Advanced treatment of genetic disorders and its translation

Roman Jerala

Department of synthetic biology and immunology
National institute of chemistry
Ljubljana, Slovenia

Kleefstra Syndrome Conference, Ljubljana, June 2nd, 2023

Scientific expertise & technologies at the Department of Synthetic Biology and Immunology at NIC

- We cover the research range from atoms to organisms
- Structural biology (molecular modelling, protein design, bioinformatics, cryo-EM)
- Biophysics (CD, fluorescence, luminescence, DLS, SEC-MALS, SAXS, FCS, ITC, SPR…)
- Molecular/synthetic biology/genetics (gene regulation, RNA post-processing, genome editing with CRISPR, chemical regulation)
- Cell biology: flow cytometry, confocal fluorescence microscopy, BSL2 labs, lentiviruses, AAV, LNP delivery
- Animal experiments – imaging, preparation of genome engineered animals, complete biochemical analysis

Research: gene and cell therapy technologies

CTGCT
CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

Cutting-edge scientific results at
the National Institute of Chemistry
in areas relevant for translation into therapeutics

nature chemical biology
ARTICLES
https://doi.org/10.1038/s41589-022-01136-x

Check for updates

Chemically inducible split protein regulators for mammalian cells

Erik Rihtar[1,2,6], Tina Lebar[1,6], Duško Lainšček[1], Katarina Kores[3], Samo Lešnik[3], Urban Bren[3,4] and Roman Jerala[1,5]✉

nature COMMUNICATIONS

Coiled-coil heterodimer-based recruitment of an exonuclease to CRISPR/Cas for enhanced gene editing

Duško Lainšček[1,2,7], Vida Forstnerič[1,7], Veronika Mikolič[3,4], Špela Malenšek[1,4], Peter Pečan[1,4], Mojca Benčina[1,2], Matjaž Sever[3,5], Helena Podgornik[3,6] & Roman Jerala[1,2]✉

nature COMMUNICATIONS

Regulation of protein secretion through chemical regulation of endoplasmic reticulum retention signal cleavage

Arne Praznik[1,2], Tina Fink[1], Nik Franko[1], Jan Lonzarić[1], Mojca Benčina[1,3], Nina Jerala[1,4], Tjaša Plaper[1,2], Samo Roškar[1] & Roman Jerala[1,3]✉

nature chemical biology
ARTICLES
https://doi.org/10.1038/s41589-019-0443-y

A tunable orthogonal coiled-coil interaction toolbox for engineering mammalian cells

Tina Lebar, Duško Lainšček, Estera Merljak, Jana Aupič and Roman Jerala*

CellPress OPEN ACCESS
Cell

Article

TDP-43 condensation properties specify its RNA-binding and regulatory repertoire

Martina Hallegger,[1,2,10,*] Anob M. Chakrabarti,[1,3,10] Flora C.Y. Lee,[1,2,10] Bo Lim Lee,[4] Aram G. Amalietti,[1,2,5] Hana M. Odeh,[4] Katie E. Copley,[4,6] Jack D. Rubien,[4] Bede Portz,[4] Klara Kuret,[5] Ina Huppertz,[7] Frédérique Rau,[1,2,11] Rickie Patani,[1,2] Nicolas L. Fawzi,[8] James Shorter,[4,6] Nicholas M. Luscombe,[1,3,9] and Jernej Ule[1,2,5,12,*]

nature neuroscience

Characterizing the RNA targets and position-dependent splicing regulation by TDP-43

James R Tollervey[1,8], Tomaž Curk[2,8], Boris Rogelj[3,8], Michael Briese[1], Matteo Cereda[1,4], Melis Kayikci[1], Julian König[1], Tibor Hortobágyi[3], Agnes L Nishimura[3], Vera Župunski[3,5], Rickie Patani[6], Siddharthan Chandran[6,7], Gregor Rot[2], Blaž Zupan[2], Christopher E Shaw[3] & Jernej Ule[1]

nature chemical biology
ARTICLES
https://doi.org/10.1038/s41589-018-0181-6

Design of fast proteolysis-based signaling and logic circuits in mammalian cells

nature COMMUNICATIONS

Designed folding pathway of modular coiled-coil-based proteins

Jana Aupič[1,7], Žiga Strmšek[1,2,7], Fabio Lapenta[1,3], David Pahovnik[4], Tomaž Pisanski[5,6], Igor Drobnak[1], Ajasja Ljubetič[1] & Roman Jerala[1,3]✉

SCIENCE ADVANCES | RESEARCH ARTICLE

SYNTHETIC BIOLOGY

Metal ion–regulated assembly of designed modular protein cages

Jana Aupič[1]†, Fabio Lapenta[1,2]‡, Žiga Strmšek[1], Estera Merljak[1,3], Tjaša Plaper[1,3], Roman Jerala[1,2]*

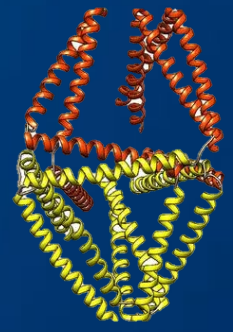
ARTICLE

nature biotechnology

Design of coiled-coil protein-origami cages that self-assemble *in vitro* and *in vivo*

Ajasja Ljubetič[1,10], Fabio Lapenta[1,2,10], Helena Gradišar[1,3], Igor Drobnak[1], Jana Aupič[1,4], Žiga Strmšek[1], Duško Lainšček[1], Iva Hafner-Bratkovič[1,3], Andreja Majerle[1], Nuša Krivec[1], Mojca Benčina[1], Tomaž Pisanski[5], Tanja Ćirković Veličković[6], Adam Round[7,8], José María Carazo[9], Roberto Melero[9] & Roman Jerala[1,3]

