inaktivace konkrétního genu. ???

Genová terapie monogenních podmíněných chorob je léčebný postup při němž je do genomu pacienta vložena sekvence DNA přičemž tato sekvence kóduje nějaký chybějící nebo nefungující protein.

Definice SÚKL

- biologické léčivé přípravky s léčivou látkou, jež obsahuje rekombinantní NK, nebo je touto kyselinou tvořena používanou nebo podávanou lidem k regulaci, opravě výměně, doplnění nebo odstranění genetické sekvence, přičemž léčebný, preventivní nebo diagnostický účinek těchto léčivých přípravků se vztahuje přímo na sekvenci rekombinantní NK nebo na produkt genetické exprese této sekvence; léčivé přípravky pro genovou terapii nezahrnují vakcíny proti infekčním onemocněním

https://www.mzp.cz/C1257458002F0DC7/cz/verejna_schuze_gmo_prezentace/$FILE/oeres-novinky_lecive_pripravky_GMO-20170613.pdf (10-12-2017)

Milníky a píky genové terapie

1990 PRVNÍ GT
ADA-SCID

Figure 3: Number of gene therapies in active development

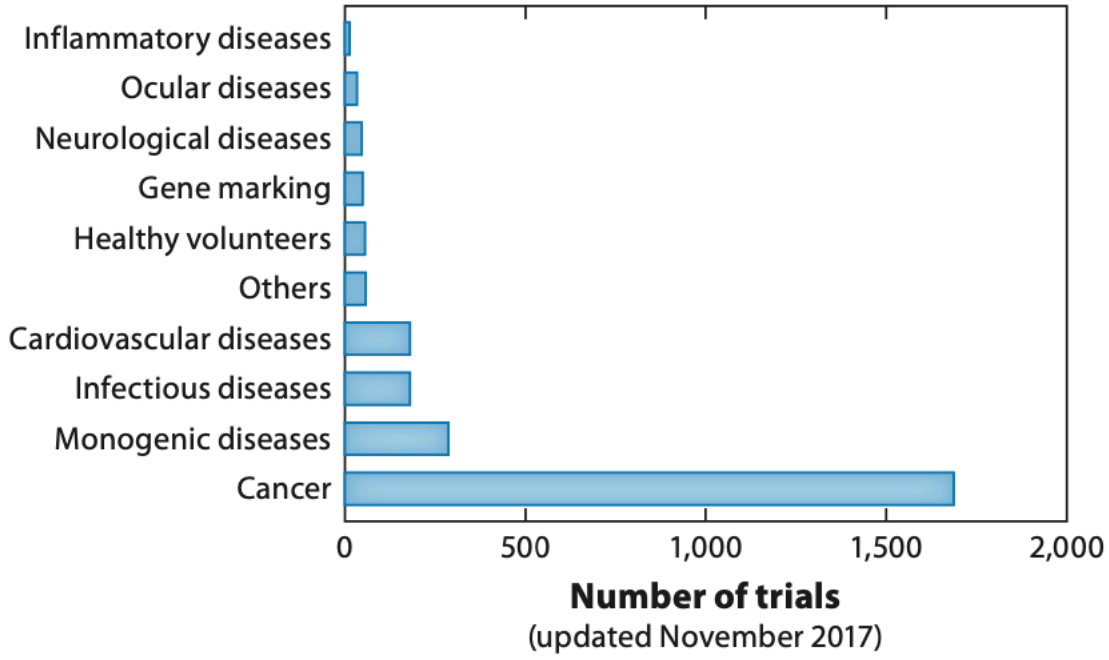

Figure 1

Indications in gene therapy clinical trials. The bar graph classifies clinical gene transfer studies by disease. Adapted from Reference 1.

Source: Ph

This growth is support
investigational new dr
cellular and gene thera

He died of systemic inflammatory response syndrome – immune reaction to adenovirus vector

The leukemia was caused by insertion of retrovirus near proto-oncogenes and activation of these proto-oncogenes by retroviral switches

he number of applications for
device exceptions (IDE) relating to
nce 2010 (Figure 4).

ren has just been

world's first gene therapy
ght by the European
vere combined
at can be fatal in a very short

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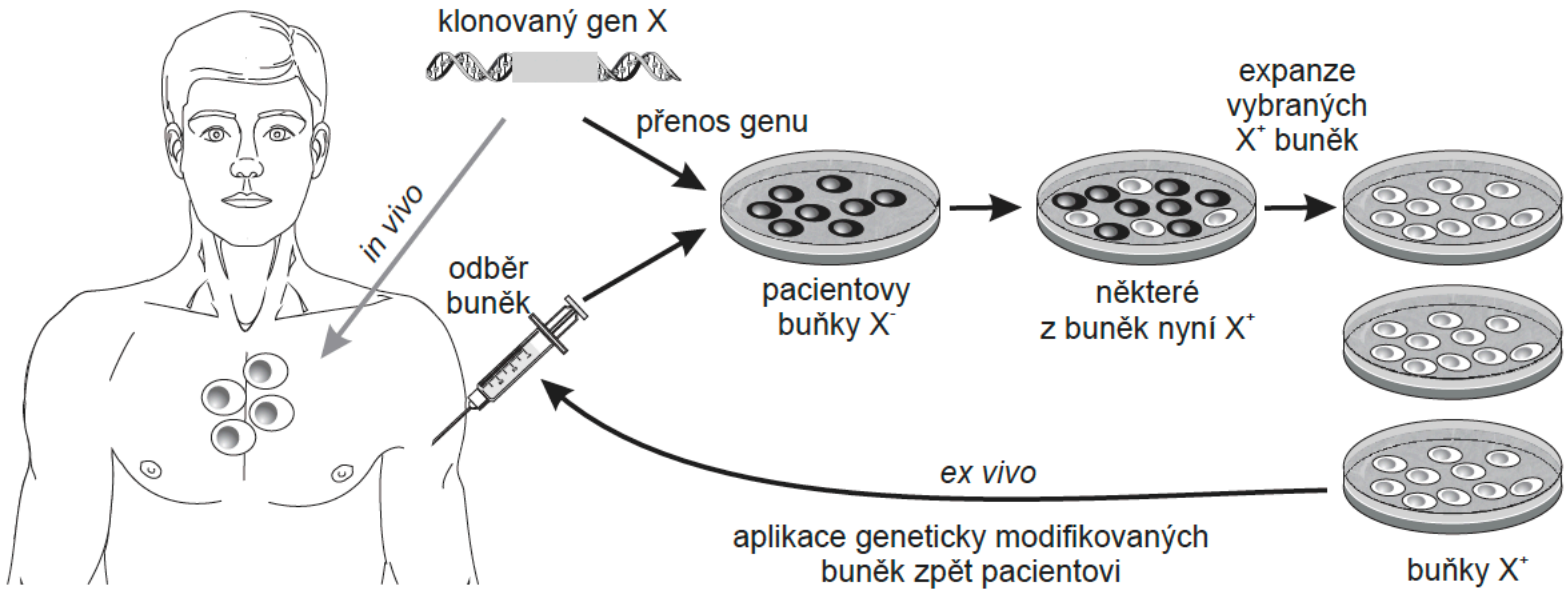
val granted to

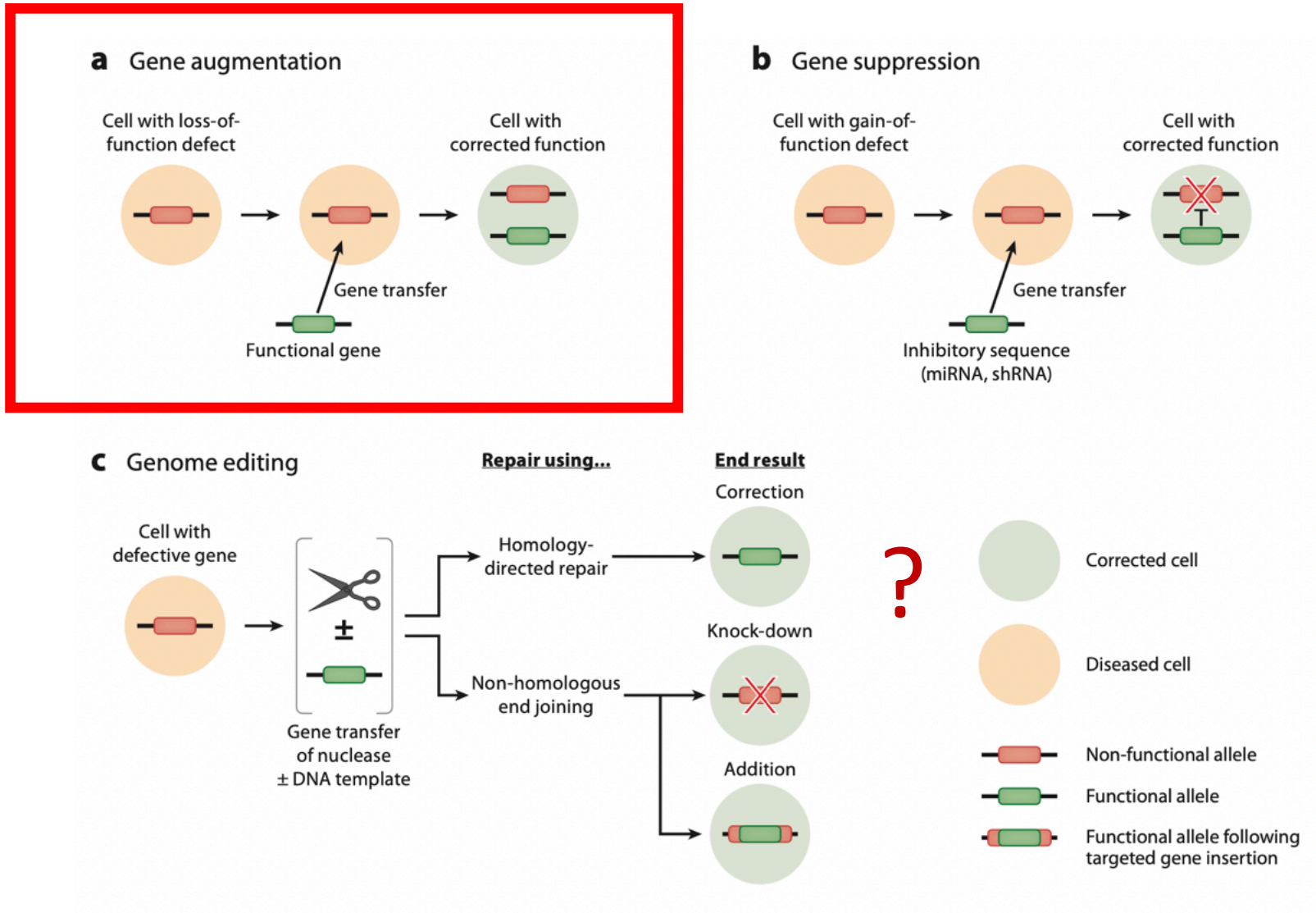
Základní typy genové terapie

Genová terapie somatických buněk vs. ~~Genová terapie zárodečných buněk~~ (celosvětové moratorium)

