Gene Therapies

Roche in Germany´s Biotech expertise is key to unlock Gene Therapies full potential

Performed by a student of the National University of Pharmacy : Inna Mischenko
Group LDm22(1.5)-4, 1st course

Functioning of Gene Therapies

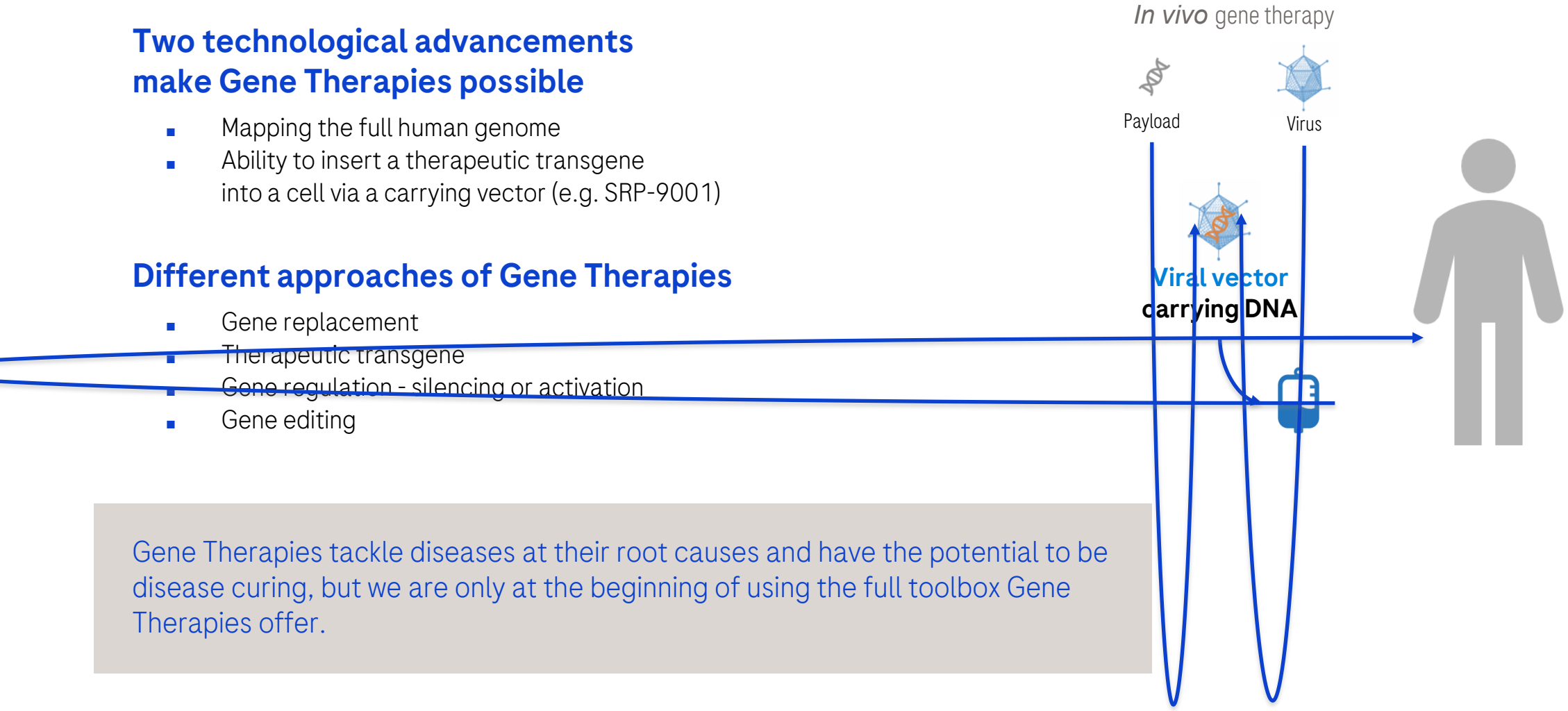
Opening up a new world of treatment options

Two technological advancements make Gene Therapies possible

- Mapping the full human genome
- Ability to insert a therapeutic transgene into a cell via a carrying vector (e.g. SRP-9001)