2 ERC projects for established biomedical scientists



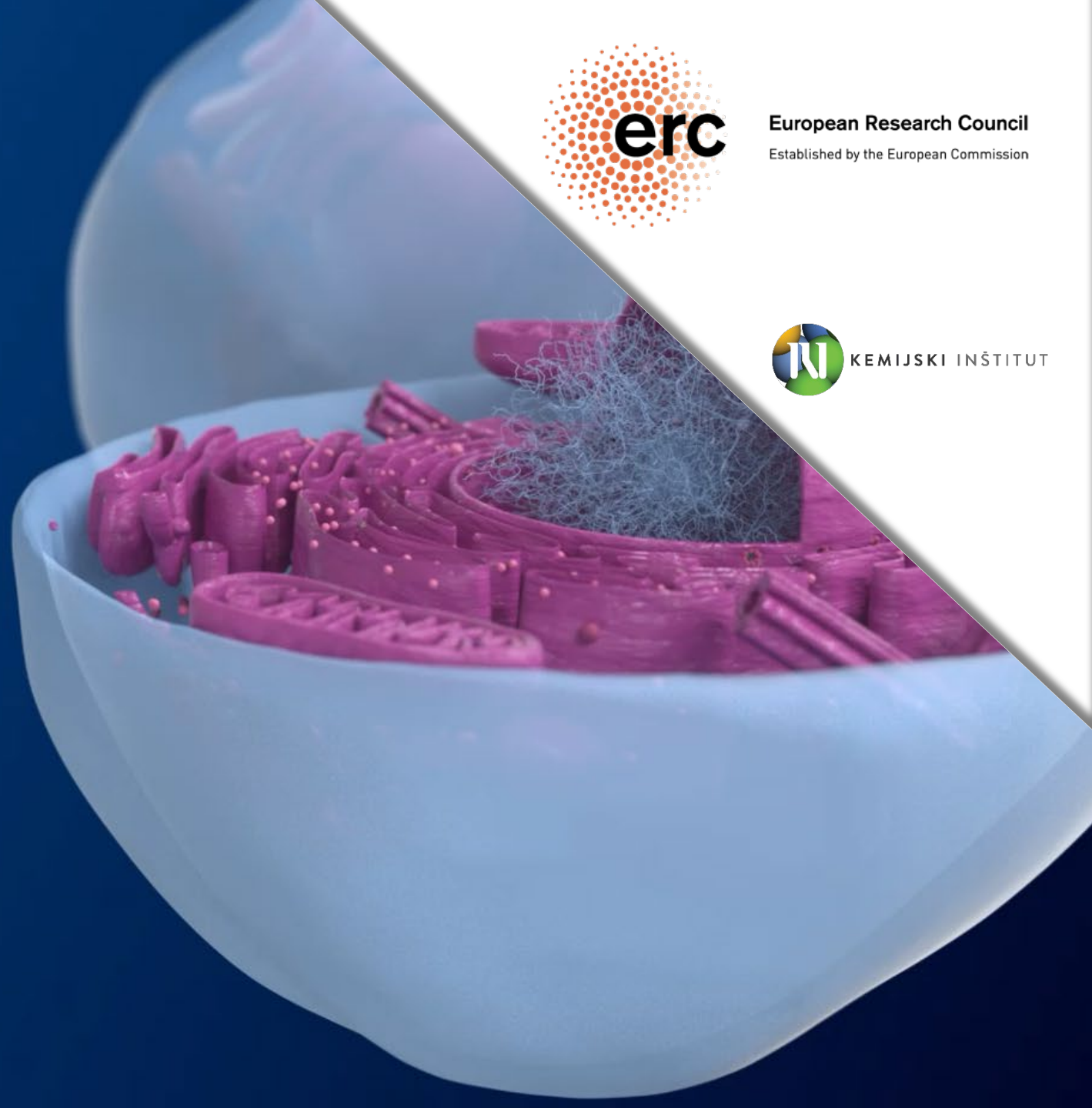
Roman Jerala

Jernej Ule

ERC proof-of-concept project

CCEdit

Enhanced gene editing



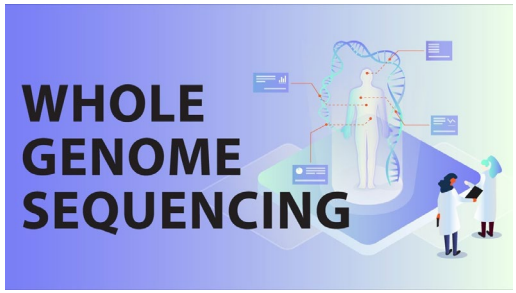
European Research Council

Established by the European Commission

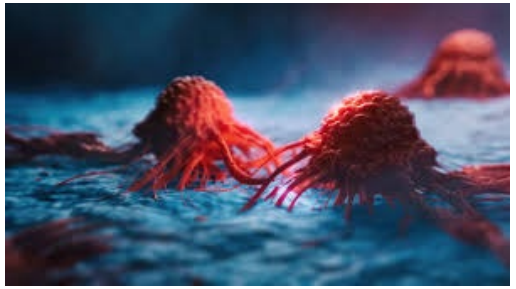
KEMIJSKI INŠTITUT

Revolutionary breakthroughs in the life sciences

CTGCT
CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY



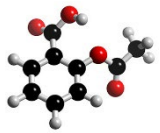
- Whole genome sequencing
- Structure of biological macromolecules (200,000 experimentally determined, 200 million 3D models)
- Genome repair (CRISPR and similar tools)
- Synthetic biology tools
- Programming human cells for therapy
- Translation into personalized medicine



Pillars of pharmacological therapies

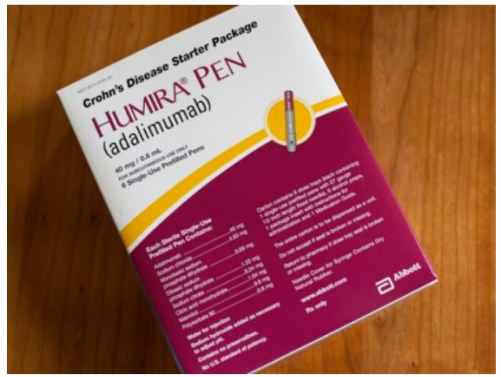
First pillar
Small molecules

ASPIRIN®



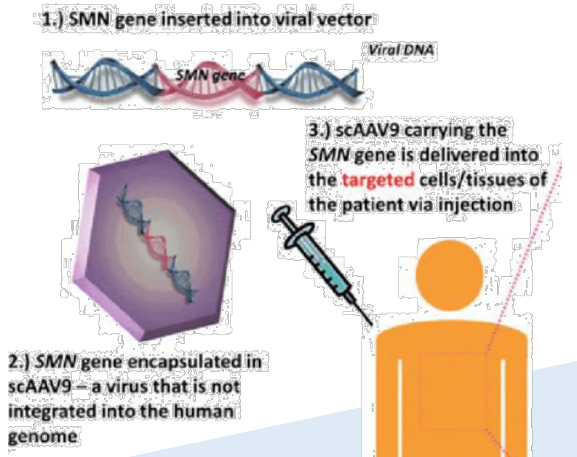
Second pillar
Biological drugs

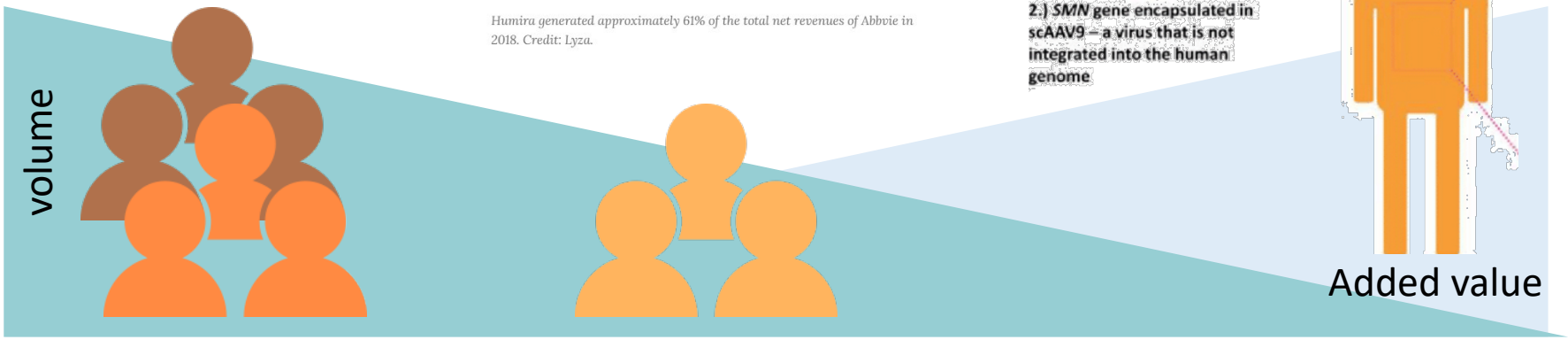
1. Humira (adalimumab) – $19.9bn



Humira generated approximately 61% of the total net revenues of Abbvie in 2018. Credit: Lyza.

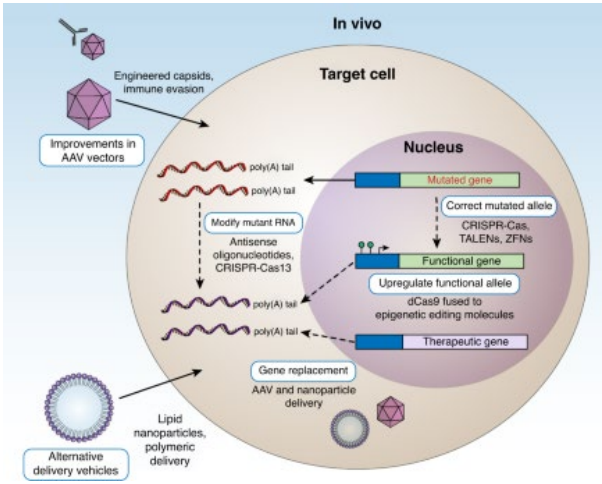
Third pillar
Cell and gene therapy





Therapeutic options for genetic disorders

- **Replacement gene** (delivery via viruses, AAV)
- **Genome editing** (CRISPR, ZnFingers, TALEs – for selected types of mutations)
- **Inactivation of dominant-negative gene** (RNAi, gapmer ASOs)
- **Upregulation of the healthy allele**

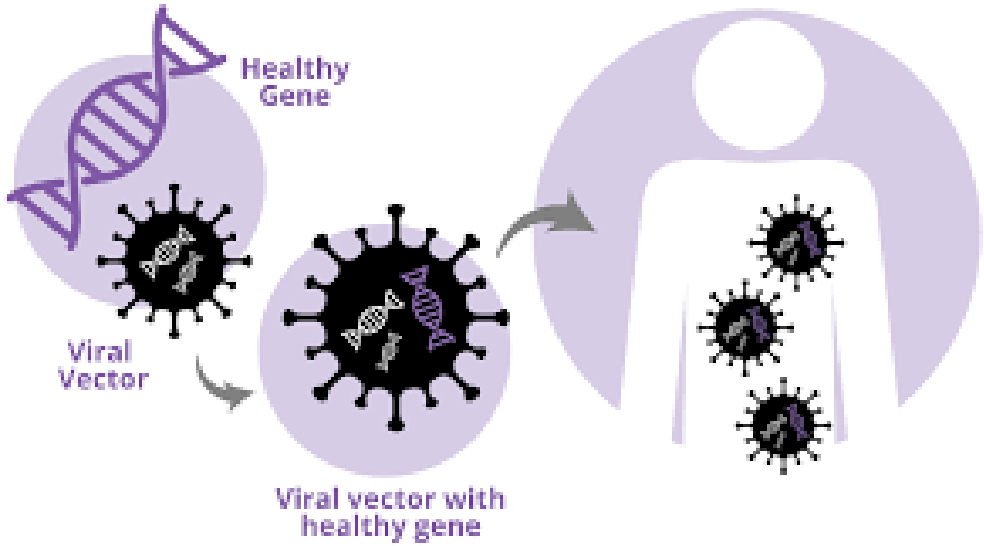


Gene replacement therapy

- Wild (healthy) copy of a gene, typically delivered through AAV
- Several therapies have been approved: Zolgensma, Luxturna, Almondys 45,

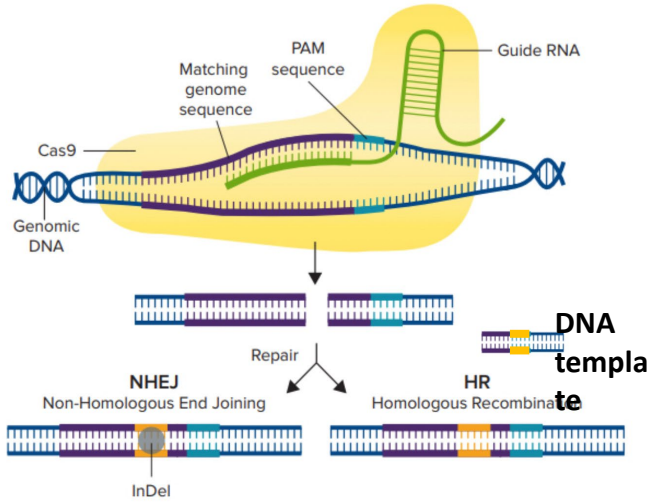
and many more already in clinical trials

