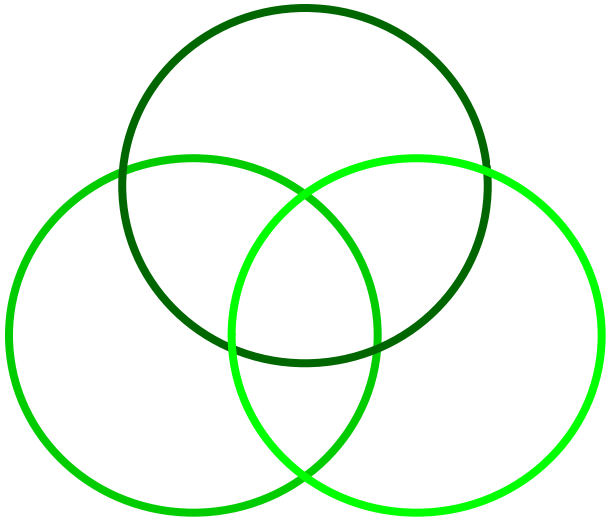
Environmental risk assessment for the market authorization of the gene therapy product Glybera



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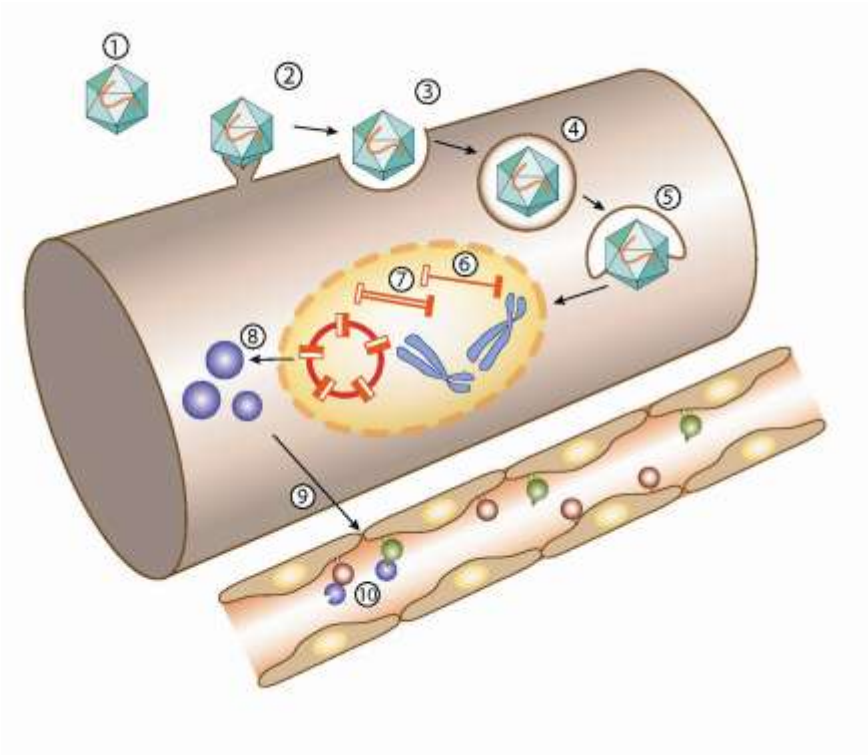
Florence Salmon, UniQure, Amsterdam, The Netherlands

Glybera by UniQure

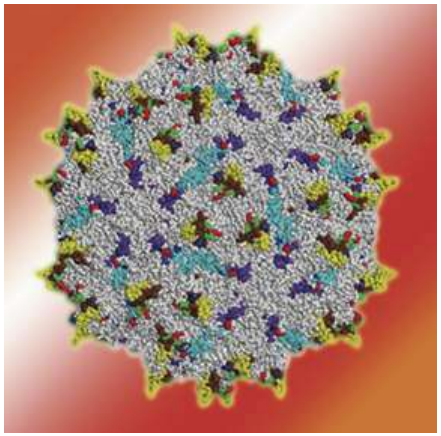
- First gene therapy medicinal product admitted for market authorization by the European Commission on 25 October 2012.
- Designed for long term compensation of lipoproteinlipase deficiency (LPLD), a seriously debilitating inherited lipid metabolism disorder due to the failure of triglyceride catabolism causing recurrent pancreatitis and possibly death.
- Glybera consists of an adeno-associated viral vector with an insert coding for a human, naturally occurring, truncated variant of the LPL^{S447X} gene.

