A PEPTIDKÉMIAI MUNKABIZOTTSÁGTÓL AZ INNOVATÍV MEDICINA KEZDEMÉNYEZÉSIG:

a peptidek szerepe a gyógyszerfejlesztésben

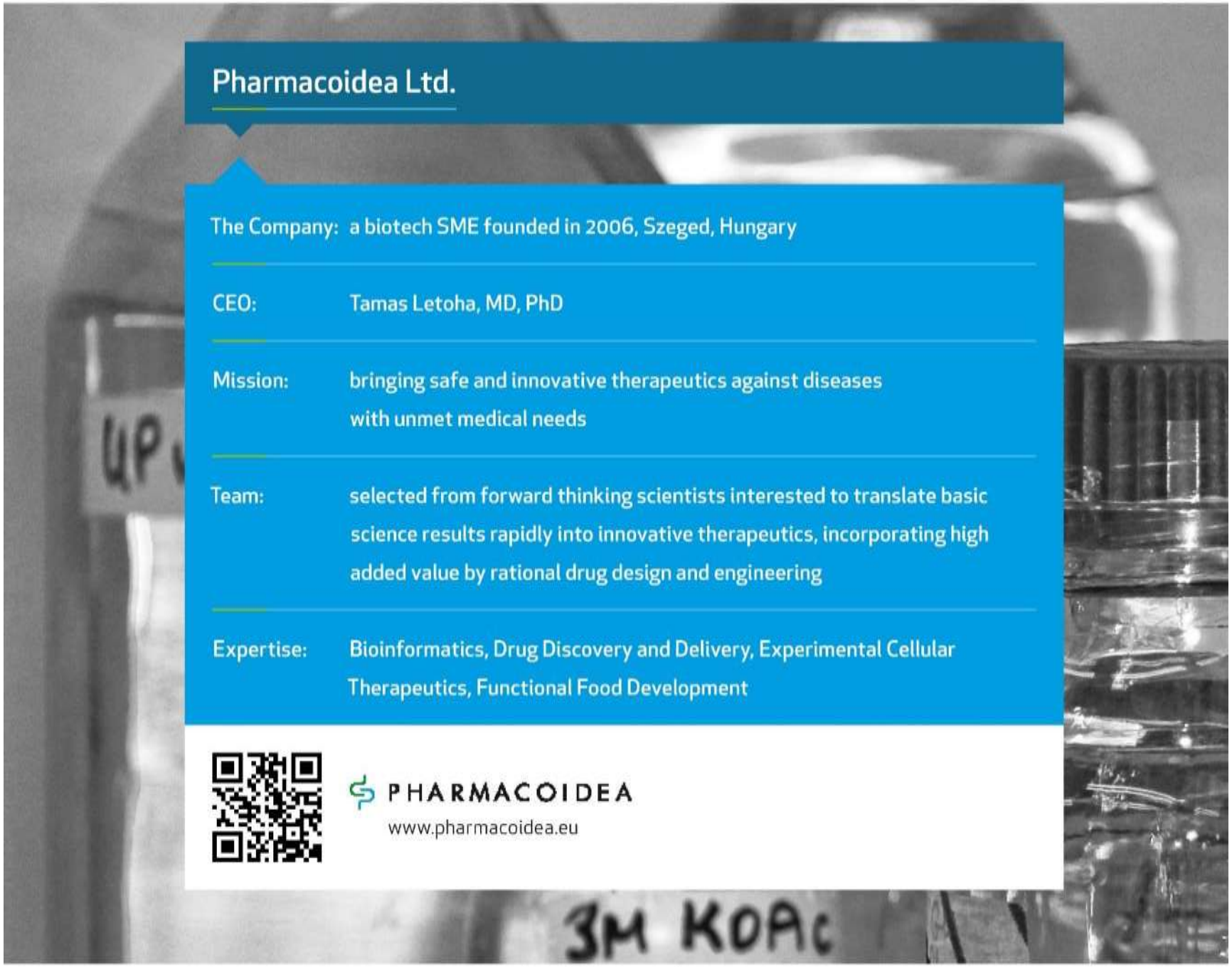
Dr. Letoha Tamás

2016.

COLLABORATION ON THE OPTIMISATION OF MACROMOLECULAR PHARMACEUTICAL ACCESS TO CELLULAR TARGETS

Innovative Medicines Initiative

A MAGYAR TUDOMÁNY ÜNNEPE



Pharmacoidea Ltd.

The Company:	a biotech SME founded in 2006, Szeged, Hungary
CEO:	Tamas Letoha, MD, PhD
Mission:	bringing safe and innovative therapeutics against diseases with unmet medical needs
Team:	selected from forward thinking scientists interested to translate basic science results rapidly into innovative therapeutics, incorporating high added value by rational drug design and engineering
Expertise:	Bioinformatics, Drug Discovery and Delivery, Experimental Cellular Therapeutics, Functional Food Development

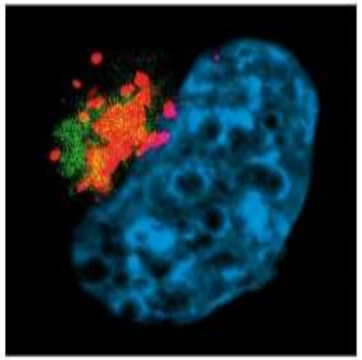
PHARMACOIDEA

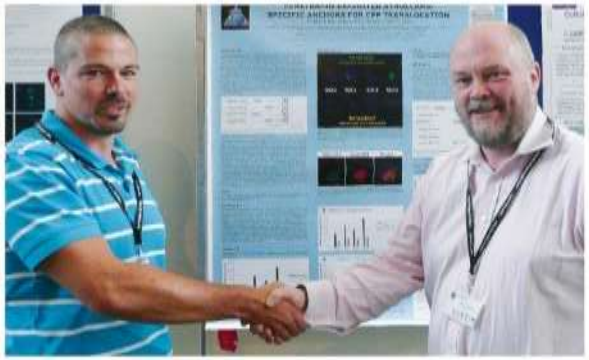
www.pharmacoidea.eu

Novel Drug Delivery Technologies

Intracellular Targeting of Molecules (PCT/IB2007/052787):

- A novel approach for the delivery of membrane impermeable drugs
- New drug target against viral infections
- 2008. June, Cardiff, UK – Cellular Delivery of Therapeutic Macromolecules, Drug Discovery Today Award
- 2009. September, Montpellier, France 3th Conference of Intracellular Delivery of Therapeutic Molecules: From Bench to Bedside, Award of the French Innovation and Transfer Office
- 2012. Innovative Medicines Innitiative: Pharmacoldea is part of the COMPACT Consortium in the Call of „Drug Delivery"

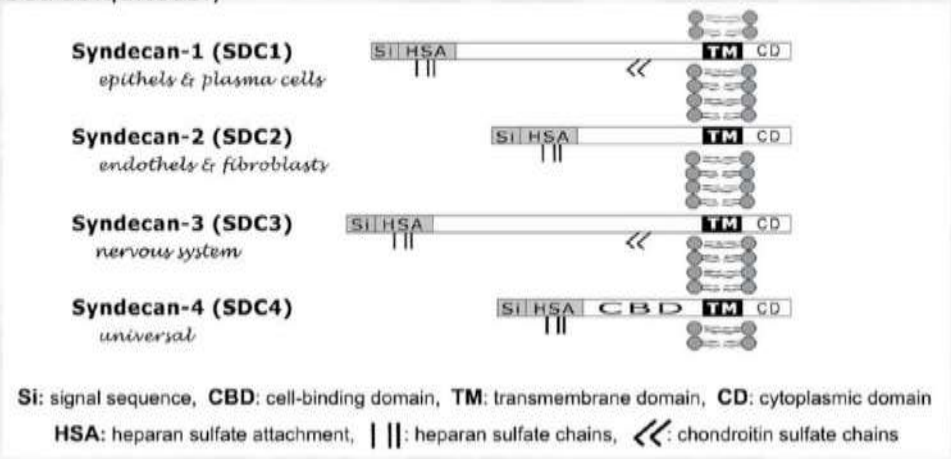




Drug delivery through syndecans

Syndecan (SDC) family of proteoglycans

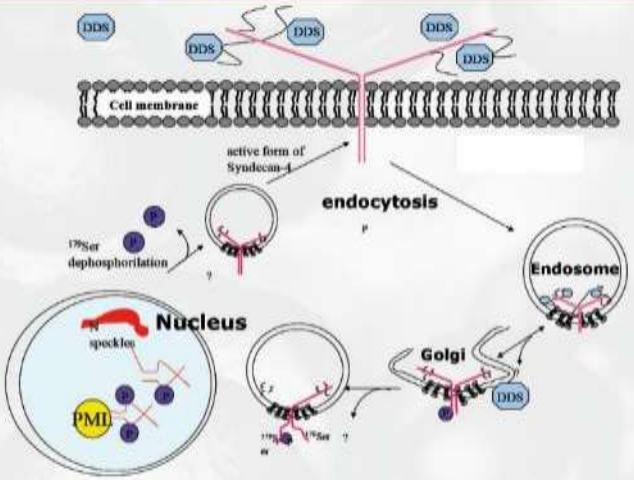
- Type I transmembrane proteins bearing heparan sulfate side chains chains on their extracellular domains
- Sharing a similar structure: a cytoplasmic domain, a highly conserved single-span transmembrane domain (TM), and a divergent extracellular domain with glycosaminoglycan (GAG) attachment sites for three to five HS or chondroitin sulfate (CS) chains
- Cell-, tissue-, and developmental stage-specific expression pattern: SDC1, 2 and 3 are most abundant in epithelial cells, fibroblasts, and neuronal tissues, respectively, while SDC4 is expressed ubiquitously



Si: signal sequence, **CBD**: cell-binding domain, **TM**: transmembrane domain, **CD**: cytoplasmic domain
HSA: heparan sulfate attachment, | ||: heparan sulfate chains, <<: chondroitin sulfate chains