Duration of the effect, alternative vectors

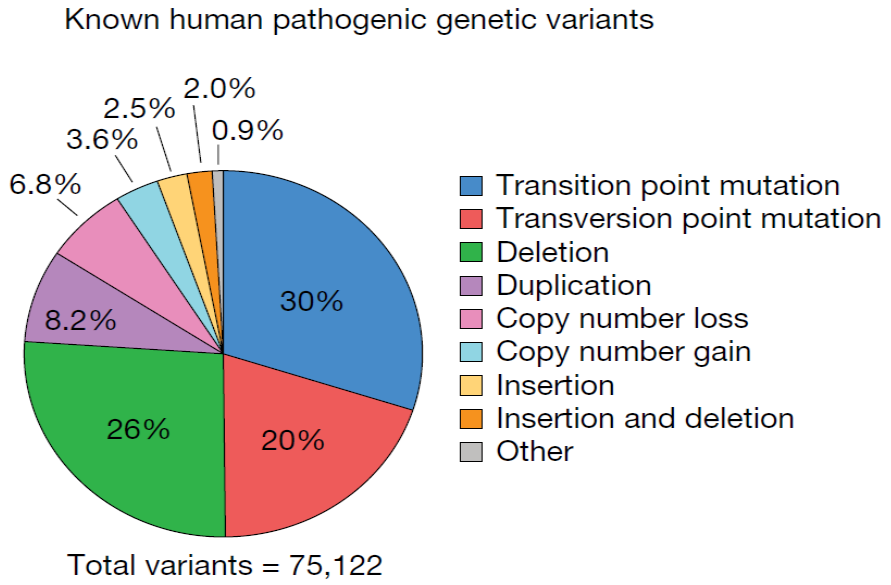


Inborn errors of metabolism	Neurologic
• Lipoprotein lipase deficiency • Canavan disease • Batten disease • Mucopolysaccharidosis type I, II, IIIA, IIIB, and VI • Metachromatic leukodystrophy • Aromatic L-amino acid decarboxylase deficiency • Familial hypercholesterolemia • Acute intermittent porphyria • Crigler-Najjar syndrome • Tay-Sachs disease • Pompe disease • Galactosialidosis • Ornithine transcarbamylase deficiency • Glycogen Storage Disease Type I	• Parkinson's disease • Alzheimer's disease • Spinal muscular atrophy • Amyotrophic lateral sclerosis • Temporal lobe epilepsy • Charcot-Marie-Tooth neuropathy type 1A
Musculoskeletal	**Ocular**
• Rheumatoid arthritis • Osteoarthritis • Muscular dystrophies (Duchenne, Becker, and Limb-Girdle) • Digital flexor tendon injury	• Leber congenital amaurosis • Leber hereditary optic neuropathy • Choroideremia • Age-related macular degeneration • Achromatopsia (Color Blindness) • Retinitis pigmentosa • X-linked retinoschisis
Pulmonary	**Cancer related**
• Cystic fibrosis	• Gastric cancer • Prostate cancer • Nasopharyngeal carcinoma • Multiple myeloma • Malignant melanoma • Non-Hodgkin lymphoma and B cell acute lymphoblastic leukemia • Irradiation-induced parotid salivary

CRISPR/Cas-based genome editing

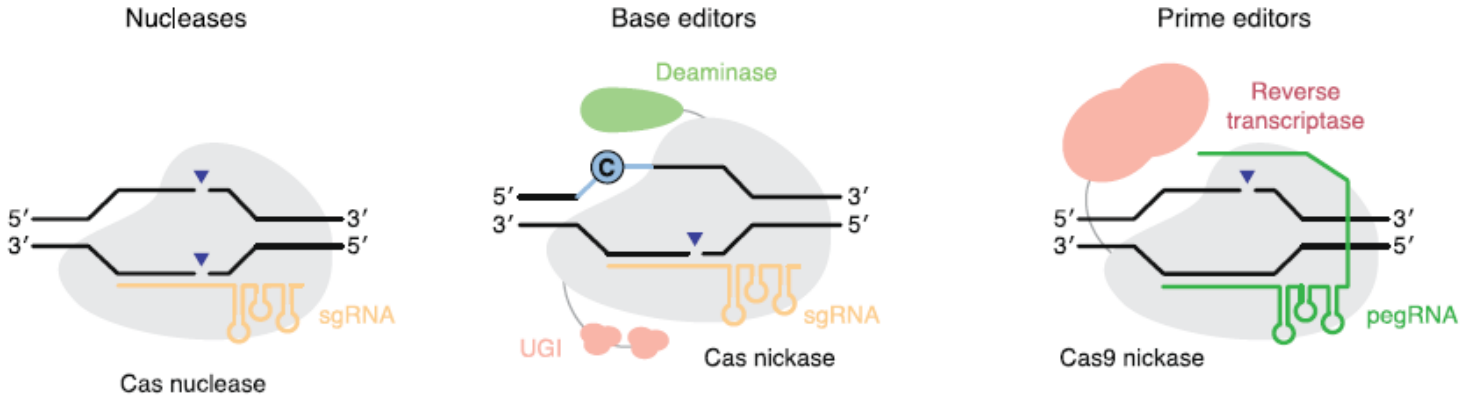


Gene disruption **Gene editing**



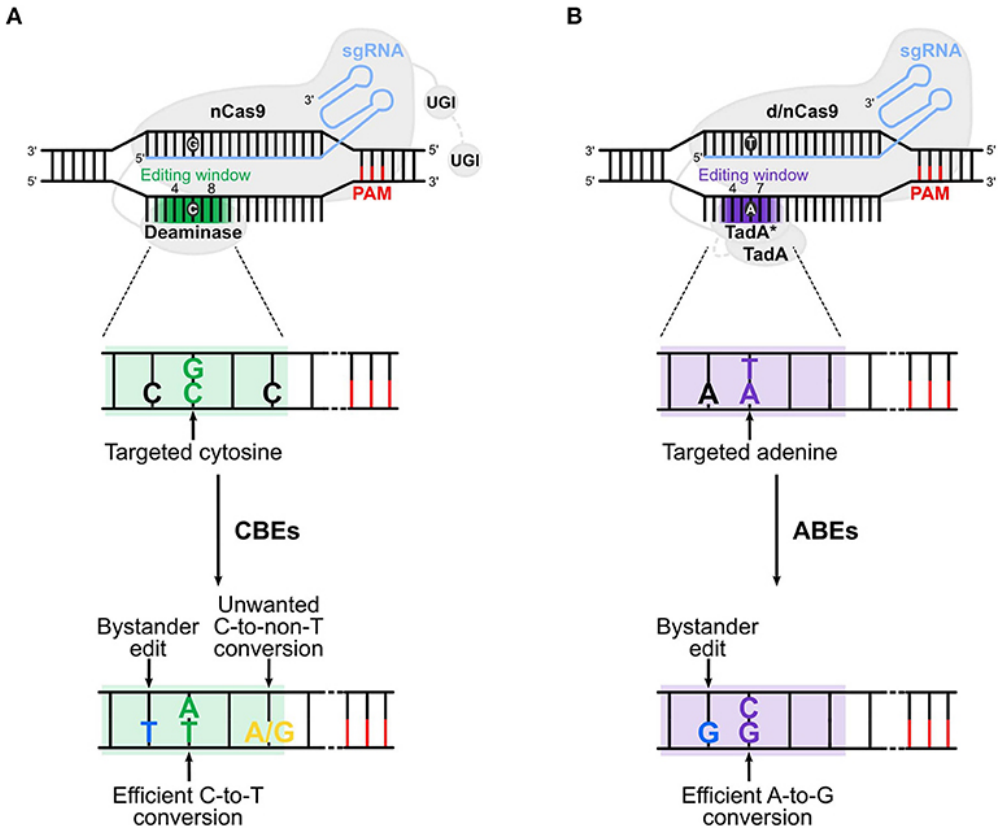
More than 100 ongoing clinical trials, based on CRISPR:

- Leukemias (ALL. AML..)
- Angelman Syndrome
- Alzheimer's disease
- Atopic dermatitis
- Beta thalassemia
- Infection diseases (COVID, B.pertusis, hepatitis, HIV etc.)
- Cystic fibrosis
- DM
- DMD
- Huntington's disease etc.