Genová terapie EX VIVO vs. IN VIVO



Tři základní principy genové terapie

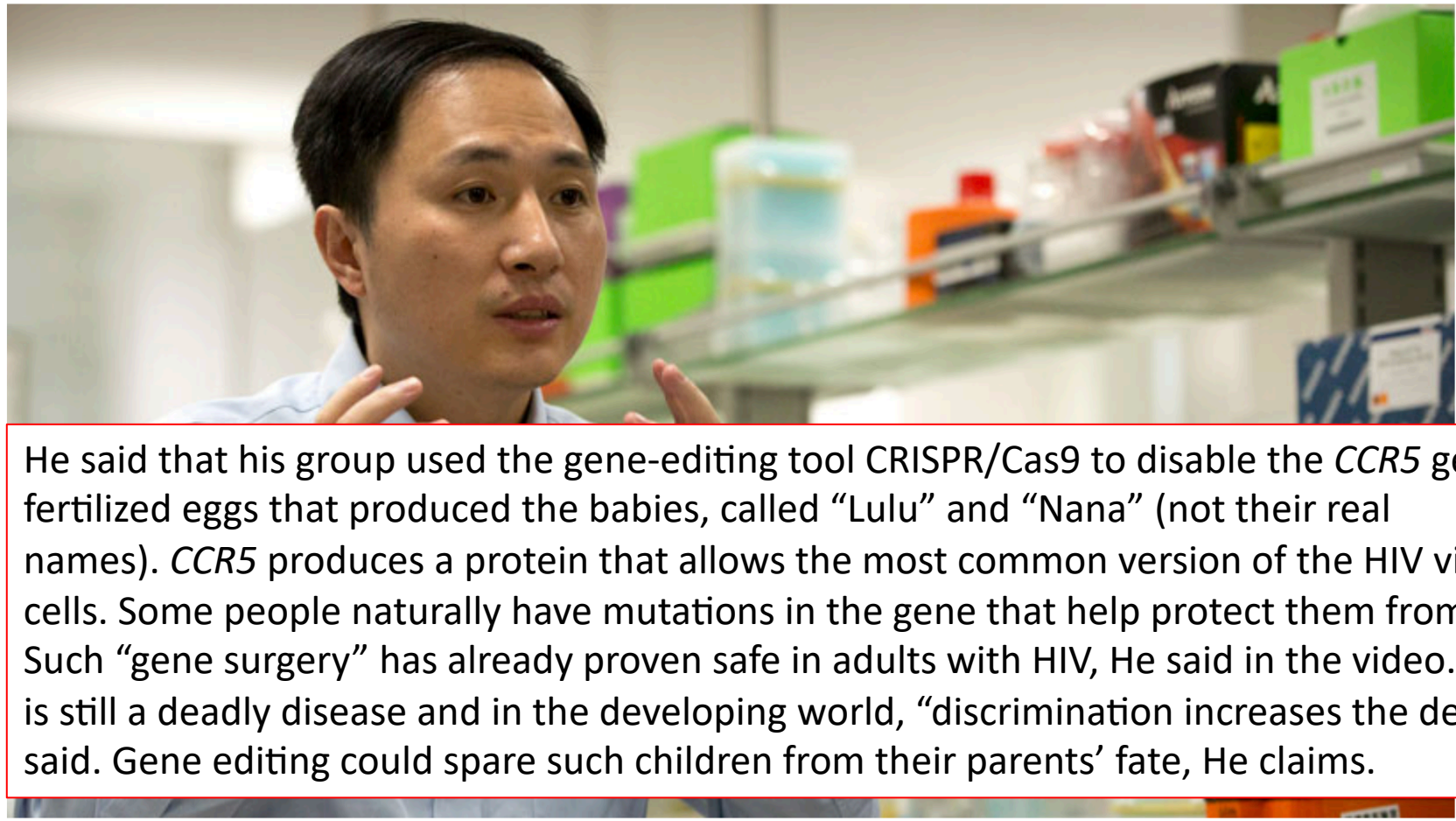


Anguela XM, Annual Review of Medicine, 2019

NEWS GENETICS

Chinese scientists raise ethical questions with first gene-edited babies

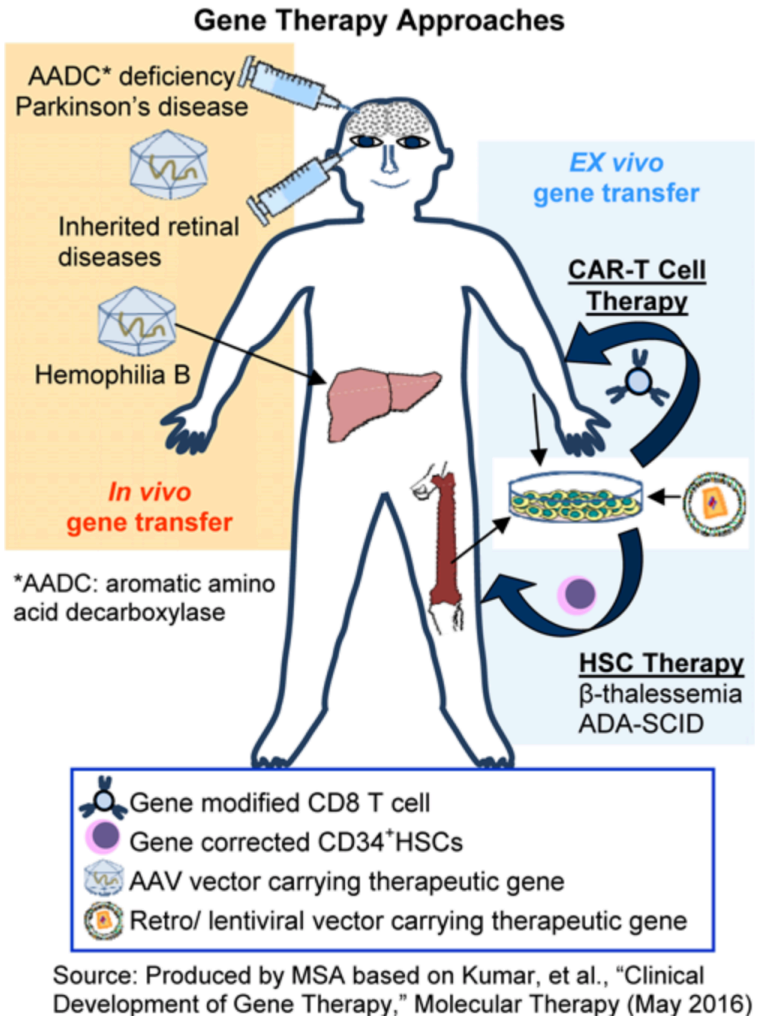
Researchers used CRISPR/Cas9 to alter a gene involved in HIV entry into cells



He said that his group used the gene-editing tool CRISPR/Cas9 to disable the *CCR5* gene in the fertilized eggs that produced the babies, called “Lulu” and “Nana” (not their real names). *CCR5* produces a protein that allows the most common version of the HIV virus to enter cells. Some people naturally have mutations in the gene that help protect them from HIV infection. Such “gene surgery” has already proven safe in adults with HIV, He said in the video. HIV infection is still a deadly disease and in the developing world, “discrimination increases the devastation,” He said. Gene editing could spare such children from their parents’ fate, He claims.

GENE EDITOR Jiankui He, shown in a lab in Shenzhen, China, on October 10, has announced the birth of the world’s first gene-edited babies.

Metody dopravení DNA do tkáně



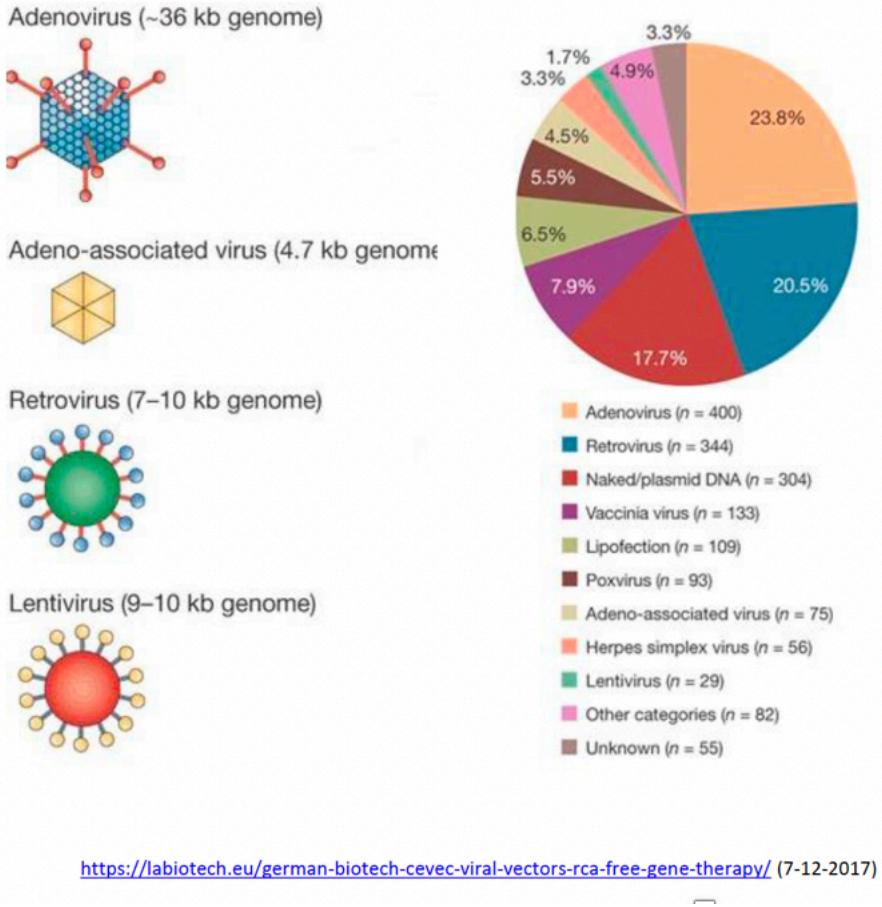
Source: Produced by MSA based on Kumar, et al., "Clinical Development of Gene Therapy," Molecular Therapy (May 2016)

Pomocí
-virových vektorů
-nevirově

Tab. 11.6.1. Srovnání nevirových strategií a metod genové terapie. Plazmidová DNA obsahující transgen bývá aplikována injekčně, pomocí fyzikálních metod (elektroporace, sonoporace), nebo metodami chemickými.

Strategie	Metoda	Výhody	Nevýhody
nahá DNA	injekční podání *in vivo*	snadné použití	malá efektivita
		možné použití pro genetické vakcíny	dočasný efekt
			internalizace jen do svalových a srdečních myocytů a antigen prezentujících buněk
fyzikální metody	elektroporace	snadná aplikace do kosterního svalstva a kůže, invazivní i do ostatních orgánů	malá efektivita
			dočasný efekt
			limitované spektrum aplikací
	sonoporace	neinvazivní aplikace	malá efektivita
			dočasný efekt
chemické metody	lipozomy	snadná aplikace	malá efektivita
	kationtové lipidy	snadné použití	dočasný efekt
	kationtové polymery		

Metody dopravení DNA do tkáně – virové vektory



https://labiotech.eu/german-biotech-cevec-viral-vectors-rca-free-gene-therapy/ (7-12-2017)

Table 1. Overview of common viruses used for generating gene therapy viral vectors.

