The Long and Winding Road: How the Human Genome Project and Gene Therapy Research Led to the First Gene Therapies for Genetic Disease

Katherine A. High, M.D.
President and Head of R&D, Spark Therapeutics
Keynote address, LCA Family Conference
Philadelphia, PA – July 27, 2019

N-GT-US-490049

Forward-looking statements

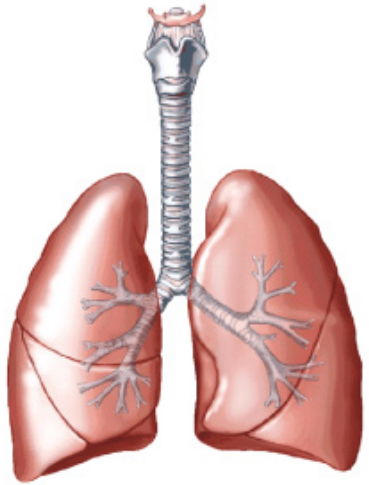
This presentation contains "forward-looking statements" within the meaning of the Private Securities Litigation Reform Act of 1995, including statements regarding the company's product and product candidates, including LUXTURNA® (voretigene neparvovec-rzyl), *SPK-7001*, *SPK-9001, SPK-3006, SPK-8011* and *SPK-8016*. The words ''anticipate,'' ''believe,'' ''expect,'' ''intend,'' ''may,'' ''plan,'' ''predict,'' ''will,'' ''would,'' ''could,'' ''should,'' ''continue'' and similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words. Any forward-looking statements are based on management's current expectations of future events and are subject to a number of risks and uncertainties that could cause actual results to differ materially and adversely from those set forth in, or implied by, such forward-looking statements. These risks and uncertainties include, but are not limited to, the risk that: (i) our preliminary clinical results for our product candidate, *SPK-8011*, for hemophilia A may not be sustained; (ii) our implementation of a prophylactic approach to steroid administration for subjects participating in our *SPK-8011* clinical trials may not sufficiently prevent immune responses; (iii) we may not successfully initiate a multinational Phase 3 clinical trial for *SPK-8011* and the timing and design of such trial may vary from our expectations; (iv) our initial evaluation of safety in non-inhibitor patients for *SPK-8016* may not be successful; (v) our clinical equivalence testing for *SPK-8011* resulting from changes in our adherent manufacturing process to a suspension cell culture manufacturing process may not produce expected results; (vi) we may not achieve our expected objectives for commercialization for LUXTURNA; (vii) we may be unable to maintain or continue to enter into agreements with payers for the provision of LUXTURNA; (viii) we will not be able to reach agreement with the Centers for Medicare & Medicaid Services regarding LUXTURNA; (ix) Novartis may not be successful in commercializing or selling voretigene neparvovec in one or more markets; (x) we may not receive any additional milestone or royalty payments from Novartis, Pfizer or our other collaborators; (xi) the improvements in functional vision demonstrated by LUXTURNA in our clinical trials may not be sustained over extended periods of time; (xii) interim data from our *SPK-7001* Phase 1/2 clinical trial, including data to be generated from our recently expanded cohort, may not support further development of this product candidate; (xiii) we may not advance our *SPK-3006* program into the clinic when anticipated, or at all; (xiv) the data for *SPK-3006* IND-enabling studies may not be sustained; (xv) our preliminary results of scale-up to non-human primates supporting the initiation of clinical studies of *SPK-3006* in humans may not be sustained; (xvi) actual cash burn does not reflect expectations; and (xvii) any one or more of our product candidates in preclinical or clinical development will not successfully be developed and commercialized. For a discussion of other risks and uncertainties, and other important factors, any of which could cause our actual results to differ from those contained in the forward-looking statements, see the "Risk Factors" section, as well as discussions of potential risks, uncertainties and other important factors, in our Annual Report on Form 10-K, our Quarterly Reports on Form 10-Q and other filings we make with the Securities and Exchange Commission. All information in this presentation is as of the date of the presentation, and Spark undertakes no duty to update this information unless required by law.

Burden of genetic disease

- Unmet medical need: genetic diseases

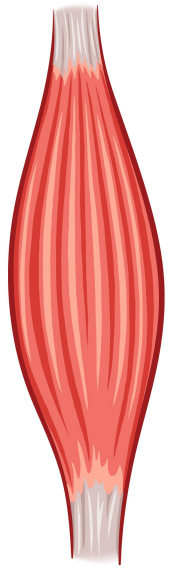
~25 million Americans with rare genetic diseases

Cystic Fibrosis



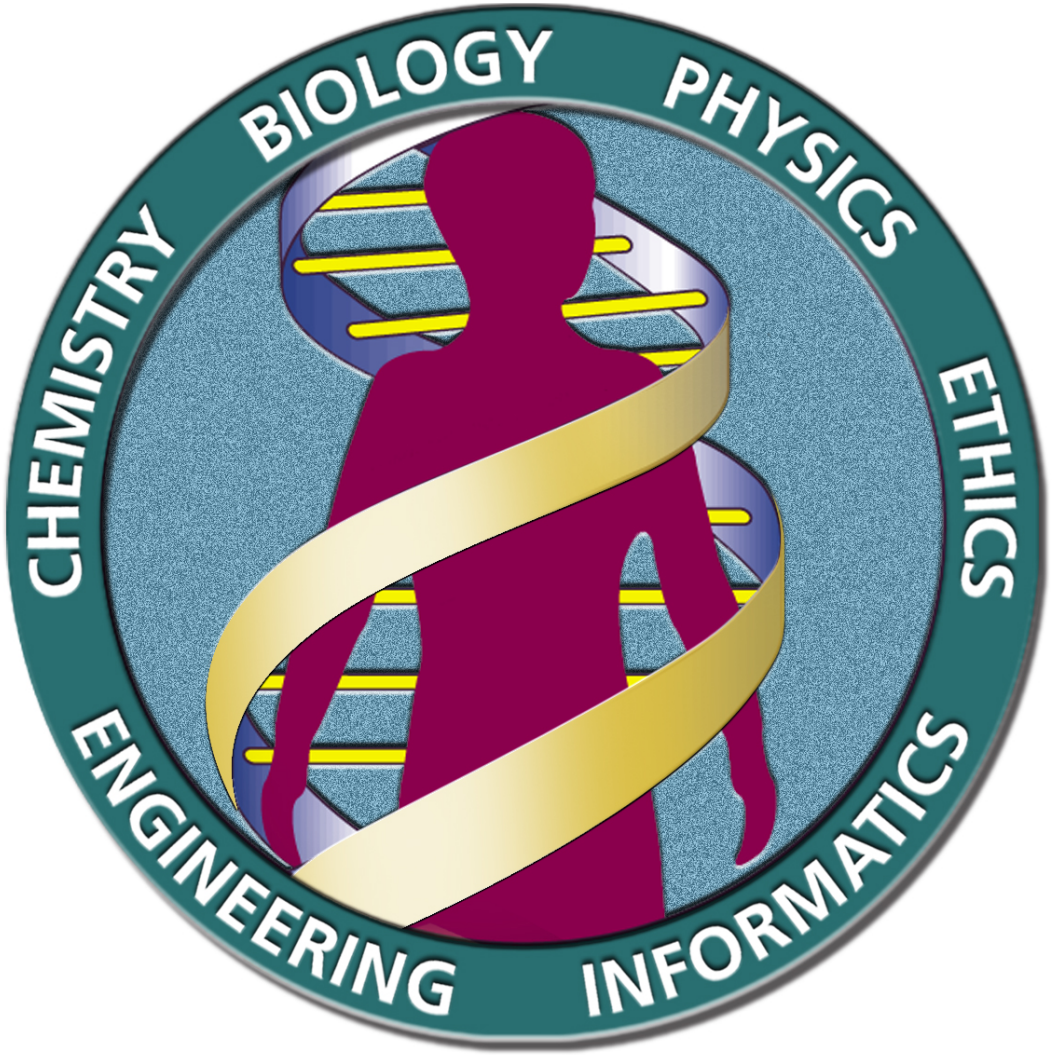
Sickle Cell Anemia

Muscular Dystrophy



https://globalgenes.org/rare-diseases-facts-statistics/

The Human Genome Project



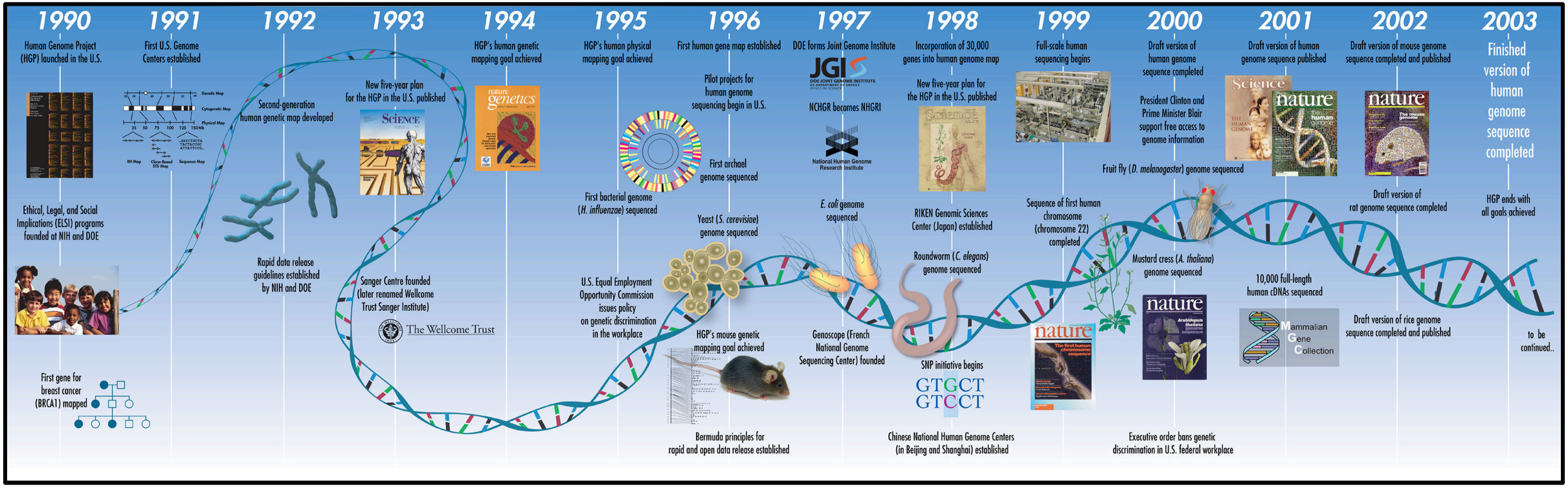
The Human Genome Project

- The Human Genome Project (HGP) began in 1990
- Mission of the HGP: The quest to understand the human genome and role it plays in both health and disease