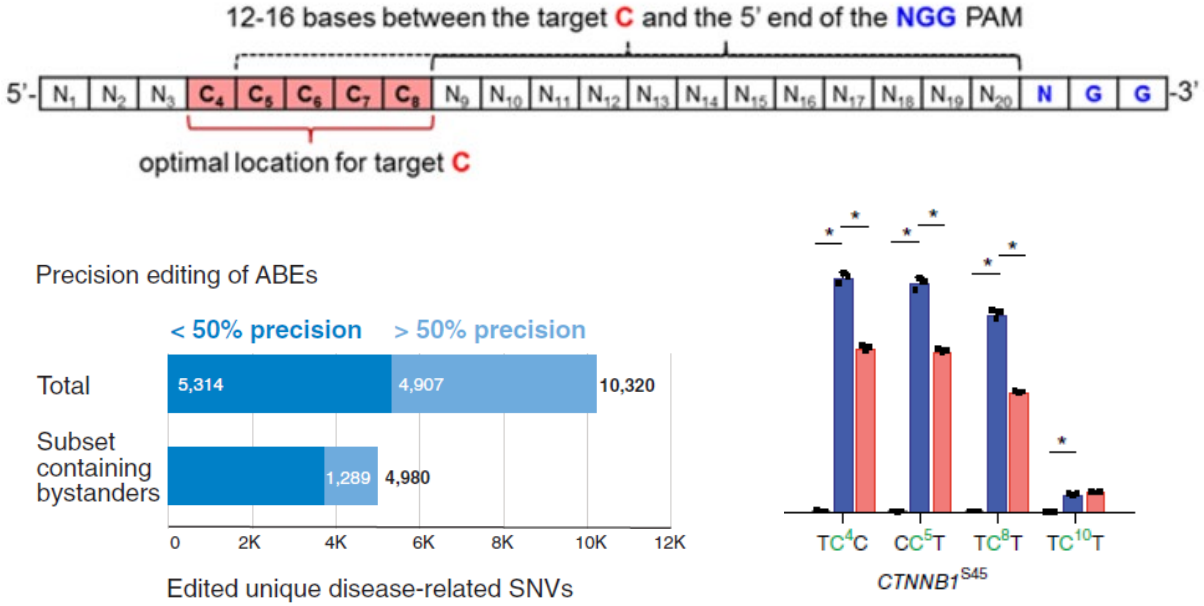


Anzalone Nature 2019

Base editors (cytosine, adenosine); C→T; A→G, (G→R)



Antoniu.2021.Frontiers

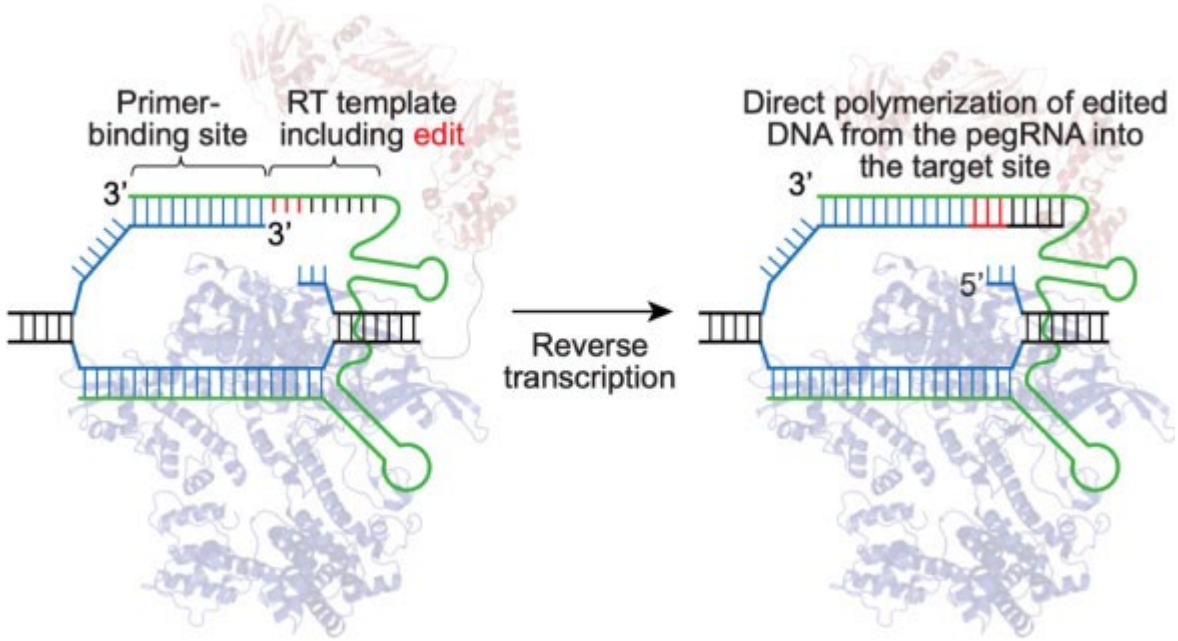


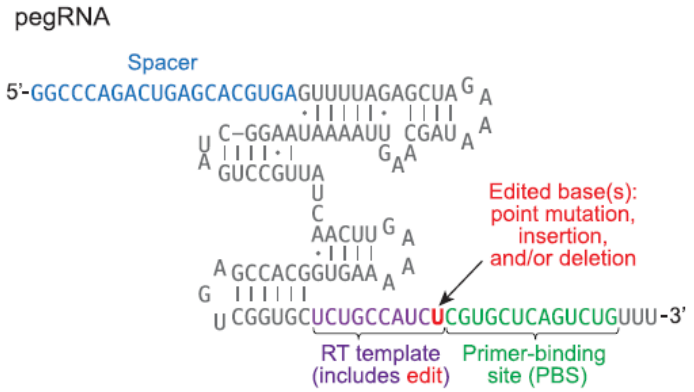
Zafra.2018.NatBiotech

Table 1 | **Selected base editors in development**

Drug (sponsor)	Mechanism	Indication	Delivery	Status
VERVE-101 (Verve)	*PCSK9* silencing	Heterozygous familial hypercholesterolaemia	In vivo LNP	Phase Ib
BEAM-101 (Beam)	Activation of fetal haemoglobin	Sickle cell disease; β-thalassemia	Ex vivo HSCs	IND approved
BEAM-102 (Beam)	Correction of HbS mutation	Sickle cell disease	Ex vivo HSCs	IND-enabling studies
BEAM-201 (Beam)	Multiplexed silenced CD7 CAR-T	T cell ALL; CD7⁺ AML	Ex vivo T cells	IND-enabling studies
Unnamed candidate (Verve)	*ANGPTL3* silencing	Familial hypercholesterolaemia	In vivo LNP	Preclinical
BEAM-301 (Beam)	Correction of R83C mutation	Glycogen storage disease 1a	In vivo LNP	Preclinical
Unnamed candidate (Beam)	Correction of G1961E mutations	Stargardt disease	In vivo AAV	Preclinical
Unnamed candidate[a] (Wave)	Correction of mutation in *SERPINA1* mRNA	α-1 antitrypsin deficiency	Subcutaneous	Preclinical

Prime editors (all possible base changes, small insertions, deletions, combinations)

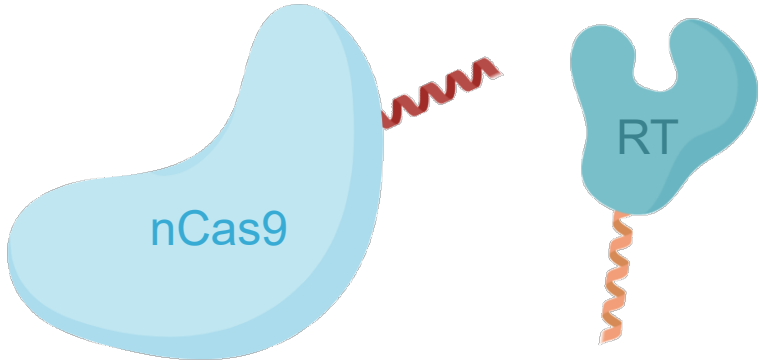


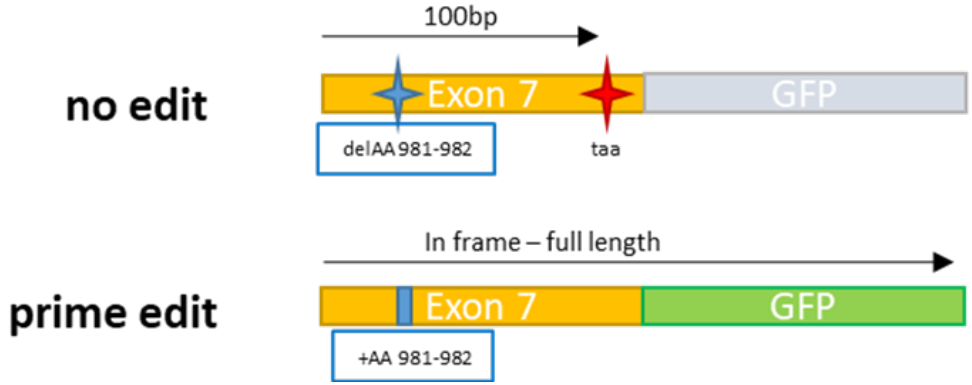


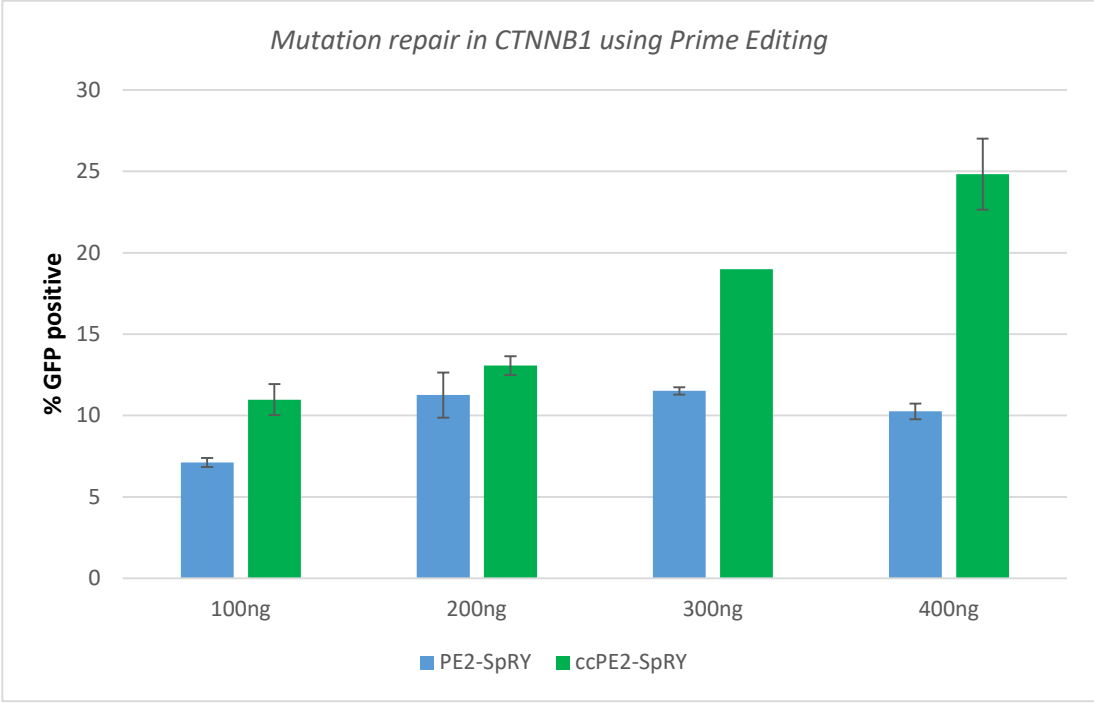
Clinical trials:
liver, eye, skin, muscular, neurodegenerative hereditary diseases, cystic fibrosis, beta-thalassemia, X-linked severe combined immunodeficiency and cancer.

