PROGRAMME

ORAL & POSTER ABSTRACTS

International Conference of Pharmacology, Drug Research and Development

HUPHAR 2026

MATRAHAZA **3-5, JUNE**

HUPHAR
Hungarian Society for Experimental and Clinical Pharmacology

BioTech innovative × QIAGEN

Sample to Insight

From sample to ready-to-interpret data

From February 2026,
BioTech Hungary Ltd. has taken over the official representation of QIAGEN in Hungary in the fields of research, diagnostics, and HID (Human Identification).

This partnership marks a significant milestone, as QIAGEN is globally recognized for its integrated "*Sample to Insight*" solutions — covering the entire workflow from biological sample collection to ready-to-interpret data.

QIAGEN technologies enable laboratories to achieve:

- **more reliable results**
- **higher reproducibility**
- **greater efficiency**

while providing comprehensive support across the entire workflow.

Key areas of the QIAGEN portfolio

- DNA/RNS izolation
- PCR /qPCR /dPCR
- NGS
- Data analysis and bioinformatics

BioTech Hungary Kft. is committed to providing Hungarian laboratories with tools and services that deliver real value in everyday practice.

We look forward to your inquiries at the contact details below

BioTech Hungary Kft.
A QIAGEN hivatalos forgalmazója Magyarországon

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www.ibiotech.hu

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WELCOME

Dear Colleagues,

On behalf of the organizing committee, we are pleased to inform you and all interested colleagues that the Hungarian Society of Experimental and Clinical Pharmacology (HUPHAR) organize the **HUPHAR2026 -International Conference of Pharmacology, Drug Research and Development** in Mátraháza, Hungary, between 3-5 June 2026.

We pay special attention to young researchers: provide great opportunities to present and discuss their results. Besides the outstanding scientific program, there will be recreational and social activities too.

THE MAIN TOPICS OF THE CONFERENCE ARE:

Pharmacovigilance
Clinical pharmacology: regulatory and patient safety
Innovative drug delivery systems: nanoparticles
Extracellular vesicles
Neuropharmacology
Novel approaches, analytics and *in vitro* models in pharmacokinetics
Immunopharmacology
Psychopharmacology
Oncopharmacology
Toxicology
Antimicrobials
Pain and inflammation
Cardiovascular pharmacology
Gene- and cell therapy
Respiratory pharmacology
Artificial intelligence in drug research and development
Medicinal chemistry

Information on Gyoftex/ Oftex accreditation scores for participant with registration numbers will be provided later.

ORGANIZER OF THE CONFERENCE:

HUNGARIAN SOCIETY FOR EXPERIMENTAL AND CLINICAL PHARMACOLOGY

CONFERENCE CHAIRS:

Katalin Monostory
Conference Chair, HUN-REN Research Centre for Natural Sciences

Zsuzsanna Helyes
President of HUPHAR, University of Pécs

Péter Ferdinandy
Conference Chair, Semmelweis University

ORGANIZING COMMITTEE:

Kata Csekő *(University of Pécs)*
Éva Szőke *(University of Pécs)*
Zsófia Gulyás-Ónodi *(Semmelweis University)*
Valéria Tékus (*University of Pécs)*
Gábor Mórotz *(Semmelweis University)*
Zoltán Varga *(Semmelweis University)*

SESSION ORGANIZERS:

Zoltán Giricz *(Semmelweis University)*
Zsolt Némethy *(Richter Gedeon)*
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Renáta Papp *(Semmelweis University)*
András Perczel *(Eötvös Loránd University)*
Miklós Soós *(Auro-Science Ltd.)*

VENUE OF THE CONFERENCE

Mátraháza

HOTEL ÓZON & LUXURY VILLAS**** superior

TECHNICAL ORGANISER OF THE EVENT

Altagra Business Services and Travel Agency Ltd.

www.altagra.hu

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PRECÍZIÓS ESZKÖZÖK A PREKLINIKAI KUTATÁSOKHOZ

Megbízható. Etikus. Innovatív.
Megoldásaink elősegítik a tudományos eredmények elérését és az állatjólét javítását.

PREKLINIKAI MODELLEK

- Rágcsáló- és zebradánió rendszerek
- 3R elvek szerinti etikus megoldások

NEUROFIZIOLÓGIA & VISELKEDÉS

- Videóalapú viselkedéskövetés
- EEG, optogenetika, fiber photometria
- Reflex- és fájdalomvizsgáló eszközök

ÉLETTANI MONITOROZÁS & KERINGÉSKUTATÁS

- Légzés, EKG monitorozás
- Véráramlás- és mikrokeringés-monitorozás
- Non-invazív hemodinamikai rendszerek

BIOANALITIKA & LABOR

- Perfúziós rendszerek és állatházi kiegészítők
- Kisállat altatógépek
- Metabolikus és farmakológiai mérőeszközök

AZ 3R ELVEK TÁMOGATÁSA

Etikus megoldások az állatjóllét elősegítésére és az állatok számának csökkentésére.

INTEGRÁLT MEGOLDÁSOK

Teljes körű rendszerek a kutatás minden lépéséhez.

KUTATÓKNAK TERVEZVE

Tudósok által féjlesztett eszközök, tudósok számára.

KÉPZÉS & TÁMOGATÁS

Szakértői képzések és dedikált támogatás a kutatás minden szakaszában.

EGYÜTTMŰKÖDŐ PARTNEREINK

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Találkozzunk a HUPHAR 2026 konferencián!

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+36 70 431 4441
www.animalab.hu

AKRONOM Kft.
1149 Budapest, Angol u.34.
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E-mail: info@ akronom.hu

Az **AKRONOM Kft.** a magyar állatkísérleti kutatási piac egyik meghatározó szereplője. Az általunk üzembe helyezett termékek szervizét garancia időn belül és azon túl is saját, akkreditált, szakképzett szervizünk végzi.