Different approaches of Gene Therapies

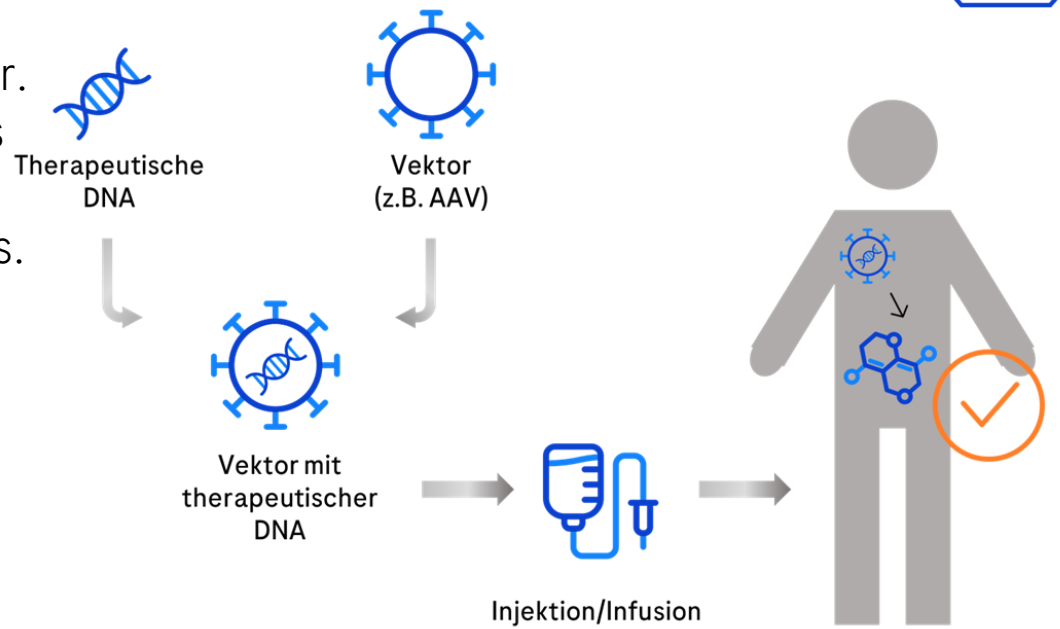
- Gene replacement
- Therapeutic transgene
- Gene regulation - silencing or activation
- Gene editing



Gene Therapies tackle diseases at their root causes and have the potential to be disease curing, but we are only at the beginning of using the full toolbox Gene Therapies offer.

GT – Transduction using Vectors

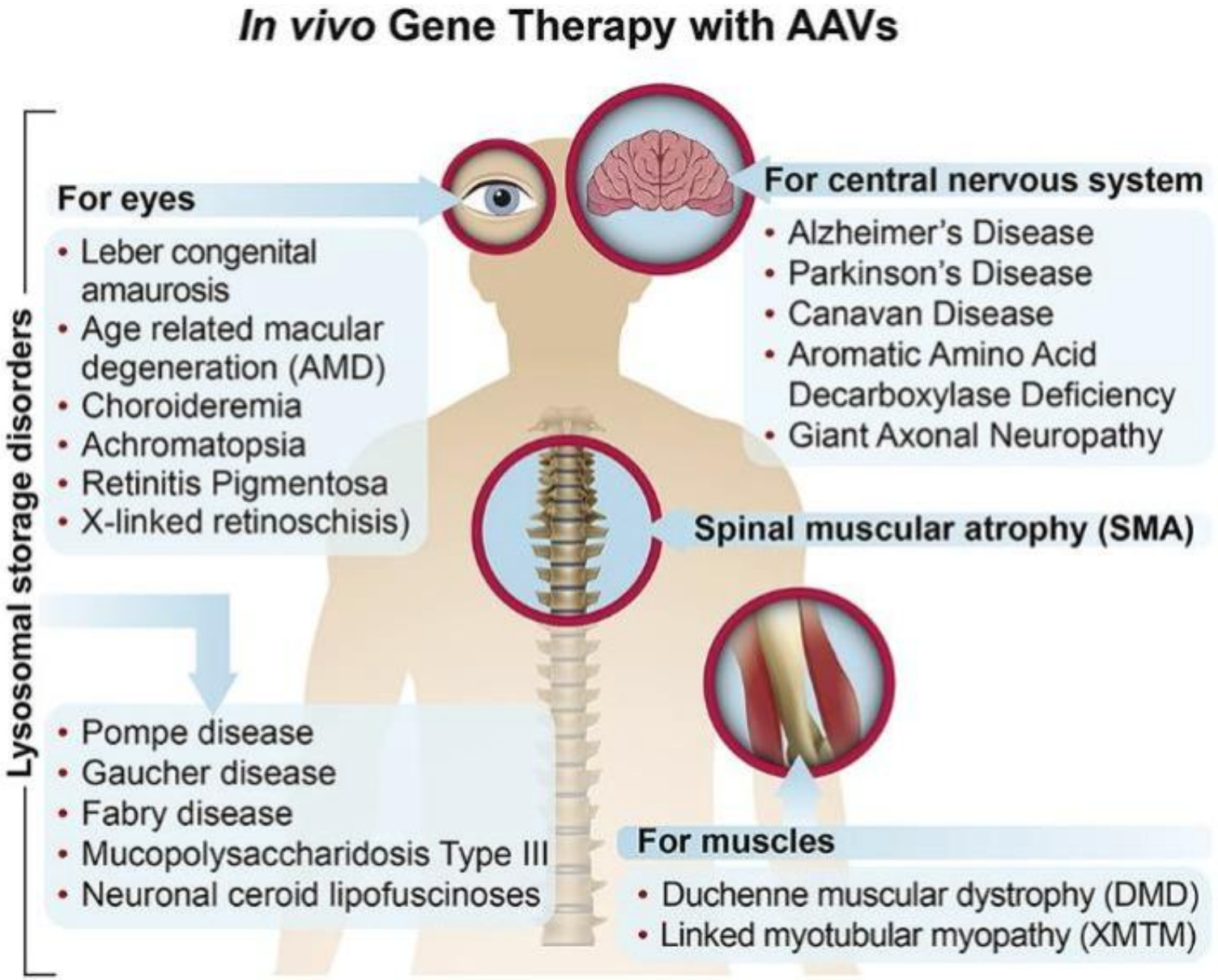
- The therapeutic DNA is introduced into the body cells via a vector. A vector can be many things, for example a part of a certain virus from which all pathogenic components have been removed. This serves as a means of transport from the puncture site to the cells.
- Thanks to its many desirable properties, adeno-associated viral vectors (AAV-V) have become the predominant vectors used to deliver the gene of interest 3