Parameter	Retrovirus	Lentivirus	AAV	Adenovirus
Coat	Enveloped	Enveloped	Non-enveloped	Non-enveloped
Packaging capacity (Kb)	8	8	~4.5	7.5
Tropism/infection	Dividing cells	Broad	Broad excluding hematopoietic stem cells	Broad
Inflammatory potential	Reduced	Reduced	Reduced	High
Host genome interaction	Integrating	Integrating	Integrating/ non-integrating	Non-integrating
Transgene expression	Long lasting	Long lasting	Potentially long-lasting	Transient or long-lasting depending on immunogenicity

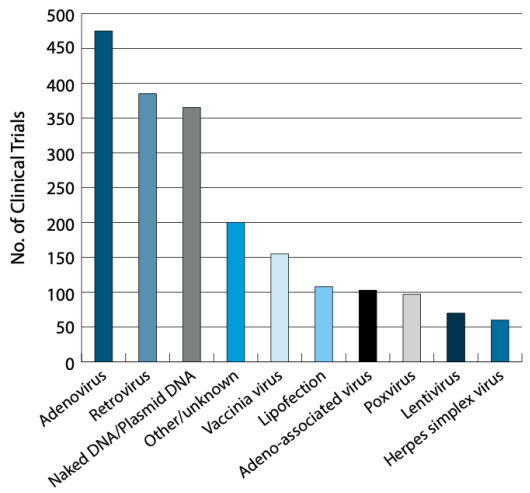
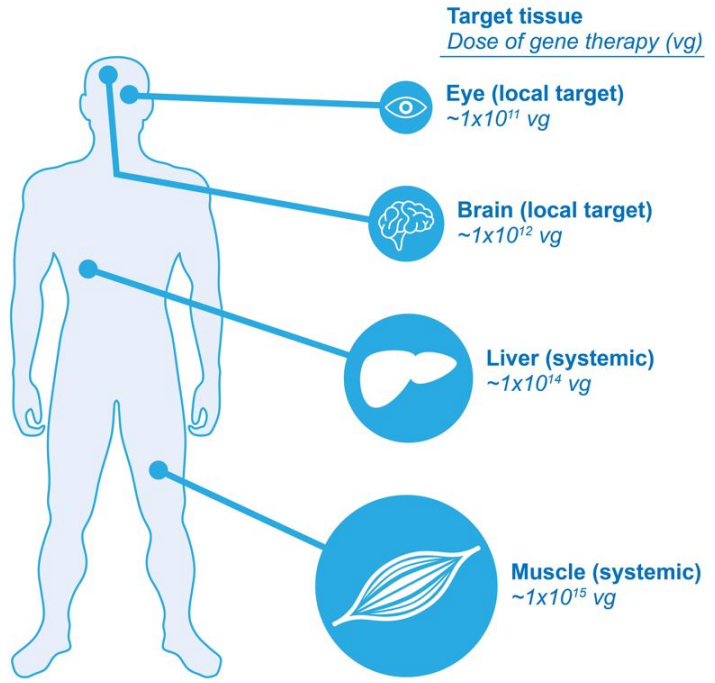


Figure 4. *Leading vectors used in gene therapy clinical trials. Data from Gene Therapy Clinical Trials Worldwide provided by the* Journal of Gene Medicine. *http://www.wiley.com//legacy/wiley chi/genmed/clinical/. Updated June 2014.*



RETROVIRY

-jsou schopny atakovat **pouze dělící se buňky** (neprochází kompaktní jadernou membránou)
- napadají především T-lymfocyty
- pouze *ex-vivo* terapie a náhodná integrace do hostitelského genomu
- riziko inzerční mutageneze
- maximální velikost transgenu do 7.5 kbp
- retroviry nezpůsobují lyzi buňky

LENTIVIRY

mohou nahradit klasické retroviry, jelikož jsou schopny infikovat a integrovat svůj genom do **nedělících buněk** např. neurony, makrofágy, **hematopoetické kmenové buňky** a buňky svalů a jater
delece v LTR, není schopen produkovat infekční částice, ale schopen integrace

ADENOVIRY

dsDNA viry
napadají všechny typy buněk
virová DNA se neintegruje do genomu hostitele
exprese je silná ale pouze dočasná (max. měsíc)
způsobují **silnou imunitní odpověď** (exprese wild-type proteinů virové částice)
nová generace adenovektorů neobsahující přirozené geny
nejsou vhodné pro léčbu nádorových buněk, protože ty na ně nemají receptory
Většina lidí má protilátky, 90% vektorů je degradováno během prvních 24 hodin od podání

ADENO-ASOCIOVANÉ VIRY (AAV)

dsDNA viry
Většina odovozena od serotypu 2 (AAV2)
nikdy nespojovány s lidskými nemocemi
napadají všechny typy buněk
virová DNA se integruje do genomu hostitele na specifickém místě (u člověka na konci 19tého chromozomu)
Není riziko inzerční mutageneze
exprese je ale slabá a vektory mají malou kapacitu 4kb
nezpůsobují silnou imunitní odpověď

Kdy je doporučeno dlouhodobé sledování záleží na použitém vektoru

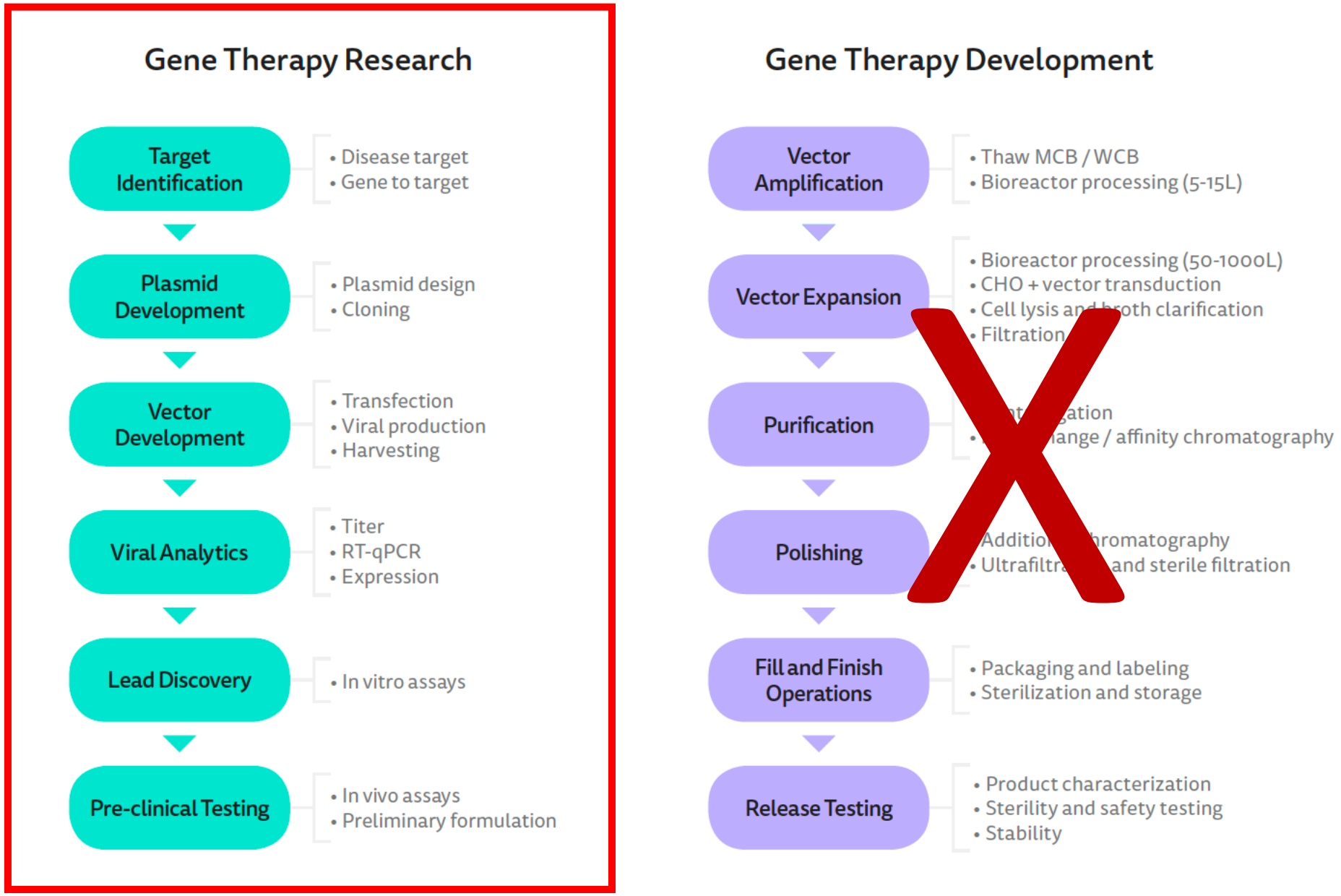
Vector	Pros	Cons	Long-Term Follow-Up Recommended?
 Adenovirus	► Capable of holding large amounts of DNA ► Non-integrating, less risk of mutations	► Only the cells that are directly modified will express the gene of interest – long-term expression is unlikely ► Can induce immune response	No*
 Adeno-associated virus (AAV)	► Not known to cause human diseases, so very little risk of immune response ► Integrates at specific site so limited risk of mutations	► Can only hold a small amount of DNA ► Transient expression	Product specific (2-5 years of follow-up)
 Retroviruses (e.g. lentivirus, gammaretrovirus)	► Long-term expression of the gene due to DNA integration ► Can infect both dividing and non-dividing cells	► Non-specific insertion site can cause high risk of mutations ► Potential for activation of proto-oncogenes and risk of malignancies	Yes
 Nonviral Vectors (e.g. plasmids, RNA)	► Capable of holding large amounts of DNA ► No immune response	► Less efficient at modifying cells	No

*Specific circumstances that indicate persistent expression of the transgene, in the absence of integration or genome editing, may require LTFU observations to mitigate long term risks to subjects.