Termékköreink:

1. ***Laborállat tartó eszközök és berendezések***
   - komplett állattartó ketrec rendszerek, aktivitás ketrecek laborállatok számára
   - komplett „Aquatic" állattartó rendszer (zebra danio, afrikai karmosbéka)
   - szellőztetett szekrények szűrőtetős ketrecek számára (HEPA- és szénszűrővel)
   - egyedi szellőztetett (IVC) ketrec rendszerek, transzport és tárolós kivitelben is
   - hermetikusan záródó egyedi szellőztetett (DCC, ISOcage) ketrec rendszerek BSL2 BSL3 és BSL4 felhasználók számára speciális biztonsági munkavédelmi szekrénnyel
   - diuresis és metabolikus ketrecek, tartóállványokkal, hűtőtárolóval
   - állatcserélő biztonsági munkavédelmi szekrények (LAF box)
   - alomürítő berendezések; központi alom elszívó és töltő rendszer
   - laborállat tápok, almok és környezetgazdagító eszközök (fészekanyagok, papírcsövek)
2. ***Laborállat tartó eszközök mosásával és fertőtlenítésével* foglalkozó *berendezések***
   - palackmosó, ketrecmosó, állványmosó, műszermosó berendezés, mosóalagút
   - palacktöltő rendszer a fél-automatától a teljesen automatizált ürítő-mosó-töltő rendszerig
   - rozsdamentes acél átadó- és fertőtlenítő kabin a barrier sávhoz
   - autoklávok (25 litertől 8.712 literig), szárítószekrények (417 litertől 5.040 literig), gőzgenerátorok (16 kg/h-tól 1.450 kg/h-ig - pirogénmentes nagytisztaságú gőzt előállító is)
   - H_2O_2 HPV generátorok és rendszerek és fertőtlenítő kamra
3. ***Laborállat forgalmazás***
   - ENVIGO laborállatok forgalmazása
4. ***Labortechnológiai berendezések***
   - laborállat altatógépek (gáz vagy folyadék alapú), lélegeztető gépek, eutanázia berendezések
   - szövetbank, plazma és gyors fagyasztók -86ºC-ig, vérlemezke inkubátorok és keverők
   - laborhűtők +4ºC, kombinált labor hűtő-fagyasztók +4ºC/-20 ºC
   - plazma és őssejt kiolvasztók
   - laboratóriumi jégkészítők, hordozható hűtő-fagyasztó dobozok, inkubátorok széles választéka
   - klimatizált kamrák, szárító szekrények +130ºC-tól +300ºC-ig
   - légzuhanyok
5. ***Laboratóriumi és tisztatéri bútorok és mobíliák***
   - teljes műtői és vizsgálói rozsdamentes bútor és mobília választék, átadóablakokkal
   - laborbútor rendszer fém SPF falrendszerrel, nyílászárókkal
6. ***Laboratóriumi és tisztatéri fal- és padló rendszer***
   - tisztatéri fal rendszer (minden szükséges kiegészítővel, pl. reteszelt nyílászárókkal)
   - tisztatéri padló rendszer (fal és lefolyó illesztéssel, 5 rétegben, különböző igényekre)
7. ***Tisztító, mosó- és fertőtlenítőszerek forgalmazása***

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Hatékony mintatárolás rack rendszerben, optimalizált helykihasználással

Cryogenic Canister Freezers

Biztonságos, hosszú távú kriogén tárolási megoldások

Vapor Shippers

Mintaszállítás biztonságosan, folyékony nitrogén gőzfázisban

Dewars

Megbízható LN_2 tárolás laboratóriumi és ipari felhasználásra

High Capacity • High Efficiency • Advanced Cryogenic Technology

GENERAL INFORMATION

Onsite Registration

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza – Hotel Lobby

3rd June 2026, from 10 am

The organisers kindly ask participants to please come to the registration desk first. They are also kindly requested to wear their name badges until the end of the conference.

Presentations

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza, Kékes Room ("A" and "B")

Participants wishing to present a lecture are requested to prepare their presentation in MS PowerPoint format in English and hand it to the technician in the lobby of the auditorium at the latest during the last coffee break before the presentation. Presentations cannot be modified at this point. The presentations will be copied onto the computer by the technician colleague who will be available to the presenters during their presentation. The language of the presentation can be either English or Hungarian, depending on the needs of the audience and the presenter. Technicians will provide a control monitor, a wireless presenter including a laser pointer and a countdown timer monitor each room.

Poster session

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza, Galya Room and Foyer of the Galya Room on the 1st floor

Posters should be exhibited until 12.00 on Wednesday 3rd June and kept outside until 5.00 pm on Friday 5th June, the end of the Conference. Organisers will provide poster stands and all necessary equipment to hang the posters on it. Poster size: 90 cm (width) 150 cm (height).

The poster discussion will take place on Thursday afternoon, 4th June 2026. Poster presenters are kindly requested to be present in person during the Poster Session to answer questions from interested parties.

The Best Youth Poster Presentation Awards, selected by the Scientific Committee, will be presented during the Conference's closing ceremony.

Industrial Exhibition, coffee break

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza, Foyer of Kékes Room and Foyer on the 2nd floor

Lunches and Dinners

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza, Mátra Restaurant

Breakfast

All guests will be able to have breakfast in their own accommodation

Social programme

Morning refreshment run with the **Greiner Bio-One team** on 4th and 5th June at 7:00 am, meeting at the reception of HOTEL ÓZON & LUXURY VILLAS****.

Exhibitors' Gamification- 3rd and 4th June at the Industrial Exhibition areas

Thursday, 4th June 2026, 20:00 pm, Mátra Restaurant: **Gala dinner and dance**

Arrival, departure

To check in at the hotels, you must present your ID and address card. This rule applies to all accommodation in Hungary. Please make sure you are aware about your accommodation check in and check out time. These are available on the webpage. Check-out after the official check out time will incur an additional charge. Hotels will provide luggage room on both days.