Anzalone. 2019. Nature

Refinement of PE at NIC

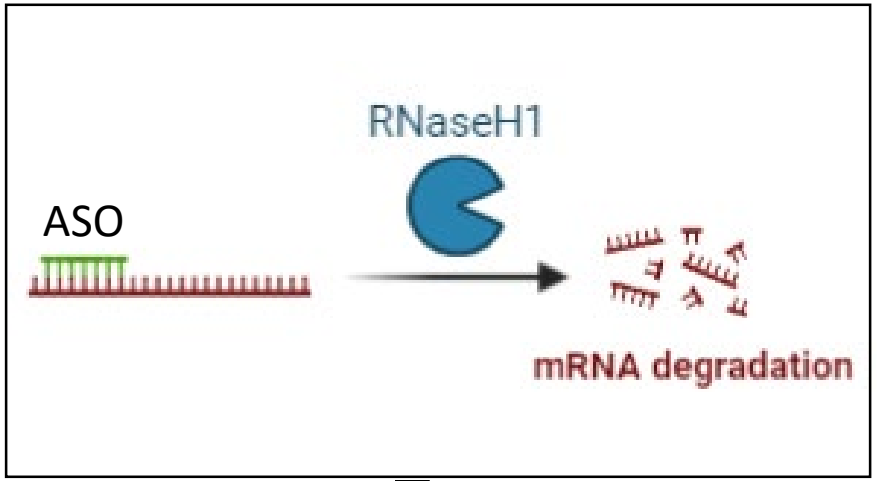






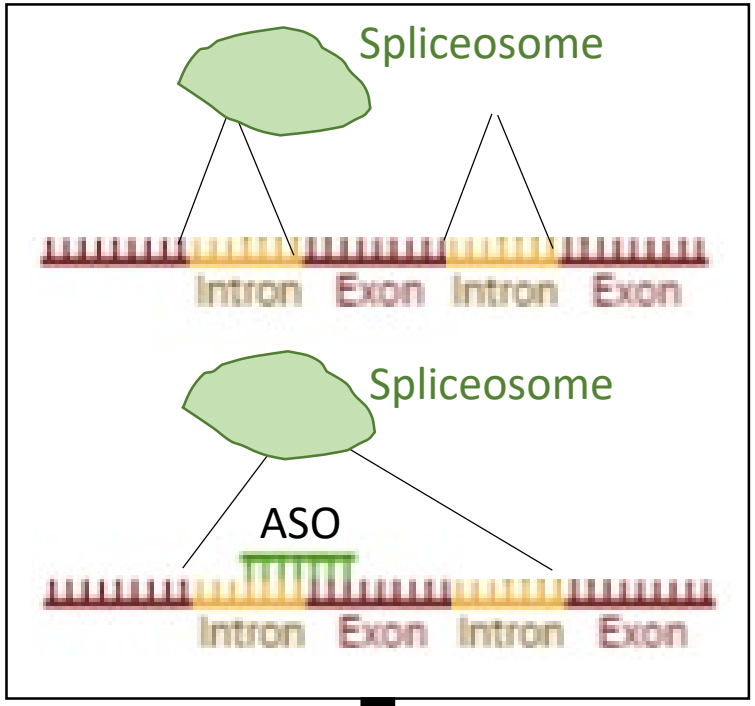
Mechanisms of action of antisense nucleotides (ASOs)

RNA degradation



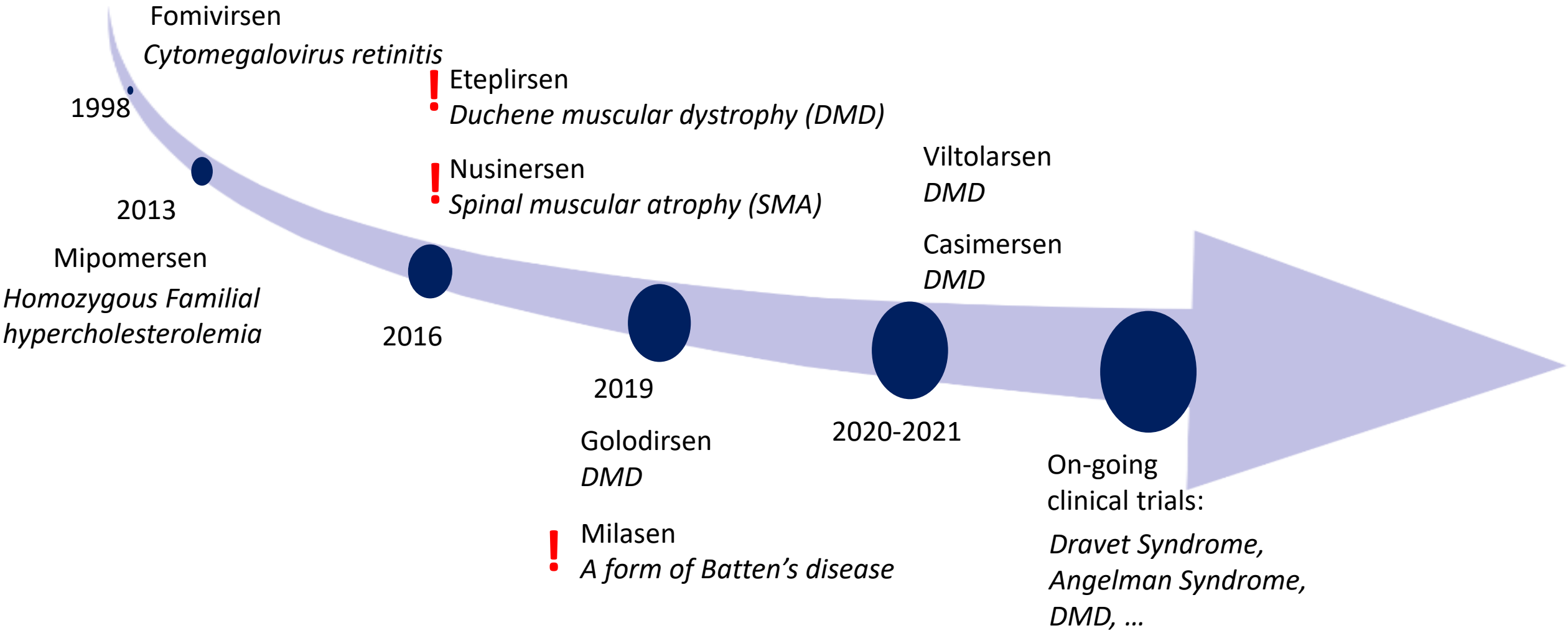
Downregulated protein expression

Splicing modulation
(exon skipping or inclusion)

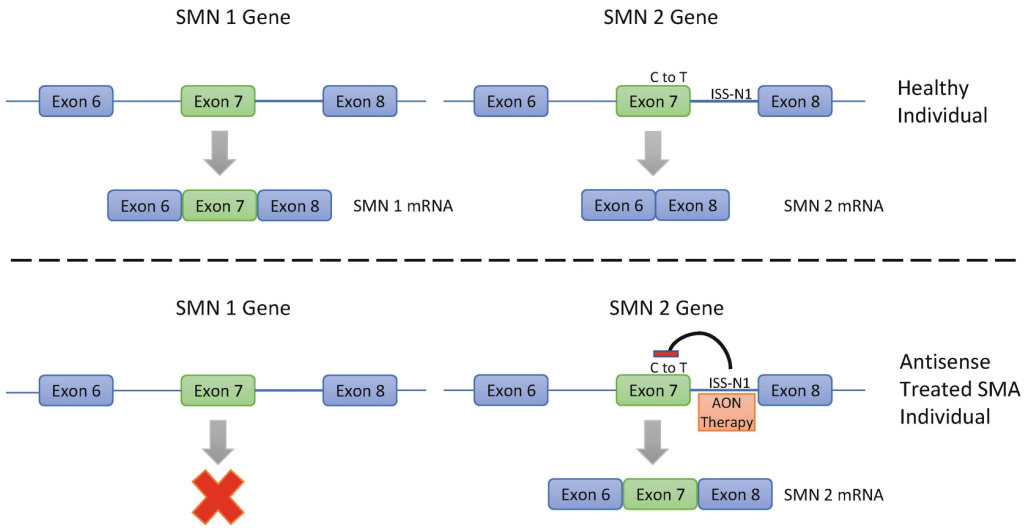


Altered protein (upregulation or downregulation)