"The true payoff from the HGP will be the ability to better diagnose, treat, and prevent disease."
-- *Francis Collins, Director of the HGP and the National Human Genome Research Institute (NHGRI)*

https://www.genome.gov/10001772/all-about-the--human-genome-project-hgp/
National Human Genome Research Institute: https://www.youtube.com/watch?v=slRyGLmt3qc

Human Genome Project: From concept to reality



Credit: Darryl Leja, NHGRI.
https://www.genome.gov/image-gallery#nanogallery/nanogallery2/0/26964377742

Twenty-two years from first approved trial in western world to first approved product

1990

Four-year-old became the first patient enrolled in a gene therapy trial, NIH

2012

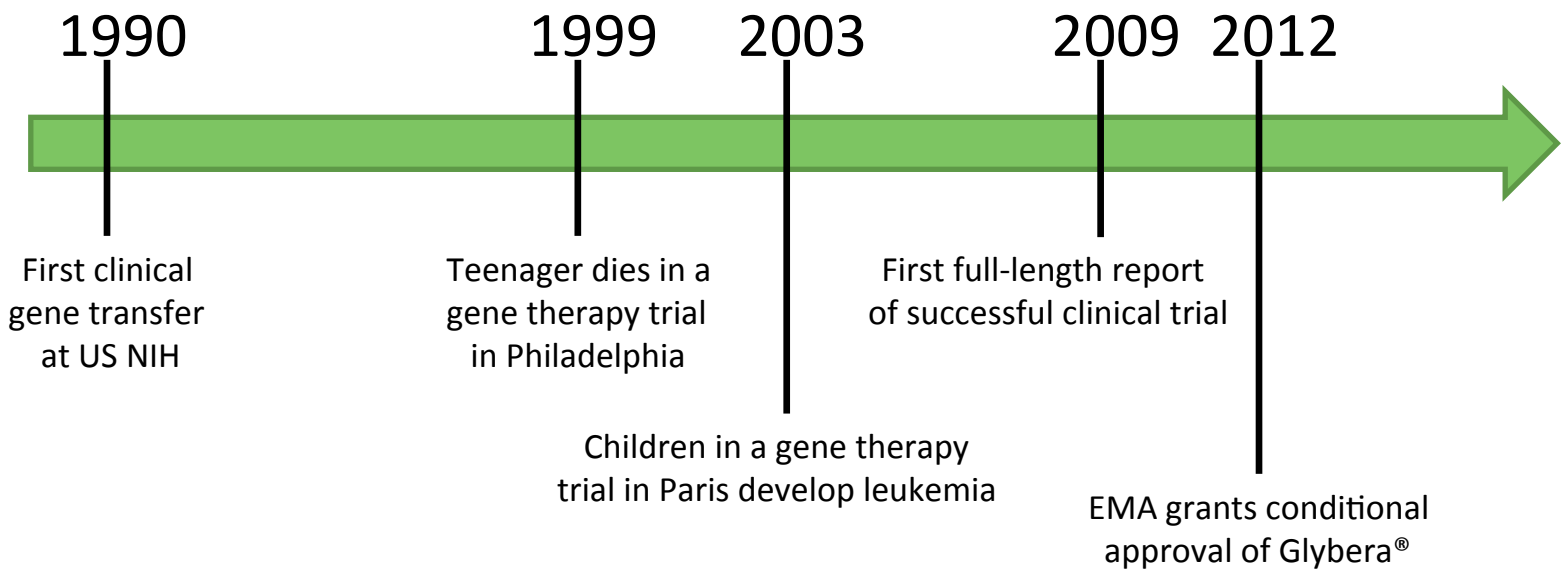
Glybera® (alipogene tiparvovec): the first European Medicines Agency (EMA) licensed gene therapy drug*

https://history.nih.gov/exhibits/genetics/sect4.htm

*The marketing authorization in Europe for Glybera® expired on 25 Oct. 2017 and was not renewed by uniQure.uniQure N.V. Press Release, 20 April 2017.

Glybera® is a registered trademark of UniQure N.V.

Human gene therapy: Starts in the clinic at the same time



High profile adverse events at turn of century (millenium)

- General sense of those outside the field was that gene therapy was not well-understood enough to be developed as a therapeutic – “Not ready for prime time”
- Decline in numbers of gene therapy trials
- Decline in subjects willing to participate in trials
- Marked decline in interest from large pharmaceutical companies, other investors
- Concern among best regulatory talent – was moving to or staying in the FDA’s Office of Cell and Gene Therapy a good career move?

Spark THERAPEUTICS

Gene therapy was sustained by

- Academic societies such as ASGCT, ESGCT, and other national societies
- NIH, including the Program of Excellence in Gene Therapy which evolved into the Gene Therapy Resources Program
- Academic medical centers, few in number, that continued to invest resources in gene therapy

Spark THERAPEUTICS™

ASGCT: American Society of Gene and Cell Therapy
ESGCT: European Society of Gene and Cell Therapy

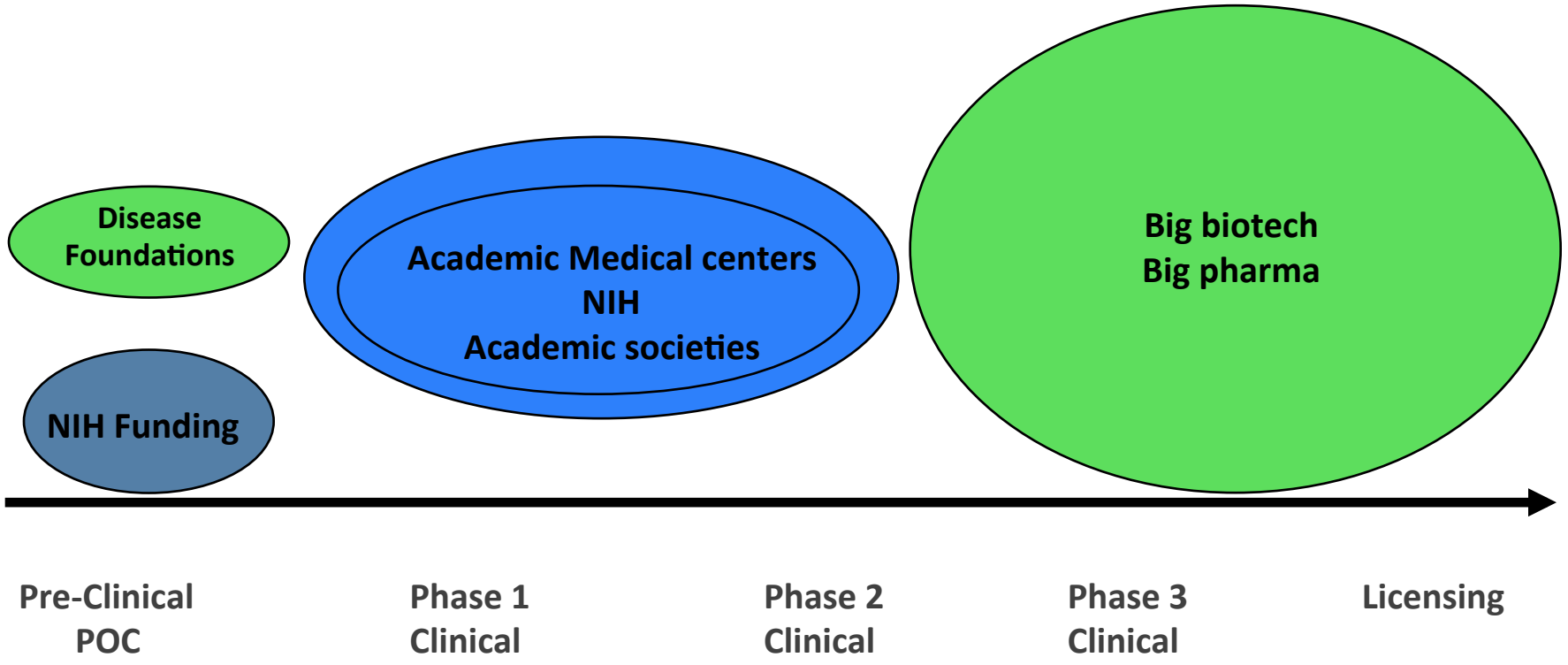
These investors…

- Allowed investigators to develop therapeutics based on best science, not commercial considerations
  - *RPE65* blindness trials

- Provided investigators the opportunity to work out problems without pressures related to timelines or total cost
  - Turn every obstacle into a PPG

Spark™ THERAPEUTICS

Hurdles in Gene Therapy



WSJ.com THE WALL STREET JOURNAL. ONLINE

Free Dow Jones Sites

Gene Therapy Still Lacks Breakthrough

By Sharon Begley, The Wall Street Journal, February 21, 2005

Katherine High thought she was making progress. She and her team had managed to take the human gene that produces Factor IX, a blood-clotting substance that people with hemophilia lack, and slip it into a harmless virus called AAV. The virus played Trojan horse, carrying the gene into lab mice and dogs. There, it worked its way into the animals' DNA and produced enough clotting factor to cure their hemophilia.

But when Dr. High, a professor of pediatrics at Children's Hospital of Philadelphia, and her colleagues launched human trials in 2001, the picture darkened. They injected the gene-carrying viruses into the livers of seven patients. Two produced measurable amounts of Factor IX, 3 percent and 12 percent of the normal level. But within a few weeks the levels became undetectable, Dr. High reported last year. The patients' immune systems had apparently killed the cells containing the inserted gene.

Dr. High had some ideas about how to fix that. But in May the biotech company sponsoring her work bailed out.

So it has gone for gene therapy, the great hope of the genetic revolution. The idea was simplicity itself: Use a safe virus to carry a healthy gene into the cells of patients who suffer from a genetic disease -- sickle cell, cystic fibrosis, hemophilia -- because their own version of that gene is kaput. Rather than treating the downstream effects of the broken DNA, gene therapy would fix what was actually broken.

If only. Since 1989 there have been more than 350 gene-therapy trials world-wide intended to help patients. Of the thousands in the trials, about a dozen, all children with a rare immune disorder, have been cured.

"There was so much optimism and naivete," says Donald Kohn of Childrens Hospital Los Angeles, who conducts gene-therapy trials with bone-marrow cells. "What worked so well in mice in the 1980s -- the reason for the optimism -- turned out to be very difficult to translate into humans."