Drug delivery through syndecans

Exploiting HSPG mediated drug delivery for COMPACT

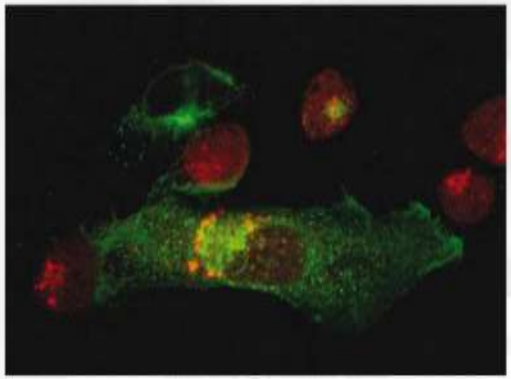


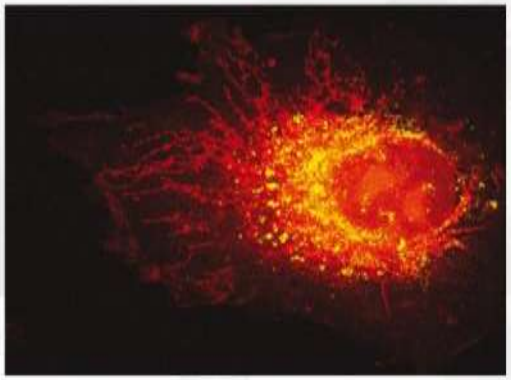
- Development of more rational and specific drug delivery methods or systems
- Development of short peptides, organic molecules to bind to HSPGs and stimulate endocytosis
- Study the retrograde transport
- Discover how delivered drug could be released from the cellular compartments to the cytoplasm.
- Reveal, which mechanism helps the HSPG-drug complex to enter the nucleus
- Figure out to which region of nuclei the HSPG-drug complex is guided

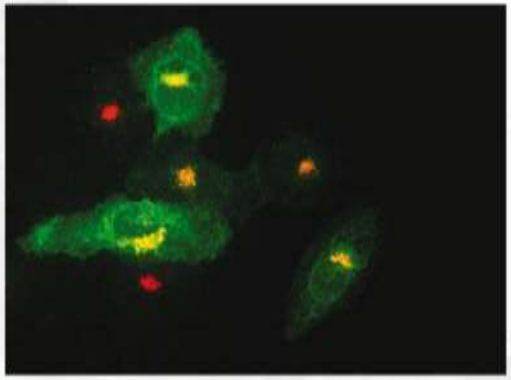
Drug delivery through syndecans

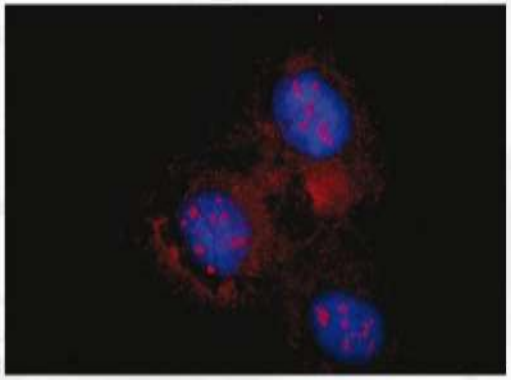
Endocytosis of syndecans

Ligand or antibody mediated clustering leads to redistribution of SDCs to membrane rafts and stimulation of a lipid raft-dependent, but clathrin-independent endocytosis of the SDC core protein.



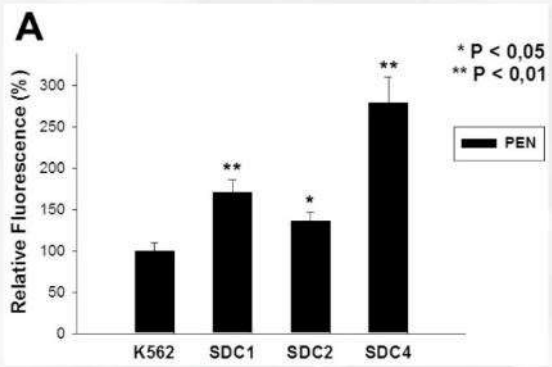


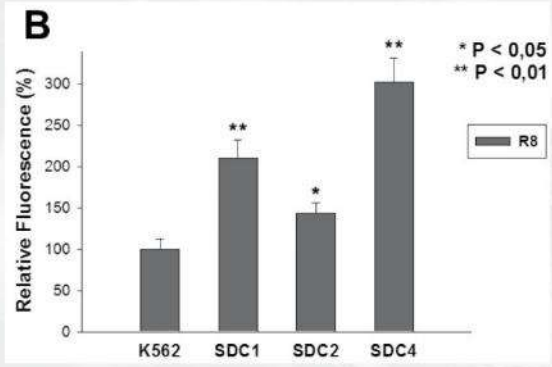


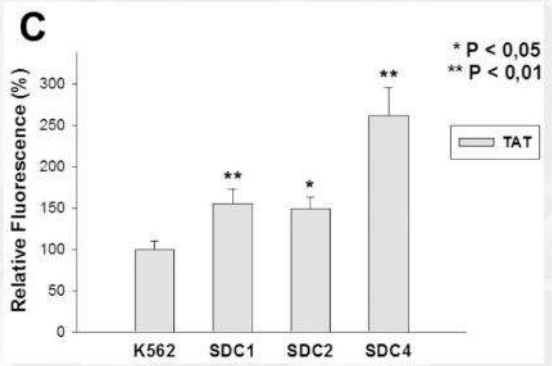


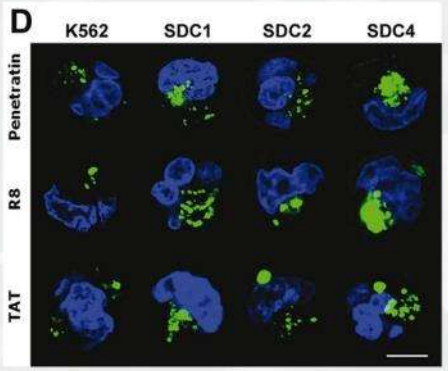
Drug delivery through syndecans

Contribution of syndecans to CPP uptake



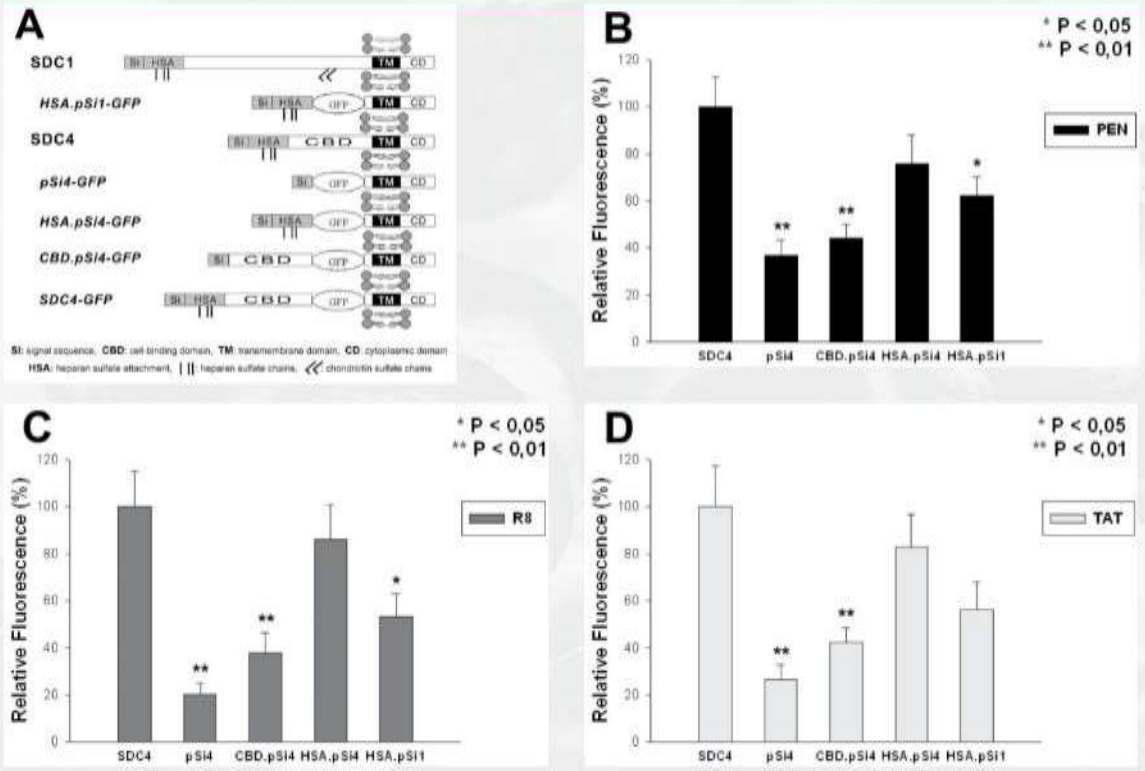






K562 cells and transfectants of human SDC1, SDC2 and SDC4 were incubated with the FITC-labeled CPPs (penetratin, R8 and TAT, respectively) at a concentration of 5 µM for 60 min at 37 °C. After 60 min of incubation, cellular uptake was analyzed with flow cytometry or confocal microscopy. (**A-C**) Flow cytometric analyses of CPP uptake. (**D**) Confocal microscopic visualization of CPP entry into SDC transfectants. Scale bar = 10 µM.

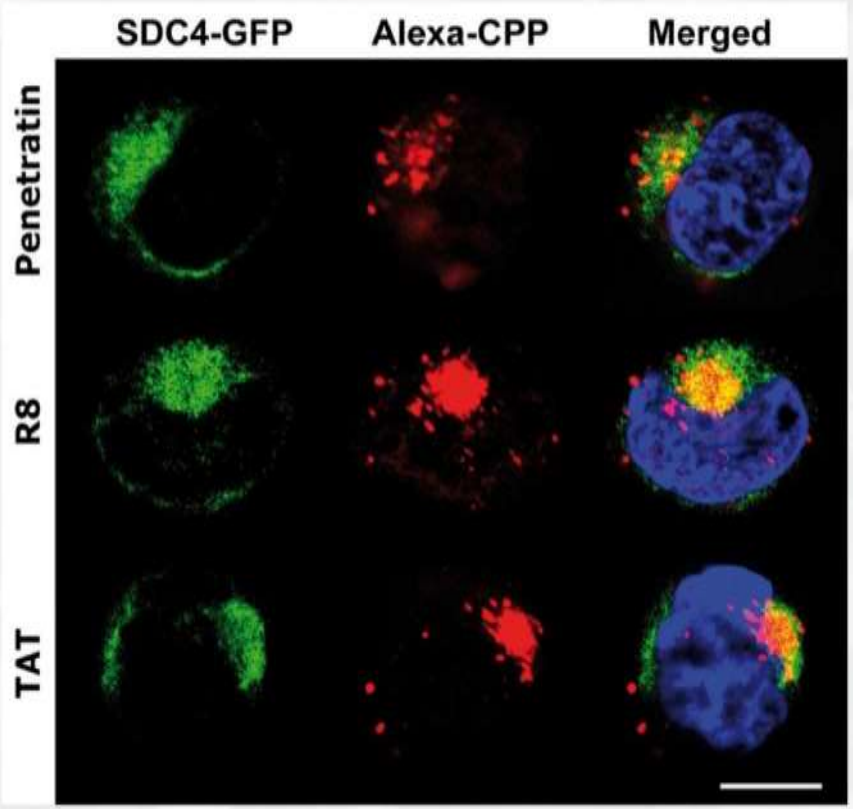
Mutational analysis of syndecan-mediated CPP uptake



(**A**) Schematic representation of SDC1 and SDC4 deletion mutants used in the study. (**B-D**) Results of flow cytometric measurements. Detected fluorescence intensities are normalized to Alexa555-CPP treated wild-type SDC4 transfectants as standards. The bars represent mean ± S.D. of six independent experiments. *p<0.05 vs wild-type SDC4 transfectants; **p<0.01 vs wild-type SDC4 transfectants.