- Various serotypes are used in the production of the corresponding serotypes, which, for example, have a specificity towards certain organs and thus enable targeted therapies.

AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9	AAV10
Muscle CNS Heart Retina	Cancer cells CNS Kidney Liver Retina	Liver Cancer cells	Lung CNS Retina	Muscle Liver CNS Retina	Heart Lung Muscle Liver Spinal cord	Liver Muscle	Pan-creas Heart Muscle Liver Retina	CNS Heart Muscle Liver Lung	CNS Muscle Liver

Application



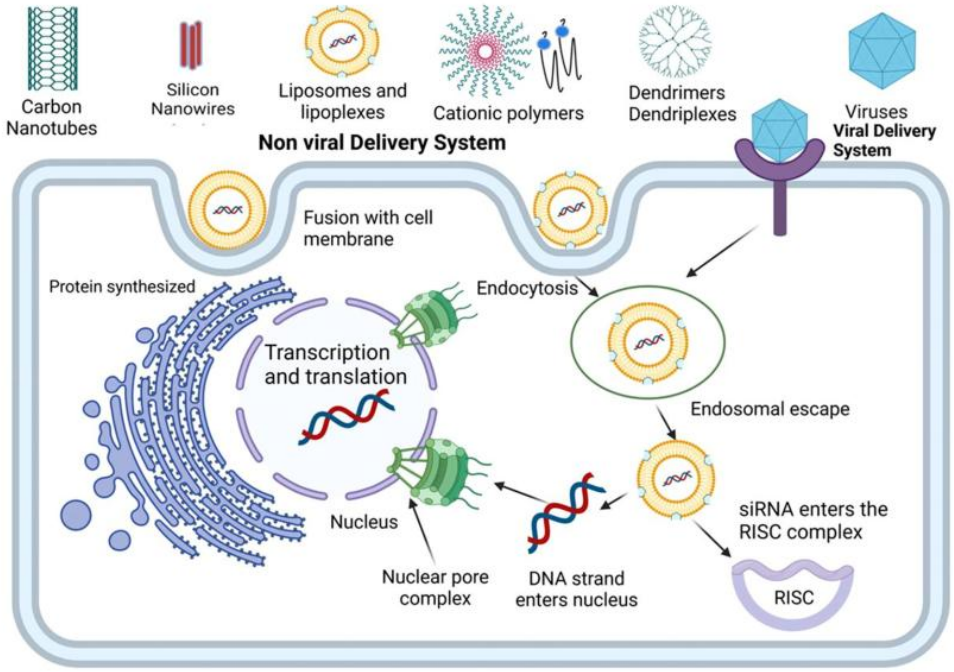
- Diseases that can theoretically be with gene therapy using AAV vectors.
- Therapies have already been approved for some, while clinical trials are underway for others