Program code explanation

First letter of the name of days: W-Wednesday, T-Thursday, F-Friday

Second letter is the venue of the lectures: **K**- Kékes Room **KA**- Kékes Room A, **KB**- Kékes Room B

Numbers: 1st number: number of the section within a day, 2nd number: serial number within a section

Let’s start the day with us!

GREINER 5K Morning Run

HUPHAR2026

Come and run with us on **June 4th and 5th** in the beautiful natural surroundings of **Mátraháza.**

Departure is at 07:00 AM both days, meeting at the reception of Hotel Ózon. The program includes a refreshing run or jog of about 30 minutes.

Every participant will receive a gift!

For more information, visit the Greiner stand.

The Greiner team warmly welcomes everyone.

greiner

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THINCERT® 6-96 WELL SEJTTENYÉSZTŐ INZERTEK

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- Nagy áteresztőképességű transzport vizsgálatok és kokultúrák
- Kemotaxishoz és inváziós vizsgálatok
- Levegő-folyadék interfész (ALI) modellezés

M3D 6-1536 WELL MÁGNESES SEJTTENYÉSZTŐ RENDSZEREK

ALKALMAZÁS:

- Sceening /toxiciás vizsgálatok
- Daganat kutatás
- Szövet modellezés
- Őssejt kutatás
- Szövet rekonstrukció

TOVÁBBI INFORMÁCIÓÉRT KERESSE KOLLÉGÁINKAT AZ ALÁBBI ELÉRHETŐSÉGEKEN:

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Highlights 2025 – Edition: #02

standards

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- World Standard Month Webinar Series On Demand available now! **Special webinar series** featuring a distinguished line-up of speakers from leading pharmacopoeias, metrology institutes, and industry authorities. Engage with leading voices on quality, compliance, and evolving best practices relevant to laboratories, regulators, and professionals across sectors.
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columns

New Ascentis Express C18 pH+ and Bioshell Å120 Oligo C18 UHPLC columns

- **Ascentis Express C18 pH+** UHPLC columns are now available to enhance separation of basic coumpounds:
  - pH stability from 2- 12
  - Superficially Porous Particles SPP technology for supreme efficiencies at reduced back pressure
  - Suitable for all USP L1 applications
  - In analytical and guard columns
- **Bioshell Å120 Oligo** C18 UHPLC columns enable you superior separations of oligonucleotides at elevated pH:
  - pH stability from 2- 12
  - Special inert stainless steel housing to minimize interactions with the oligos
  - SPP technology for supreme efficiencies at reduced back pressure
  - Suitable for all USP L1 applications
  - In analytical and guard columns

solvents

Solvents solutions

- Top (U)HPLC **solvents now include serialized Data Matrix Codes** (DMCs) for unique bottle-by-bottle traceability:
  - One-click scan integration with your LIMS to register batch number, product ID, and expiry date for each bottle
  - Secure automated compliance documentation and real-time inventory management
- **New UHPLC-MS solvents** for PFAS allowing most sensitive and first-timeright analyses:
  - For all PFAS analytes by the EPA 533, EPA 537.1, and EPA 1633
  - For all these analytes their levels in our solvents are < LCMRL !
  - Superb APCI/ESI (+) and (-), metal, and PEG specifications

analytical solutions

Unlocking Insights for Your Success

MK_FL14945EN Ver. 1.0 66871 10/2025

CALL FOR EXHIBITORS' GAMIFICATION

Dear Participant,

Join our exhibitor prize draw for a chance to win one of **three valuable gift packages**, to be raffled during the Gala Dinner on 4th June.

How to participate:

1. Visit all exhibition stands on the 2nd and 3rd floor during the event.
2. Collect a stamp or signature from each exhibitor representative.
3. Based on the information you gather at the stands, answer the exhibitor-related questions on the entry form.
4. Submit your completed form at the Conference Registration Desk **by 5:30 p.m. on Thursday.**

Entry forms are available throughout the exhibition area and at the registration desk.
Participants who collect all required stamps/signatures and correctly complete the questionnaire will be entered into the prize draw.
Please note: prizes can only be claimed by winners who are personally present at the draw.

Thank you for participating, and we wish you an inspiring and enjoyable conference experience!

The organizers

NOVOLAB

NORMA Icon-3

Készülék jellemzői:

- Teljes vérkép egy percen belül
- Könnyen használható, egyszerűen kezelhető
- Kompakt méret, alacsony reagensfogyasztás és kis mintatérfogat
- Betegágy melletti (PoC) alkalmazás: intenzív osztályok, mentőautók, sürgősségi osztályok
- Kapilláris mintákból is képes mérni
- WIFI csatlakozási lehetőség (WIFI adapterrel)
- Remote Access: készülékdiagnosztikai célú, online távoli hozzáférés

Analitikai készülékeinket módszerfejlesztéssel támogatjuk a gyógyszergyártás szolgálatában

FEPTEST

A KROMAT CSOPORT TAGJA

PRECÍZIÓS TECHNOLÓGIÁK A KUTATÁS SZOLGÁLATÁBAN

Bio kasztel

A BIO-KASZTEL a HUPHAR2026 konferencián innovatív élettudományi és gyógyszerkutatási megoldásokat mutat be akadémiai, klinikai és ipari kutatások számára.

Kiemelt fókuszban a Bio-Rad Laboratories molekuláris biológiai technológiái állnak, melyek magyarországi kizárólagos disztribútora 2026-tól a BIO-KASZTEL.