Transduction of a muscle cells by Glybera

1) AAV1-capsid particles with AAV2-derived attenuated vector genome with the LPL insert
2) I.m. injection (muscles are natural location of LPL expression)
3) Complementation ssDNA to form dsDNA and circular concatemers, persisting as extra-chromosomal DNA in the nucleus.
4) Expression of LPL and transport from the muscle cell to the epithelium of nearby capillaries.



Adeno-Associated Virus

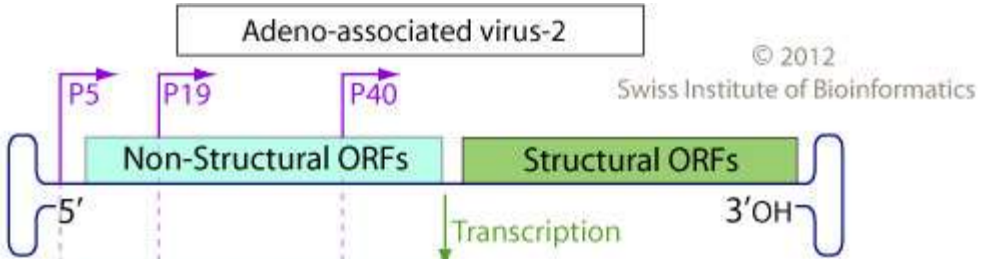


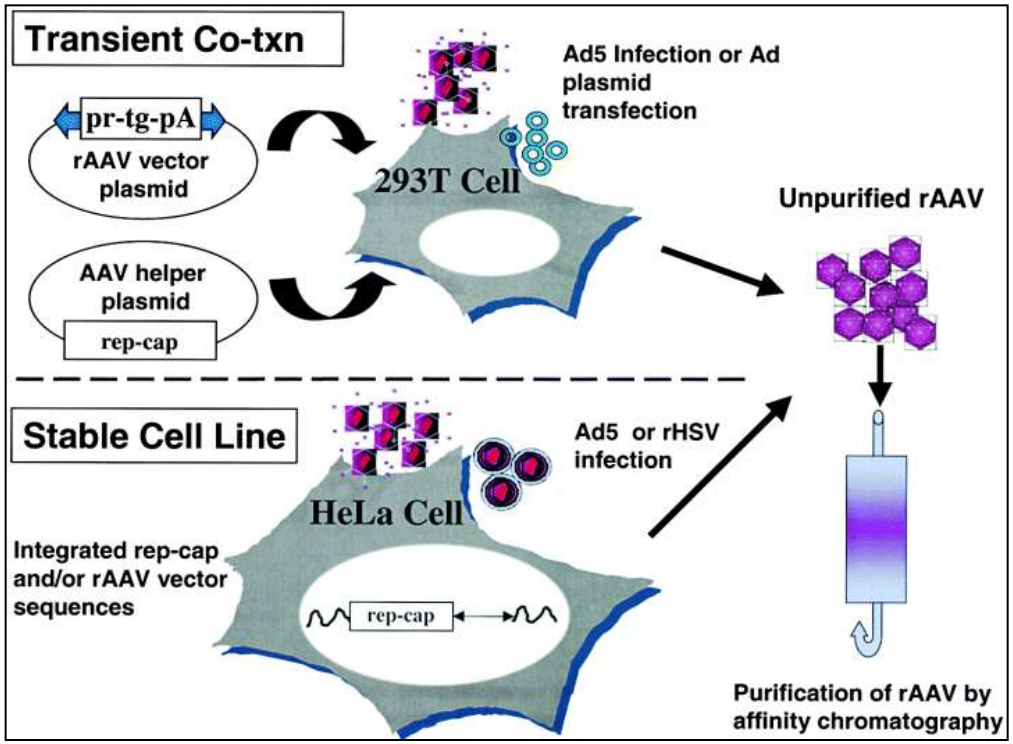
- Parvoviridae/Dependovirus
- Small ssDNA virus
- Infection of humans (AAV2) and some non-human primates (AAV1 and 3) an a wide range of vertebrates (other clades)
- **Not known to cause disease**
- Very mild immune response, **most adults are seropositive**
- Persist in **extra-chromosomal state** but can stably integrate into the host cell genome at a specific site of chromosome 19 (1%)
- **Replication-defective**, can be only produced in the presence of **helper virus** such as Adenovirus, Herpes-Simplex-Virus, Cytomegalovirus or Human Herpes Virus-6
- Non-enveloped