Approved Therapies

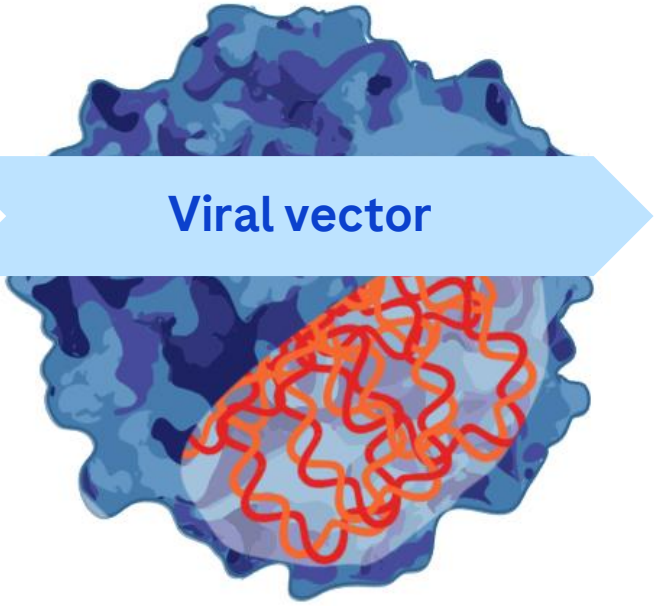
ABECMA (idecabtagene vicleucel) Celgene Corporation
BREYANZI Juno Therapeutics, Inc., a Bristol-Myers Squibb Company
CARVYKTI (ciltacabtagene autoleucel) Janssen Biotech, Inc.
IMLYGIC (talimogene laherparepvec) BioVex, Inc., a subsidiary of Amgen
KYMRIAH (tisagenlecleucel) Novartis Pharmaceuticals Corporation
LAVIV (Azficel-T) Fibrocell Technologies
LUXTURNA Spark Therapeutics, Inc. a subsidiary of Roche Inc.
PROVENGE (sipuleucel-T) Dendreon Corp.
RETHYMIC Enzyvant Therapeutics GmbH
SKYSONA (elivaldogene autotemcel) bluebird bio, Inc.
STRATAGRAFT Stratatech Corporation
TECARTUS (brexucabtagene autoleucel) Kite Pharma, Inc.
YESCARTA (axicabtagene ciloleucel) Kite Pharma, Incorporated
ZYNTEGLO (betibeglogene autotemcel) bluebird bio, Inc.
ZOLGENSMA (onasemnogene abeparvovec-xioi) Novartis Gene Therapies

Emerging Technology - GT

- In the first half of 2020 alone, 350 clinical studies for in vivo gene therapy1 were running
- The FDA predicted that by 2025 it will be approving 10 to 20 new cell and gene therapy products annually2
- The FDA defines gene therapy as "a technique that modifies a person's genes to treat or cure a disease."
  - To do this, a disease-causing gene can be inactivated or replaced with a healthy gene.
- Alternatively, a new gene can be introduced into the body for therapeutic purposes
- In addition to physical (e.g. microinjection) and chemical methods (e.g. liposomes) for gene transfer, transduction with vectors is the most common method.



The molecular complexity of Gene Therapies implies challenges for industrialization