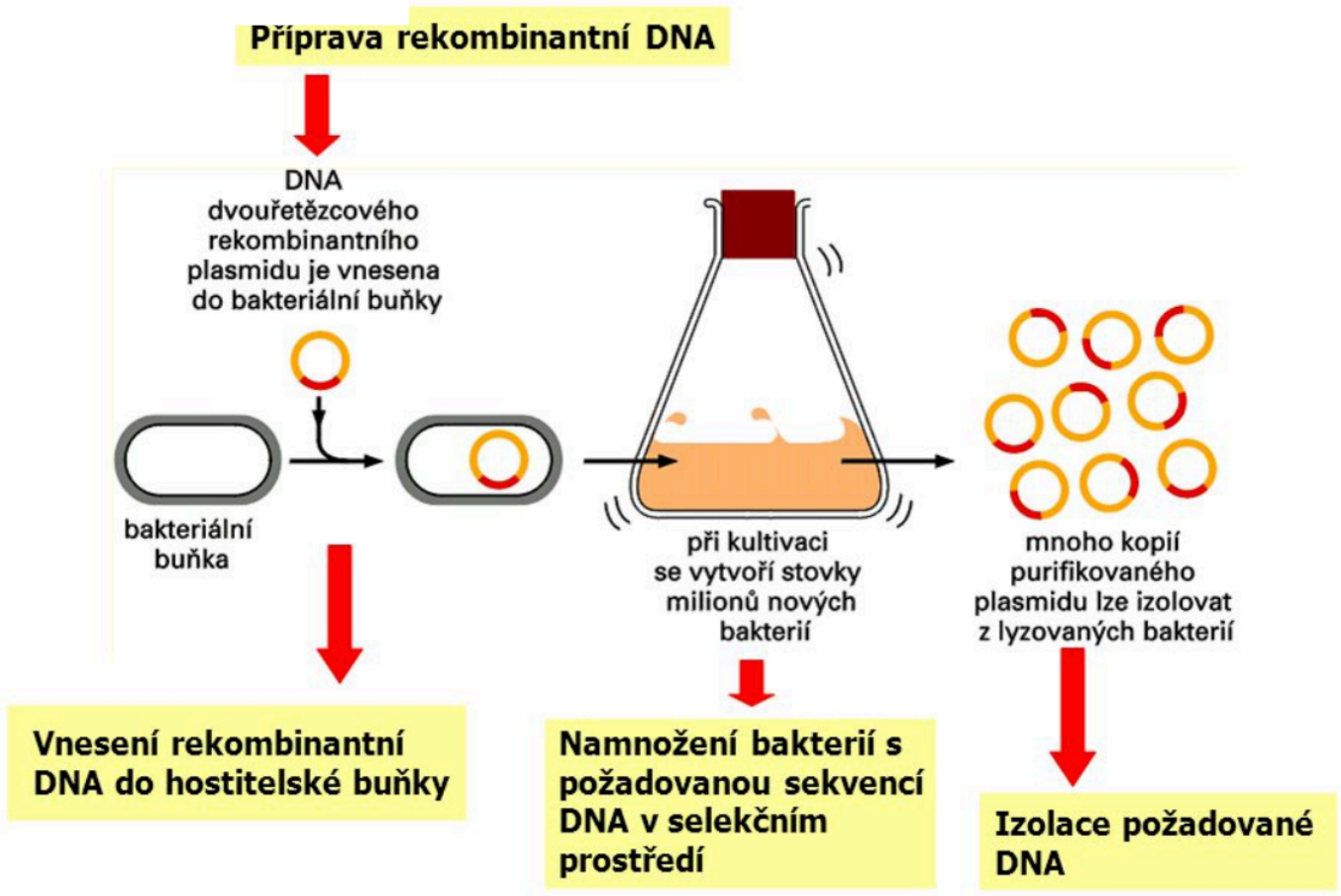
Jaké vlastnosti by měl mít ideální vektor

- Bezpečný (bez vedlejších účinku, neimunogenní)
- Cílený na příslušné buňky
- Snadno získatelný (produkce, purifikace apod.)
- Stabilní
- Efektivní v doručení DNA
- Snadno aplikovatelný
- Schopný chránit genetickou informaci, kterou nese
- Nesmí obsahovat –sekvence homologní s funkčními geny, jiné čtecí rámce, informace pro produkci toxického produktu apod.

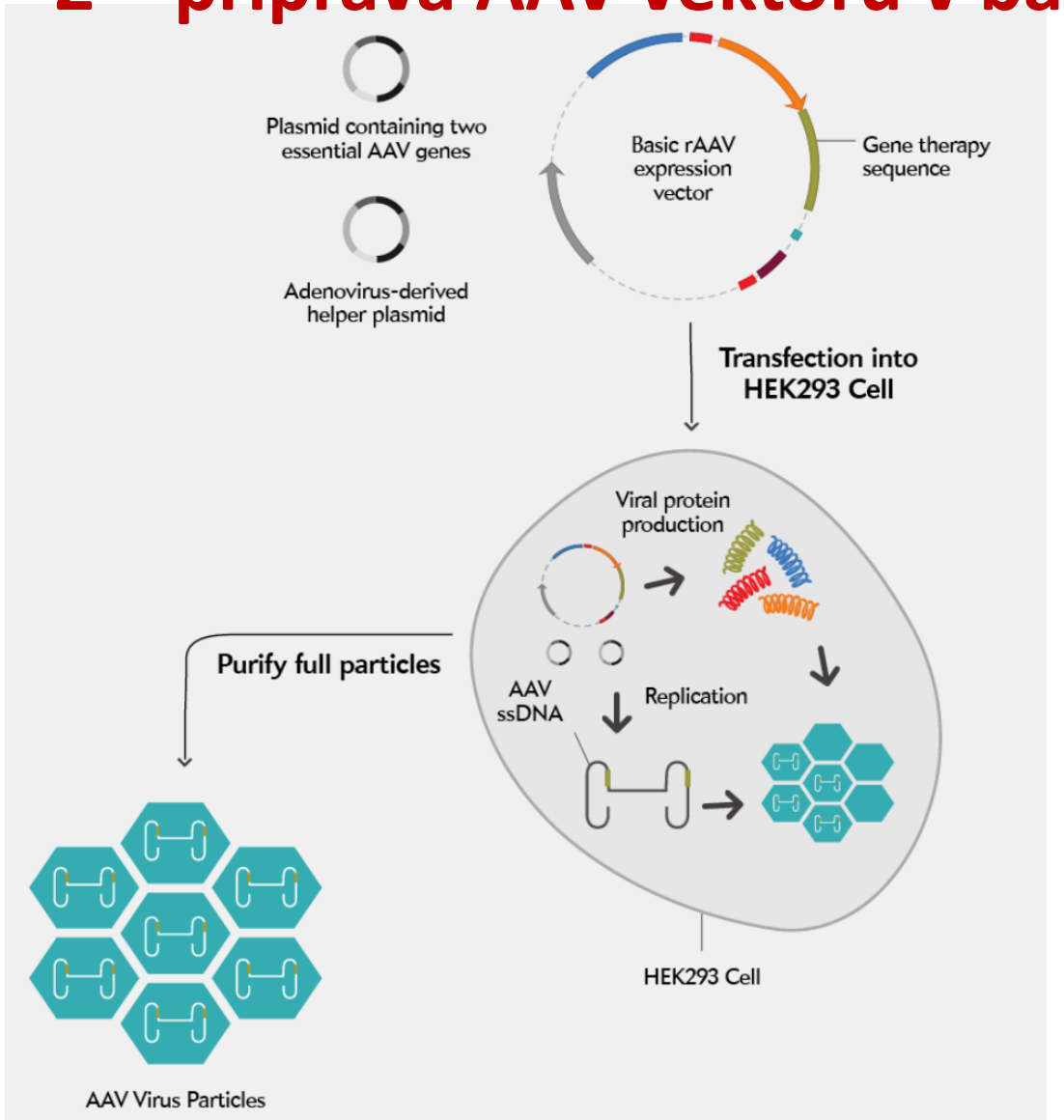
Workflow při vývoji genové terapie



Workflow při vývoji genové terapie
1 – klonování plasmidů v bakteriálním systému



Workflow při vývoji genové terapie – příklad AAV 2 – příprava AAV vektoru v balících buňkách