Kiemelt technológiák

- Mutációkimutatási és biomarker kutatási megoldások
- Génexpressziós és onkológiai kutatási alkalmazások
- Fehérjeanalízis és képalkotó rendszerek
- Antitestek és immunológiai reagensek
- ELISA és biokémiai assay kitek
- Kis molekulájú vegyületek és vegyületkönyvtárak

QX700 Droplet Digital PCR System
Nagy érzékenységű ddPCR technológia precíziós kutatásokhoz és fejlett molekuláris analízishez.

PTC Harmony Thermal Cycler
Megbízható és intuitív PCR workflow kutatási és biopharma laboratóriumok számára.

Találkozzunk a HUPHAR2026 konferencián!

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Élvonal személyre szabva

Global Technology.
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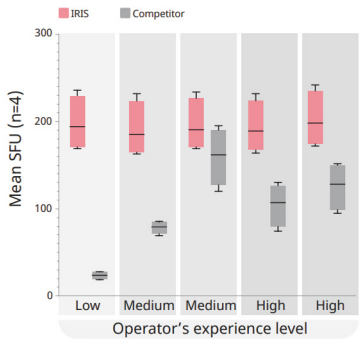
Spot your next discovery with Biomedica & Mabtech

- Increase your understanding of immune responses
- FluoroSpot and ELISpot technology is used to quantify the secretion profile under physiological conditions
- Cells in suspension are added to the wells of a plate and the secreted proteins of each individual cell are captured by specific antibodies immediately after secretion and throughout the stimulation process.

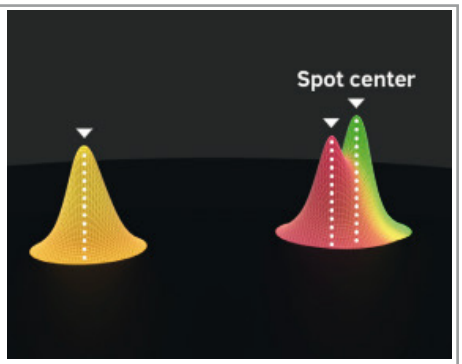
Fluorospot and ELISpot assays are best experienced with the Mabtech IRIS™ and Mabtech ASTOR™ readers

- Both readers are robust, have high throughput capabilities and are manufactured in Sweden
- Plug and play: Automated reader configuration and default analysis settings minimize user-defined subjective input and thus reduce bias of the operator
- User-friendly software: Easy to operate and train new users
- Multiplex ready: Up to 4 plex with Fluorospot
- CFR21 part 11 compliance



The secretion profile of every single cell

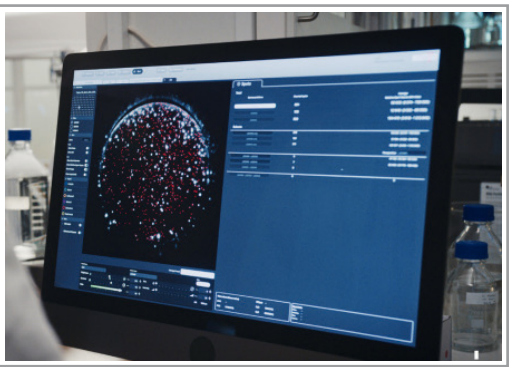
- By far the most striking feature of the readers is their novel spot-counting algorithm, RAWspot, which can identify spot centers with unseen accuracy
- The 3-dimensional model allows conclusions about the volume of each spot and provides a measurement for the amount of secreted analyte per cell, the relative spot volume (RSV).



Read once, adjust later

- Change count settings and experiment without affecting the originally captured RAW image data
- All signals from the spots are already recorded, so adjustments can be done off-line and you never have to re-read a plate

A new era of almost-too-easy!



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PROGRAMME

International Conference of Pharmacology, Drug Research and Development

HUPHAR 2026

MATRAHAZA **3-5, JUNE**

June 3, 2026 (Wednesday)

10:00-20:00		**Registration**
12:00-13:00		**Snack Lunch**
13:00-13:45		**Opening Ceremony** **Issekutz Lecture and Award Ceremony** **Knoll-Szolcsányi Award Ceremony** **Young Investigators Award Ceremony**
13:45-14:45		**"Drug of the Year" scientific presentation and award ceremony**
14:45-15:30		**Coffee break – Industrial Exhibition**
15:30-16:10	**W-KB1**	**PLENARY LECTURE 1 – Janne Backman** [1]Janne Backman [1]University of Helsinki, Finland ***Story of the Clinical Significance of the CYP2C8 Enzyme***
16:10-16:40	**W-KB2**	**PLENARY LECTURE 2 – Zoltán Giricz** [1,2]Zoltán Giricz [1]Semmelweis University, Hungary, [2]Pharmahungary Group, Hungary ***Research and development potential of extracellular vesicles***
16:50-17:50	**W-KA1**	**THE NEUROINFLAMMATIONAL RESEARCH NETWORK AROUND RICHTER - HOW OPEN INNOVATION COULD FACILITATE DRUG RESEARCH** Chair: Zsolt Némethy
	W-KA1-1	**[1]Zsolt Némethy (8')** [1]Department of Pharmacology and Drug Safety Research, Laboratory of Systems Biology, Gedeon Richter Plc., Hungary ***Scientific networks around Richter: collaborative efforts between basic and industrial science***
	W-KA1-2	**[1]Ádám Dénes (10'+3')** [1]HUN-REN Institute of Experimental Medicine, Hungary ***Exploring checkpoints of neuroinflammatory cascades in CNS disease states***
	W-KA1-3	**[1]Szilvia Benkő (10'+3')** [1]University of Debrecen, Faculty of Medicine, Department of Biochemistry and Molecular Biology, Inflammation Biology Research Group, Hungary ***In vitro macrophage models for drug research in neuroinflammation***