Approval of ASOs for treatment of neurodevelopmental diseases



Data from Amanat et al., 2022



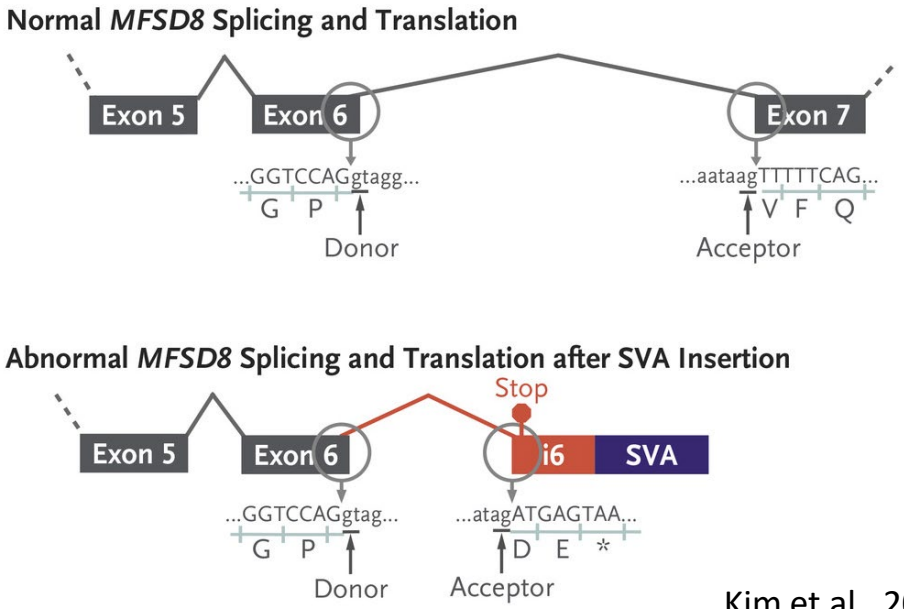
Goodkey et al., 2018

Nusinersen

Spinal muscular atrophy

Disease:
SMN1 gene mutations lead to its loss of function.

Mechanism:
Upregulation of a SMN1 gene homolog - SMN2 which is poorly expressed due to silenced splicing.



Kim et al., 2019

Milasen (first personalised ASO)

Batten´s disease

Disease:
Insertion of retrotransposon into the MFSD8 gene gives rise to alternative splicing which shifts the reading frame.

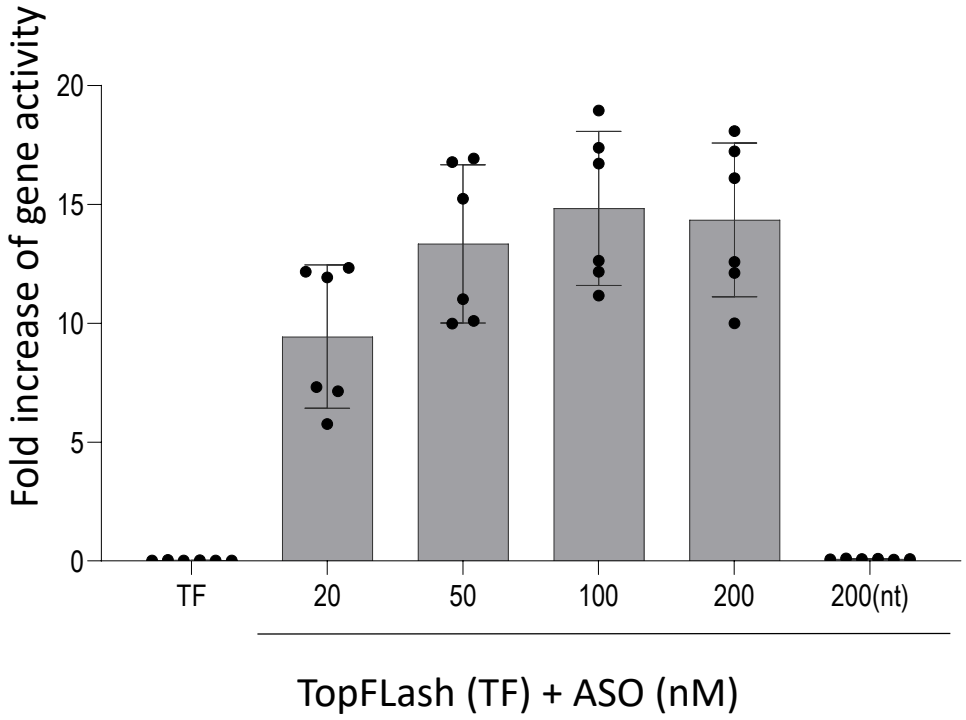
Mechanism:
Masking of alternative intronic splice site to skip retrotransposon.

Our ASO pipeline for CTNNB1

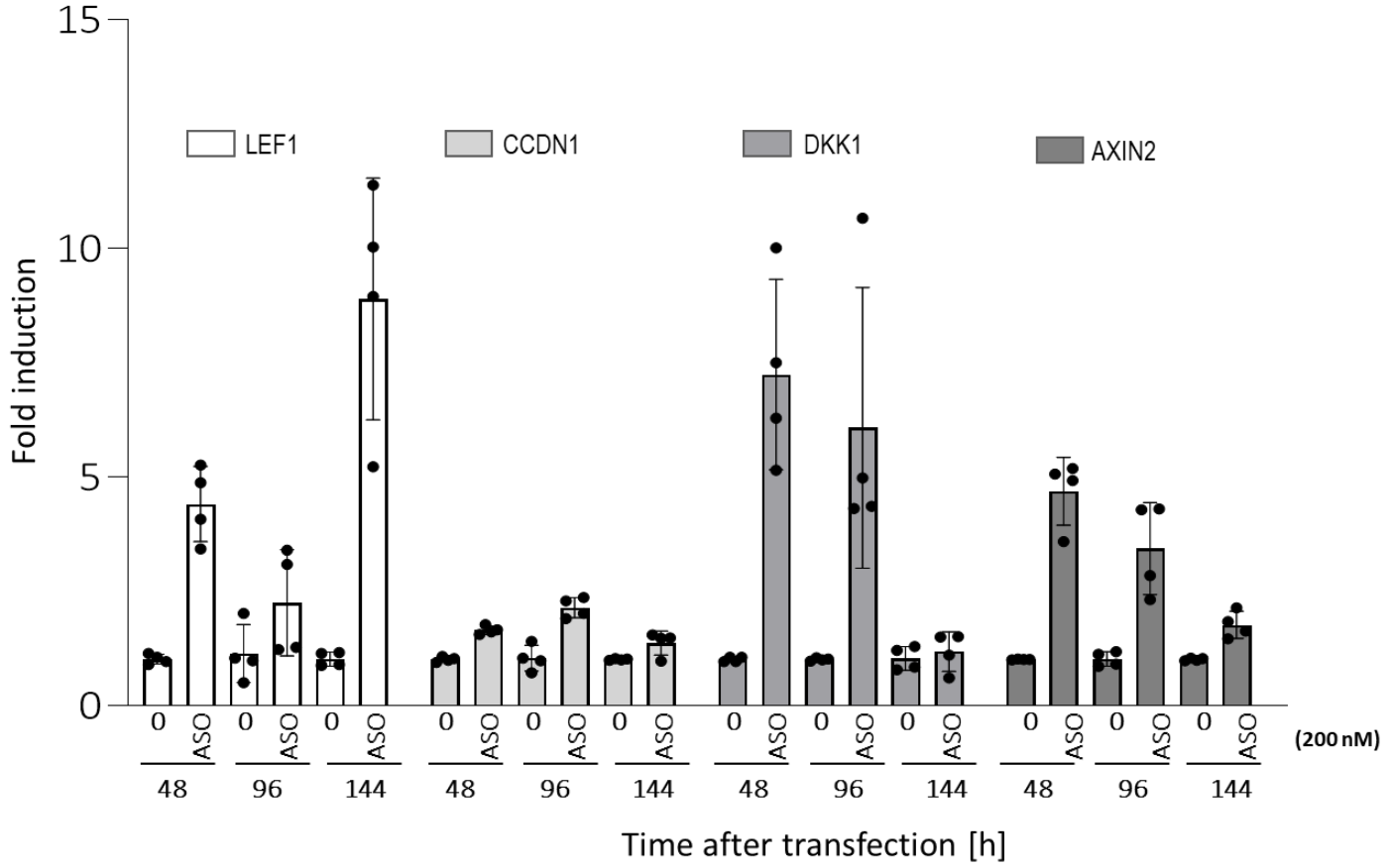
Design of ASO candidates targeting the Wnt signalling pathway

Effect on β-catenin activity (TopFlash reporter system)

Effect on Wnt transcriptional programme



Dose-dependent increase in β-catenin activity



Upregulation of Wnt target genes

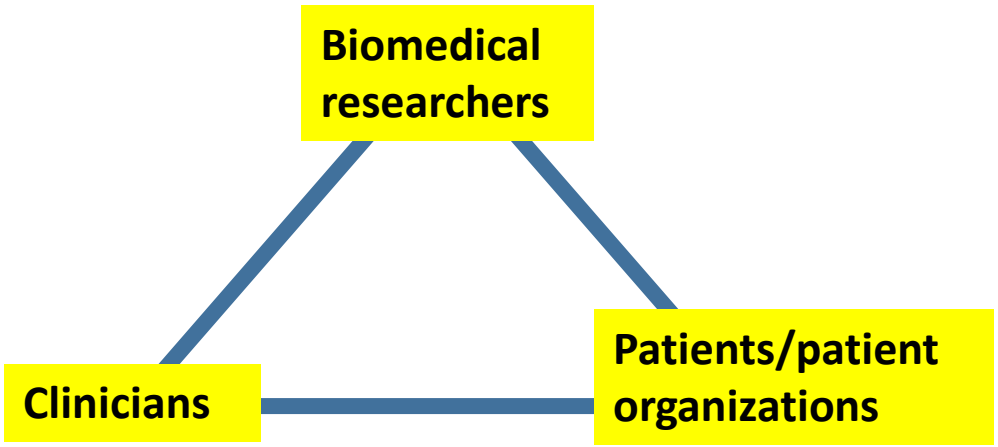
Collaboration between researchers, clinicians and patient organizations

CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

The existing collaborations through joint projects and PhD students with haematologists, pediatric neurologists and internists.