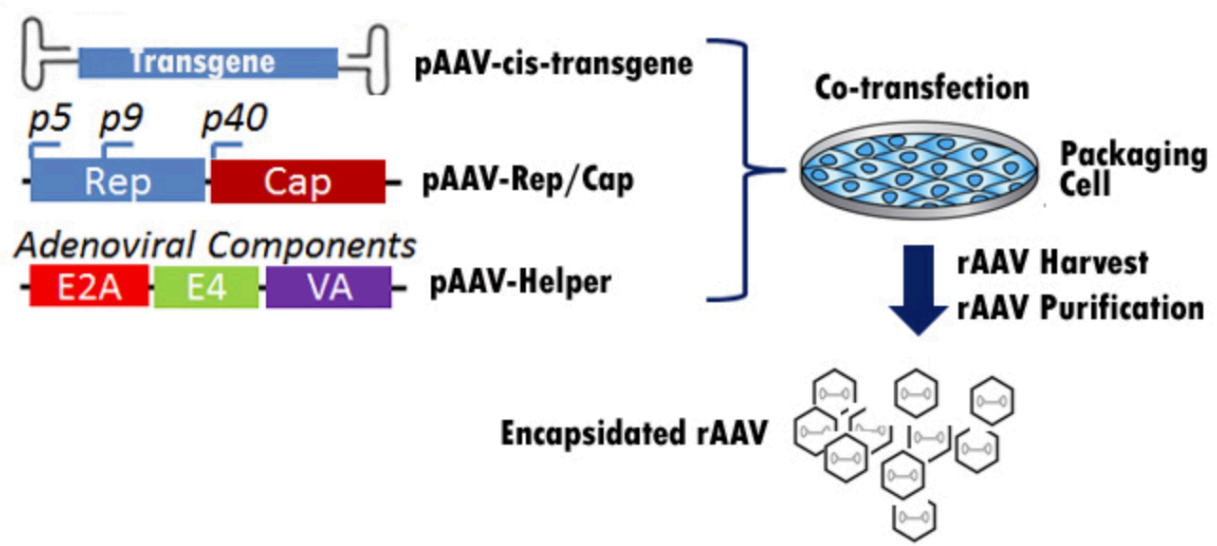
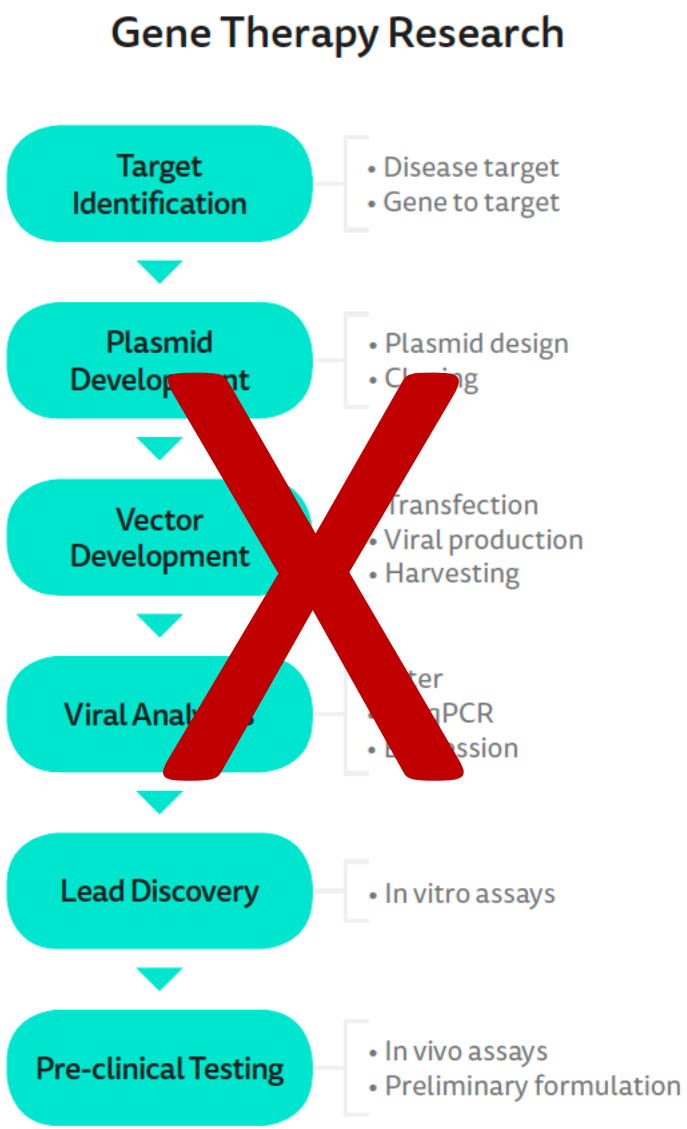
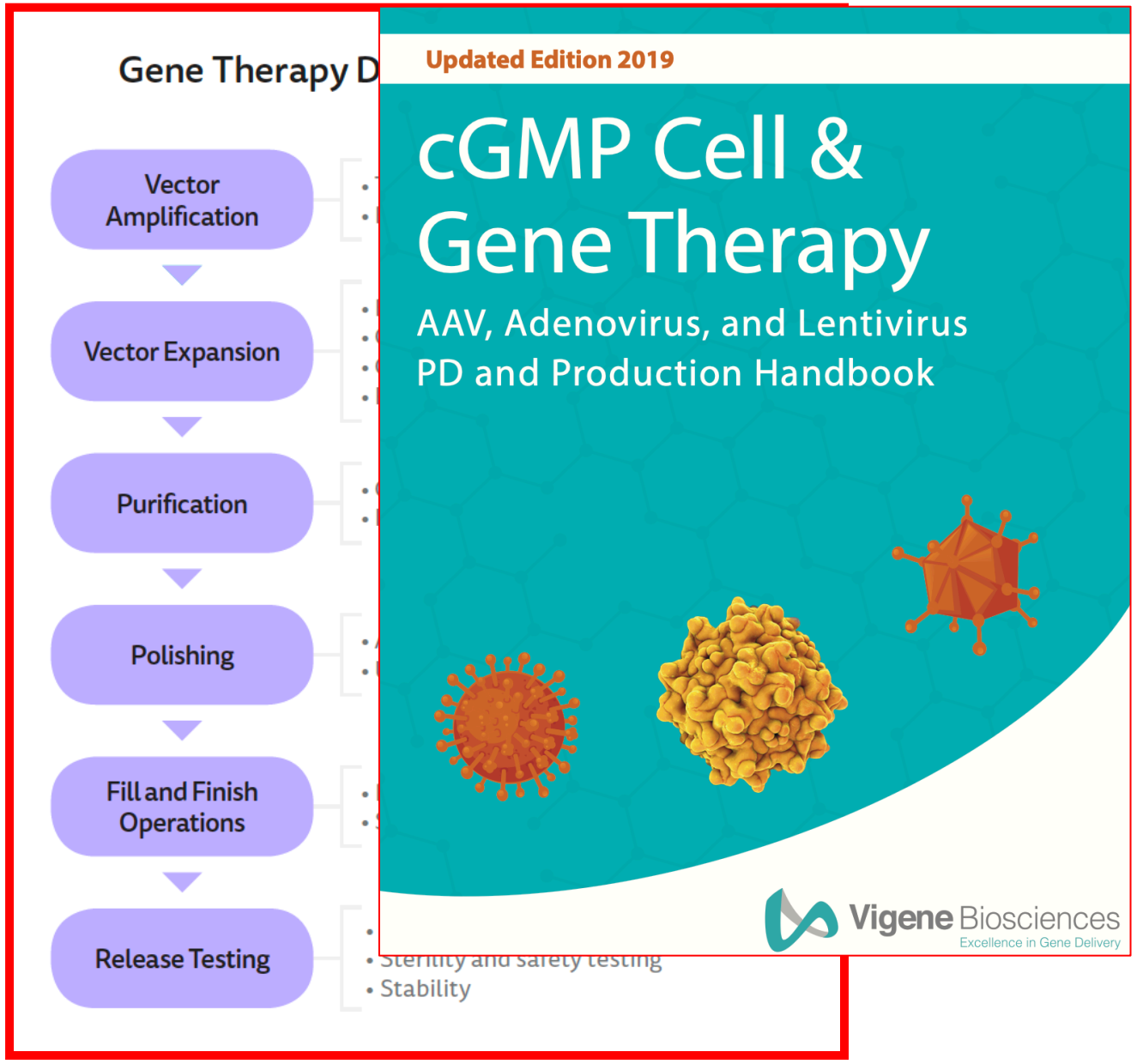


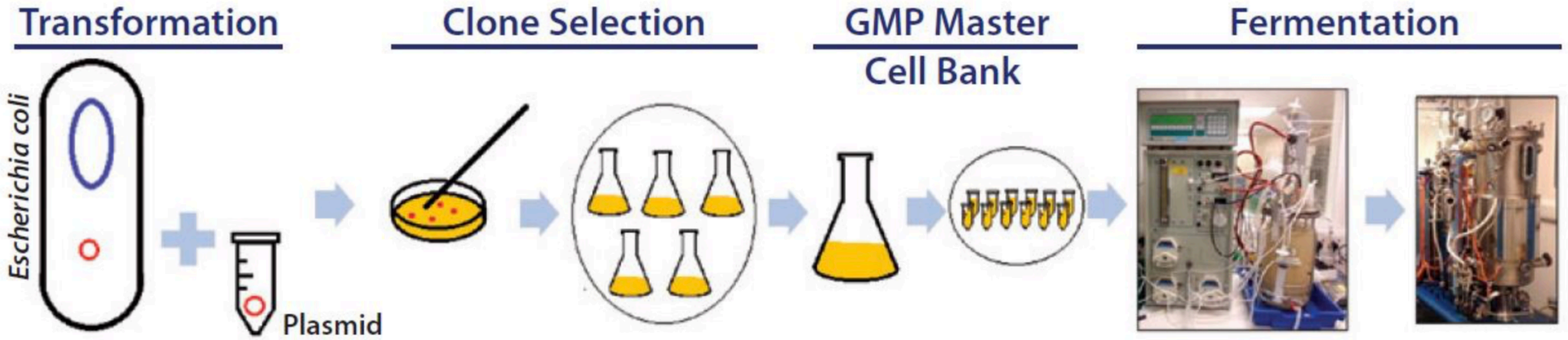
Figure 1. A cartoon showing how rAAV is packaged with a helper free system.

Workflow při výrobě přípravku genové terapie





Workflow při vývoji genové terapie
1 - příprava plasmidů v bakteriálním systému



Optimized harvest and transfection process

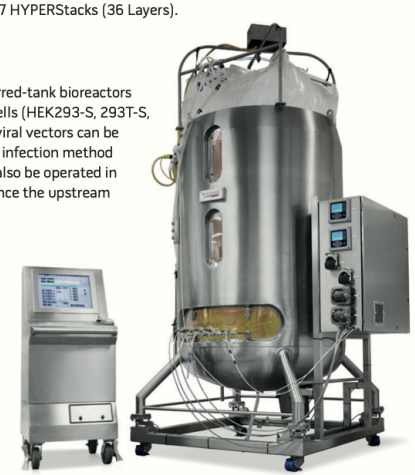
Recovery and stabilization of crude

Concentration, formulation, and filling of vectors

Cell Culture Process

Scale-up of adherent cell culture
The scale-up of adherent cell culture using HYPERstacks or roller bottles can be very labor intensive and inefficient. Vigene uses iCELLis 500 (Pall Life Sciences), the world's first fully-integrated, single-use high-density adherent cell culture bioreactor to scale up adherent HEK293, 293T and HeLa culture processes. Central to the iCELLis bioreactor technology is the use of a compact fixed-bed, filled with custom macrocarriers. This matrix provides up to 500 m^2 available area for cell growth, which is surface-equivalent of 3,000 Roller Bottles (1700 cm^2) or 277 HYPERStacks (36 Layers).

Scale-up of suspension cell culture
Vigene uses 200L - 500L single-use stirred-tank bioreactors for high density culture of suspension cells (HEK293-S, 293T-S, HeLa-S and sf9 insect cells). High titer viral vectors can be produced through either transfection or infection method in batch mode. Bioreactor systems can also be operated in fed-batch and perfusion modes to enhance the upstream process and increase volumetric yield.