For one thing, in mice the target cells tend to divide constantly and "easily take up the foreign gene," he explains. It wasn't unusual to get the gene into 50 percent of certain mouse cells. Not so in humans, where 0.1 percent is a triumph. In fact, in what seems like sheer spite on nature's part, human bone marrow cells that take in the foreign gene are also the ones that function for only a few weeks, leaving no lasting therapeutic benefit, explains Dr. Kohn; human cells in which the gene has a good chance of functioning over the long run resist taking it in.

No wonder the field seems cursed.

In 1999, 18-year-old volunteer Jesse Gelsinger died when he experienced a fatal inflammatory reaction to gene

NEWS FEATURE

Gene therapy: cursed or inching towards credibility?

Can gene therapy ever live down its setbacks and live up to its initial promise? A chastened but determined group of pioneers believes it can, and they are pointing to a new generation of products to back up that claim. Malorye A. Branca investigates.

Future gene therapy candidate? Nine-year-old child with Crigler-Najjar syndrome, a genetic disease that causes elevated bilirubin levels, sleeps under UV lights every night.

Most agree a pause for reflection was warranted, and that the result has been a much better understanding of the underlying science. But some investigators feel the new regulatory restrictions go overboard, unduly shackling those already using appropriate caution, by adding steps to an already difficult process.

Ironically, although Paul Gelsinger, Jesse's father, does believe the technology was hyped prematurely, he doesn't blame gene therapy for his son's death. "The problem wasn't gene therapy," he says. Rather he faults certain individuals and the system (**Box 2**). Others saw it differently, however, and gene therapy was branded as an unusually risky field within the already volatile biotechnology sector. Some companies shifted their focus to new fields, or recast their work.

"Because gene therapy has such a nasty reputation, people tried to rename it or call it 'new and improved' to free themselves of the stigma," says Michael Zasloff, an analyst with Ferris, Baker Watts of Washington, DC. "That may fool the public, but the market sees through it," he says. Whenever genetic manipulation is involved, no matter for how long or where in the body, investors typically treat it like gene therapy, despite what the product's developers may say.

Safety and efficacy

It is not just safety problems that have dogged gene therapy, efficacy has been much harder to achieve than expected.

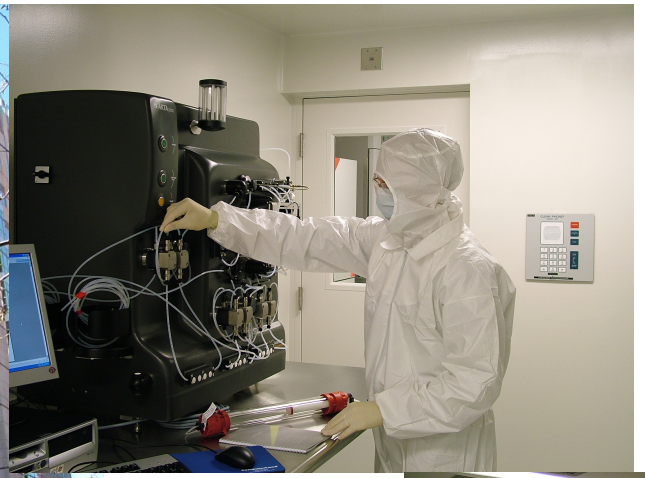
Katherine High, of Children's Hospital of

Spark THERAPEUTICS

The Children's Hospital of Philadelphia®

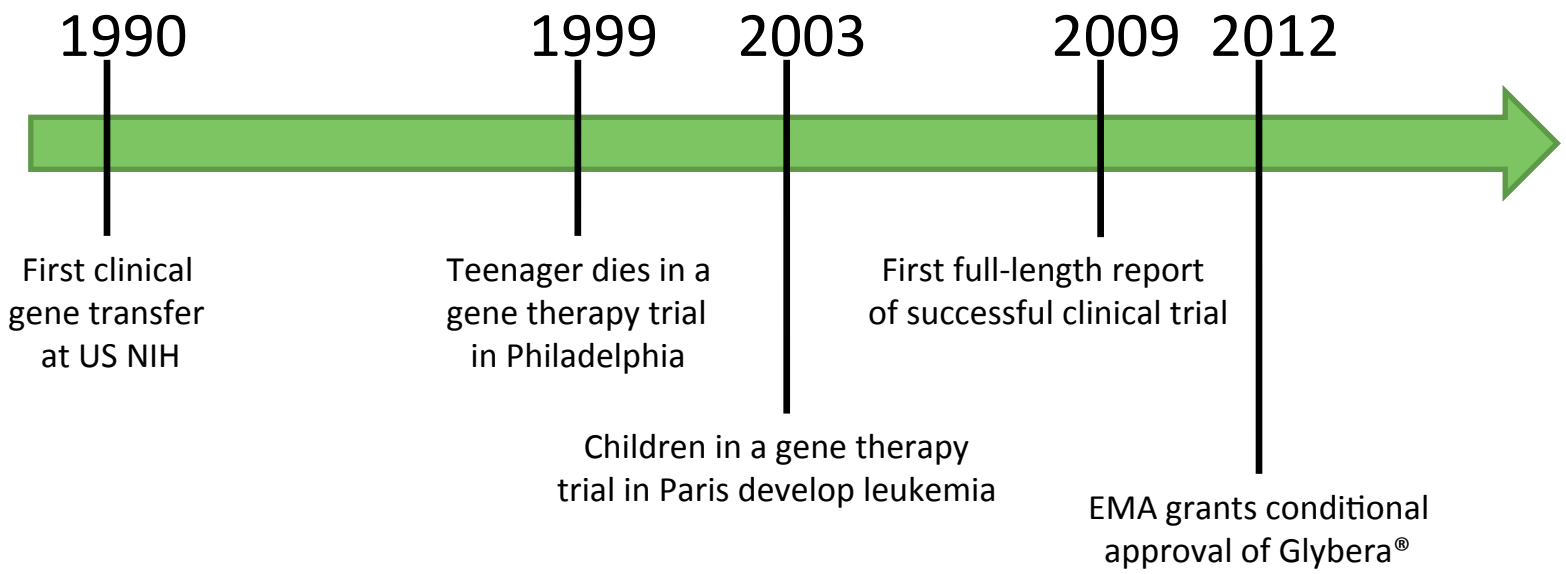
RESEARCH INSTITUTE

CENTER FOR CELLULAR & MOLECULAR THERAPEUTICS

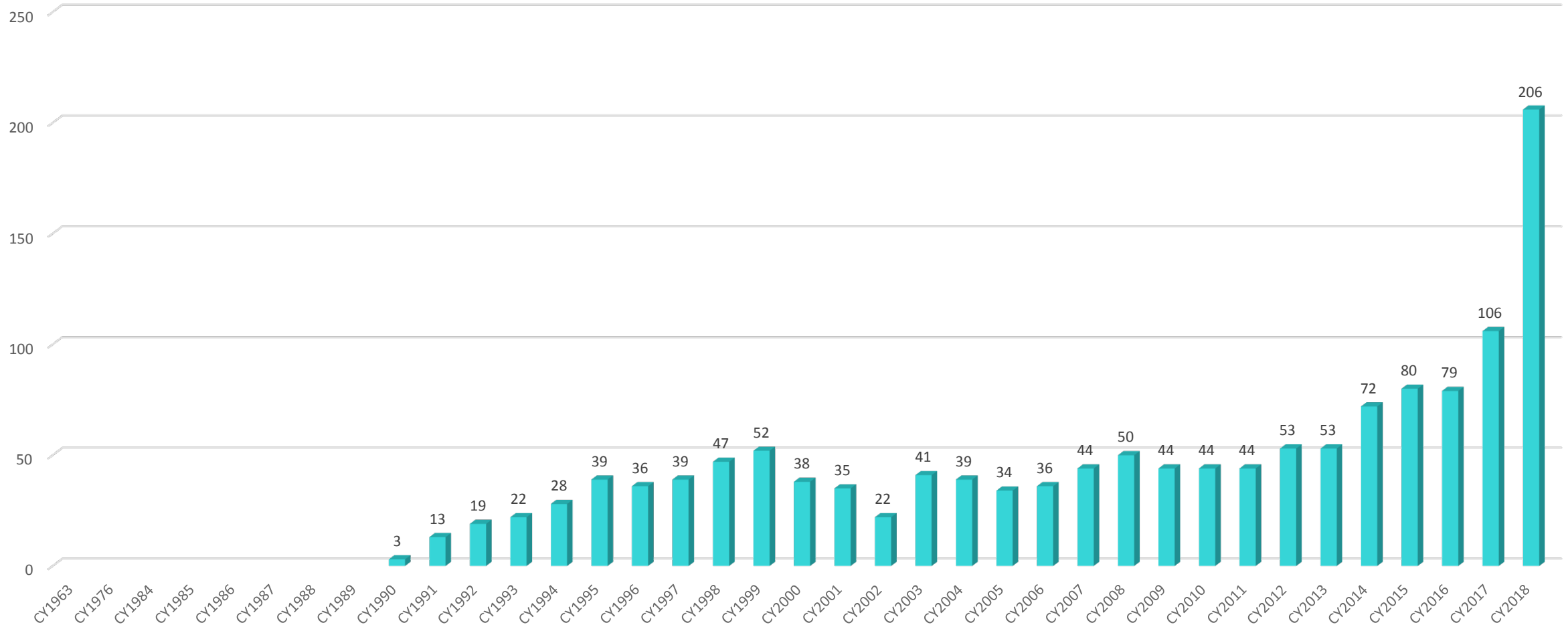


Spark THERAPEUTICS

Human gene therapy: begins to accumulate positive results



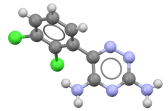
IND Submissions with Gene Therapy Products, CY 1963 – 2018



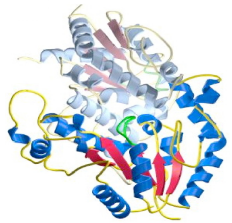
Spark THERAPEUTICS

www.fda.gov

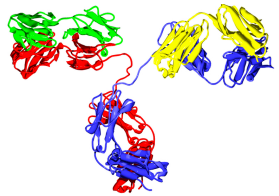
- Small Molecules



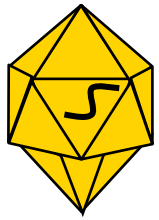
- Recombinant Protein



- Monoclonal Antibody



- Viral Vector



Pace of licensing of new products

- Monoclonal antibodies (MAbs)
  - 1986 OKT3
  - 1994 ReoPro® (abciximab)
  - 1997 Rituximab, Daclizumab
  - 1998 Four products
  - By 2007, 8 of top 20 biotech drugs were MAbs

ReoPro® is a registered trademark of Janssen Pharmaceutical Companies

Scolnik PA. MAbs 2009; 1(2):179-84.
http://www.actip.org/products/monoclonal-antibodies-approved-by-the-ema-and-fda-for-therapeutic-use/

Goals of gene transfer for genetic disease

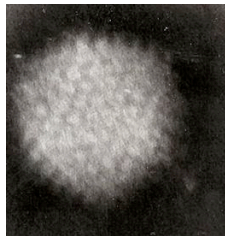
- Therapeutic levels of transgene expression
- Long-term safety and efficacy

Spark THERAPEUTICS

Mingozzi & High. Nat Rev Genet 2011; 12(5):341-55.