- Collaboration on the development of therapy for CTNNB1 syndrome.
- Launch of CAR T therapy in Slovenia, expansion to additional cancer types & improvements

Fundacija CTNNB1



+

- Facilities/companies for the production of GMP reagents
- Regulatory agencies
- Health insurance companies/agencies

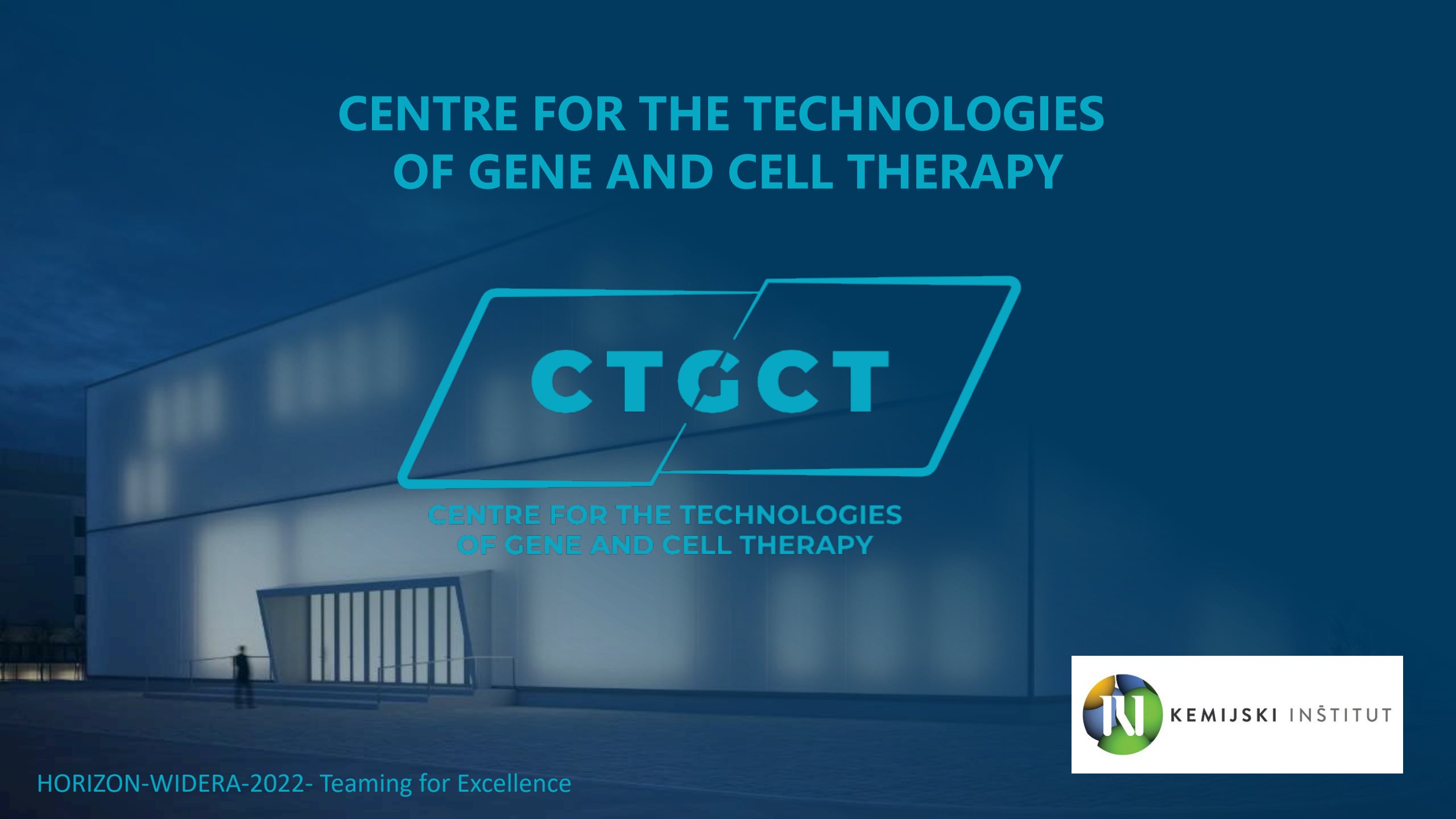
Research group and collaborators

Duško Lainšček
Petra Sušjan
Matea Maruna
Vida Forstnerič
Tina Fink
Erik Rihtar
Anja Golob
Hana Vokač
Ajasja Ljubetič
Mojca Benčina
Iva Hafner Bratkovič

Samo Zver
Matjaž Sever
Dept. Hematology, UKC

Špela Miroševič
Damijan Osredkar
Dept Neurology, UKC

CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY



KEMIJSKI INŠTITUT

HORIZON-WIDERA-2022- Teaming for Excellence

Collaboration between clinicians and patients

CTGCT
CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

Existing collaborations through joint projects and PhD students with haematologists, paediatric neurologists and internists.

- Preparation for the launch of CAR T therapy in Slovenia.
- Collaboration on the development of a cure for CTNNB1 syndrome.

The missing link is an organization, to translate biomedical research and prepare reagents for clinical testing.

Establishing a Centre to translate innovative scientific breakthroughs in synthetic biology and genetics for the design of future gene and cell therapies into the clinic.

Establishment of CTGCT

CTGCT
CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

Modern technologies make it possible to **treat** the direct cause of an increasing number of genetic diseases.

Mission

CTGCT Centre of Excellence will **develop** gene and cell therapy **technologies**, and work to **prepare innovative drugs** for clinical trials for diseases for which we do not yet have effective treatments.

Aim

To provide Slovenian patients and clinicians with access to modern effective treatments and to increase their availability (high cost).

Improve survival possibilities and quality of life for patients.

116 applications (Phase 1 application, October 2021),
36 invited (Invitation to 2nd Phase, April 2022),
12 projects selected

www.ctgct.si

The Vision

CTGCT
CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

- development of advanced technologies;

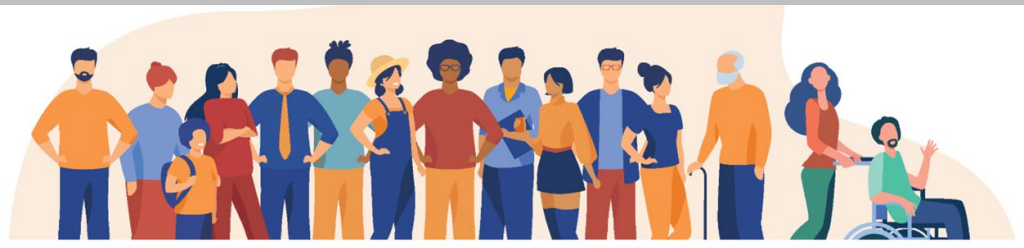
focus on genetic neurological and rare diseases and cancer immunotherapy

- bringing biomedical research to patients

preparation of reagents according to Good Manufacturing Practice (GMP) criteria

- training for researchers
- encouraging the creation of biotech start-ups
- bringing together researchers, clinicians and patients

In 2021, >1300 developers were working on gene therapy and related technologies, raising more than €22 billion.



THE HEALTHCARE LANDSCAPE

CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

Modern technologies make it possible to treat the direct cause of an increasing number of genetic diseases.

RNA dCas

Use of dCasRx to correct splicing defects in amyotrophic lateral sclerosis and frontotemporal dementia, a devastating neurodegenerative disease, serve as **a roadmap for new therapies in the nervous system**.

CRISPR

Non-viral delivery, genome editing, and targeted insertion of therapeutic genes using CRISPR (CCexo) technology. Improving the efficiency of existing technologies and preparing the technology for industrial production.

Synthetic biology

CAR T-cell

Developing CAR-T technologies for safer and more effective cancer therapies (INSPIRE, INSRTR, CCCtag). The Health Council supported the introduction of CAR-T therapies.

Impact

CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

SCIENTIFIC	Breakthrough scientific discoveries.
HEALTH	Improvements to the health of patients affected by diseases directly addressed by CTGCT as well as others via partners applying similar technological platforms.
ECONOMIC	More therapies at lower costs; increased national funding, patient organizations' support for fundraising.
ECONOMIC/TECHNOLOGICAL	A new market for advanced technologies for GCTs and connections with pharmaceutical companies' open-innovation programmers.
SOCIETAL	Improved quality of life for patients; increased societal acceptance of novel gene-related technologies; establishment of a new type of institutional organization that will support scientific and technological excellence.

Organization

CTGCT
CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

The establishment of the CTGCT is coordinated by the National Institute of Chemistry.