Drug delivery through syndecans

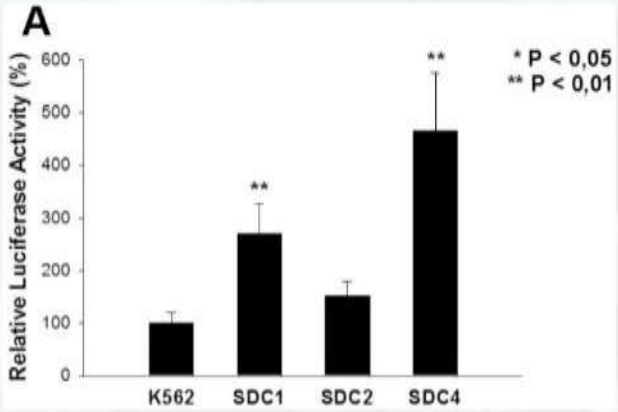
Intracellular colocalization of SDC4 and cationic CPPs

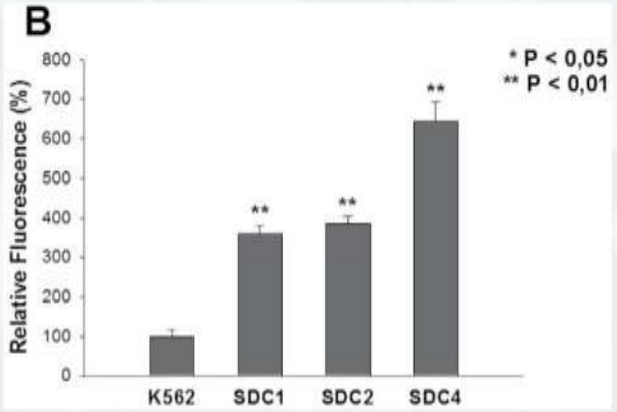


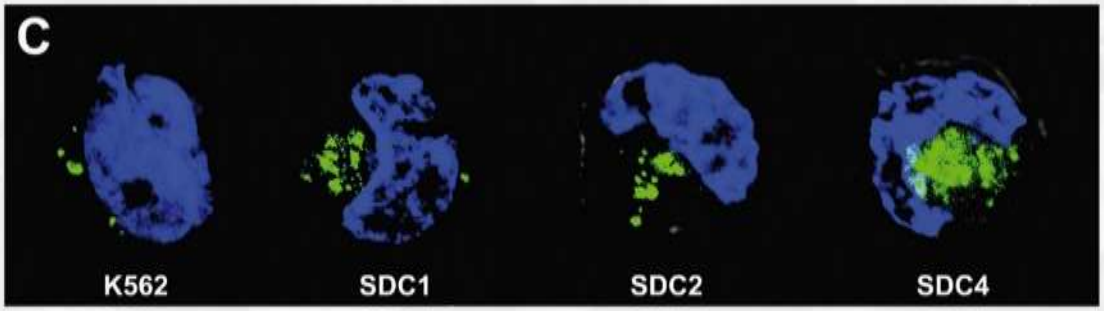
Transfectants expressing GFP-tagged SDC4 with the Alexa-labeled CPPs (penetratin, R8 and TAT, respectively) at a concentration of 5 μM for 60 min at 37 °C. After 60 min of incubation, cellular uptake was immediately analyzed with confocal microscopy. Scale bar = 10 μM.

Drug delivery through syndecans

Contribution of syndecans to lipoplex-mediated gene delivery



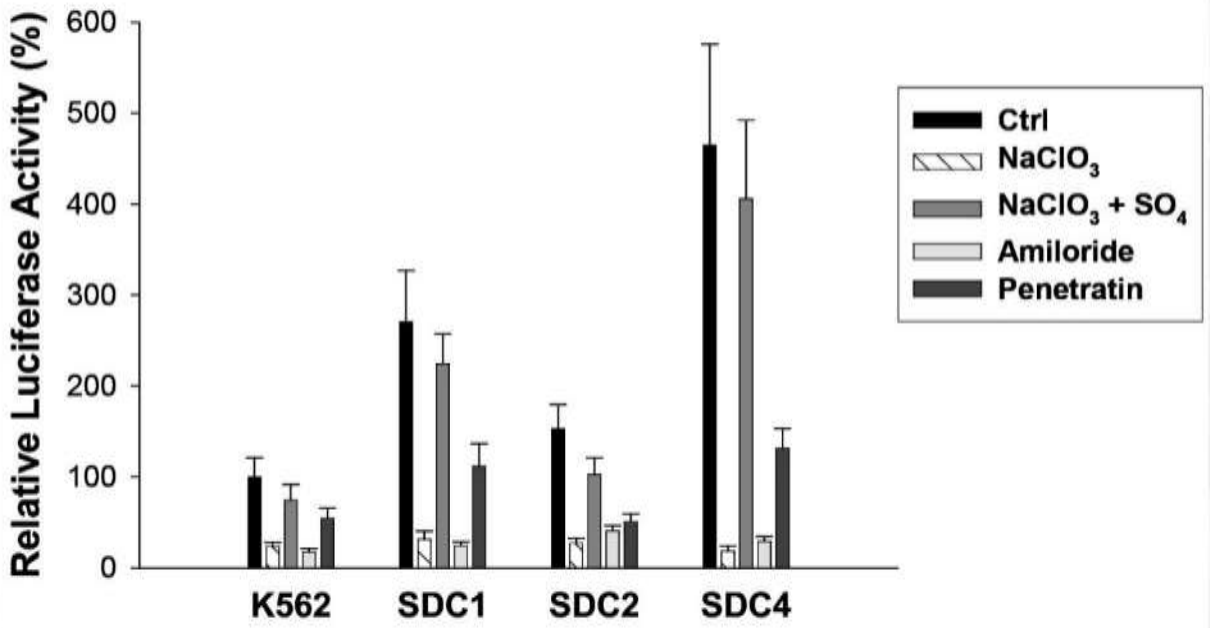




(**A**) K562 and SDC clones were transfected with pEGFP using DMRIE-C reagent. Forty-eight-hour post-transfection, the expression of EGFP in K562 cells and SDC transfectants was examined with flow cytometry. A minimum of 10,000 events per sample was analyzed. (**B**) Internalization of the fluorescently labeled (YOYO-1) plasmid DNA using DMREI-C into K562 cells and SDC transfectants visualized by confocal laser scanning microscopy.

Drug delivery through syndecans

Effect of endocytosis inhibitors on syndecan-mediated lipoplex uptake



Wild type and SDC overexpressing K562 cells and transfected with pGLuc-Basic plasmid using the DMRIE-C reagent. Forty-eight hours later, the activity of luciferase expression was quantified. Some cells were incubated with sodium azide (NaN3), others with sodium chlorate (NaClO3), Gö 6796, amiloride or the penetratin prior to transfection at 37 °C. Detected luciferase activities were normalized to lipoplex-treated wild-type K562 cells as standards. The bars represent mean ± S.D. of six independent experiments.

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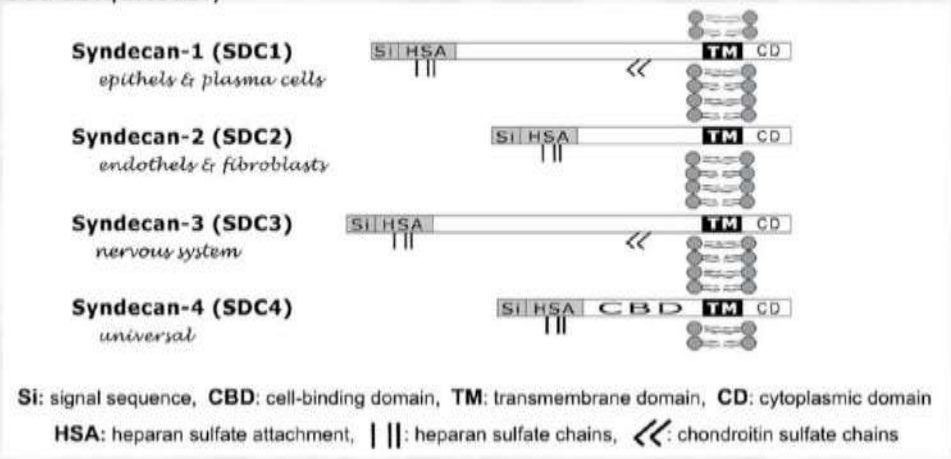

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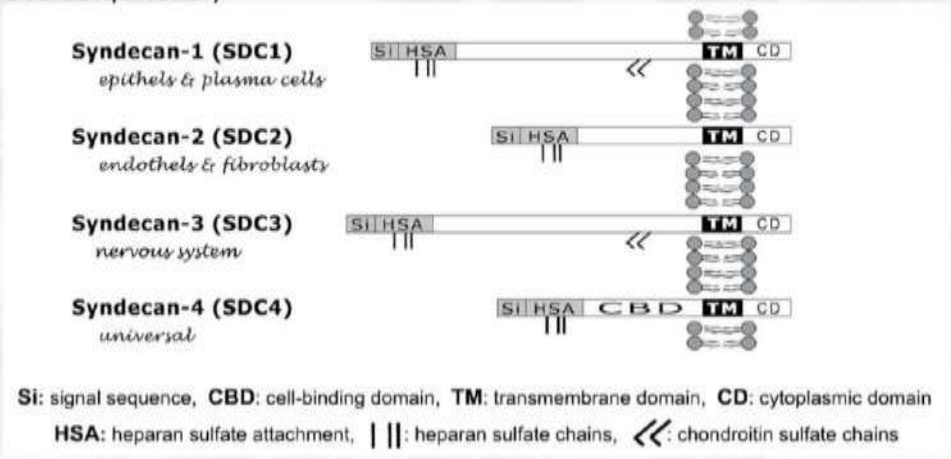