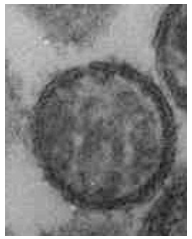
Gene therapy for genetic disease

- Critical components of any gene transfer strategy
  - Transgene
  - Vector: viral/non-viral
  - Target tissue

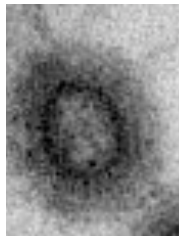


Adenovirus

AAV



Lentiviral



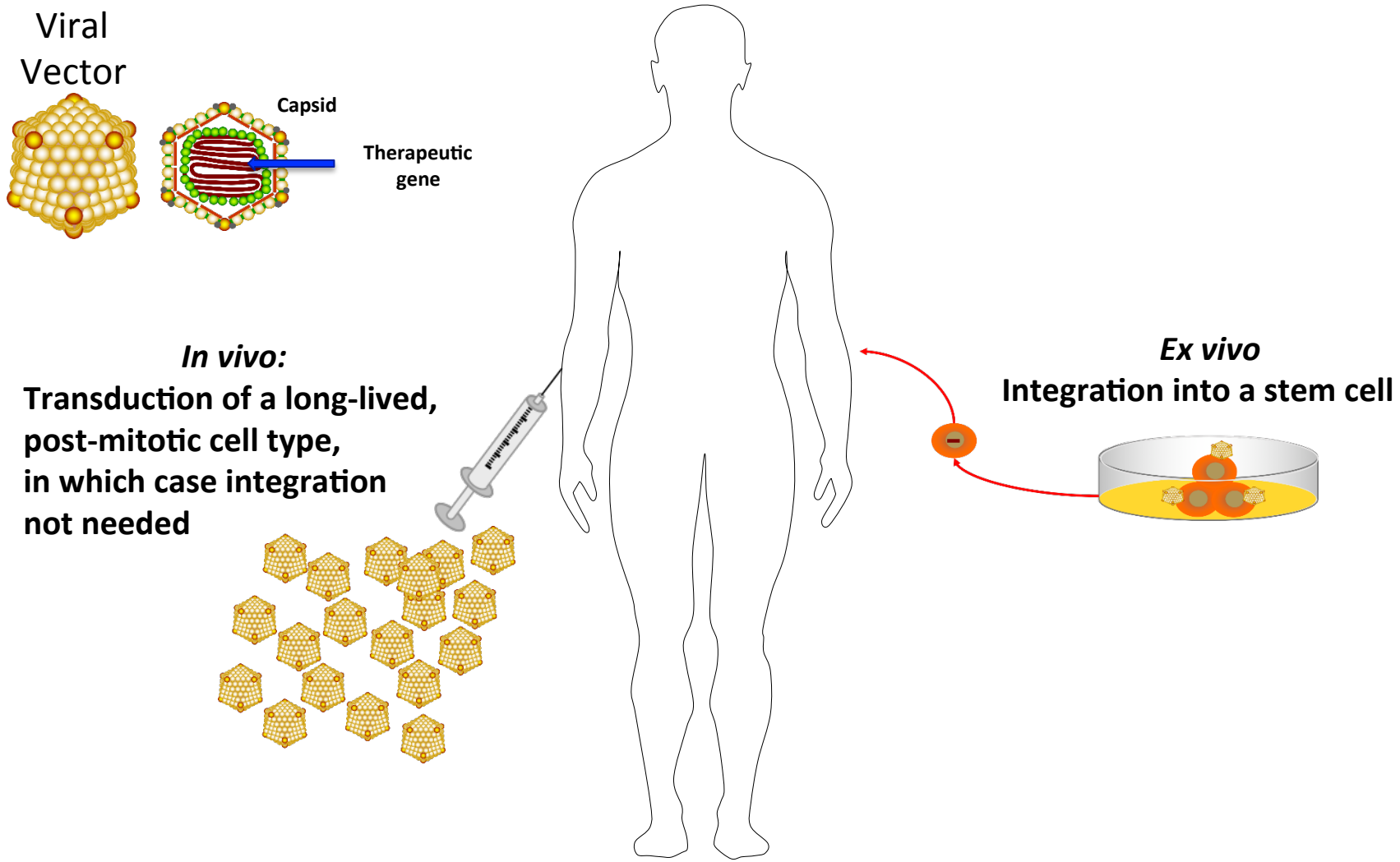
Retroviral

Spark THERAPEUTICS

AAV: adeno-associated virus

Mingozzi & High. Nat Rev Genet 2011; 12(5):341-55.

Two basic strategies to achieve long-term expression



Current status of gene therapies for genetic disease

Glybera® (alipogene tiparvovec)
AAV1-LPL
Conditional approval by EMA 2012*

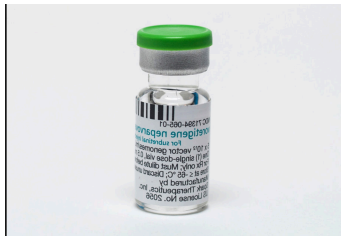
Glybera® is a registered trademark of UniQure N.V.

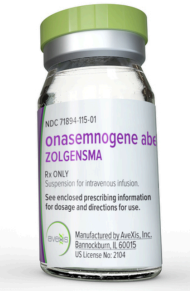
Strimvelis® (GSK2696273)
RV-ADA
Approved by EMA May 2016

Strimvelis® is a registered trademark of GlaxoSmithKline

LUXTURNA® (voretigene neparvovec-rzyl)
AAV2-RPE65
Approved by FDA in 2017; by EMA in 2018

LUXTURNA® is a registered trademark of Spark Therapeutics, Inc.





Zolgensma® (onasemnogene abeparvovec-xioi)
AAV9-SMN1
Approved by FDA 2019

Zolgensma® is a registered trademark of AveXis, Inc.

zynteglo™
(autologous CD34⁺ cells encoding $\beta^{A\text{-}T87Q}$-globin gene)

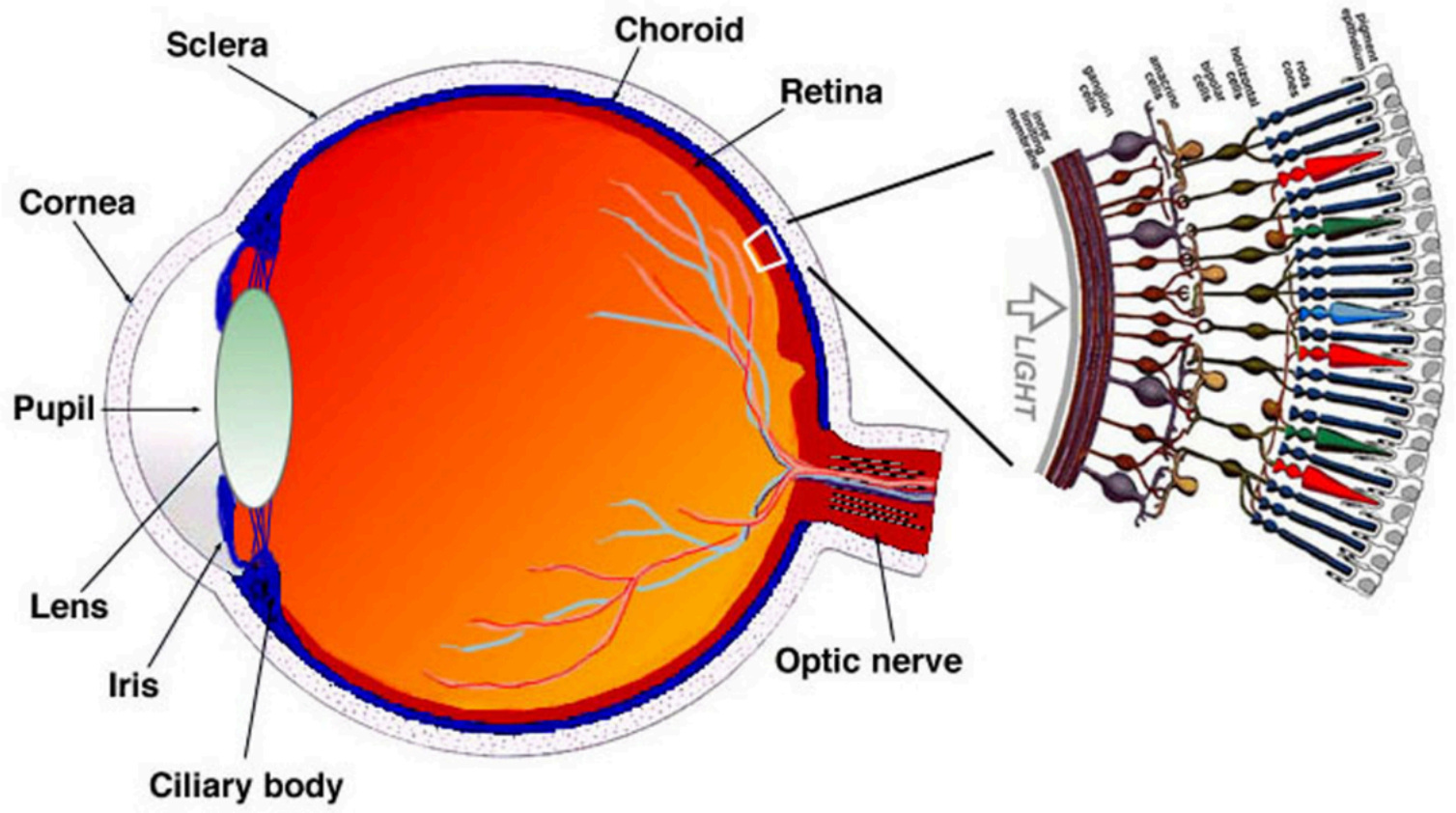
Zynteglo® (autologous CD34⁺ cells encoding β^{A}-T87Q-globin gene)
LV-β-globin
Conditional approval by EMA 2019

Zynteglo™ is a trademark of bluebird bio, Inc.