CTGCT partners with experience in bringing new technologies to patients: University College London (UCL), Charité University Hospital Berlin, Utrecht University Medical Centre and Dresden University of Technology

UCL
UMC Utrecht
Utrecht University
CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN
TECHNISCHE UNIVERSITÄT DRESDEN

www.ctgct.si

Bridging gaps in translation

1400 m^2 of a new research infrastructure linked to the research environment of the National Institute of Chemistry

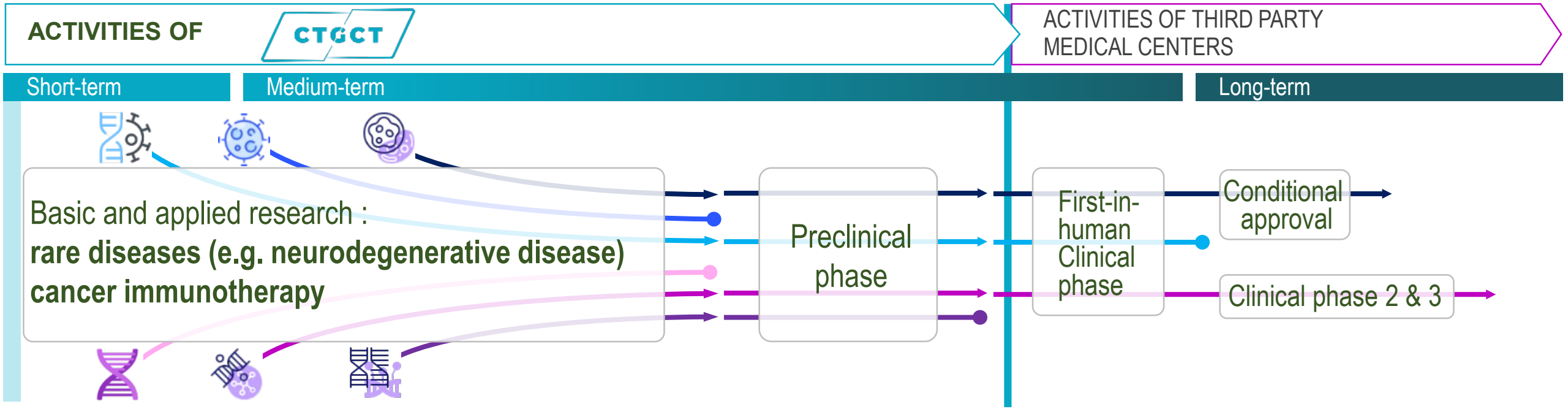
CTGCT
CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

Organization

private non-profit research institution with a public interest status

www.ctgct.si

WORKFLOW and PARTNERS CONTRIBUTION



Timeline

CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

- 2023

investment documentation
project documentation

- 2024

construction documentation

- 2025

execution of GOI works
technical inspection

- 2026

final activities

The Centre will become fully operational within 5 years.

THE TEAM BEHIND THE PROJECT

NATIONAL INSTITUTE OF CHEMISTRY

PROF DR ROMAN JERALA
National Institute of Chemistry
Innovative use of synthetic biology for the programming of molecules and cells for improved efficacy and safety of advanced treatments

PROF DR JERNEJ ULE
National Institute of Chemistry and UK Dementia Research Institute at King's College London
Research liaison between Slovenia and Great Britain

PROF DR MOJCA BENČINA
National Institute of Chemistry
Ultrasound applications in conjunction with synthetic biology to regulate molecular biological cell processes

DR DUŠKO LAINŠČEK
National Institute of Chemistry
Expert in the field of genome modification

BARBARA TIŠLER
National Institute of Chemistry
Project office

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

PROF PETRA REINKE
Berlin Center for Advanced Therapies (BeCAT) Charité
Enhance CTGCT's capabilities for refined transfer of research results to the first-in-human clinical practice and further accessibility of the ATMP as a treatment option for patients

UCL

PROF GIAMPIETRO SCHIAVO AND PROF PIETRO FRATTA
Queen Square IoN
Application of gene therapies to neurologic diseases

STEPHANIE SCHORGE
GeneTxNeuro facility at the UCL School of Pharmacy
Viral vector production

PROF QASIM RAFIQ
UCL Department of Biochemical Engineering
Production technology development for GCT bioprocessing

PROF EMMA MORRIS
Institute of Immunity & Transplantation, UCL
Development of immunotherapies

DR JANE KINGHORN AND DR PAMELA TRANTER
Translational Research Office, UCL

UMC Utrecht

Utrecht University

PROF JURGEN KUBALL
Department of Hematology, Cancer Center at UMC Utrecht and OncodePACT
Therapeutic T-cells and the valorisation of CAR T-cell development

ASSOC PROF ZSOLT SEBESTYEB
OncodePACT
Building a preclinical development infrastructure to de-risk and accelerate the drug development process and leads DARE-NL platform for cancer specific ATMP research

TECHNISCHE UNIVERSITÄT DRESDEN

PROF EZIO BONIFACIO
Center for Regenerative Therapies Dresden, TU Dresden
SaxoCell association
Expertise, technology and equipment for gene editing and regeneration towards new therapies such as neurodegenerative and haematological diseases

Acknowledgements

CTGCT
CENTRE FOR THE TECHNOLOGIES OF GENE AND CELL THERAPY

Colleagues at the Institute of Chemistry

Tina Fink
Duško Lainšček
Tomaž Bizjak
Barbara Tišler
Ivica Ilić

KEMIJSKI INŠTITUT

University Medical Centre

Samo Zver
Damijan Osredkar

univerzitetni klinični center ljubljana

MIZŠ

Division for Science

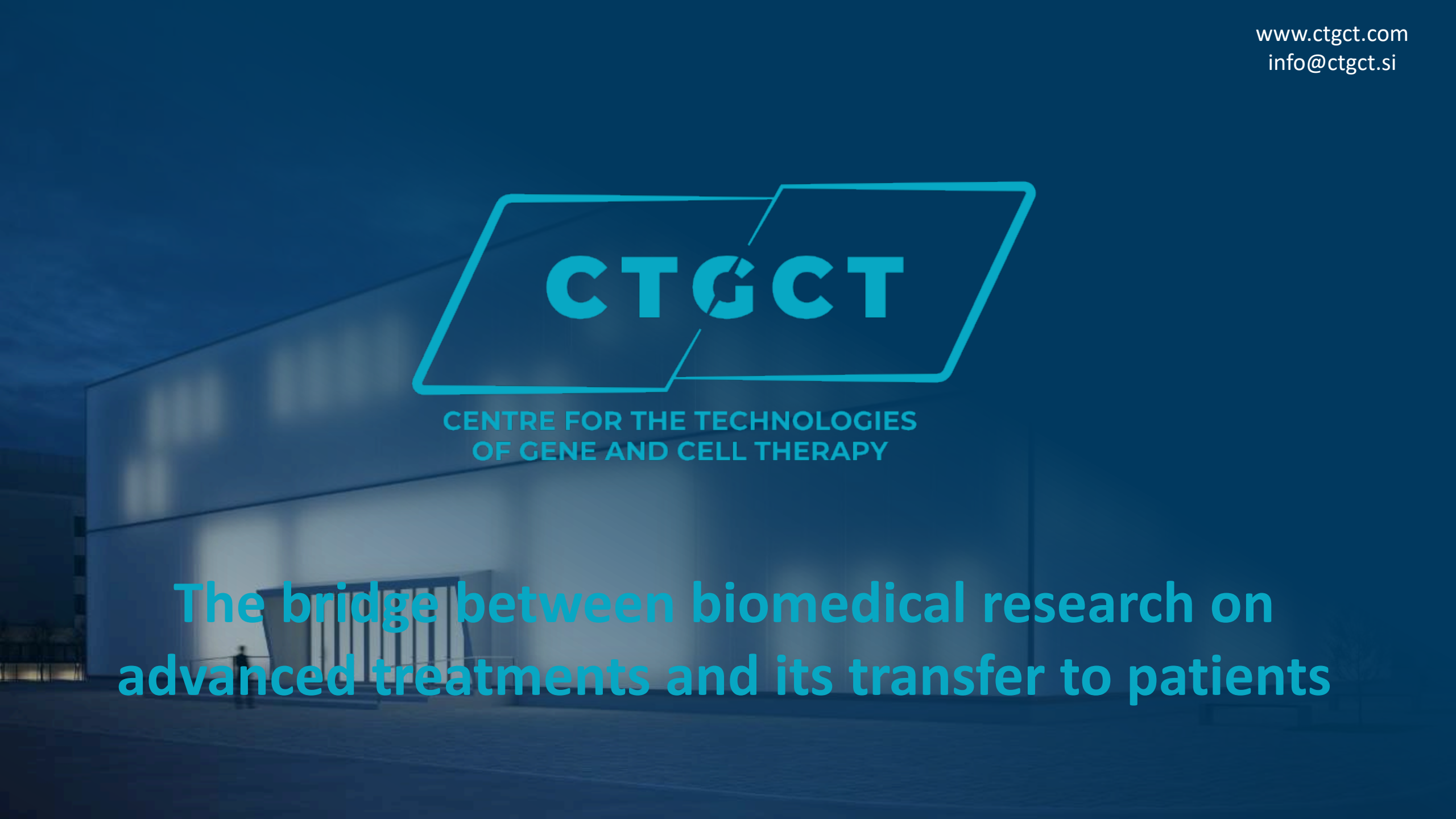
REPUBLIKA SLOVENIJA
MINISTRSTVO ZA IZOBRAŽEVANJE, ZNANOST IN ŠPORT

Patient organisations

TO ALL SUPPORTERS OF THE CTGCT

The CTGCT will be an opportunity to recruit motivated colleagues who want to tackle the challenges of bringing biomedical research to patients.

www.ctgct.com
info@ctgct.si



The bridge between biomedical research on advanced treatments and its transfer to patients