W-KA1-4 **[1]László Cervenák (10'+3')**

[1]Cell Biology and Cell Therapy Group, Department of Internal Medicine and Haematology, Semmelweis University, Hungary

Suppression of neuroinflammation by mesenchymal stem cells

W-KA1-5 [1]Eszter Geiszelhardt, [1,2]Melinda Gazdik, [1]Maissa Ben Mahmoud, [3,4]Júlia Tárnoki-Zách, [3,4]András Czirók, [1,2]Attila Szűcs, [1]Katalin Schlett, [1]**Krisztián Tárnok (10'+3')**

[1]Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University, Hungary, [2]Institute of Clinical Pathophysiology, Semmelweis University, Hungary, [3]Department of Biological Physics, Institute of Physics and Astronomy, Eötvös Loránd University, Hungary, [4]BioPhys-Concepts Kft., Hungary

Autism in petri-dish: investigations in 2D and 3D models

16:50-18:00 **W-KB3** **EXTRACELLULAR VESICLE THERAPEUTICS: BUBBLE OR REALITY?**
Chair: Csenger Kovácsházi

W-KB3-1 **[1]Pieter Vader (25'+5')**

[1]University Medical Center Utrecht, The Netherlands

Extracellular vesicles for nucleic acid delivery: from mechanistic insights towards therapeutic applications

W-KB3-2 **[1]Tamás Beke-Somfai**, [1]Tünde Juhász, [1]Katja Vasmatics, [1]Kamal et Battioui, [1]Sohini Chakraborty, [1]Imola Szigyártó, [1]Kamilla Ujvári, [1]Benjámin Kovács, [1]Tasvilla Sonallya, [1]Vignesh U. Nagaray asvilla Sonallya, [1]Tünde Juhász, [1]Imola Cs. Szigyártó, [1]Kinga Ilyés, [1]Zoltán Varga **(10'+5)**

[1]HUN-REN Research Centre for Natural Sciences, Hungary

Supramolecular antibiotics with vesicle manipulating action mechanisms

W-KB3-3 **[1,2]Csenger Kovácsházi**, [1,2]Dóra Kapui, [1,2]Yavuz Artuner, [1,2]Szabolcs Hambalkó, [1,2]Richárd Rácz [1,2]Teresa Hung, [1,2]Bennet Y Weber, [1,2]Kenan Aloss, [1,2]Tamás G Gergely, [3]Dániel Dobák, [4,5]Gemma Chiva-Blanch, [6]Joost PG Sluijter, [7,8,9]Edit I Buzás, [1,2,10]Péter Ferdinandy, [1,2,10]Zoltán Giricz **(10'+5)**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary, [3]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Hungary, [4]Health Sciences Faculty, Universitat Oberta de Catalunya (UOC), Spain, [5]Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Spain, [6]Department of Cardiology, Laboratory of Experimental Cardiology, Circulatory Health Research Center Utrecht, Regenerative Medicine Center Utrecht, Transplantation Center Utrecht, University Medical Center Utrecht, Utrecht University, the Netherlands, [7]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Hungary. [8]HCEMM-SU Extracellular Vesicle Research Group, Hungary, [9]HUN-REN-SU Translational Extracellular Vesicle Research Group, Hungary. [10]Pharmahungary Group, Hungary

Extracellular vesicle-based diagnostics and prognostics: currently applied technologies and unmet needs – insights from a scoping review on heart failure

W-KB3-4

[1,2]**Dóra Kapui**, [1,2]Szabolcs Hambalkó, [1,2]Yavuz Artuner, [1,2]Orsolya Szalai, [1,2]Sándor Zsidai, [1,2]Richárd Rácz, [1,2,3]Péter Ferdinandy, [1,2]Csenger Kovácsházi, [1,2,3]Zoltán Giricz **(8'+2')**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary, [3]Pharmahungary Group, Hungary

Separation of plasma extracellular vesicles from lipoproteins using density gradient ultracentrifugation and size exclusion chromatography for diagnostic applications

18:00-19:00 **Dinner - Mátra Restaurant**

19:00-21:00 W-KB4 **YOUNG INVESTIGATOR SESSION**
Chair: Éva Szőke, Zoltán Varga

W-KB4-1

[1]**Andrea Nehr-Majoros,** [1,3]Valéria Dezső-Tékus, [1]Rita Börzsei, [1,2]Csaba Hetényi, [1,2,3]Zsuzsanna Helyes, [1,2,3]Éva Szőke (10'+5')

[1]Department of Pharmacology and Pharmacotherapy, Medical School & Centre for Neuroscience, University of Pécs, Hungary, [2]National Laboratory for Drug Research and Development, Hungary, [3]Hungarian Research Network, Chronic Pain Research Group, Hungary

Lipid raft disruption as a novel analgesic strategy: multilevel evidence from neuropathic pain, cellular cholesterol dynamics and in silico modelling

W-KB4-2

[1,2,3]**Tünde Molnár**, [1,2,3]Viktória Szabó, [1,2,3]Boldizsár Jójárt, [1,2,3]Noémi Csákány-Papp, [4]Péter Hegyi, [1,2,3]Petra Pallagi, [1,2,3]József Maléth **(10'+5')**

[1]Department of Internal Medicine, Albert Szent-Györgyi Medical School, University of Szeged, Hungary, [2]Momentum Epithelial Cell Signaling and Secretion Research Group, Hungarian Academy of Science – University of Szeged, Hungary, [3]Molecular Gastroenterology Research Group, Hungarian Centre of Excellence of Molecular Medicine – University of Szeged, Hungary, [4]Centre for Translational Medicine, Semmelweis University, Hungary

Plasminogen activator inhibitor-1 as a therapeutic target in chronic pancreatitis

W-KB4-3

[1,2]**Rasha Ghanem Kattoub**, [1]Miklós Bege, [1]Erika Hevesi-Mező, [1]Anikó Borbás **(10'+5')**