Spark THERAPEUTICS

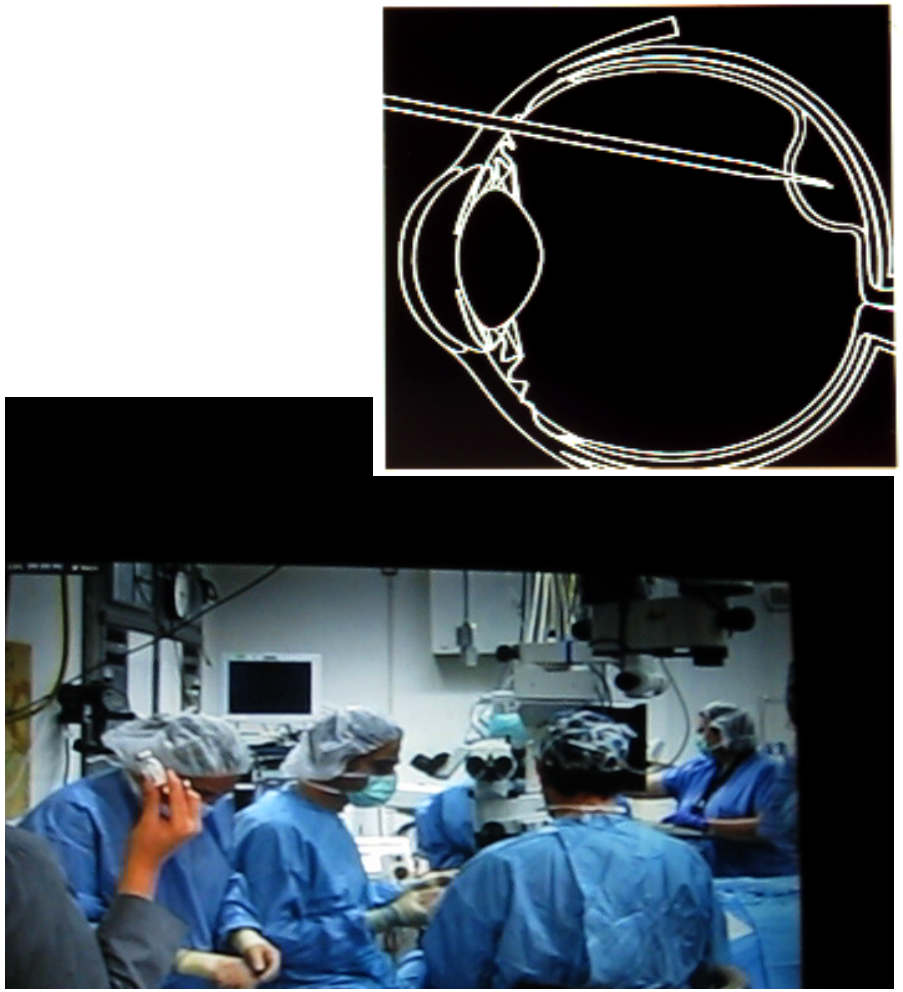
*The marketing authorization in Europe for Glybera® expired on 25 Oct. 2017 and was not renewed by uniQure.
uniQure N.V. Press Release, 20 April 2017.

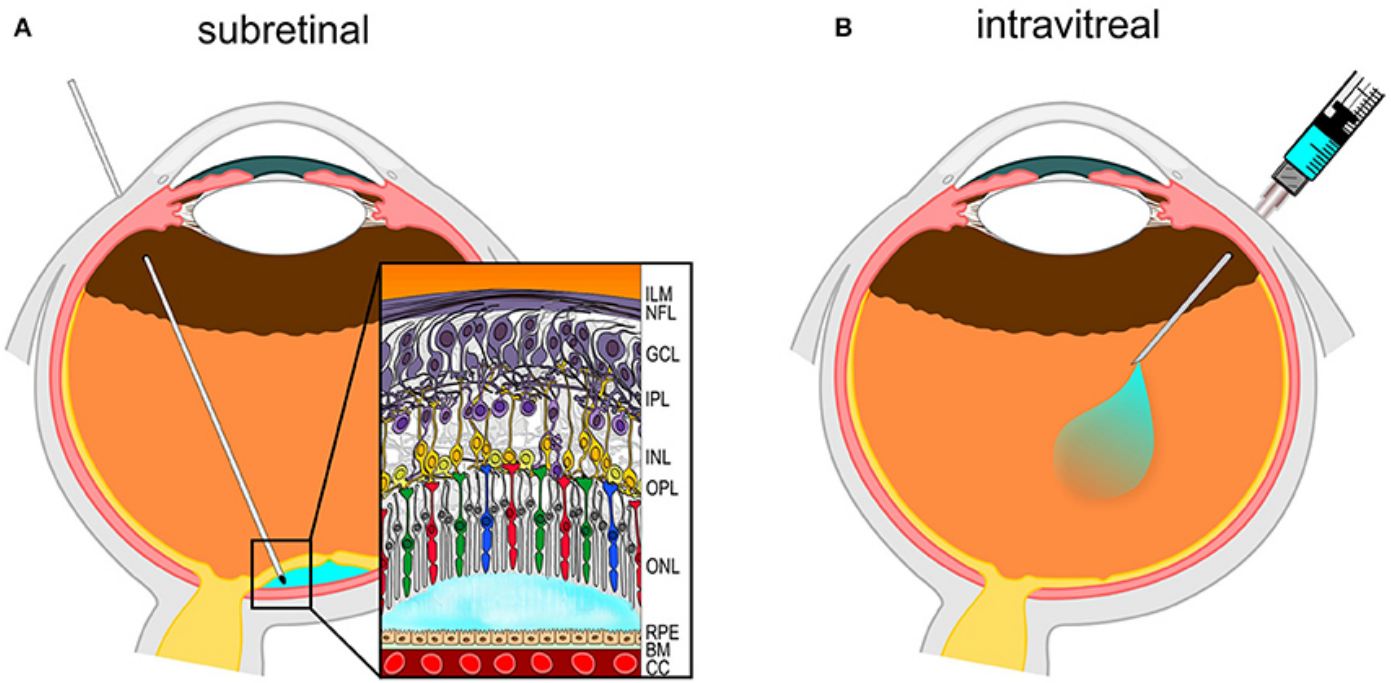
Human retina has many different types of cells



https://webvision.med.utah.edu/wp-content/uploads/2018/05/sagschem.jpg

Gene therapies are being researched as direct injections to the eye

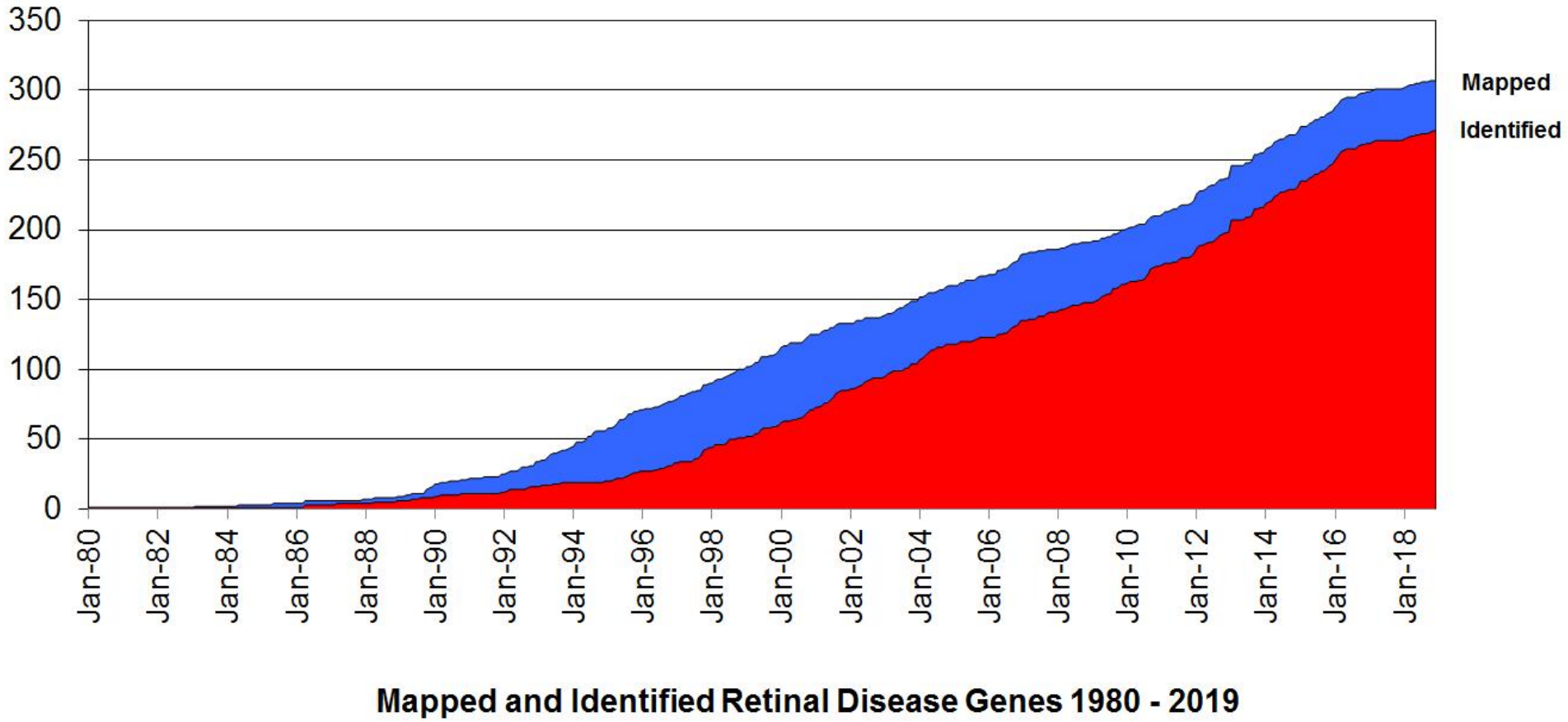




Spark THERAPEUTICS

Maguire AM, et al. N Engl J Med 2008; 358(21):2240-8.
Ochakovski GA, et al. Front Neurosci 2017; 11:174.