Production of AAV vectors

- **Separation of the components**
  - ***Trans-acting sequences***
    - a plasmid encoding Rep and Cap
    - or a cell line encoding Rep and Cap
  - ***Cis-acting sequences***
    - the vector plasmid carrying the transgene to be packed into the new particles

- **Cell line needs to contain Adenovirus E2 and E4 sequences**





Success in Biotechnology through Science, Safety and Security

"Going off the beaten path" with unconventional ideas or unpredictable discoveries

Safety for the Environment

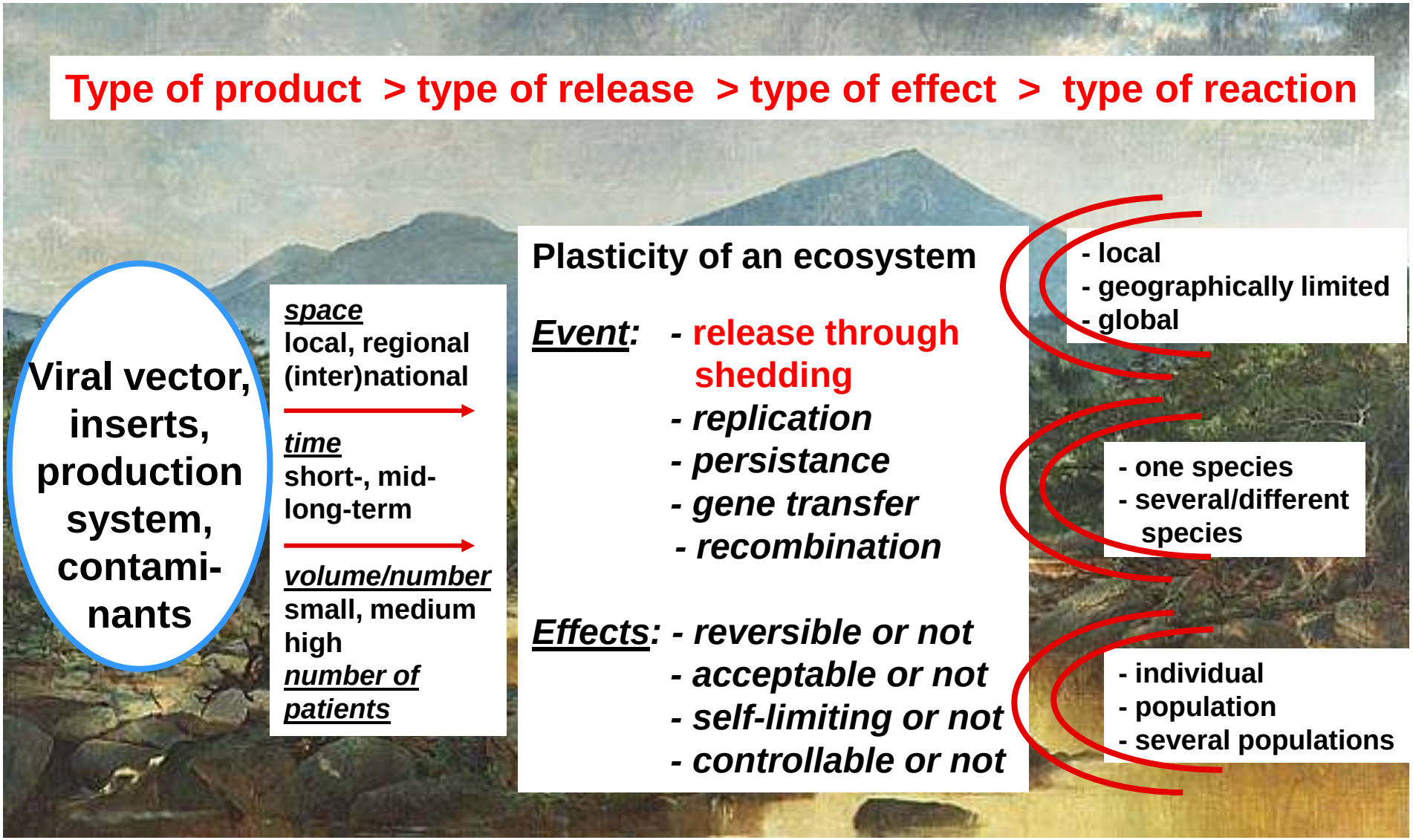
Safety for patients and people at large

Maximize advantages & minimize disadvantages for society

EC requires an environmental risk assessment for *in vivo* and ex vivo gene therapy products