[1]Department of Pharmaceutical Chemistry, University of Debrecen, Hungary, [2]Doctoral School of Pharmaceutical Sciences, University of Debrecen, Hungary

New class of uridine- and adenosine-based morpholino-nucleosides as potential anti-sars-cov-2 agents

W-KB4-4

[1]**Mohammad Izzeldeen Ali Bani Ata**, [1]Dima Fayiz Barakat Alsoub, [1]Minjin Ganbold, [1]Emese Ritter, [1]Eszter Kepe, [1]Máté Harmat, [5]Rainer Schulz, [5]Klaus-Dieter Schlüter, [6]Péter Ferdinandy, [1,2,3,4]Zsuzsanna Helyes, [1,2]Valéria Tékus **(10'+5')**

[1]University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy, Hungary, [2]PharmInVivo Ltd., Hungary, [3]Hungarian Research Network-University of Pécs, Chronic Pain Research Group, Hungary, [4]National Laboratory for Drug Research and Development, Hungary, [5]Institute of Physiology, Justus-Liebig University, Germany, [6]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary

UCP2 deletion exacerbates mechanical but not cold hypersensitivity in a chronic restraint stress rat model

W-KB4-5 [1]**Richárd Rácz**, [1]Szabolcs Hambalkó, [1]Nóra Wagner, [2]Jessica Whelan, [2]Junpeng Luo, [1]Dr. Zoltán Giricz **(10'+5')**

[1]Semmelweis University, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, Hungary, [2]University College Dublin, Biomanufacturing Research Group, Ireland

2D and 3D microcarrier grown mesenchymal stem cell derived extracellular vesicles comparison, functional study and their utilization for the reduction of chemotherapeutics-caused cardiotoxicity

W-KB4-6 [1,2,3]**Márk E. Jakab**, [1,2,3]Zsombor I. Hegedűs, [1,2,3]Tamás Gergely, [1,2,4]Péter Ferdinandy, [1,2,3]Zoltán V. Varga **(10'+5')**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [2]Center for Pharmacology and Drug Research and Development, Semmelweis University, Hungary, [3]MTA-SE Momentum Cardio-Oncology and Cardio-Immunology Research Group, Hungary, [4]Pharmahungary Group, Hungary

Cardiovascular safety of vista immune checkpoint inhibition: evidence from human heart failure and murine ischemia models

W-KB4-7 **Lecture by the Winner of the YoungPharm Oral Session**

June 4, 2026 (Thursday)

8:00-20:00		**Registration**
9:00-9:40	**T-KB1**	**PLENARY LECTURE 3 – Cherry Wainwright** **[1]Cherry L Wainwright** [1]School of Pharmacy, Applied Scences and Public Health, Robert Gordon University, UK ***GPR55 in cardiometabolic control: the good, the bad and the ugly***
9:40-10:00	**T-KB2**	**INDUSTRIAL LECTURE - BioTech Hungary Ltd.** **[1]Josef Uskoba** [1]BioTech a.s., Czech Republic ***Flow induced dispersion analysis (FIDA): A core technology for protein characterization and kinetics in-solution***
10:00-10:30		**Coffee break – Industrial Exhibition**
10:30-11:50	**T-KA1**	**UTILIZING 3D MODELS IN PHARMACOLOGY AND DRUG DISCOVERY** Chair: Ágota Apáti
	T-KA1-1	**[1,2]András Lakatos (20'+5')** [1]Department of Clinical Neurosciences, University of Cambridge, United Kingdom, [2]Cambridge University Hospitals, Cambridge Biomedical Campus, United Kingdom ***A 3D human CNS organoid platform for translational and therapeutic discoveries in neurodegeneration***
	T-KA1-2	**[1]József Maléth (15'+5')** [1]Momentum Epithelial Cell Signalling and Secretion Research Group; Department of Translational Medicine; University of Szeged, Hungary ***Gastrointestinal organoid culture in basic and translational studies***
	T-KA1-3	**[1]Fruzsina R. Walter**, [1]Judit P. Vigh, [1]Dorottya Kocsis, [1]Anna E. Kocsis, [1]Nóra Kucsápszky, [1]Ármin Szeles, [1]Emese Bató, [1,2]Sheila S. G. Fortes, [1,2]Luíza S.B.B. Góes, [1]Melinda Pirity, [1]András Dér, [1]Mária Deli **(15'+5')** [1]HUN-REN Biological Research Centre, Hungary, [2]Instituto de Química de São Carlos, Universidade de São Paulo, Brasil ***Advances in 3D blood-brain barrier models and brain organoids in drug testing across the blood-brain barrier***

T-KA1-4 [1,2]Alexandra Sándor, [3]Éva Bakos, [4]Attila Karsai, [1]**Kornélia Szebényi (10′+5′)**

[1]Metabolic Drug-interactions Research Group, Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Hungary, [2]Doctoral School of Molecular Medicine, Semmelweis University, Hungary, [3]Drug Resistance Research Group, Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Hungary, [4]Microtrade 2002 Kft., Hungary

Cerebellar organoids in live-cell screening applications

10:30-11:50 **T-KB3** **DRUG DEVELOPMENT AND MANUFACTORING**
Chair: Ildikó Bácskay

T-KB3-1 [1]**Dimitrios Lamprou (10′+5′)**

[1]Queen's University Belfast, UK

Microfluidic manufacturing of nanoparticles for drug delivery

T-KB3-2 [1]Oliwia Kordyl, [2]Antoni Białek, [1]Monika Wojtyłko, [2]Zuzanna Styrna, [3]Jolanta Dlugaszewska, [3]Dorota Kaminska, [4]Agata Nowak, [4]Barbara Jadach, [1]Anna Froelich, [5]Dariusz Młynarczyk, [6]Ariadna Nowicka, [7]Michał Gackowski, [8,9]Joanna Budna-Tukan,[10]Elżbieta Buczkowska, [10]Valentyn Mohylyuk, [11]Bozena Michniak-Kohn, [12]**Tomasz Osmałek (10′+5′)**