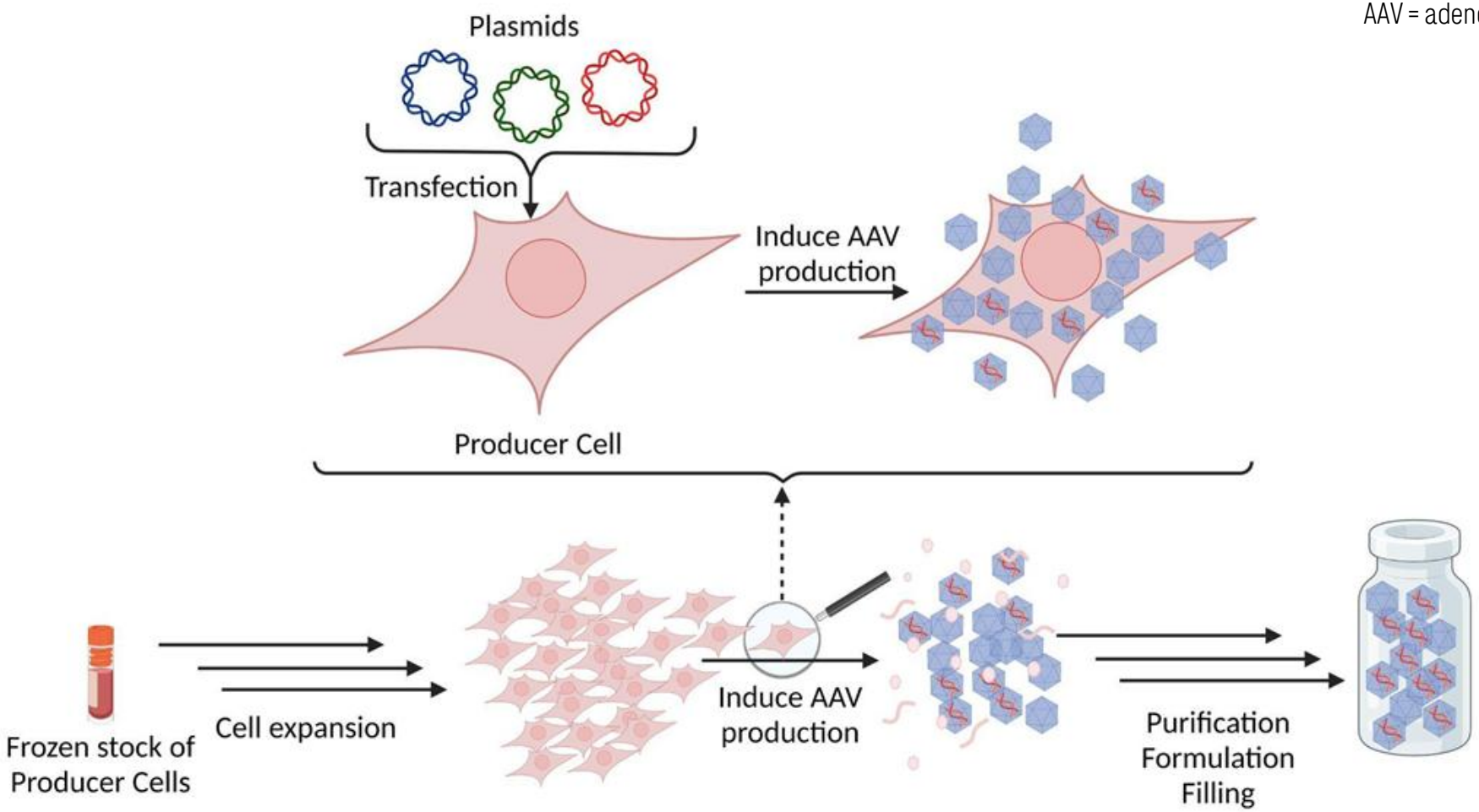


	Small Molecule	Biologic	Viral vector
			
Size	Aspirin	Avastin	SRP9001
Complexity	21 atoms	20,067 atoms	~ 180,000 atoms
			

Background: The manufacturing process at a glance

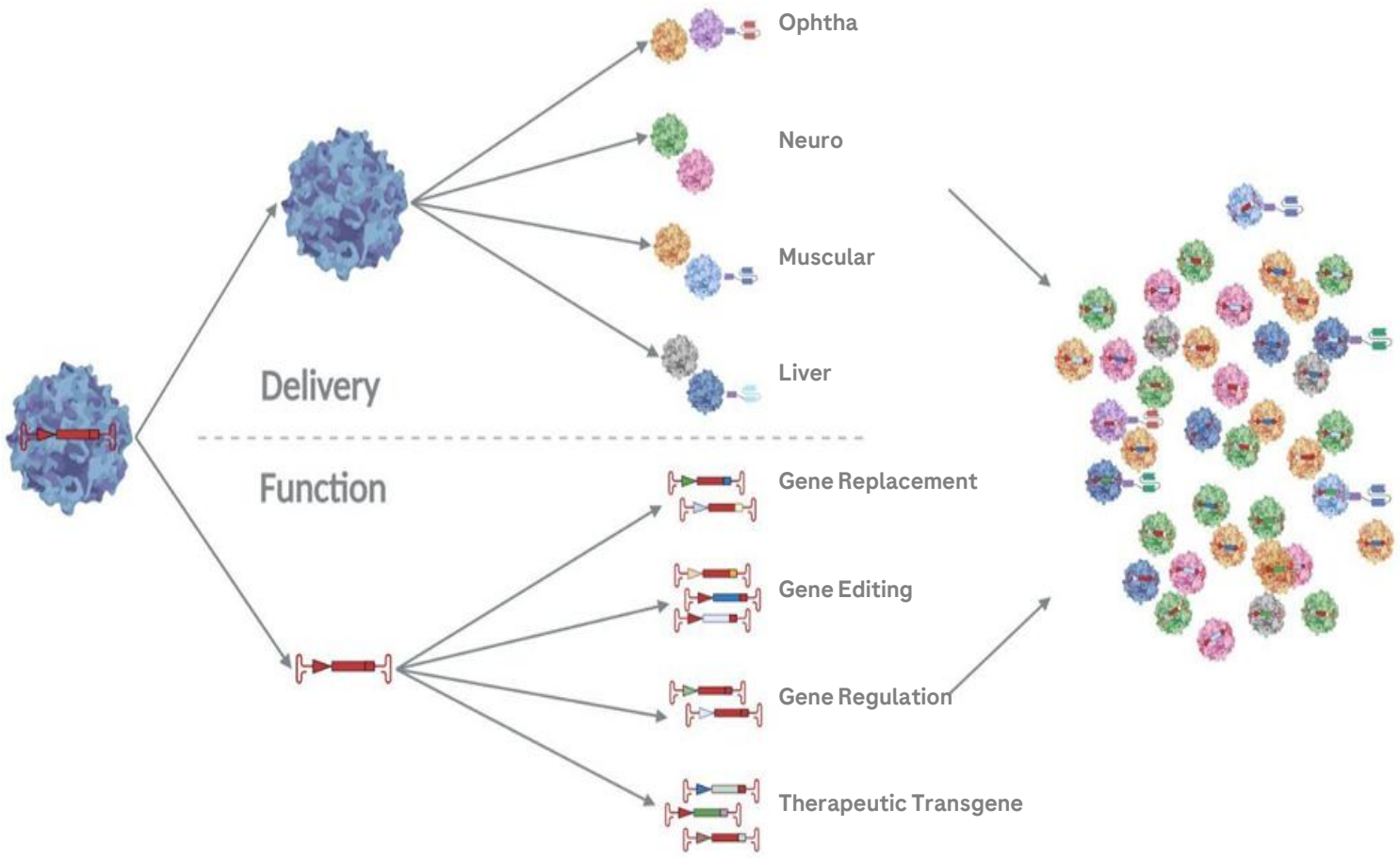
Producer cells as the “factory in the factory”, Plasmids providing the information to produce AAVs