- Master cell bank and cell expansion in adherent cell culture

Cell Expansion

Plasmid Transfection

- Transfection with 2–4 plasmid vectors

- 1- or 2-Step purification process ahead of DS formulation

Purification

Drug Product Production

- 0.2µm filtration and vial filling

Cell expansion and production using adherent cell factories

Purification on affinity, charge, and size-based processes

Workflow při výrobě přípravku ex vivo genové terapie

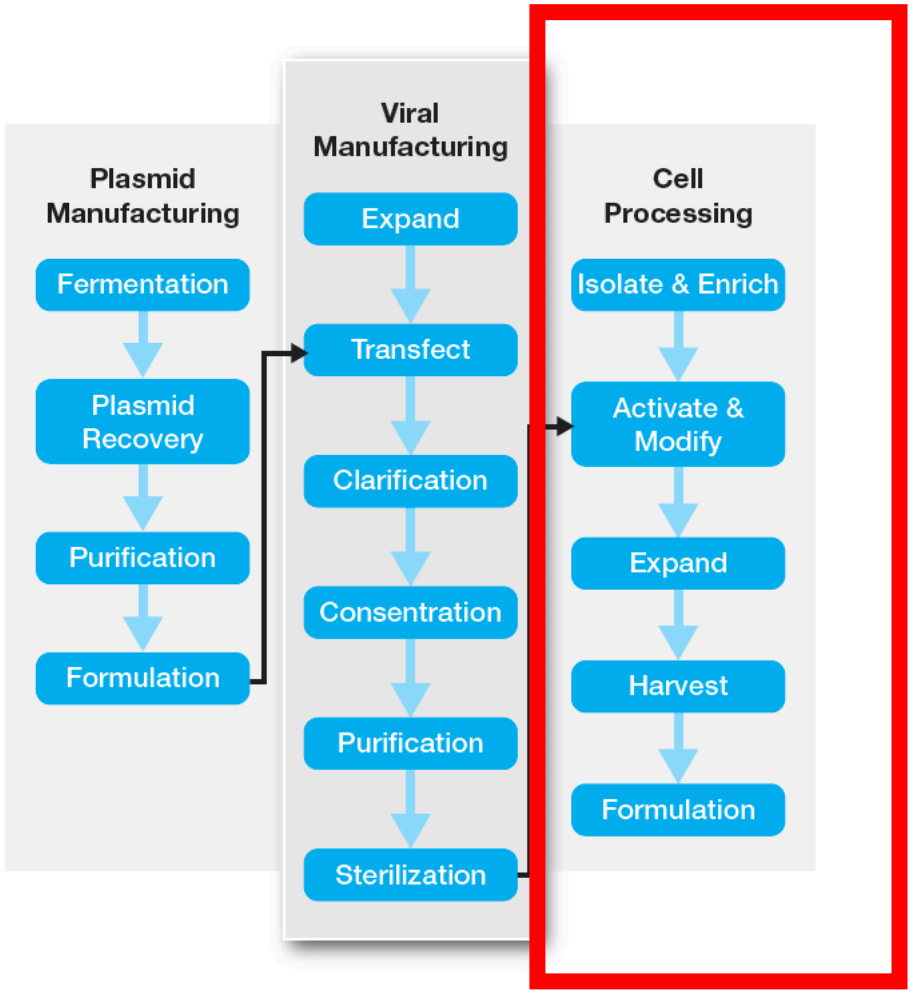
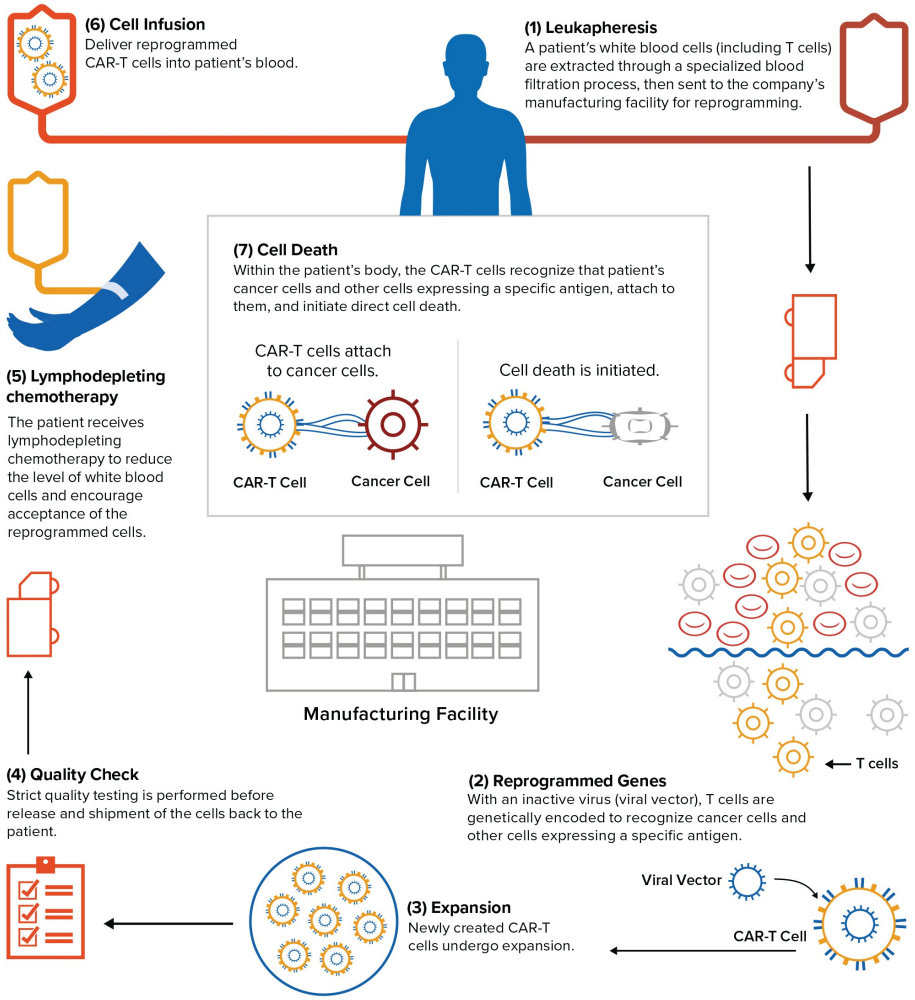


Figure 2. Example of 3 manufacturing platforms for the generation of modified cells for *ex vivo* gene therapy via viral vectors produced by transfection.

Příprava CAR T-lymfocytů



Příprava T lymfocytů s chimerickým antigenním receptorem (CAR) - Ex vivo genová terapie

I Was Planning My Funeral. This Therapy Was My Last Chance.

blogs.webmd.com/my-experience/2017/08/i-was-planning-my-funeral-this-therapy-was-my-last-chance.html

dailymail.co.uk/health/article-3821187/Terminally-ill-patient-cancer-free-raising-400-000-just-2-days-hope-treatment-US.html

Terminally ill patient is now 'cancer free' after raising £400,000 for 'last-hope' Tx

THE CAR-T REVOLUTION:

Gilead's $12B Acquisition of Kite Pharma & FDA Approval of Novartis' Kymriah Signal Tipping Point for Promising Cancer Immunotherapies

evobiotalent.com

EVOLUTION
GLOBAL TALENT ATTRACTION

GILEAD

Kite Pharma

NOVARTIS

emilywhitehead.com

Why Are Cell and Gene Therapies Challenging?

Monoclonal antibodies	Ex vivo gene therapy (e.g. CAR-T)
• Starting materials well categorized	• Starting material has high variability
• Single or limited number of GMP-compliant sources for API	• Multiple sources of starting material (apheresis centres)
• Single lot = 1000s of patients; make-to-stock is possible	• Single lot = 1 patient; therapy manufactured in real time
• Product is not personalised	• Needle-to-needle traceability required – patient must receive therapy manufactured from his/her own cells
• Long and large clinical trials – long term safety/efficacy data; time to build global experience and plan commercial supply chains	• Short and small clinical trials – limited data means long term safety studies may be required; less time to plan and develop commercial supply chain
• Simpler reimbursement models based on massive amount of commercialization experience	• High cost, potentially curative therapies (Kymriah $475k; Yescarta $375k). Reimbursement models potentially more complex and based on outcomes
• Shorter term follow-up if treatment is successful	• Long term follow-up, even if treatment is successful or curative
• Can be scaled up without scaling out	• Scale-up only achieved by scaling out, increasing supply chain complexity

Přehled schválených přípravků genové terapie

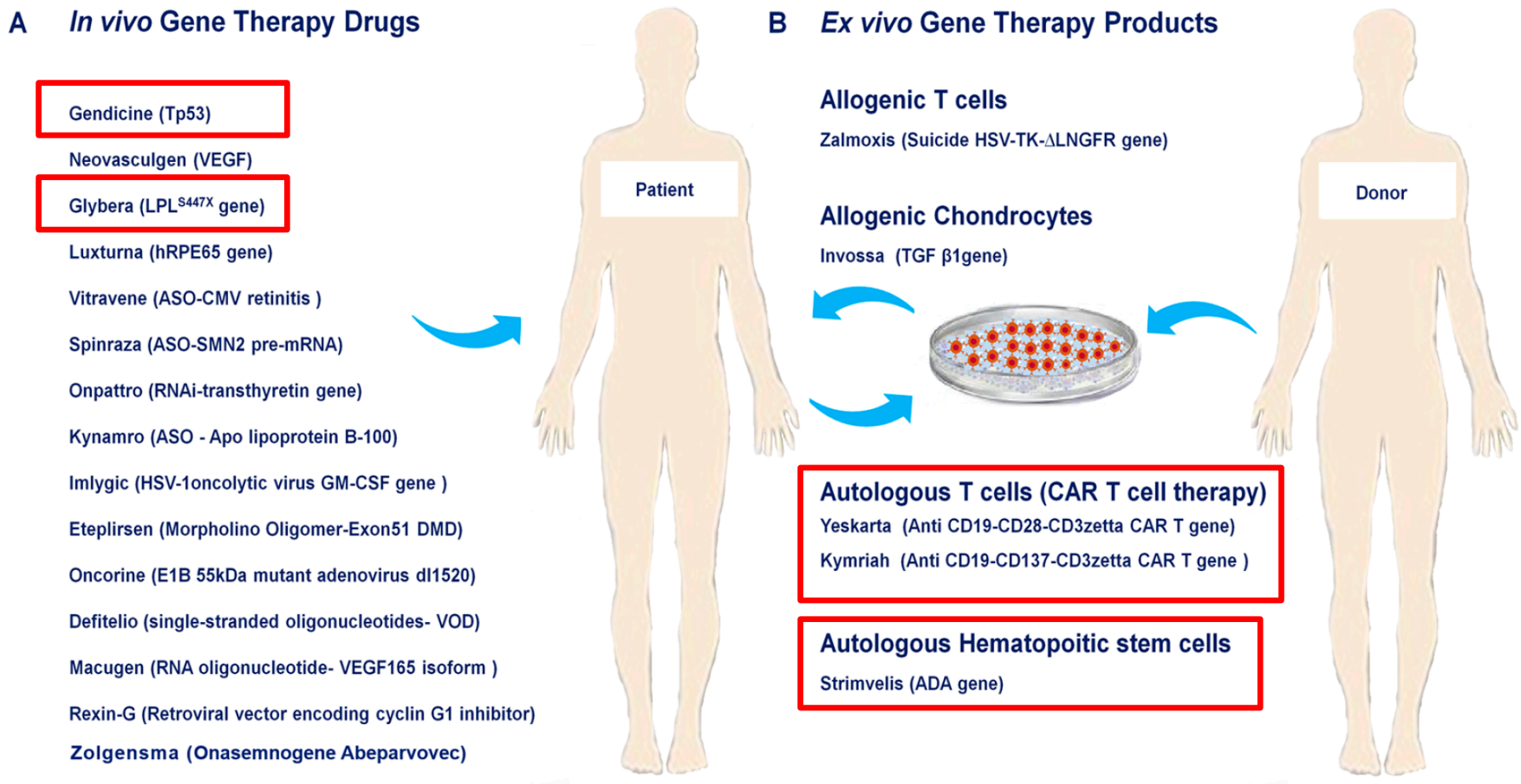
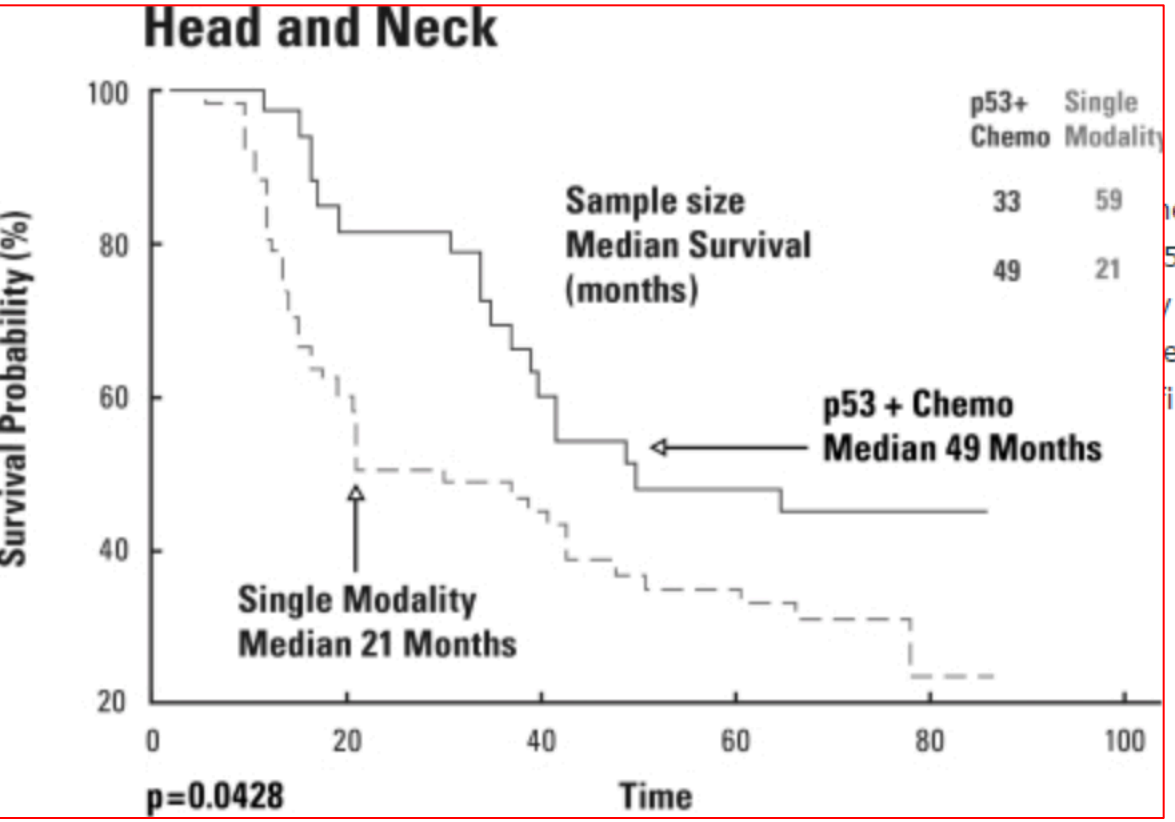