Genes involved in vision

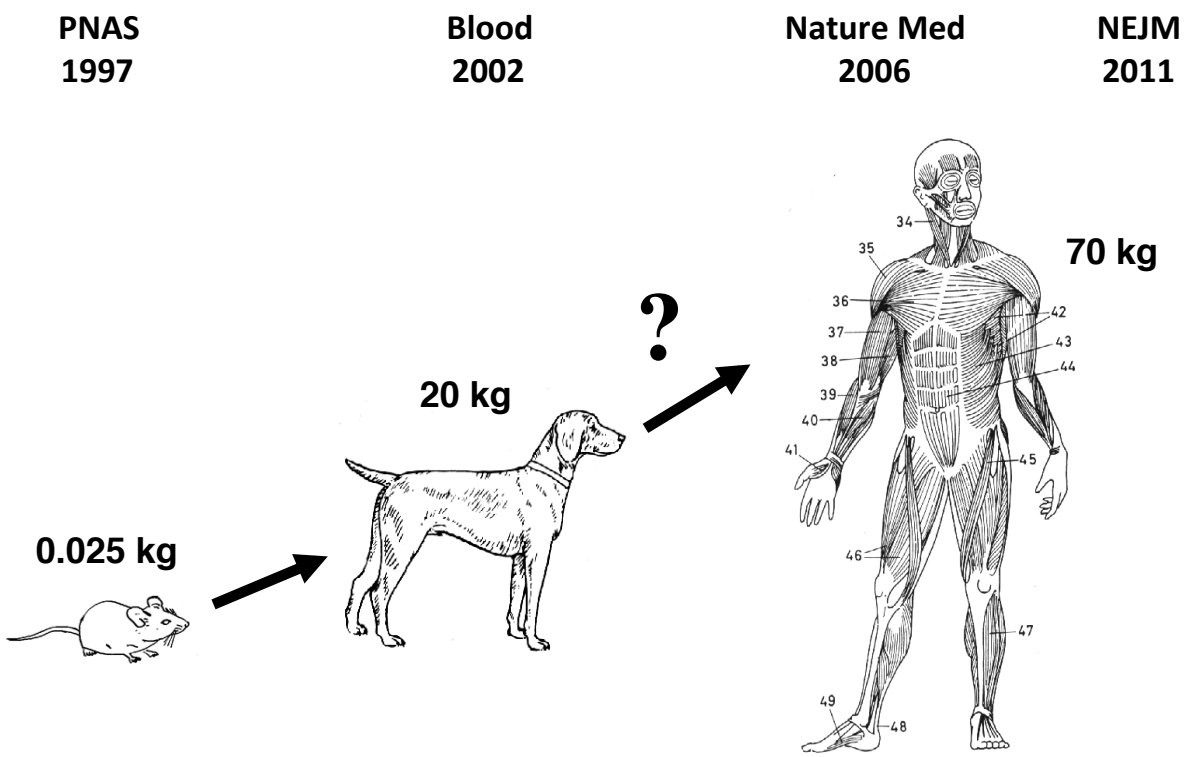


Mapped and Identified Retinal Disease Genes 1980 - 2019

RetNet: Summaries of Genes and Loci Causing Retinal Diseases – Updated 26 Mar 2019
https://sph.uth.edu/retnet/sum-dis.htm#D-graph

How does any drug go from being an experimental treatment to a licensed product?

Paradigm for development of novel therapeutics in genetic disease



Herzog RW, et al. PNAS 1997; 94(11):5804-9.
Mount JD, et al. Blood 2002; 99(8):2670-6.
Manno CS, et al. Nat Med 2006; 12(3):342-7.
Nathwani AC, et al. N Engl J Med 2011; 365(25):2357-65.

Drug Development

- Preclinical Studies
  - Pre-Investigational New Drug (IND) Application
- Clinical Trials
  - IND Submission
  - Phase 1, 2, and 3 Studies
  - Licensing Application (NDA/BLA)
- Product Licensure
  - Phase 4/Post-Marketing Surveillance

Spark THERAPEUTICS™

NDA: New Drug Application; BLA: Biologics License Application
https://www.fda.gov/patients/learn-about-drug-and-device-approvals

- Can drugs be licensed through extensive testing in animals?

No, because animal studies accurately predict side effects in humans only ~70% of the time

Drug Development – Clinical Trials

- Phase 1 – Designed to evaluate safety and dose ranging
  - Typically small group of healthy volunteers (20-100)
- Phase 2 – Designed to evaluate efficacy and side effects
  - Typically a larger group, including patients (20-300)
- Phase 3 – Expanded study, additional information on efficacy and safety
  - Typically randomized, controlled, multicenter trials on large patient groups (300-3,000+)
- Licensing Application
  - "Adequate and well-controlled studies" 21 CFR 314.126

Spark THERAPEUTICS

https://www.fda.gov/patients/drug-development-process/step-3-clinical-research

These paradigms were developed for small molecule drugs that are used to treat common diseases

- Changes have been made to adapt this structure for use in rare diseases and with novel classes of drugs such as gene therapy vectors

Drug development for rare diseases using gene therapeutics

- Phase 1/2 studies are combined and data are developed in a patient population
- Phase 3 challenges may include absence of established endpoints, and dearth of natural history data for rare disorders

Spark THERAPEUTICS

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-rare-diseases

https://www.fda.gov/industry/developing-products-rare-diseases-conditions

In the US, the clinical phase of drug development begins when a sponsor files an investigational new drug application

What goes into an IND?

- General investigational plan
  - Name of the drug and its active ingredient(s)
  - Disease indication to be studied
  - Route of administration
  - General outline of the clinical study
  - Estimated number of patients
  - Any serious risks that are anticipated

https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/information-submitting-investigational-new-drug-application

https://www.fda.gov/drugs/types-applications/investigational-new-drug-ind-application

21 CFR 312.23

What goes into an IND?

- Results of testing the drug in animals
  - Did it accomplish the desired effect in an animal model of the disease? Efficacy
  - In animals, what dose does the drug need to be delivered at to achieve efficacy? What is likely to be a safe starting dose in humans?
  - With drug manufactured in the same way as the material to be used in the clinical trial, what is the safety profile of the drug in animals, at a range of doses including doses higher than those to be used in the trial? Usually conducted as GLP studies

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/preclinical-assessment-investigational-cellular-and-gene-therapy-products

https://www.fda.gov/drugs/types-applications/investigational-new-drug-ind-application

21 CFR 312.23

GLP: Good Laboratory Practice

What goes into an IND?

- Clinical protocol, which must include:
  - The goals of the study
  - The qualifications of the physicians conducting the study
  - Criteria for patient selection and exclusion
  - Description of the type of study, including the designation of control groups, methods to minimize bias, and number of subjects to be enrolled

https://www.fda.gov/drugs/types-applications/investigational-new-drug-ind-application

21 CFR 312.23

What goes into an IND?

- Clinical protocol
  - The doses to be administered, and the route of administration
  - Description of the observations and measurements to be made to establish efficacy
  - Description of tests for safety

https://www.fda.gov/drugs/types-applications/investigational-new-drug-ind-application

21 CFR 312.23

What goes into an IND?

- Chemistry, manufacturing and control information
  - Composition, manufacture, and control of the drug substance
  - General method of preparation, including acceptable limits and analytical methods used to ensure the identity, strength, potency, stability, and purity of the material
  - Gene therapies are among the most complex therapeutic agents yet studied

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/content-and-review-chemistry-manufacturing-and-control-cmc-information-human-gene-therapy

https://www.fda.gov/drugs/types-applications/investigational-new-drug-ind-application

21 CFR 312.23

Food and Drug Administration

FDA's primary objectives in reviewing an IND are, in all phases of the investigation, to assure the safety and rights of subjects, and, in Phase 2 and 3, to help assure that the quality of the scientific evaluation of drugs is adequate to permit an evaluation of the drug's effectiveness and safety.

21 CFR 312.22 (a)

Spark THERAPEUTICS

FDA & Drug Development

- Average of at least 10 years for product approval

- About 1 in 10,000 potential drug compounds investigated preclinically receives FDA approval

Van Norman GA. JACC Basic Transl Sci 2016; 1(3):170-9.

From FDA Summary Basis of Regulatory Action

Date	Regulatory Milestone
9/20/2005	Pre-IND meeting
6/14/2007	IND 13408 Submission
3/25/2016	Pre-BLA meeting
4/27/2016 2/22/2017 5/16/2017	BLA 125610 Submission (rolling submission) -Preclinical data -Clinical data -CMC data
7/14/2017	BLA 125610 Filed, priority review
10/12/2017	Cellular, Tissue, and Gene Therapies Advisory Committee Meeting
1/12/2018	PDUFA* Action Due Date

*PDUFA=Prescription Drug User Fee Act

IND: Investigational New Drug application;
BLA: Biologics License Application
https://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM592083.pdf

Take home messages

- Patience is required—can easily be 7-10 years from initiation of a clinical trial to licensing of a product
- Importance of meetings like this one
  - Funding of early studies to establish proof of concept in animal models
  - Developing natural history studies for rare diseases
  - Developing patient registries
  - Funding or participating in studies on biomarkers and/or novel endpoints

Spark THERAPEUTICS™

Questions?