AAV = adeno-associated viral vectors



Robust & fast commercialization: product & process innovation

Early Development to support diverse research approaches with robust processes

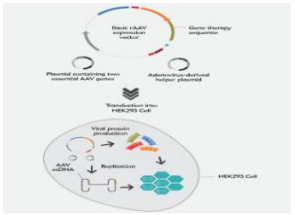


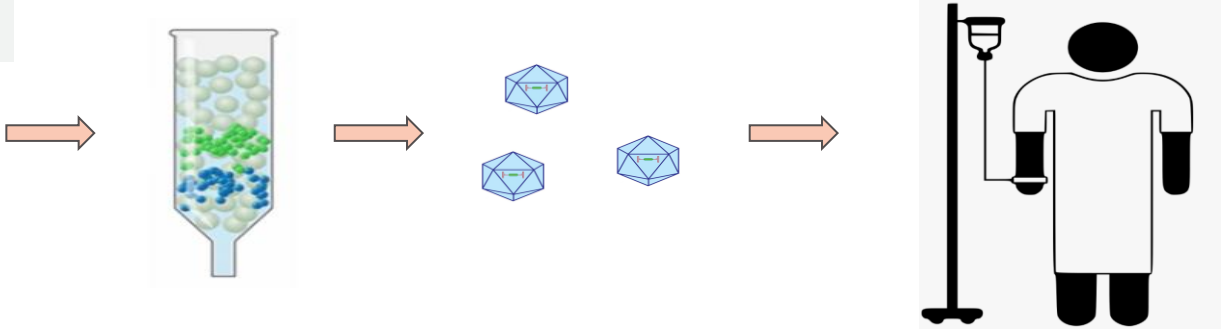
A close connection and colocation enables innovation and translating research approaches into robust processes.

This is the basis to leverage the full potential of the
GT toolbox offering diverse molecular designs.

Production Process

Production Principle





Expansion of Human Cells (HEK293)
In a bioreactor, the production cells are multiplied over several reactor stages until the target volume is reached.

Transfection of the production cells
The plasmid with the therapeutic gene, helper plasmid and cap plasmid are introduced into production cells. These now produce the viral vectors

Purification of the vectors
The vectors are separated from contamination in several steps.

Formulation of the vectors and administration to the patient
In the next step, the vectors are brought into a form that can be administered to the patient.
The disease of the patient is treated by the gene contained in the vectors.

Laboratory Layout

- Ballroom concept for high flexibility
- Cross-functional use of technical centers by all areas
- Disposable reactors and devices for increased safety, increased flexibility and minimal water and energy consumption

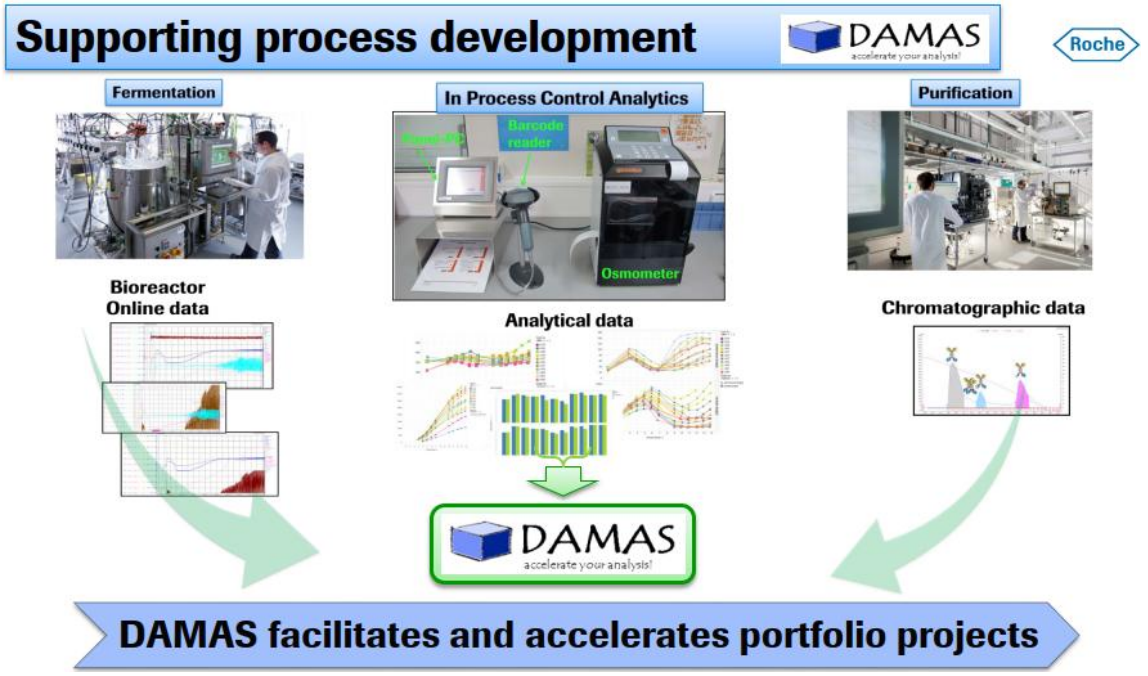
My part/contribution/side to Gene Therapies
Inna Mischenko ??