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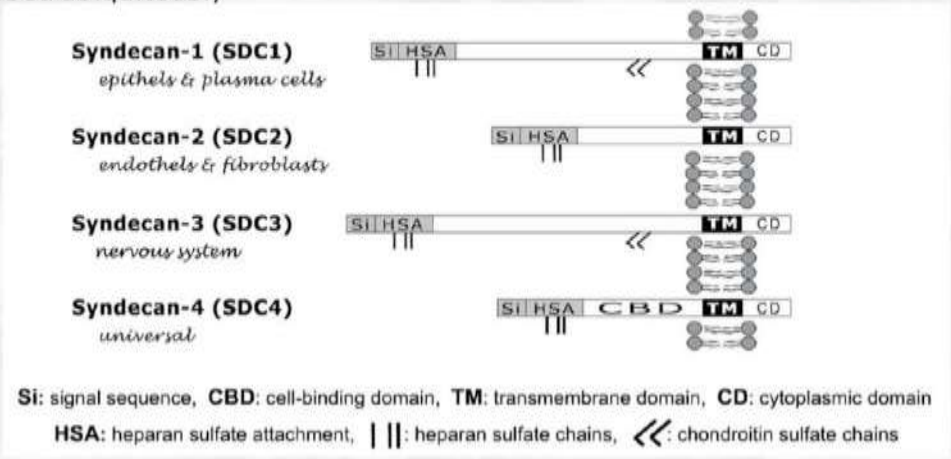

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A Public-Private Partnership to Develop Novel Delivery Systems for Biopharmaceuticals

Prof. Ekkehard Leberer (Sanofi)

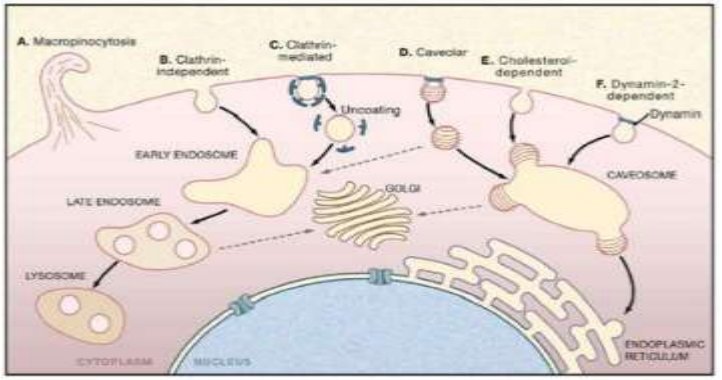
Dr. Enrico Mastrobattista (Univ. Utrecht)

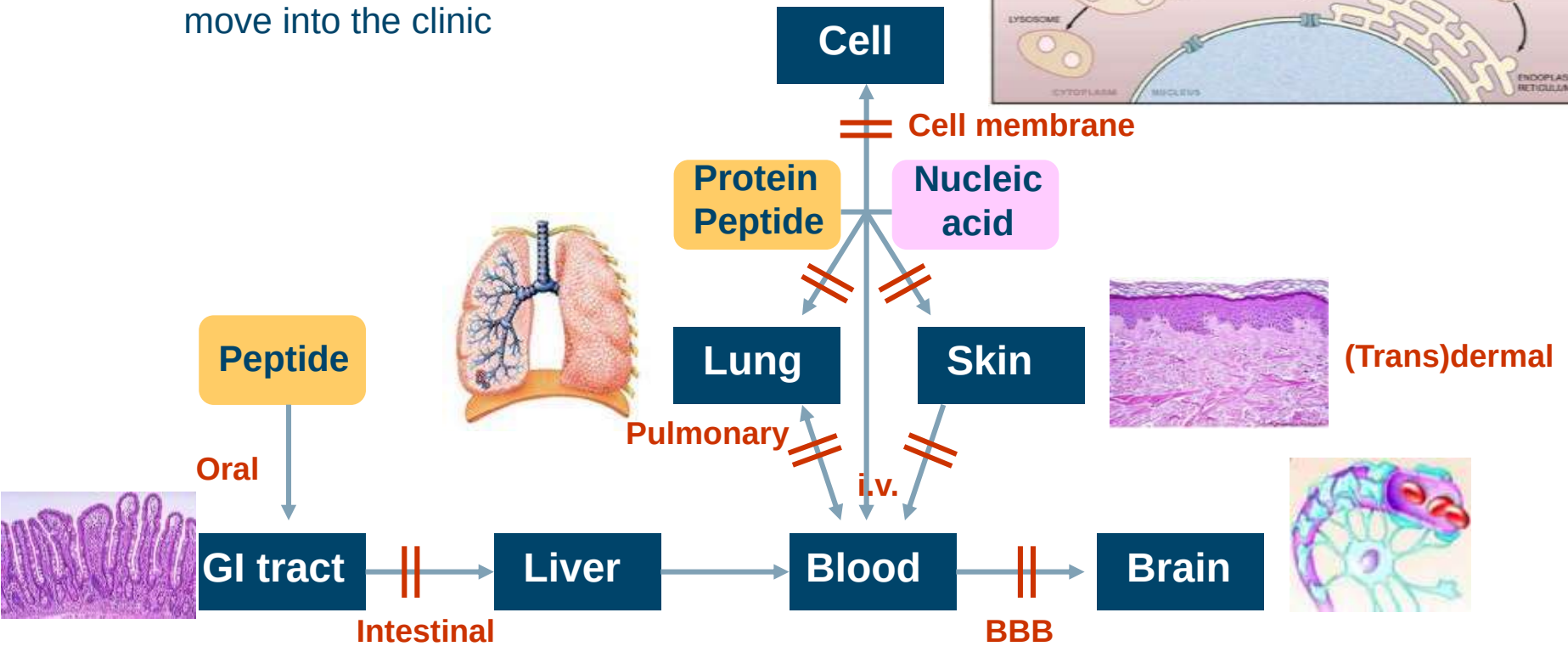
2012.11.01 – 2017.10.31

Goals

- Improve understanding of intracellular uptake and trafficking of biologics
- Develop nanocarriers to deliver biologics
  - To and across epithelial/endothelial barriers
    - Intestine; brain (BBB); lung; skin
  - Across cellular membranes into target cells
  - With drug like properties and the potential to move into the clinic

Marsh and Helenius, Cell 124, 729-40





Scope of modalities

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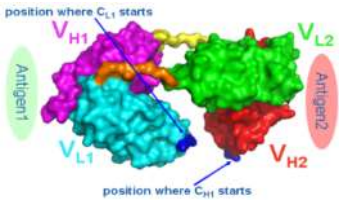
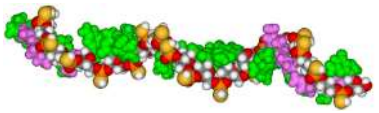
Proteins, peptides, oligonucleotides; size > 1 kDa



Antibodies
Antibody fragments

Scaffolds

Peptides



ASOs
siRNAs
miRNAs

Advantages

- Rational drug design instead of random high-throughput screening
- Opportunity ot access „non-druggable“ targets, e.g. transcription factors, protein-protein interaction
- Nucleic acid therapeutics
  - Tailored for their target sequences (ASOs, siRNAs, miRNAs)
  - Novel therapeutic modalities
    - anti-miRs, miR-mimics, long ncRNAs, mRNA replacement
  - miRNAs: Novel unexplored target space
    - Key regulators of cellular proliferation and differentiation
- Higher success rate than small molecule drugs

COMPACT strategy

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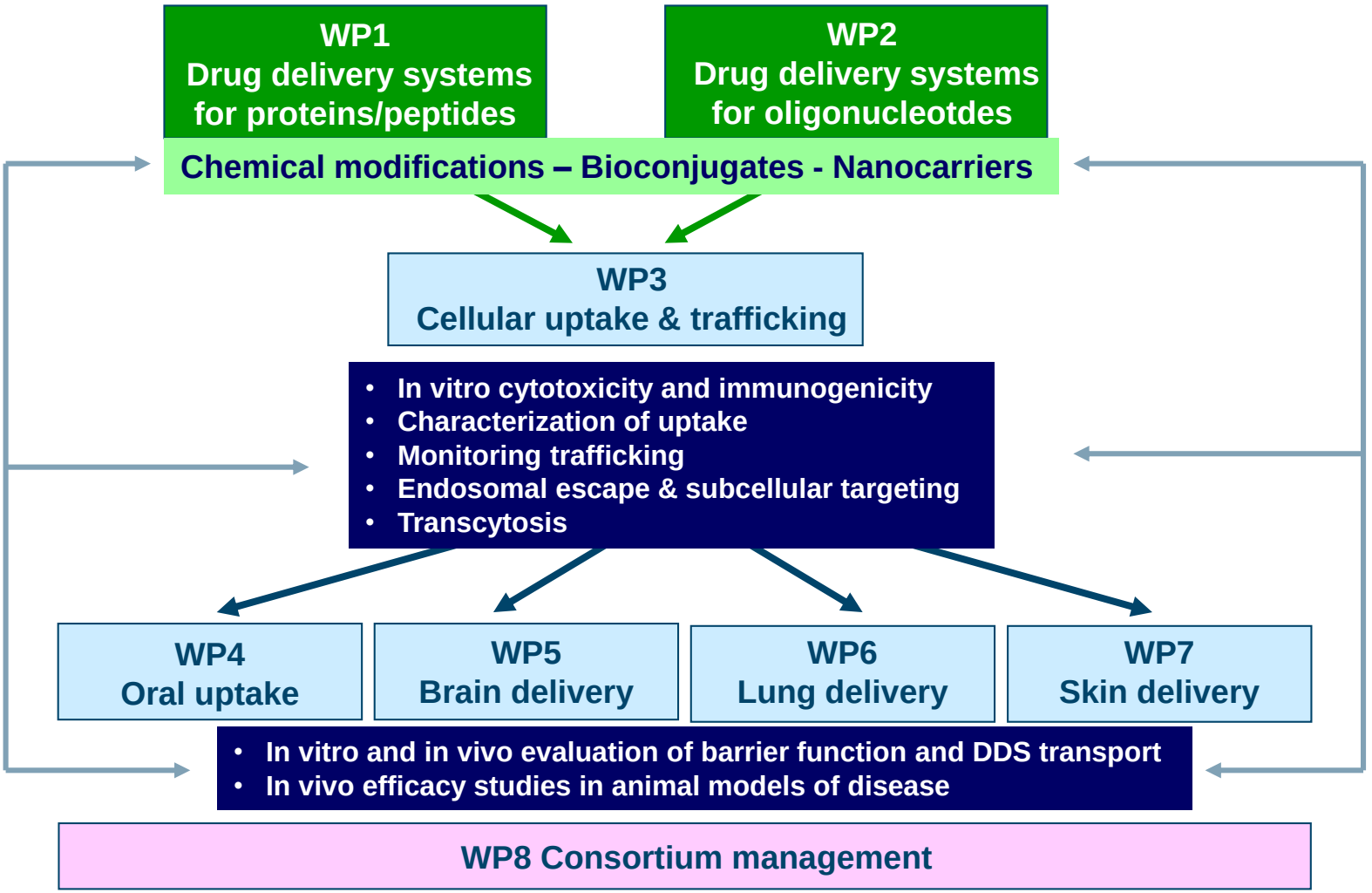
COLLABORATION ON THE OPTIMISATION OF MACROMOLECULAR PHARMACEUTICAL ACCESS TO CELLULAR TARGETS