Back up slides

Challenges in clinical development programs for rare inherited retinal disease

- Ultra-rare disease – small patient population
- Dearth of natural history data
- Best trial design for Phase 3
- Need for a novel primary endpoint

Spark THERAPEUTICS

Critical elements of delivery approach

- Administration occurs in limited number of treatment centers
  - Availability of an IRD specialist
  - Surgical and pharmacy training programs
- Continued education on role of genotyping
- Establishment of Patient Services group
- Development of novel payment and distribution models

Spark THERAPEUTICS™

Early lessons

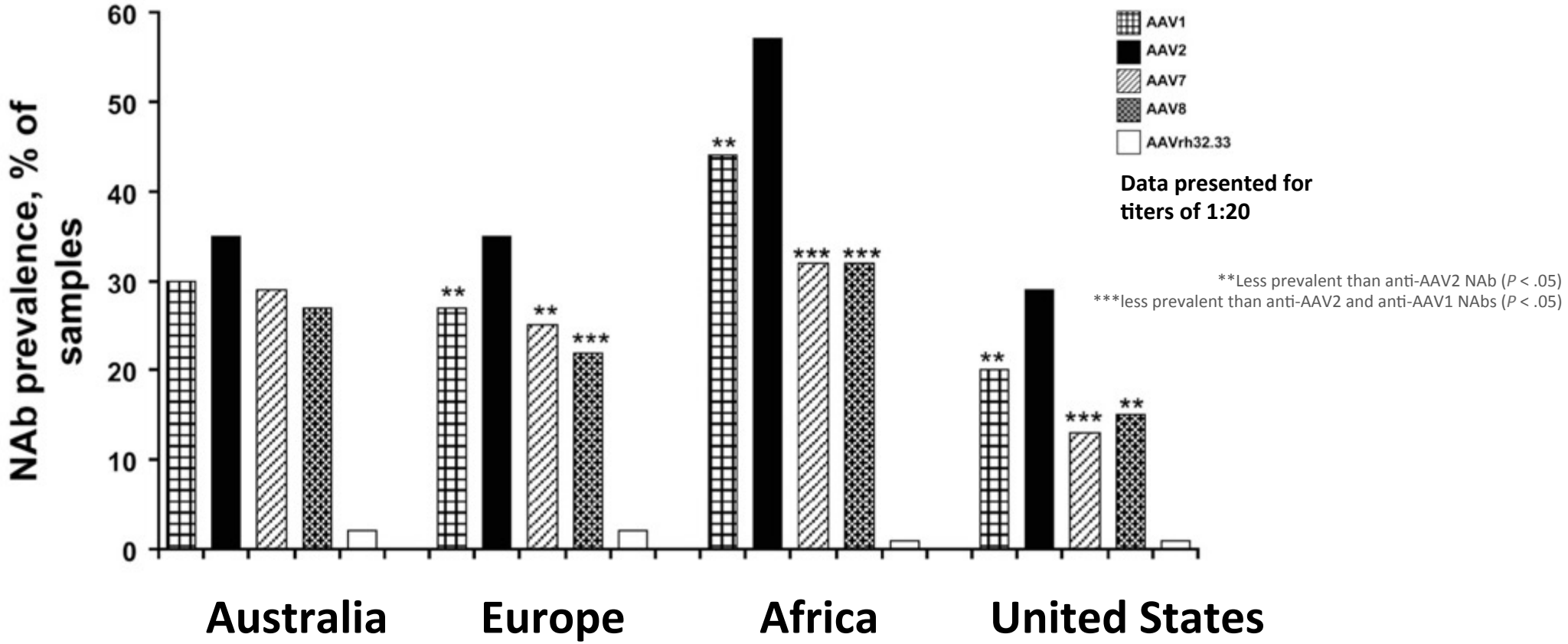
- Neutralizing antibodies
  - Most trials screen and exclude those with pre-existing antibodies
- Delayed cellular immune response – dose-dependent
  - Typically occurs in first 12 weeks after vector infusion
  - Dose-dependent
  - For certain vectors, but not others, response controlled with steroids

Spark THERAPEUTICS™

Manno CS, et al. Nat Med 2006; 12(3):342-7.
Mingozzi F, et al. Nat Med 2007; 13(4):419-22.
Nathwani AC, et al. N Engl J Med 2011; 365(25):2357-65.

Deciding who may be eligible

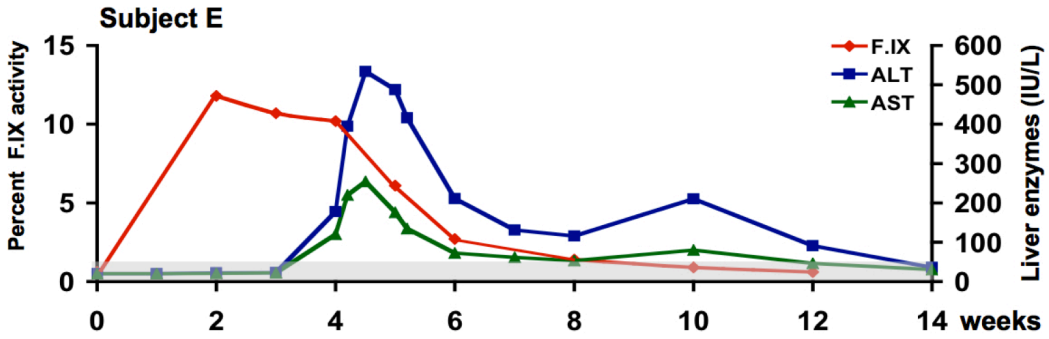
- Pre-existing neutralizing antibodies (NAbs) in 30-60% of the population

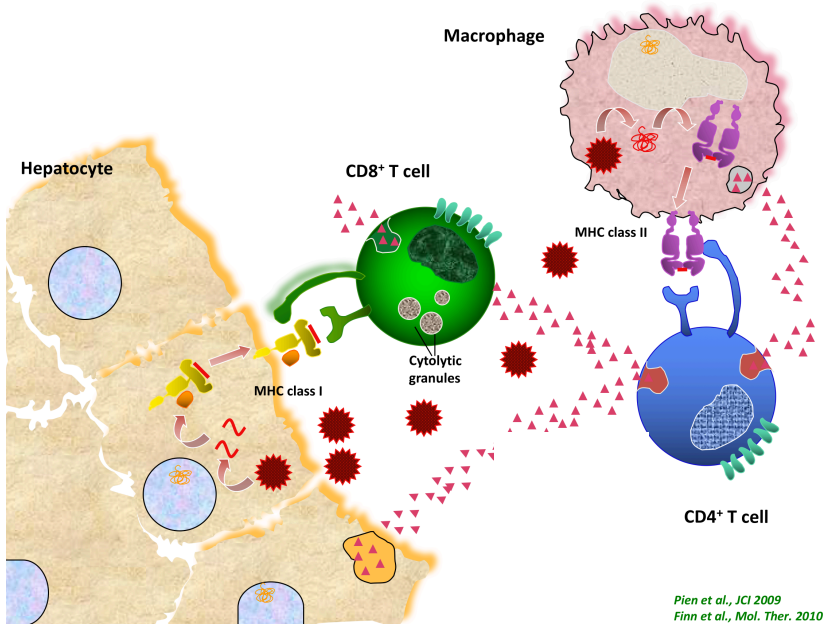


Calcedo R, et al. J Infect Dis 2009; 199(3):381-90.
Mingozzi F, et al. Sci Transl Med 2013; 5(194):194ra92.

How does the human immune response to AAV affect gene therapy to the liver?

- Dose-dependent delayed CD8$^+$ T cell response against AAV capsid that can reduce or eliminate expression if not controlled





Manno CS, et al. Nat Med 2006; 12(3):342-7.
Mingozzi F, et al. Nat Med 2007; 13(4):419-22.

Future directions

- First gene therapy products are now coming into use in US
- AAV products are in many ways like other specialty pharmaceuticals
  - Diseases that can be treated with ERT are amenable to GT approaches
- Lentiviral modification of HSCs requires a specialized cell processing facility, similar to BMT facilities
  - Genetic diseases that can be treated by BMT can be treated by GT, but reach is broader
- GT for genetic disease has the possibility to develop treatments for diseases that have previously lacked therapeutic options, and to expand therapeutic options for diseases previously treated by e.g. enzyme replacement, small molecules, or BMT

Spark THERAPEUTICS

ERT: Enzyme replacement therapy
HSC: hematopoietic stem cell
BMT: bone marrow transplant

Spark THERAPEUTICS

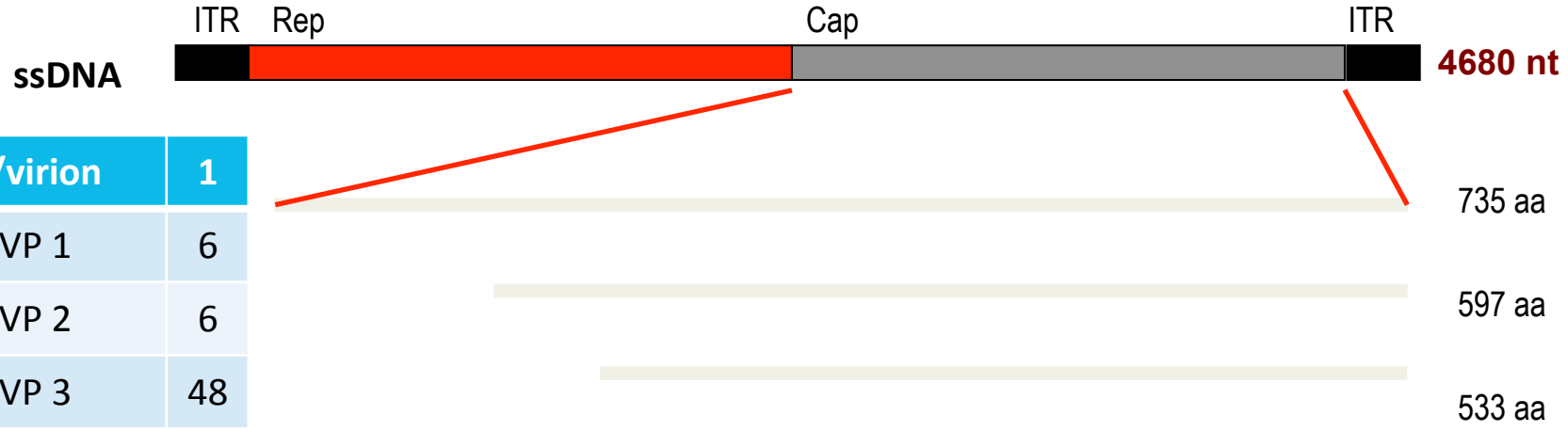
Potential complications of gene therapy

- Gene silencing
- Genotoxicity-integration events
- Phenotoxicity-overexpression of the transgene
- Immunotoxicity
- Horizontal transmission
- Vertical transmission

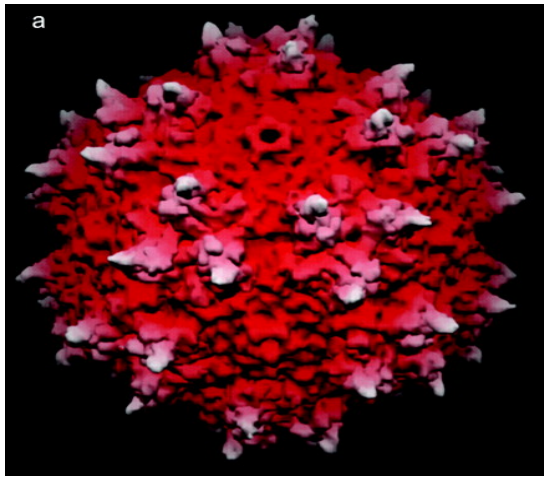
Spark THERAPEUTICS

Mingozzi & High. Nat Rev Genet 2011; 12(5):341-55.