[1]3D Printing Division, Chair and Department of Pharmaceutical Technology, University of Medical Sciences, Poland, [2]Student's Research Group of Pharmaceutical Technology, The Student Scientific Society of Poznan University of Medical Sciences, Poland, [3]Chair and Department of Genetics and Pharmaceutical Microbiology, Poznan University of Medical Sciences, Poland, [4]Division of Industrial Pharmacy, Chair and Department of Pharmaceutical Technology, Poznan University of Medical Sciences, Poland, [5]Chair and Department of Chemical Technology of Drugs, University of Medical Sciences, Poland, [6]Institute of Materials Research and Quantum Engineering, The Faculty of Materials Engineering and Technical Physics, Poznan University of Technology, Poland, [7]Doctoral School, Poznan University of Medical Sciences, Poland, [8]Department of Immunology, Poznan University of Medical Sciences, Poland, [9]Department of Anatomy and Histology, Collegium Medicum, University of Zielona Gora, Poland, [10]Leading Research Group, Faculty of Pharmacy, Rīga Stradiņš University, Latvia, [11]Center for Dermal Research and Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, USA, [12]Chair and Department of Pharmaceutical Technology, Poznan University of Medical Sciences, Poland

Microneedle platforms for topical antifungal drug delivery using 3D printing and vacuum compression molding

T-KB3-3 [1,2,3]Réka Révész, [2]Dóra Nick, [2]Tamás Görbe, [2]**Ádám Haimhoffer (10′+5′)**

[1]Doctoral School of Pharmaceutical Sciences, University of Debrecen, Hungary, [2]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, Hungary; [3]Department of Industrial Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, Hungary

Development of hormone-containing vaginal film and applicator using QbD approach

	T-KB3-4	[1]**Tamás Sohajda**, [2]Balázs Kondoros **(10'+5')** [1]CarboHyde, Hungary ***A little cyclodextrin here, a little cyclodextrin there***
	T-KB3-5	[1]**Anna Katalin Baráne Gilicze (10'+5')** [1]Nemzeti Népegészségügyi és Gyógyszerészeti Központ, Hungary ***The regulatory landscape of phage therapies***
11:50-12:50	**T-KA2**	**STATE-OF-THE-ART OBSERVATION IF IN VIVO BEHAVIOUR IN SERVICE OF PHARMACOLOGY** Chair: Viktor Román
	T-KA2-1	[1,2,3]Hanga Kelemen, [1]András Buzás-Kaizler, [1,2]Gyula Y Balla, [1]Zsolt Borhegyi, [4]Kornél Demeter, [3]Miklós Szabó, [1]**Éva Mikics (10'+5')** [1]Translational Behavioural Neuroscience Research Group, HUN-REN Institute of Experimental Medicine, Hungary, [2]János Szentágothai Neuroscience Doctoral School, Semmelweis University, Hungary, [3]Department of Neonatology, Paediatric Clinic Bókay Street Department, Semmelweis University, Hungary, [4]Behavioral Studies Unit, HUN-REN Institute of Experimental Medicine, Hungary ***Inflammatory mechanisms underlying long-term cognitive deficits induced by perinatal insults***
	T-KA2-2	[1]**Attila Gáspár**, [1]Kristóf Kelemen, [1]Diána Kostyalik, [1]Viktor Román **(10'+5')** [1]Pharmacological and Drug Safety Research Division, Laboratory of In vivo Pharmacodynamics, Cognitive Pharmacology Laboratory, Gedeon Richter Plc., Hungary ***Investigating motivation in an automated home cage system in rats***
	T-KA2-3	[1]**Írisz Szabó**, [2]Yi-Shih Lee, [2]Hanga Dormán, [2]Eszter Birtalan, [2]Anita Bánhidi, [2]Diána Balázsfi, [3]Joshua I. Sanders, [1,2]Balázs Hangya **(10'+5')** [1]Center for Brain Research, Division of Neurophysiology, Medical University of Vienna, Austria, [2]HUN-REN Institute of Experimental Medicine, Hungary, [3]Sanworks LLC, USA ***Automated mouse training for behavioral pharmacology***
	T-KA2-4	[1,2]**Rafaella Mínea Riszt**, [1]Balázs Knakker, [1]Anna Padányi, [1]Judit Inkeller, [1]Evelin Kiefer, [1]Antonietta Vitális-Kovács, [1]Boglárka Böndicz, [3]Balázs Lendvai, [1,2]István Hernádi **(10'+5')** [1]Grastyán E. Translational Research Centre, and Centre for Neuroscience, University of Pécs, Hungary, [2]Medical School, University of Pécs, Hungary, [3]Department of Pharmacology and Drug Safety Research, Gedeon Richter Plc., Hungary ***Enhancing behavioural pharmacology in non-human primates: longitudinal profiling and multidimensional testing***

11:50-12:50 **T-KB4** **ADVANCES IN HUMAN-RELEVANT MODELS FOR DRUG AND DEVICE SAFETY TESTING**
Chair: István Baczkó, Bruno Podesser

T-KB4-1

[1]**Bruno K. Podesser**, [1]Helga Bergmeister, [1]Attila Kiss, [1]Ante Jukic, [1]Barbara Kapeller, [1]Manfred Bammer **(20'+5')**

[1]Divison of Drug & Device Testing, Center for Biomedical Research and Translational Surgery, Medical University of Vienna, Austria