Work package matrix structure with iterative approach of nanocarrier generation and testing



COMPACT is in line with major trends in pharma industry

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COLLABORATION ON THE OPTIMISATION OF MACROMOLECULAR PHARMACEUTICAL ACCESS TO CELLULAR TARGETS

- **R&D expenditure shift towards external innovation**
  - Example Sanofi
    - Objective is ratio internal/external 50/50

    (Chris Viehbacher, CEO, Xconomy Jan. 17, 2012)

- **R&D portfolio continue to shift from „small molecules“ towards „biologics“**
  - Top ten drugs by sale
    - 2001: 1 biologics: Procrit
    - 2010: 3 biologics: Enbrel, Remicade, and Humira
    - 2012: 7 biologics: Humira, Enbrel, Remicade, Rituxan, Lantus, Herceptin, Avastin
      - Humira (AbbVie) was the 2nd best selling drug world wide with sales of 8.5 B $
      - Lantus (Sanofi) was the 8th best selling drug world wide with sales of 6.6 B $1

Ref.: Arrowsmith J., Nat. Rev. Drug Disc. 11, 17 – 18, 2012; www.fiercepharma.com/special-reports/15-best-selling-drugs-2012; ACS Chem. Neurosci. 4, 905-907, 2013

Funding and team

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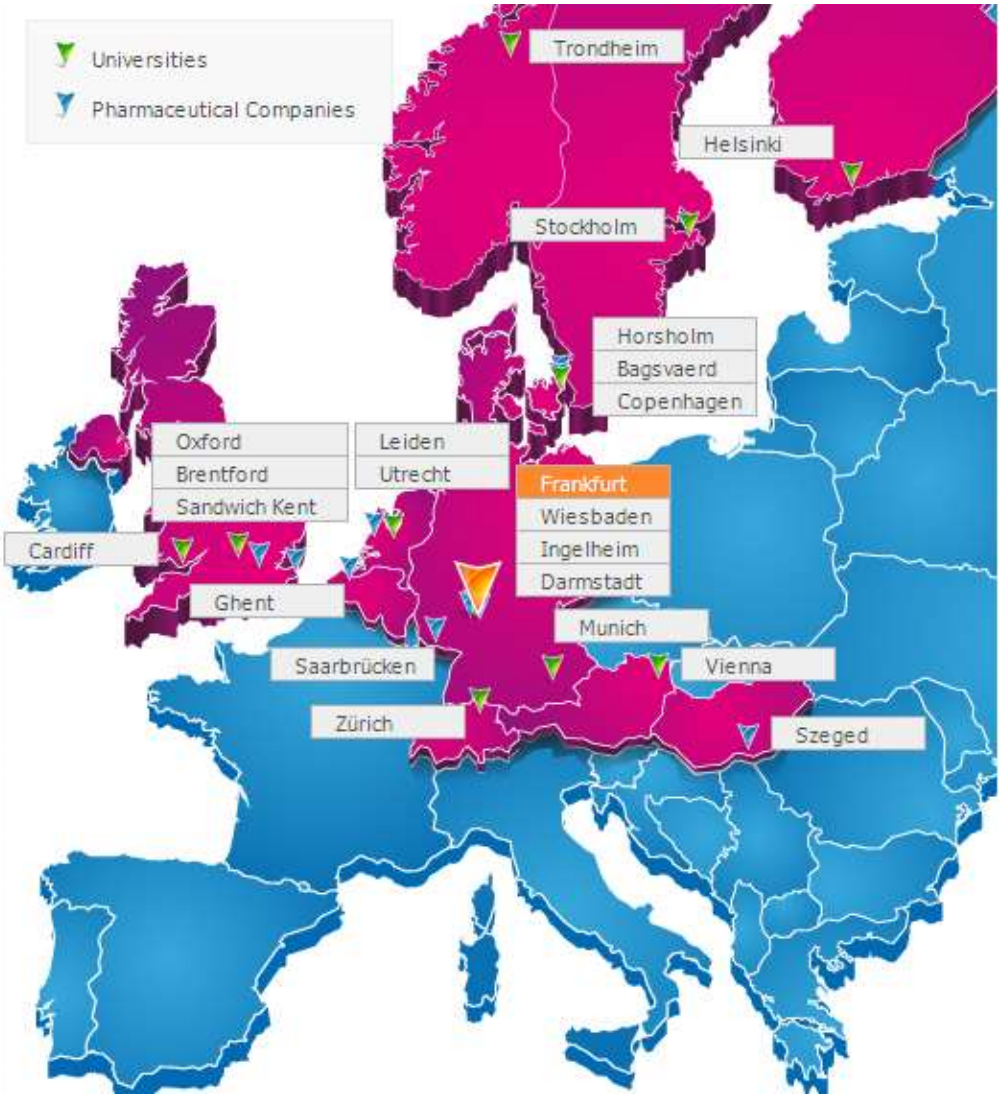
COLLABORATION ON THE OPTIMISATION OF MACROMOLECULAR PHARMACEUTICAL ACCESS TO CELLULAR TARGETS



- Term Nov 2012 – Oct 2017
- Total budget: 30 M€
  - IMI funding: 13.5 M€ (incl. 25% in kind from acad./biotech)
  - EFPIA in kind contribution: 16.5 M€
- Team
  - Currently 132 scientists
    - 82 academia/biotech
      - 35 IMI funded:
        - 9 Post Docs
        - 19 PhD students
        - 7 technicians
    - 50 EFPIA
  - Plus administrative, legal and financial support staff
  - Distributed across 12 countries

Unique opportunity to work with industry and academia

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COLLABORATION ON THE OPTIMISATION OF MACROMOLECULAR PHARMACEUTICAL ACCESS TO CELLULAR TARGETS

- **7 EFPIA partners**

SANOFI · gsk · abbvie · Boehringer Ingelheim · Merck Serono (Living science, transforming lives) · novo nordisk · Pfizer

- **14 Academic plus 2 Biotech partners**
  - Utrecht University Dept. Pharmaceutics
  - University of Copenhagen
  - Helmholtz Inst. for Pharmaceutical Research Saarland
  - Cardiff University
  - Stockholm University
  - Norwegian University of Science and Technology
  - University of Vienna
  - LMU Munich–Dept. of Pharmacy
  - University of Zurich
  - University of Ghent
  - PharmacoIdea Ltd. (Hungary)
  - BioneerPharma (Copenhagen)
  - Utrecht University Dpt.Infectious Diseases and Immunology
  - University of Helsinki
  - Leiden University
  - Oxford University

COMPACT expertise

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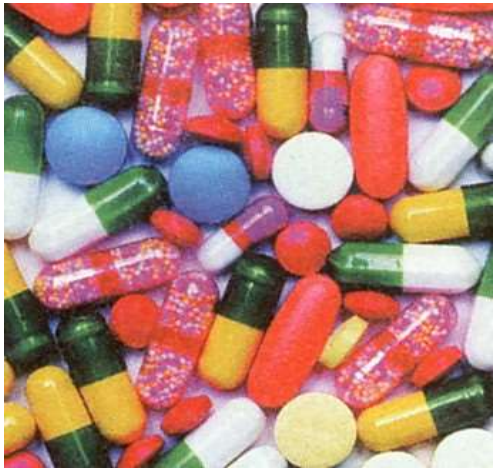
COLLABORATION ON THE OPTIMISATION OF MACROMOLECULAR PHARMACEUTICAL ACCESS TO CELLULAR TARGETS