AAV structure and composition

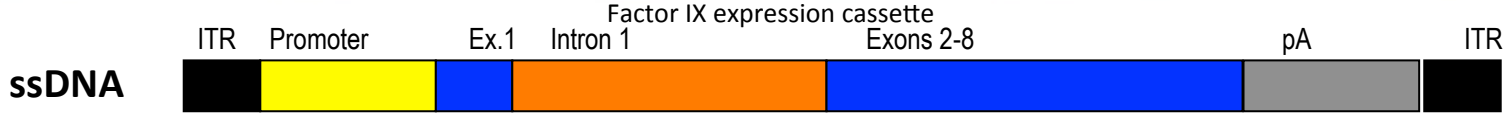


#/virion	1
VP 1	6
VP 2	6
VP 3	48

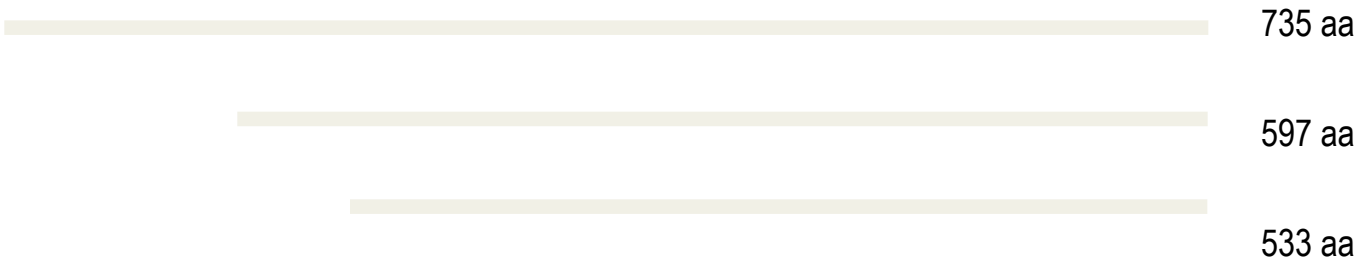


Mingozzi & High. Blood 2013; 122(1):23-36.
Xie Q, et al. PNAS 2002; 99(16):10405-10.

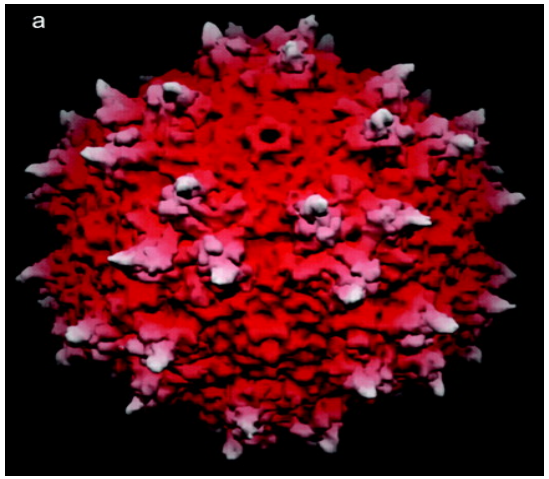
AAV structure and composition



#/virion	1
VP 1	6
VP 2	6
VP 3	48



Particle radius	25nm
Molecular weight	
protein (74%)	M_r ~ 3750 kDa
DNA (26%)	M_r ~ 1350 kDa
total virus	M_r ~ 5100 kDa

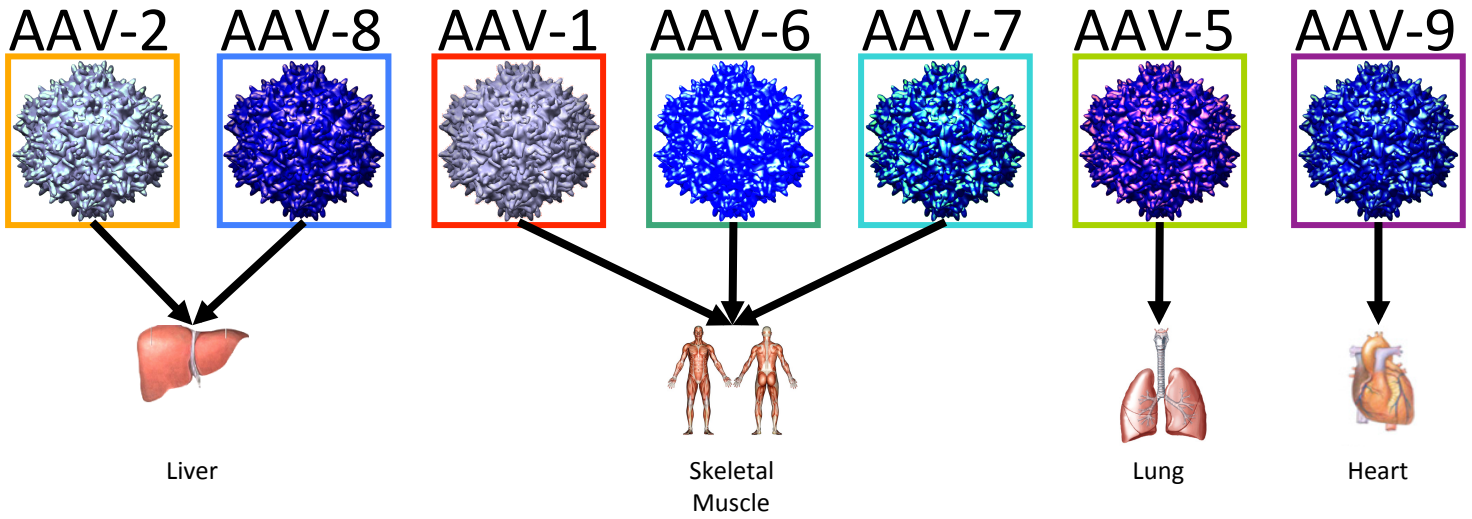


- Highly ordered set of proteins (vector capsid) containing DNA (the active agent)
- Often dosed in vector genomes/ kilograms (vg/kg)
- Maximum packaging capacity ~5 kb.

Mingozzi & High. Blood 2013; 122(1):23-36.
Xie Q, et al. PNAS 2002; 99(16):10405-10.

AAV Capsids

There are a variety of different naturally occurring (and now engineered) AAV capsids that differ in sequence and tissue tropisms




```
AAV-1 VP1   (340) --FPADVFMIPQYGYLTLNNGSQ---
AAV-2 VP1   (339) --FPADVFMVPQYGYLTLNNGSQ---
AAV-3a VP1  (339) --FPADVFMVPQYGYLTLNNGSQ---
AAV-3b VP1  (339) --FPADVFMVPQYGYLTLNNGSQ---
AAV-4 VP1   (330) --FPNDVFMVPQYGYCGLVTGNTSQQ
AAV-5 VP1   (330) --FPPQVFTLPQYGYATLNRDNTEN-
AAV-6 VP1   (340) --FPADVFMIPQYGYLTLNNGSQ---
AAV-7 VP1   (341) --FPADVFMIPQYGYLTLNNGSQ---
AAV-8 VP1   (342) --FPADVFMIPQYGYLTLNNGSQ---
```

Spark THERAPEUTICS

Mingozzi & High. Blood 2013; 122(1):23-36.
Mingozzi F, et al. Nat Med 2007; 13(4):419-22.

Recombinant AAV vector production

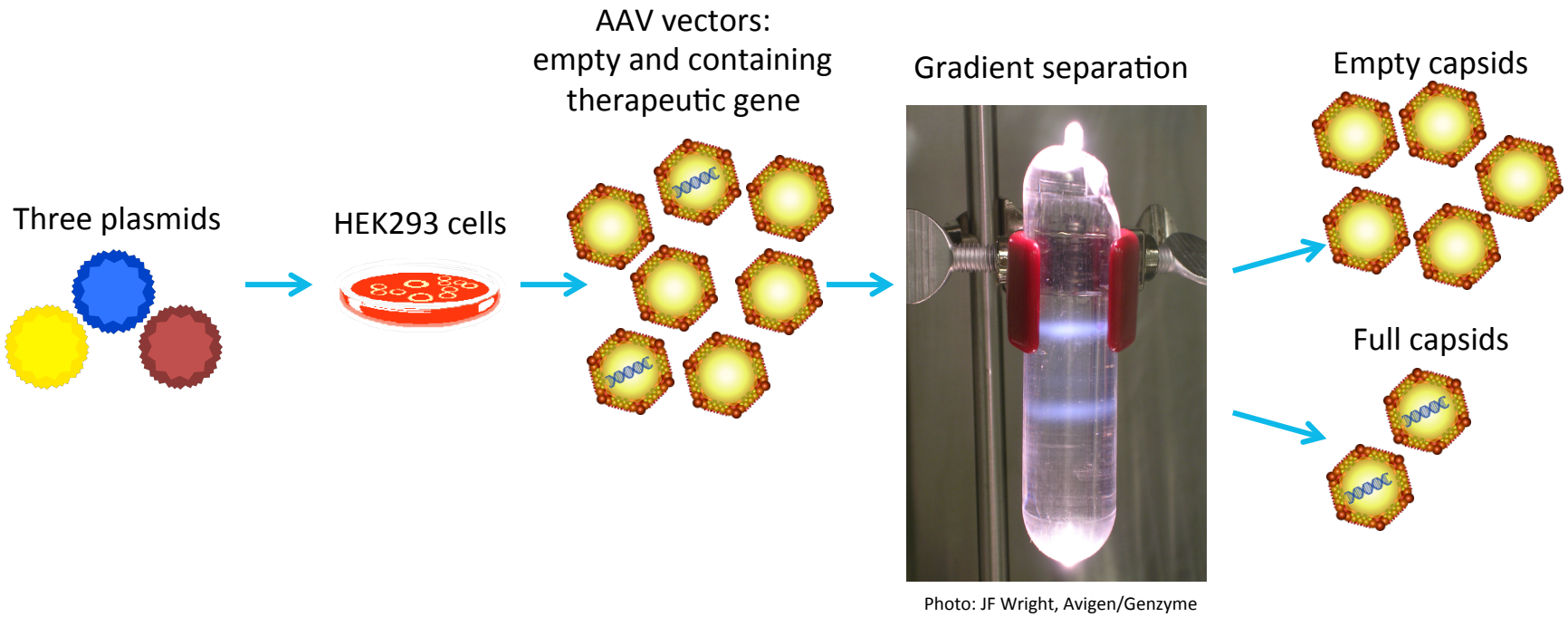


Photo: JF Wright, Avigen/Genzyme

Most preparation methods >90% empty capsid;
Final product after gradient separation <2% empty capsid

Qu G et al. J Virol Methods. 2007; 140(1-2):183-92.
Wright JF. Gene Ther 2008; 15:840-848.