FIGURE 1 | Approved human gene and cell-based gene therapy products. **(A)** *In vivo* approved gene therapy drugs such as Neovasculgen, Glybera, Defitelio, Rexin-G, Onpattro, Eteplirsen, Spinraza, Kynamro, Imlygic, Oncorine, Luxturna, Macugen, Gendicine, Vitravene as well as Zolgensma directly injected into their target tissue or organ. **(B)** *Ex vivo* gene therapy drugs include Zalmoxis as allogenic T cells, Invossaas allogenic chondrocytes, Yeskarta and Kymriahas autologous T cells (CAR T cell therapy), Strimvelisas autologous hematopoitic stem cells.

Shahryari A et al., Front Genet, 2019

Gencidine - první schválená genová terapie na světě (v Číně) – Ad vektor

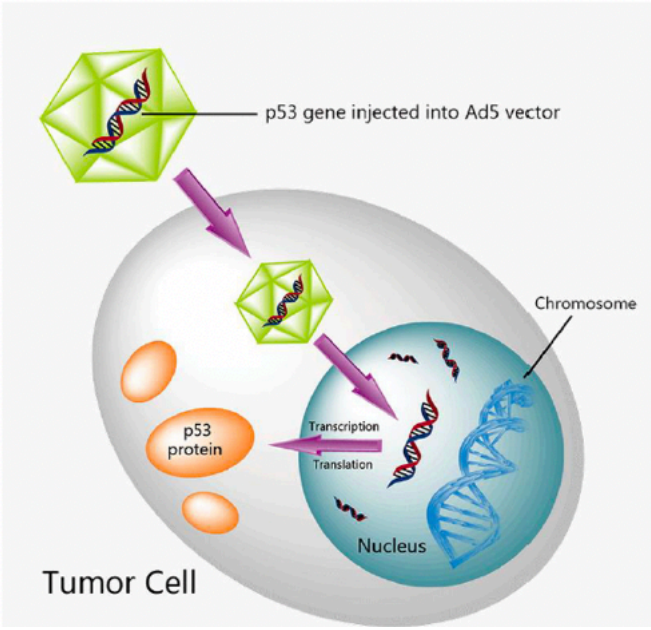


Gene Therapy for Cancer with the Recombinant Adenovirus-p53 (Gendicine®)

by Dody S. Bautista, Ph.D

[View PDF file]

ne®, a recombinant human adenovirus vector containing the 53 gene, is recognized as the world's first commercially approved product. Gene therapy products have been in clinical trials e late 1980s but it was not until 2003 when China took the credit irst country to approve a product for no less than the treatment of



2 November 2012 Last updated at 11:00 GMT

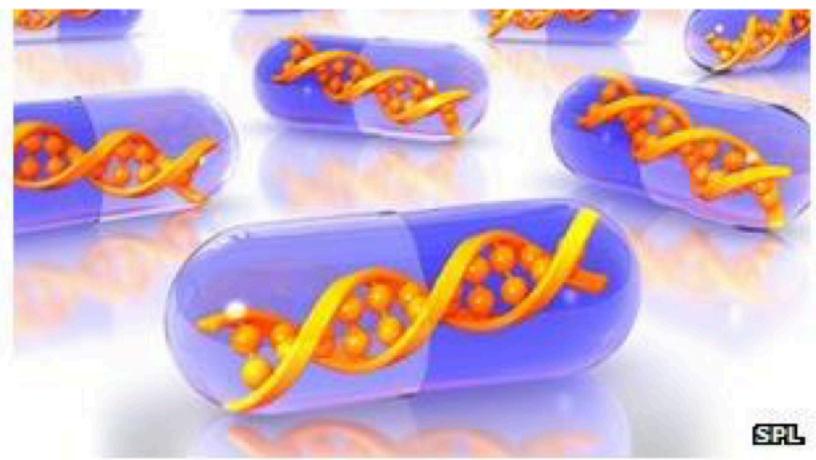
Gene therapy: Glybera approved by European Commission

By James Gallagher
Health and science reporter, BBC News

A treatment which corrects errors in a person's genetic code has been approved for commercial use in Europe for the first time.

The European Commission has given Glybera **marketing authorisation**, meaning it can be sold throughout the EU.

It is a gene therapy for a rare disease which leaves people unable to properly digest fats.



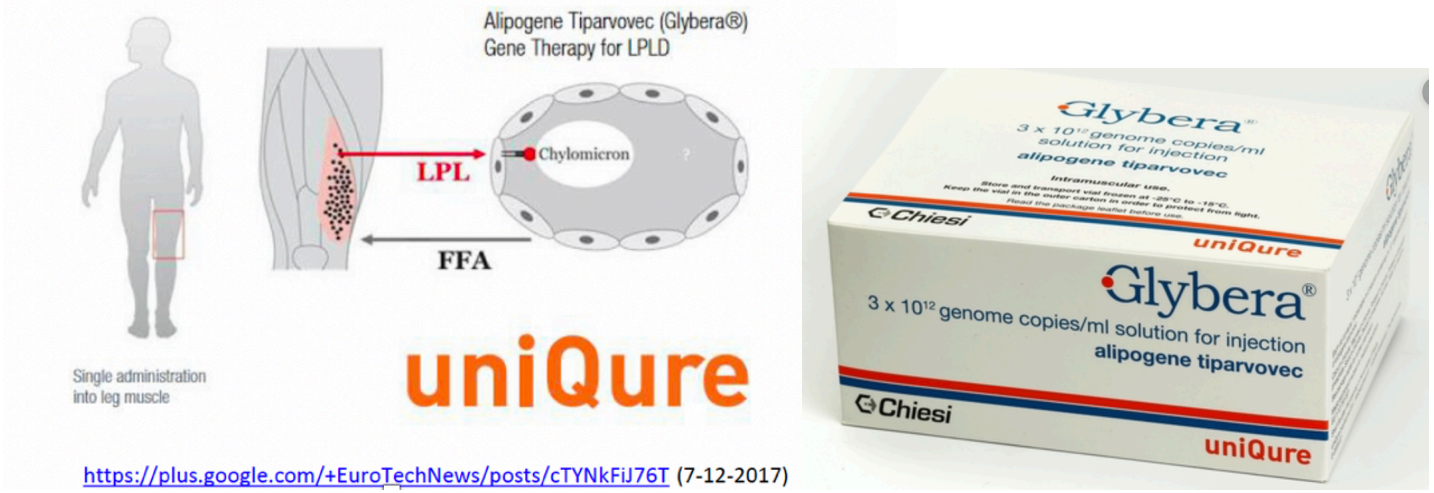
Gene therapies alter a patient's DNA

Glybera - Genová terapie deficitu lipoproteinové lipázy (AAV)

první schválenou genovou terapií v Evropě v roce 2012 byl alipogene tiparvovec
- uveden na trh pod názvem Glybera
- AAV serotyp 1 (tropismus ke svalové buňce) nesoucí kopii lidské lipoprotein lipasy
- deficience lipoprotein lipasy se vyskytuje u 1 člověka z 1-2 miliónů
- cena pro jednoho pacienta byla původně 1.6 miliónů dolarů
- v r. 2015 snížena na 1 milión dolarů
- lék byl použit jedinkrát v Německu v září 2015
- společnost uniQure oznámila, že nebude prodlužovat licenci v Evropě

až vyprší na podzim 2017

https://www.technologyreview.com/s/601165/the-worldsmost-expensiv



https://plus.google.com/+EuroTechNews/posts/cTYNkFiJ76T (7-12-2017)

uniQure

Lipoprotein lipase deficiency
Marketed in Europe 2012

Glybera is a gene therapy that is designed to restore the LPL enzyme activity required to enable the processing, or clearance, of fat-carrying chylomicron particles formed in the intestine after a fat-containing meal. The product consists of an engineered copy of the human LPL gene packaged with a tissue-specific promoter in a non-replicating AAV1 vector, which has a particular affinity for muscle cells. In order to improve activity, uniQure uses a naturally occurring variant of the LPL gene that has higher enzyme activity than the normal version of the gene that encodes the protein.

April 20, 2017

UniQure Says It Will Not Pursue EC Marketing Renewal for Glybera Gene Therapy

The World's Most Expensive Medicine Is a Bust

The first gene therapy approved in the Western world costs $1 million and has been used just once. The doctor who tried it says the price is "absolutely too high."

by Antonio Regalado May 4, 2016

The first gene therapy for children has just been approved in Europe

This is huge.

DAVID NIELD 3 JUN 2016

In a landmark moment for scientific research, the world's first gene therapy treatment for children has been given the green light by the European Commission. It's called Strimvelis, and it treats severe combined immunodeficiency (ADA-SCID) – a rare disorder that can be fatal in a very short space of time for those affected.

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Science & Environment

Gene therapy drug approval granted to GSK

By Pallab Ghosh
Science correspondent, BBC News

27 May 2016 | Science & Environment

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Strimvelis

- lék na ADA-SCID – výskyt v Evropě zhruba 15 případů ročně, 12 v USA
- ex vivo genová terapie hematopoetických kmenových buněk (HSC pomocí retrovirového vektoru s lidskou ADA
- cena byla stanovena na 594 000 € (dvojnásobek roční ceny enzymoterapie)
- schválen EK v květnu 2016

V současnosti jsou tři přípravky genové terapie schváleny FDA

August 2017

FDA approves first gene therapy (CAR-T therapy), Kymriah© (tisagenlecleucel), for treatment of children and young adults with relapsed or refractory B-cell acute lymphoblastic leukemia and for adults with relapsed or refractory diffuse large B-Cell

Cost estimate for treatment is **$373,000 - $475,000***

October 2017

FDA approves another CAR-T therapy, Yescarta© (axicabtagene ciloleucel), to treat relapsed or refractory large B-cell lymphoma administered with a one time IV infusion.