Acedemic/biotech partners

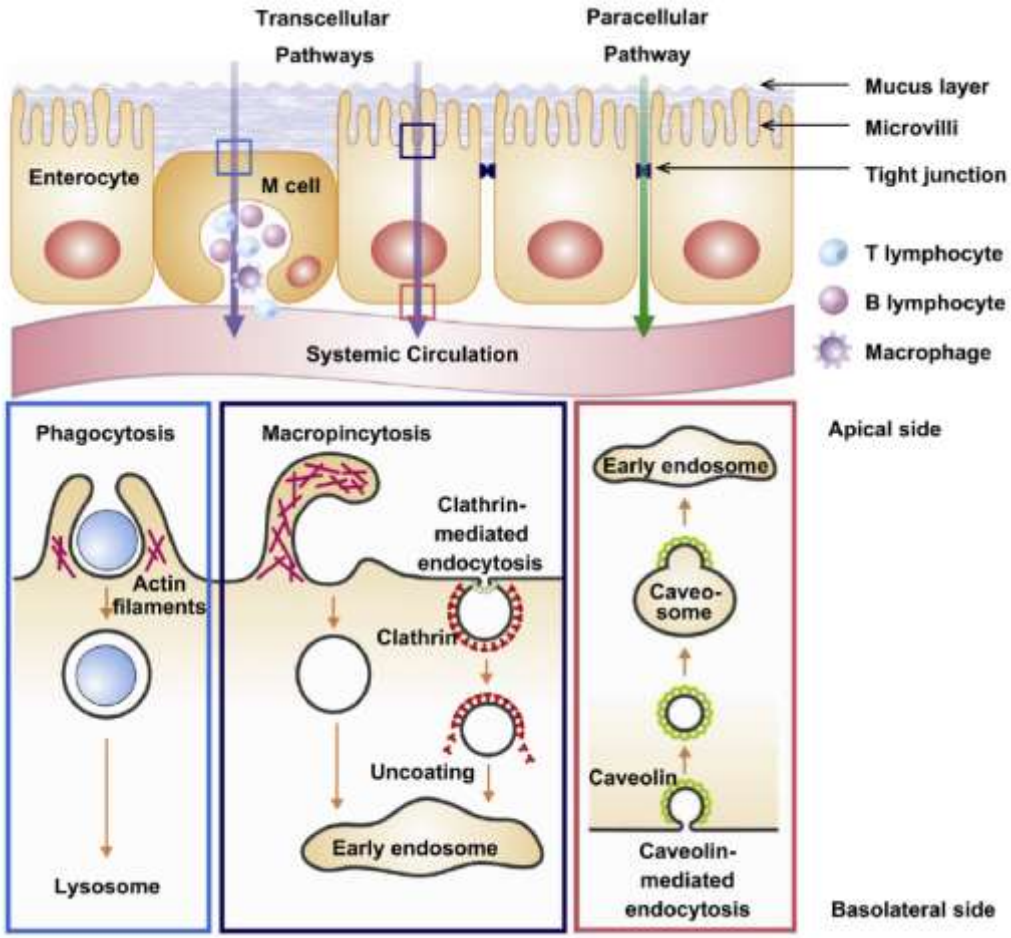
- Nanotechnology
- Biochemistry
- Molecular biology
- Cell biology
- Animal biology
- Imaging
  - *In vitro*
  - *In vivo*
- Biodistribution
- Cytotoxicity
- Immunology
- Data mining

Pharma partners

- Disease models and pharmacology
- PK
- Toxicology
- CMC
- Clinical research; Regulatory



Summary



COMPACT has initiated to establish a technology platform to

- Generate novel nanocarrier-based drug delivery systems (DDS‘s) with model payloads
- Analyze their cellular uptake and trafficking
- Follow their delivery across epithelial and endothelial biological barriers, including the intestinal barrier
- Analyze their biodistribution and pharmacokinetic properties
- Analyze the pharmacology of model payloads

The COMPACT Team

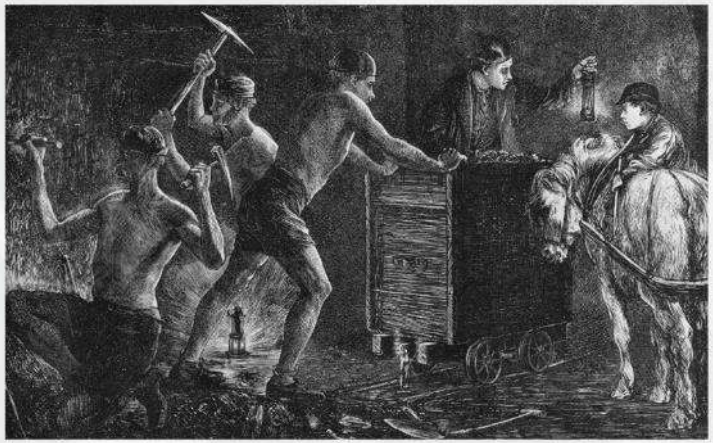
COMPACT
COLLABORATION ON THE OPTIMISATION OF MACROMOLECULAR PHARMACEUTICAL ACCESS TO CELLULAR TARGETS

www.compact-research.org

Contribution to IMI COMPACT

WPs1 & 2: Text-mining database to facilitate DDS design and optimization

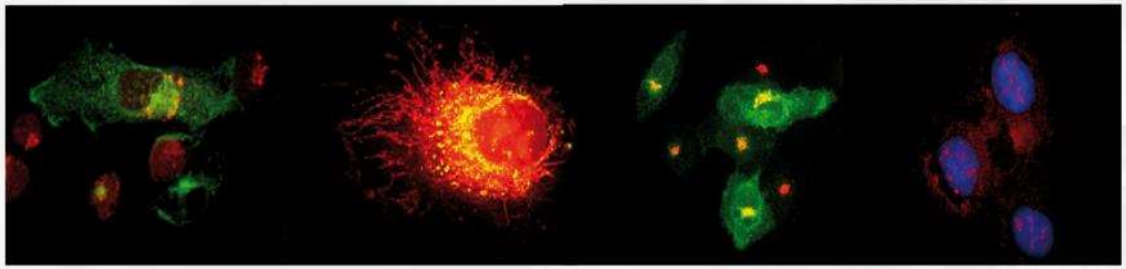
- Tasks 1.2 & 2.2 (M12), 44 FTE/WP
- Conducting searches of related scientific literature, including patents and publications to assist current research and development (search criteria should be defined!!!)
- The goal of is to extract knowledge from the DDS scientific domain in a right information structure that involves database building, data management and online updating



- Data mining is performed by highly qualified chemists and biologists using a read and extract method utilizing a predetermined taxonomy
- Database can be used by each participant of COMPACT

Contribution to IMI COMPACT

Studying cellular uptake and trafficking (WP3)

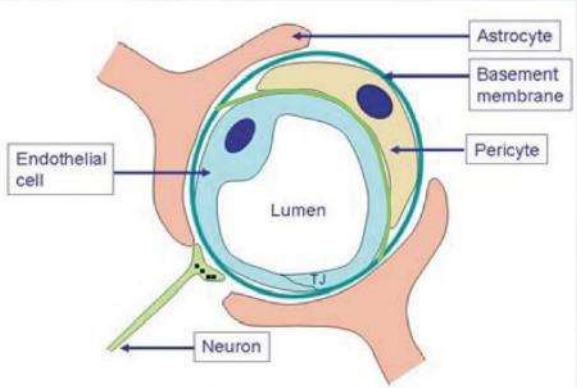


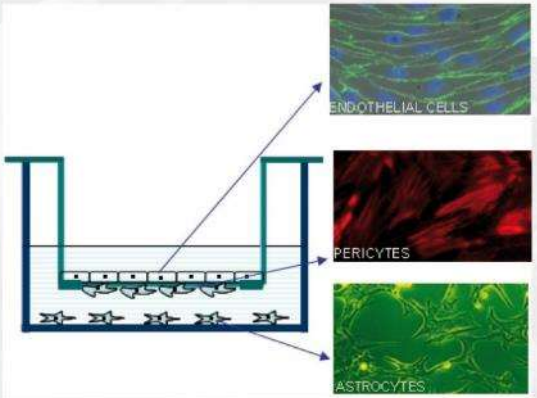
- 120 FTE , tasks include:
  - Establish methods to characterise cell binding and uptake
  - Establish methods to track payloads around the cell
  - Establish methods to evaluate Transcytosis
  - Establish whole cell reporter assays for payload delivery
  - Establish in vitro immunogenicity assay
  - Provide high capacity/ low content screens to support WP1&2 (uptake, cytotox, immunogenicity)
  - Provide Low capacity/ high content evaluations to support WP1&2 (routes, sorting, endosomal escape)

Contribution to IMI COMPACT

Delivery across the Blood Brain Barrier (WP5)

- In vitro and in vivo studies for brain delivery (32 FTE)