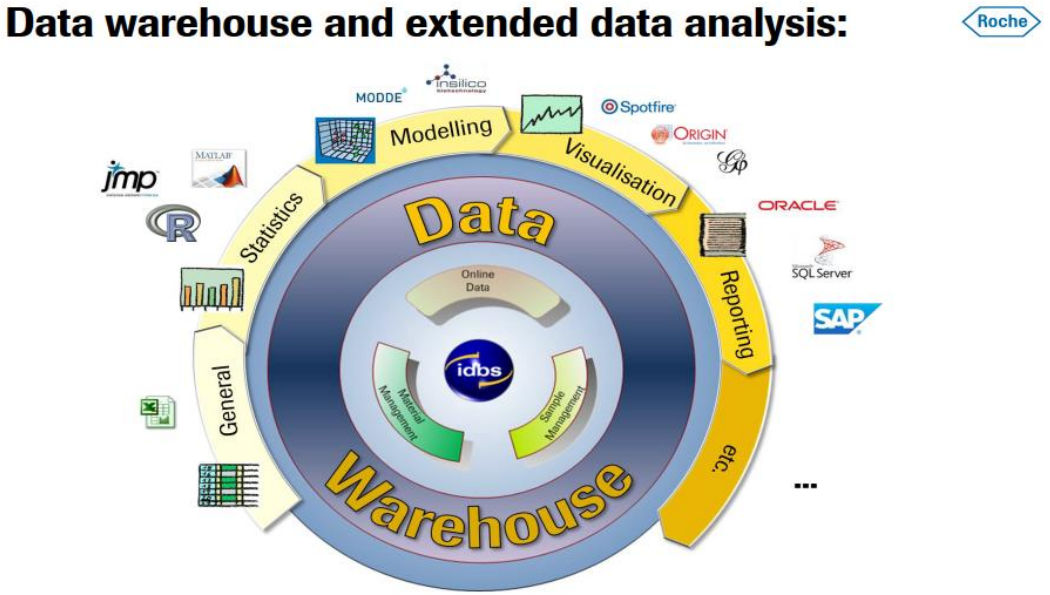
My beginning

When I started working at Roche, I had a goal - not to know the boundaries of my development.

Start was to undergo various types of training:

- introduction to Cell Culture
- onboarding and training process for industrial IT systems
- safety trainings
- use and meaning of devices, solutions, reagents, etc.





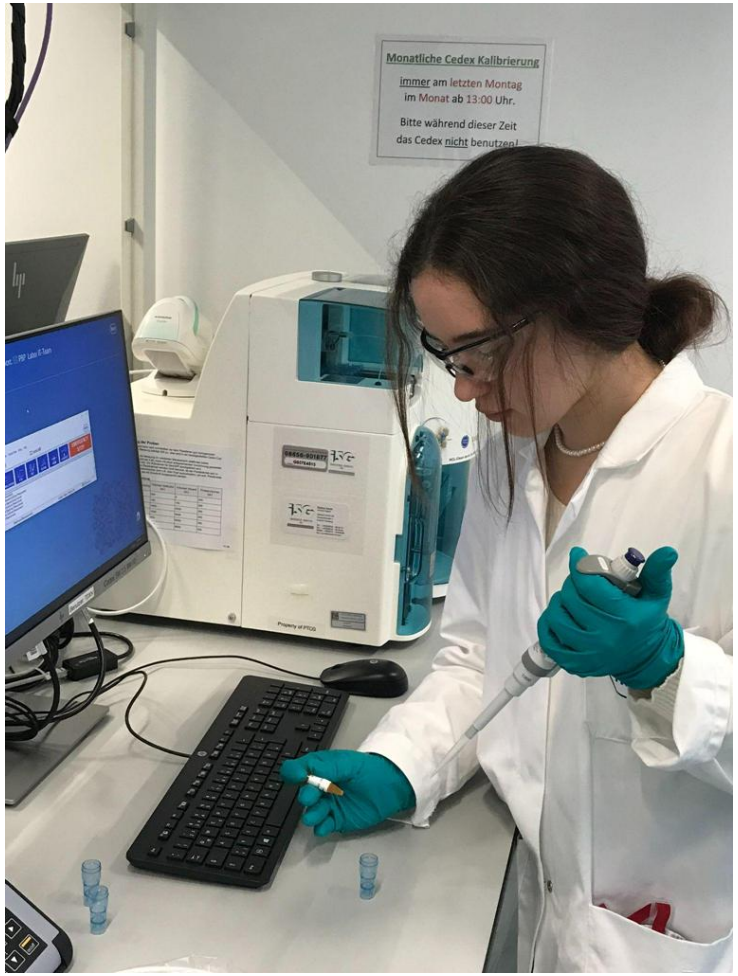
During my internship at Roche, namely in the Department of Gene Therapy, I learned the following points:

- use and application of industrial IT systems for experiment execution and data handling (like PI Osisoft™ and IDBS BioBook™)
- **preparation of media (we use the following ingredients to prepare the media, as they ensure the development and growth of human cells: (components contain powder media, growth enhancer and other components – all confidential)**
- calibration and use of pH – meter (I use it both for the preparation of the media, solutions, and for the determination of pH in the medium with cells);
- use and document data in electronic system from vendors like IDBS, Emerson and PI-Osisoft.

- transfection processes to transduce cells with plasmids to produce the viral vectors in bioreactors;
- harvest procedures for viral vectors
- setup and installation of the rocking motion single use bioreactors (to increase the volume of cells and good living conditions);
- installation of the stirred single use bioreactors for 100L and 500L;
- **cultivation of human cells and the needed cultivation parameters (biochemical and physical) in shake flasks in CO2-Incubators and bioreactors**
- turbidity measurement, ect.;

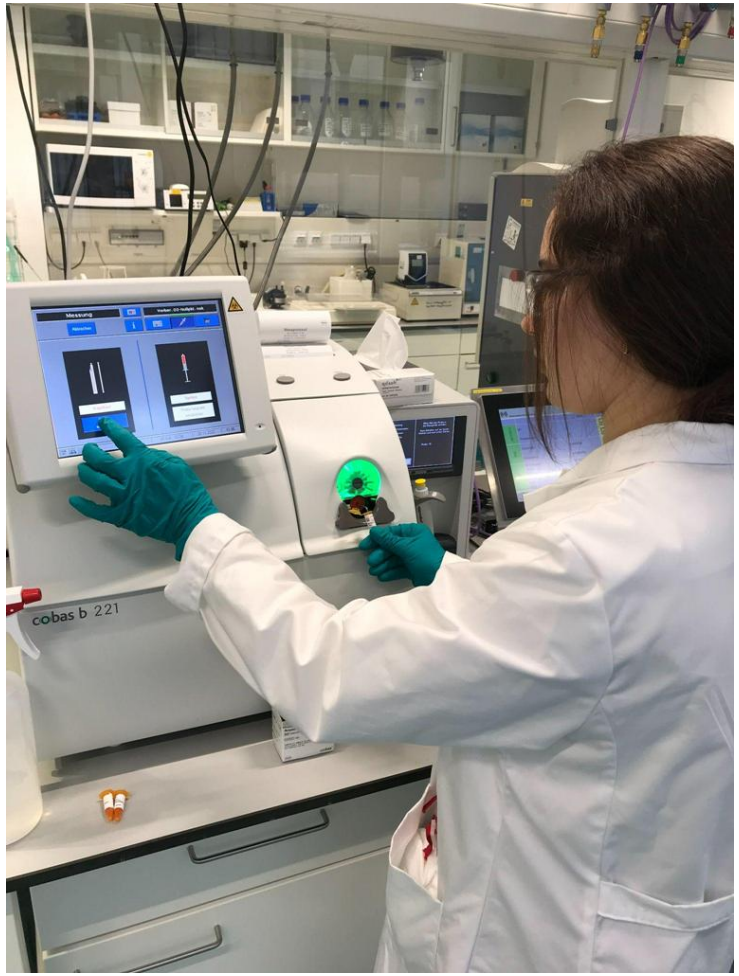
Measuring samples

Measure samples using blood gas analyzers, Osmometer and camera assisted automated cell counters (measurements are necessary because it is important to know cell viability and cultivation conditions)



camera assisted automated cell counters (Cedex)

Osmometer



blood gas analyzer (Cobas)

Storage expert

I‘m still in the process of studying the seed train and fully configuring the rocking motion single use bioreactors. I get a training to become expert for supply chain management, my duties include :

- planning of required raw materials and consumables for upcoming experiments based on my process know how (more than 50 components needed per experiment),
- checking once every two or three weeks the availability of materials & their quantity and
- communicate with vendors and initiate the ordering process

Doing now what patients need next