Cost estimate for treatment is **$373,000***

December 2017

FDA approves Luxturna© (voretigene neparvovec-rzyl) to treat RPE65 mutation-associated retinal dystrophy, an inherited form of vision loss that may result in blindness administered by Subretinal injection (one time per eye).

Cost estimate is **$475,000 per eye**

May 2018

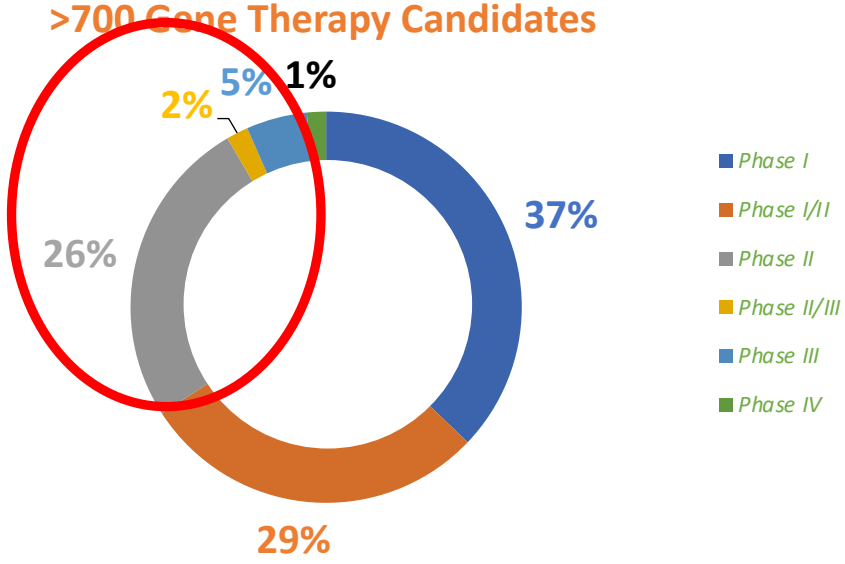
FDA approves Kymriah © for second indication, treatment of relapsed or refractory large B-cell lymphoma or leukemia administered with a one time IV infusion.

Cost estimate is **$373,000 - $475,000**

* Kymriah's cost is indication-specific. Note, price listed includes only the acquisition cost of the medication. Before receiving medication, patient must receive 3 days of conditioning chemotherapy. Product must be infused in the hospital setting and patient typically remains inpatient for a minimum of 7 days, all of which will increase total cost of care.

Přípravky genové terapie schválené nebo s očekávaným schválením FDA v roce 2019

> 1,770 Global Gene Therapy Trials Supporting
>700 Gene Therapy Candidates



May 2019 - approved

Zolgensma (AVXS-101)
Spinal muscular atrophy (SMA) Type 1
One-time IV infusion
US prevalence 500 people

Expected cost
$1-4 million

Mid/late 2019

Toca-511 Recurrent high grade glioblastoma
One-time administration followed by ER 5-fluorocystine.
US prevalence 8,000 people

Cost **TBD**

Lisocabtagene Large B-cell lymphoma
One-time IV infusion
US prevalence 1-3 per 10,000

Cost **TBD**

Late 2019

Valoctocogene roxaparvovec Hemophilia A
One-time IV infusion.
US prevalence 1 per 12,000 people

Cost **TBD**

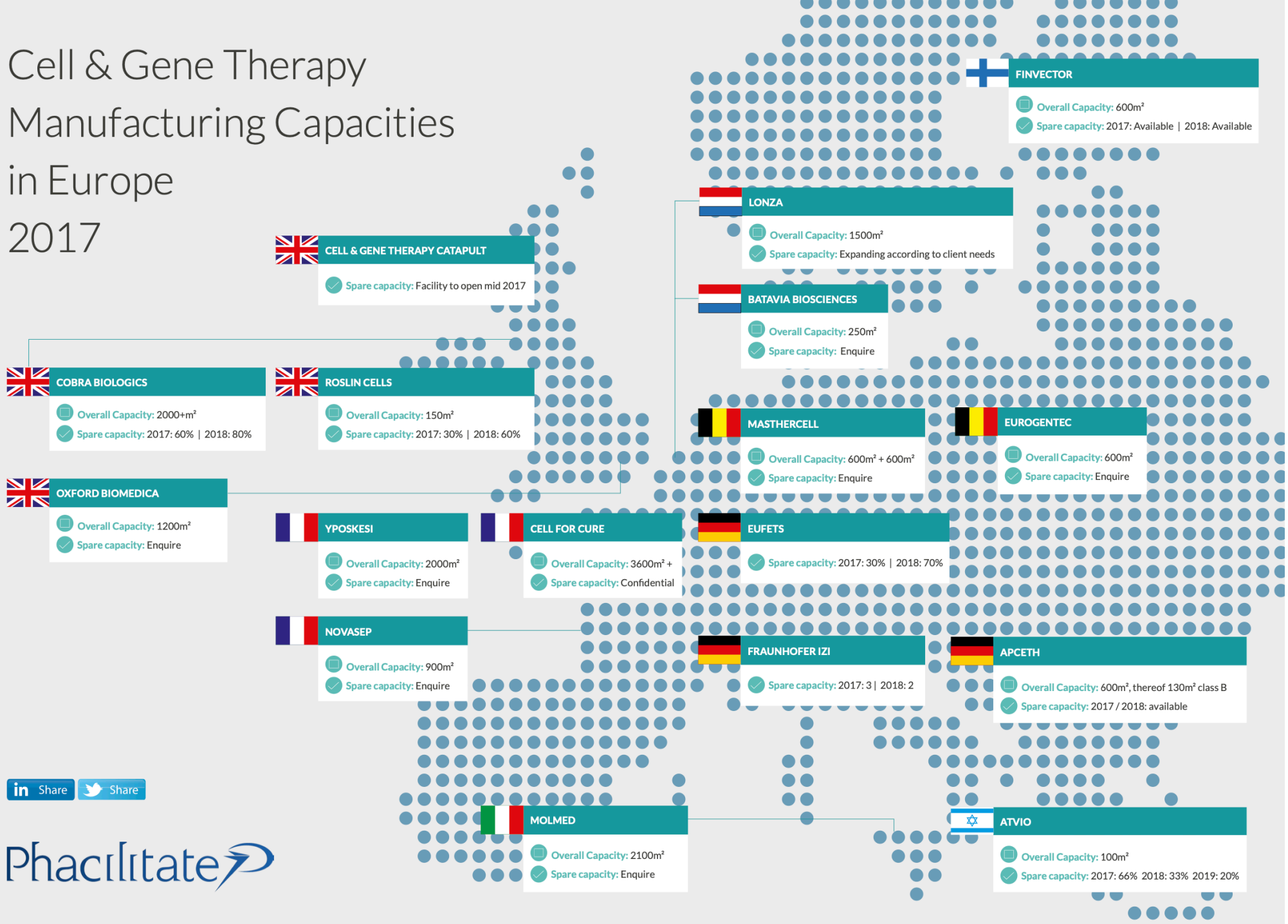
LentiGlobin Transfusion dependent beta-thalassemia
One-time IV infusion
US prevalence 1,000 people

Cost **TBD**

The number of gene-therapy developers has ballooned from 69 (2014) to 255 in 2018

- Alliance for Regenerative Medicine (ARM)

Cell & Gene Therapy Manufacturing Capacities in Europe 2017



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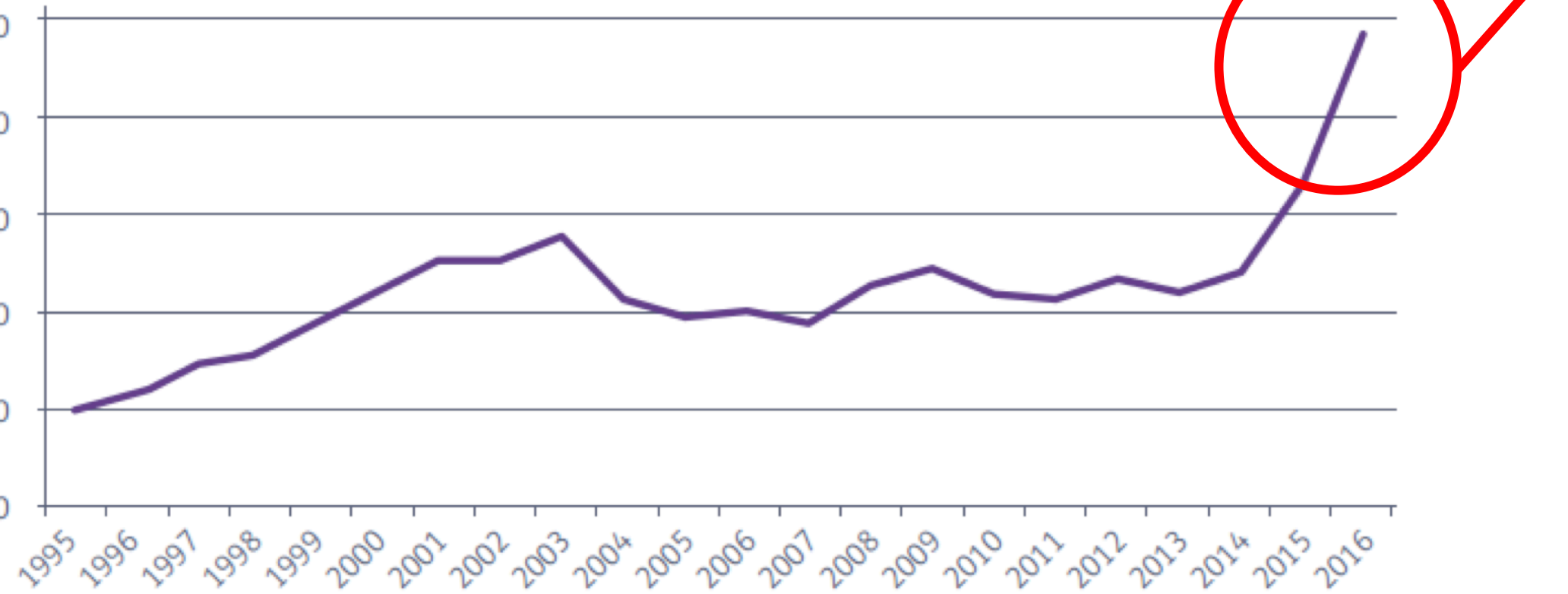
Phacilitate

Genová terapie dnes

Strimvelis®
EMA APPROVED

Figure 3: Number of gene therapies in active development



1990 PRVNÍ GT
ADA-SCID

Source: Pharmaprojects, 2016

This growth is supported by FDA evidence showing the number of applications for investigational new drugs (IND) and investigational device exceptions (IDE) relating to cellular and gene therapies has steadily increased since 2010 (Figure 4).

Děkuji za pozornost

Dante Labs Offers $199 Whole Genome Sequencing Promotion for Black Friday Week