- Regulation (EC) No 1394/2007 on **Advanced Therapy Medicinal Products** (ATMP) / *inclusion criteria*.
- Directive 2001/83/EC on the **Community Code relating to Medicinal Products for Human Use** / *definition and requirement* for *precautionary and safety measures together with an indication of potential risks presented for the environment*.
- Regulation 726/2004 on **Community Procedure for the Authorization and Supervision of Medicinal Products** / *the environmental risks shall be addressed according to* **Directive 2001/18/EC, deliberate release of GMO**, *and any* **guidance documents** *published*.

‘Things to consider’ in an Environmental Risk Assessment



Type of product > type of release > type of effect > type of reaction

Viral vector, inserts, production system, contami-nants

space
local, regional (inter)national

time
short-, mid- long-term

volume/number
small, medium high

number of patients

Plasticity of an ecosystem

Event: **- release through shedding**
- replication
- persistance
- gene transfer
- recombination

Effects: ***- reversible or not***
- acceptable or not
- self-limiting or not
- controllable or not

- local
- geographically limited
- global

- one species
- several/different species

- individual
- population
- several populations

Human exposure besides patients

Type of product > type of release > type of effect > type of reaction

Contact with health care personnel

- Spills, needle stick injuries
- Inhalation, injection, contact with mucous membrane

Contact with contact persons

- Shedding (injection site, urine, faeces)
- Contact exposure

Methodology for the ERA

- ‘Theoretical’ data derived from literature and calclulations
  - Biology of wild type AAV
  - Attenuation of AAV vector construct
  - Application scenarios for calculation of potential exposure
- Experimental data
  - General toxicity and biodistribution study in mice.
  - Pre- and post-natal reproduction toxicity study in rabbits, including analysis of vector DNA in semen and gonads.
  - Ex-vivo mechanistic integration studies of Glybera specific vector DNA.
  - During the clinical development program of Glybera, shedding was monitored in saliva, urine and semen samples of treated patients.
  - Data from quality control of the product

Characteristics of Glybera

Safety criteria	*AAV-LPL-vector*
Parental virus	Non-pathogenic AAV
Host specificity	Humans
Competence for replication	rep-deleted
Expression of viral genes	None
Recombination with viral sequences	Patients devoid of AAV, recombination with wtAAV > identical
Host genome integration	rep-deleted
Transformation capacity	No, not seen in wt AAV either
Production via helper virus	Baculovirus helper-free system
Function and origin of inserts	Human LPL, no other functional proteins expressed

Environmental exposure scenario

Scenario	Environment – 1	Environment – 2
Exposed	General environment	Waste water treatment plant
Occurrence:	Incidental spill	Shedding of product after administration by urine
Max. Exposure:	Limited to a maximum of a single dose per patient. Shipment to hospital centres in specialized absorbent packaging materials. Site decontamination in case of spill, biosafety-trained personnel.	Less than 0.000013 % of dose per day per patient in the first week after treatment, and much less thereafter. Due to rarity of the disease, 1-2 patients per week per hospital centre are unlikely to be exceeded.
Likelihood of occurrence	negligible	negligible

Human exposure scenario

Scenario	Human - 1	Human - 2
Exposed	Health care provider	Bystander / Family member
Occurence:	Spillage or accidental self-inoculation during product administration.	Contact with shedded product after administration
Max. Exposure:	Approximately 3 % of clinical target dose, due to compart-mentalised packaging and administration, personal protective equipment, adoption into pharmacovigilance plan in case of injury.	Less than 0.000013 % of dose per day, based on data from shedding studies. No germ line-transmission. Normal hygiene.
Likelihood of occurrence	small	negligible

Conclusion of the ERA

Based on the available data and the conducted assessments, the overall risk of Glybera for people and the environment was concluded to be negligible.

The long road to market authorization

- **Lessons learned from the clinical development and market authorization of Glybera**.
  Bryant L.M. *et al.* Human Gene Therapy Clinical Development 2013, 24:1-10.

- **From mutation identification to therapy: discovery and origins of the first approved gene therapy in the western world**. *Kastelein J.J.P. et al.* Human Gene Therapy 2013, 24:472-478