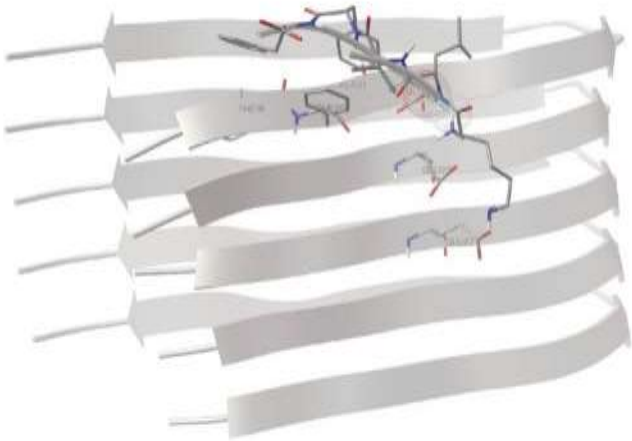


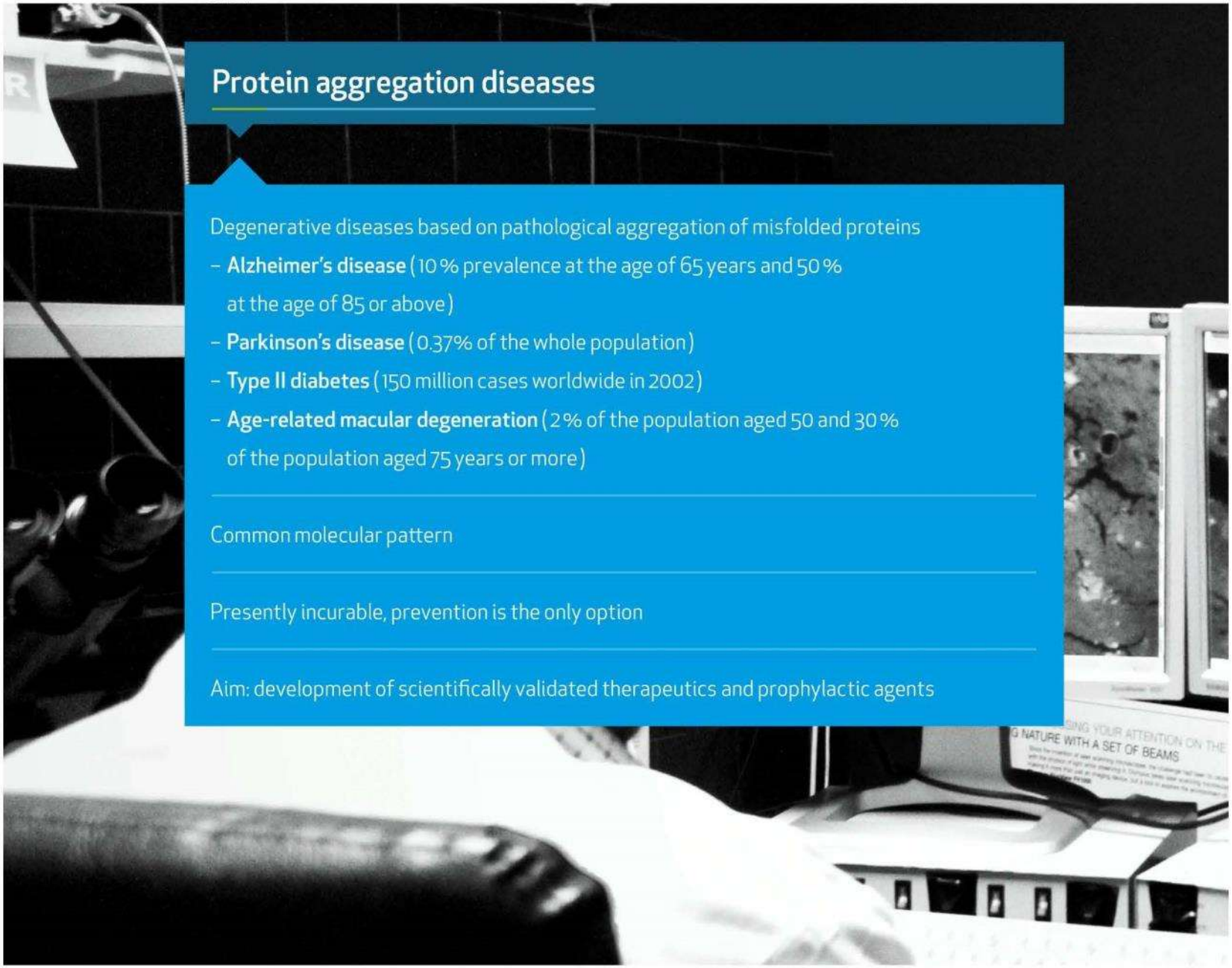


Bioinformatics

Accelerating the rate of discovery & reducing expensive lab work

- High quality, cost effective curation, literature informatics solutions and annotation
- Comprehensive database of protein-protein and protein-small molecule interactions
- Highly complex data content
- Kinetic parameters, pharmacokinetics and pharmacodynamics values, dose response details
- Simple search window to search biomedical entities
- Author and journal based filters
- In silico screening, QSAR algorithms





Protein aggregation diseases

Degenerative diseases based on pathological aggregation of misfolded proteins

- **Alzheimer's disease** (10 % prevalence at the age of 65 years and 50 % at the age of 85 or above)
- **Parkinson's disease** (0.37% of the whole population)
- **Type II diabetes** (150 million cases worldwide in 2002)
- **Age-related macular degeneration** (2 % of the population aged 50 and 30 % of the population aged 75 years or more)

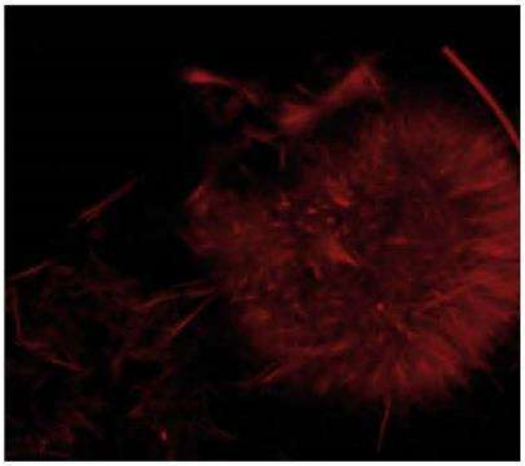
Common molecular pattern

Presently incurable, prevention is the only option

Aim: development of scientifically validated therapeutics and prophylactic agents

Protein aggregates (plaques)

β-amyloid (Alzheimer)
α-synuclein (Parkinson)
amylin (Type II Diabetes)
drusen (AMD)

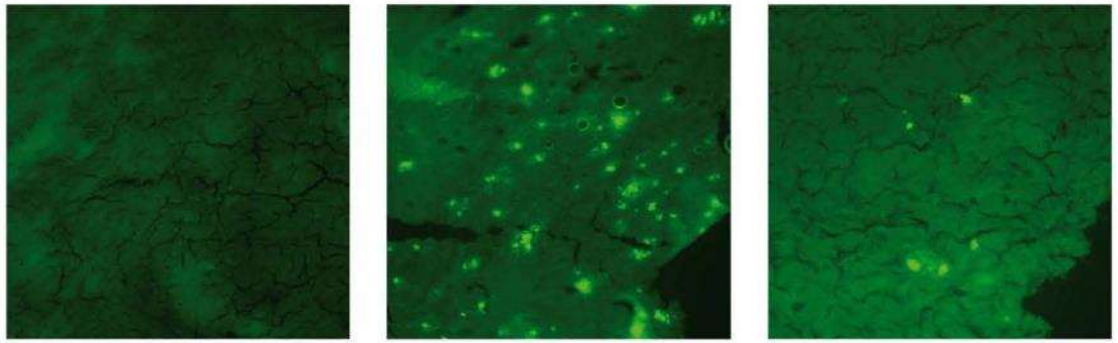


Plaque Busters

High-throughput bioassays + transgenic animal models (APPswe)
+ in silico modeling and docking algorithms + structure-activity relationships

A molecular library of new chemical entities

like thorough molecular lancets, plaque busters can specifically recognize and eliminate pathological protein aggregates/plaques (see figure below)



Normal brain

Alzheimer's brain

Alzheimer's brain
+ Plaque Busters

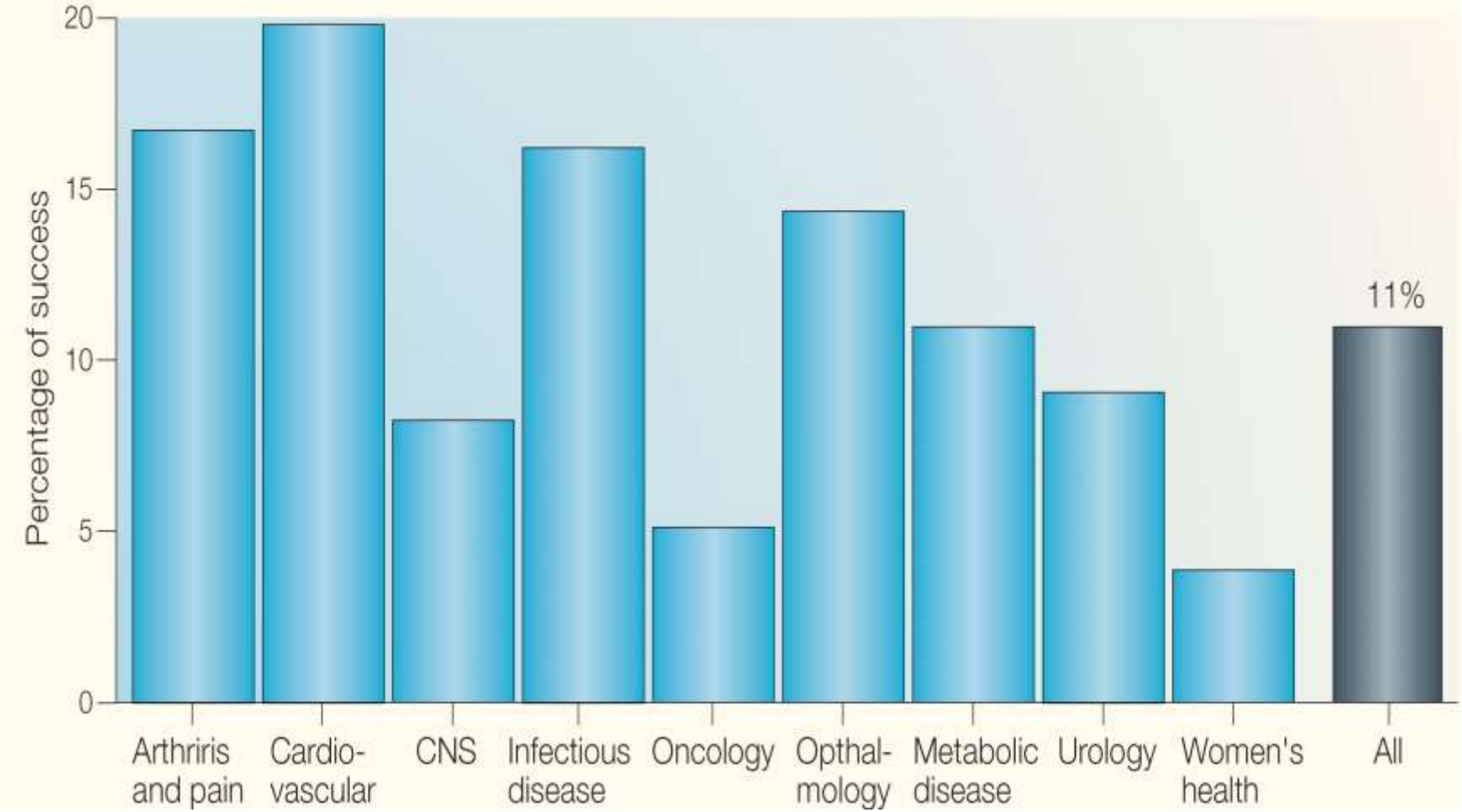
Overview on Concept and Goals

AETIONOMY

Prof. Martin Hofmann-Apitius
(Fraunhofer SCAI)

Prof. Duncan McHale
(UCB Pharma)

The Challenge



Reasons for Failure

- **Wrong target**
- **Lack of pharmacological effect in the target organ at achievable dose**
- **Lack of sufficient effect from single mechanism**
- **Disease heterogeneity**
- **Phenotypic classification**

Current Classification

- Refined version of 200 year old classification
- Based on phenotypic features of disease
- Does not necessarily reflect underlying disease mechanisms
- Where classification has become more mechanistic then new effective therapies have been developed
- Oncology is a good example

"I'm pretty sure you've got what this guy had."

What About Alzheimer's & Parkinson's?

- Archetypal phenotypic diseases
- Multiple rare families with different causative genes
- For PD there are environmental mimics
- Differing disease courses
- No disease modification therapies
- If we do not start early with the right treatment it will be too late
- If we do not have confidence in the biological rational for treatment then companies will not continue to invest
- Multiple failures in anti amyloid approaches
- There is a lot of data available in the literature and in databases we can access

AETIONOMY Project Vision

- To lay the ground for new approaches in translational research in AD and PD by paving the way for model-driven research in these indication areas.

- To establish the AETIONOMY approach as a “blueprint” for disease-specific data-, information-, knowledge- and model-integration.

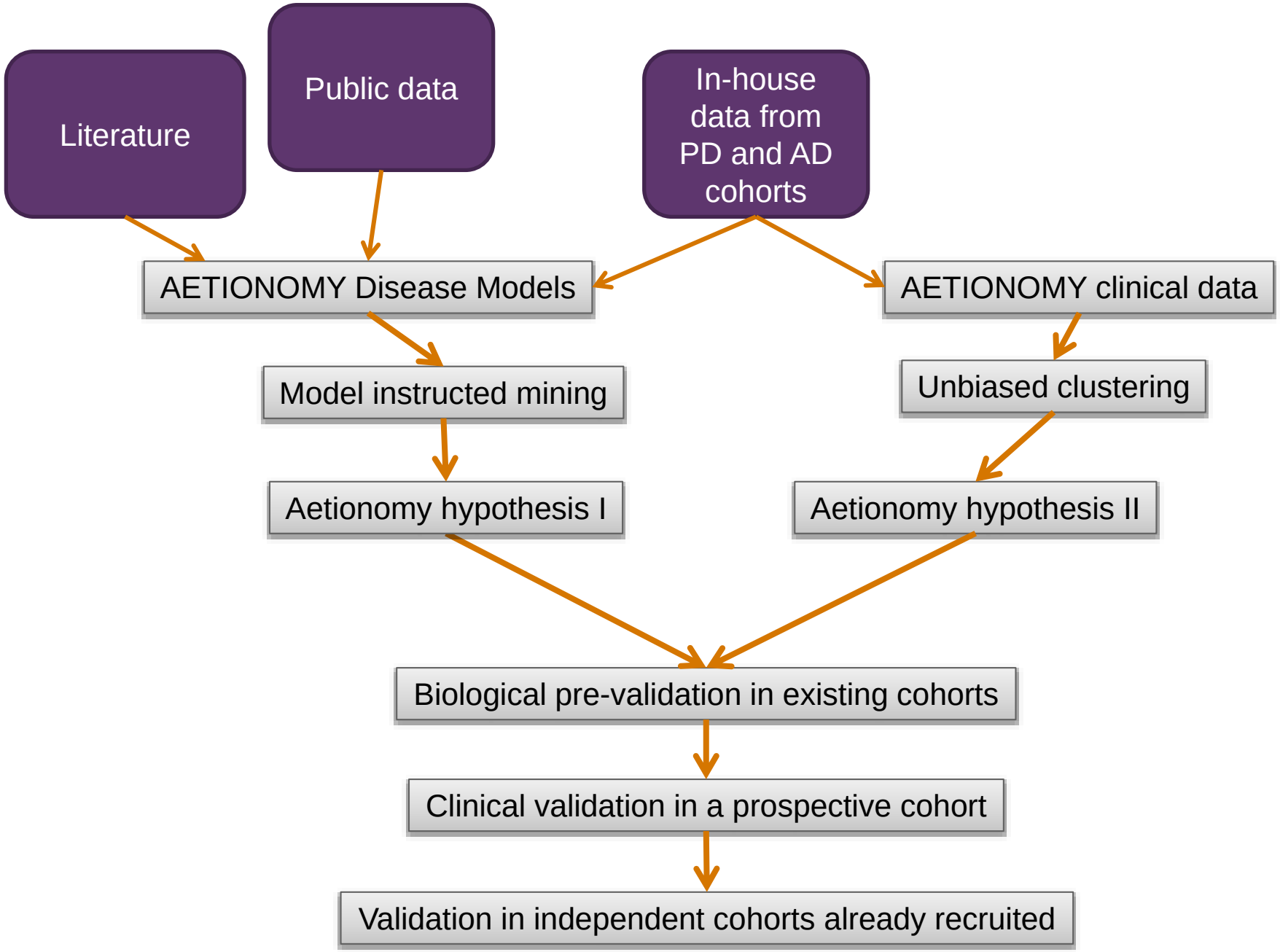
- To model disease in a way that allows for the generation of testable hypotheses on possible mechanisms underlying PD and AD.

Key Figures

- We have 5 years to demonstrate that our concept is valid
- We have a total budget of 17.8 Mio € to support the work
- In AETIONOMY, quite heterogeneous expertise comes together to address a scientific challenge that has not been solved so far by hundreds of researchers with hundreds of millions of € or $
- The expertise is distributed over 9 academic, 4 industry and 2 SME partners. 2 partners representing patient interest groups. These 17 partners will collaborate over 5 years to achieve the major goal of AETIONOMY: the development of a taxonomy of disease based on disease mechanisms and the validation of this taxonomy in the course of clinical studies.

New Approaches in Translational Research

- AETIONOMY brings together scientists who do not necessarily work together at a routine basis
- Collaboration in such a multi-disciplinary team requires a culture of listening and mutual respect
- AETIONOMY tries to take a new approach towards AD and PD. We are not going to continue with “what we did anyway”, but rather will “walk the talk” and fill the new paradigm underlying our concept with life
- AETIONOMY has the potential to turn into a lighthouse project for future AD and PD research. Already now, before it even started, the project has substantial visibility (e.g. G8 summit). We need to work closely together in order to deliver to the promise.


Literature
Public data
In-house data from PD and AD cohorts
AETIONOMY Disease Models
AETIONOMY clinical data
Model instructed mining
Unbiased clustering
Aetionomy hypothesis I
Aetionomy hypothesis II
Biological pre-validation in existing cohorts
Clinical validation in a prospective cohort
Validation in independent cohorts already recruited

Expected Impact On The R&D Process

Increased homogeneity of disease will:

- Decrease trial sizes
- Improve benefit risk profiles of drugs
- Increase speed to patients

Improved understanding of the disease

- Ensures patients can access the right treatments for them regardless of phenotypic presentation
- Increased confidence in target

Reduce drug discovery costs by impacting attrition

The AETIONOMY Team

www.aetionomy.org

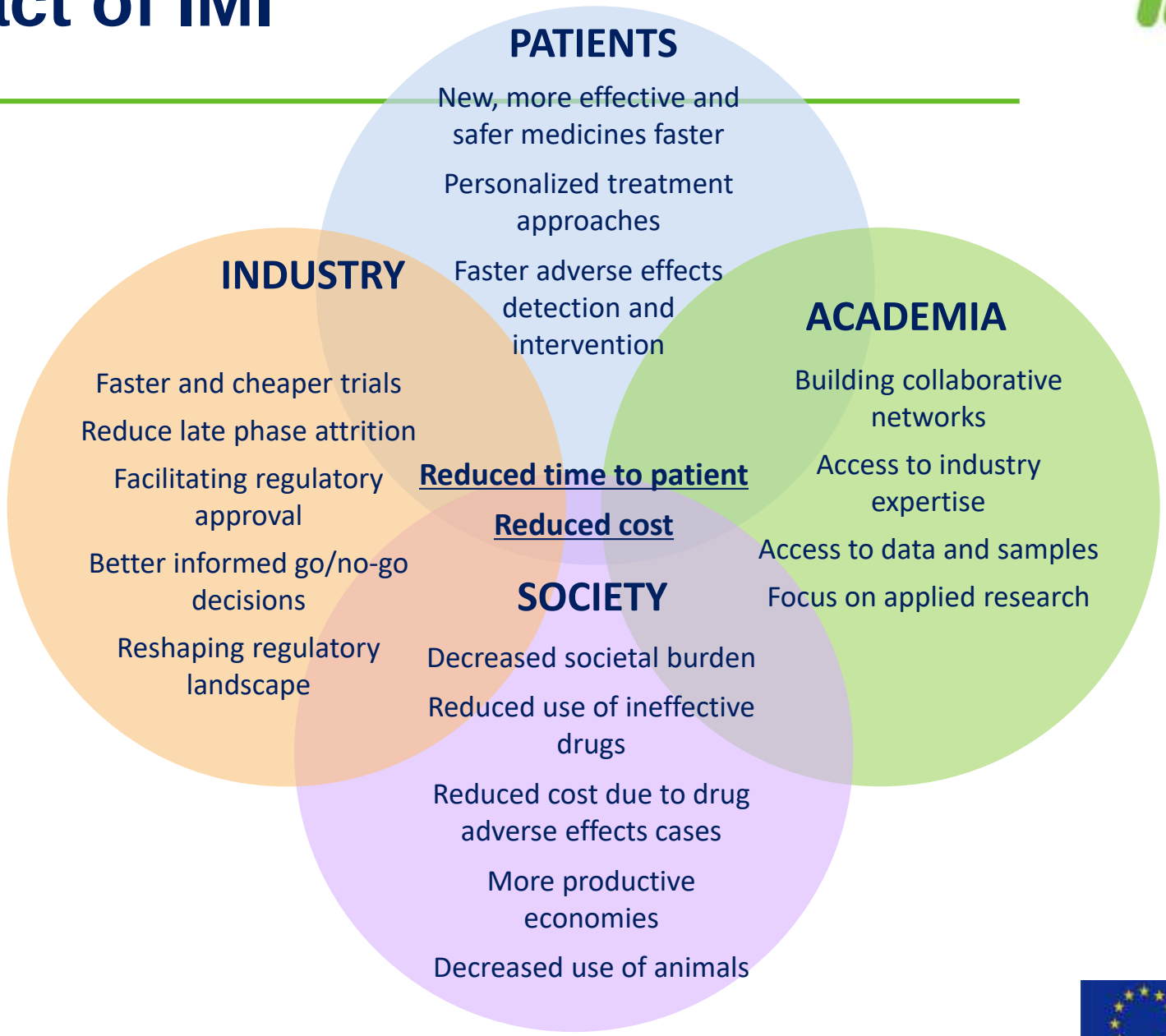
Partnership in IMI

"Non-competitive" collaborative research for EFPIA companies

Open collaboration in public-private consortia (data sharing, wide dissemination of results)

Competitive calls to select partners of EFPIA companies (IMI beneficiaries)

Impact of IMI



How will your results improve R&D productivity?

How will your project impact patients?

How will the project outcomes improve healthcare?

The Impact IMI Expect From You?

Success!

To achieve success

Communicate & Collaborate!

What Can IMI Deliver?