Advances in human-relevant models for drug and device safety testing

T-KB4-2

[1,2,3]Djemail Ismaili, [4]Paavo Virtanen, [1,3]Junsoo Im, [1,3]Paul Brunnbauer, [1,3]Umber Saleem, [1,3]Muhammed Ikbal **Sönmez,** [1,2,3]Carl Schulz, [1,3]Margaret Nandudu, [5]Joris Winters, [3,6]Cristina E. Molina, [1,3,7]Johannes Petersen, [8,9]Monika Stoll, [5]Ulrich Schotten, [1,3]Arne Hansen, [1,3]Thomas Eschenhagen, [4]Jussi T. Koivumäki, [1,3]**Torsten Christ** **(20'+5')**

[1]Institute of Experimental Pharmacology and Toxicology, University Medical Centre Hamburg-Eppendorf, Germany, [2]Department of Cardiology, University Heart and Vascular Centre, Germany, [3]DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Luebeck, Germany, [4]BioMediTech, Faculty of Medicine and Health Technology, Tampere University, Finland, [5]Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht University, The Netherlands, [6]Institute of Experimental Cardiovascular Research, University Medical Center Hamburg-Eppendorf, Germany, [7]Department of Cardiovascular Surgery, University Heart and Vascular Center, Germany, [8]Division of Genetic Epidemiology, Institute of Human Genetics, University of Muenster, Germany, [9]Department of Biochemistry, CARIM School for Cardiovascular Sciences, Maastricht University, The Netherlands

Higher sensitivity to ouabain-induced toxicity in hipsc-cardiomyocytes than human adult heart tissue despite similar na^+/k^+-atpase pump current amplitudes

T-KB4-3

[1,2]**Norbert Nagy**, [1]Gergő Bitay, [3]Kálmán Benke, [3]Ali Alex Sayour, [3]Tamás Radovits, [4]Miklós Bitay, [1,2]András Varró, [1]István Baczkó, [3]Béla Merkely **(8'+2')**

[1]Department of Pharmacology and Pharmacotherapy, Szent-Györgyi Albert Medical School, University of Szeged, Hungary, [2]Department of Pharmacology and Pharmacotherapy, Szent-Györgyi Albert Medical School, University of Szeged, HUN-REN-SZTE Research Group for Cardiovascular Pharmacology, Hungary, [3]Semmelweis University Heart and Vascular Center, Hungary, [4]Second Department of Internal Medicine and Cardiology Center, University of Szeged, Hungary

Investigating the arrhythmogenic effect of action potential alternans in human heart failure

12:50-13:50 **Lunch – Mátra Restaurant**

13:50-14:20 **T-KA3** **KEYNOTE 1 – Eeva Moilanen**

[1]**Eeva Moilanen**

[1]The Immunopharmacology Research Group, Faculty of Medicine and Health Technology, Tampere University, Finland

Transient receptor potential ankyrin 1 (TRPA1) as a factor in inflammation

13:50-14:20 **T-KB5** **KEYNOTE 2 – Wan-Wan Lin**

[1]Wan-Wan Lin

[1]Department of Pharmacology, National Taiwan University, Taiwan

Diverse functions of calcium-calmodulin dependent protein serine kinase (cask) in health and diseases

14:20-15:50 **T-KA4** **NOVEL PROMISING DRUG TARGETS IN THE TREATMENT OF NEURODEGERATIVE DISORDERS AND PAIN**
Chair: Erika Pintér, Mahmoud Al-Khrasani

T-KA4-1 [1]**Dubravka Svob Strac**, [1]Barbara Vuic, [1]Tina Milos, [1]Lucija Tudor, [1]Marcela Konjevod, [1]Matea Nikolac Perkovic, [1]Gordana Nedic Erjavec, [1]Vladimir Farkas, [1]Nikola Balic, [2]Ana Knezovic, [2]Jelena Osmanovic Barilar, [3]Adrienn Szabo, [3]Szidonia Farkas, [3]Dora Zelena, [4]Suzana Uzun, [4]Oliver Kozumplik, [4]Ninoslav Mimica, [5]Alja Videtic Paska, [5]Katarina Kouter **(15'+5')**

[1]Ruder Boskovic Institute, Croatia, [2]Medical School, University of Zagreb, Croatia [3]Medical School, University of Pécs, Hungary, [4]University Psychiatric Hospital Vrapce, Croatia, [5]Medical School, University of Ljubljana, Slovenia

DHEA(s) and BDNF in neurodegeneration: neurobiology and therapeutic potential

T-KA4-2 [1]**Diana Cash (15'+5')**

[1]The BRAIN Centre, Neuroimaging Department, Institute of Psychiatry, Psychology and Neuroscience, King's College London, United Kingdom

Preclinical brain imaging in neuroscience and translational drug discovery

T-KA4-3 [1]**Dóra Zelena**, [1]Dorottya Várkonyi, [1] Erika Eliza Kvak Kvak, [1]Evelin Szabó, [1]Choi Muyong, [1]Szidónia Farkas **(15'+5')**

[1]University of Pécs, Medical School, Institute of Physiology, Szentágothai Research Centre, Centre for Neuroscience, Hungary

NK3 receptor and termoregulation in dementia

T-KA4-4 [1]**Viktória Kormos (10'+5')**

[1]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Hungary

Challenges and alternative methods in detection of trpa1 ion channel, a potential drug target in neurodegenerative disorders

T-KA4-5 [1,2]**Mahmoud Al-Khrasani**, [1]Amir Mohammadzadeh, [1,2]Anna Rita Galambos, [1,2]Sarah K. Abbood, [1,2]Judit Mária Kirchlechner-Farkas, [1,2]Imre Boldizsár, [1,2]Kornél Király, [2,3]**Éva** Szökő, [2,3]Tamás Tábi, [1,2]Ifj. Hársing G. László, [1,4]Ferenc Zádor **(10'+5')**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [2]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary, [3]Department of Pharmacodynamics, Semmelweis University, Hungary, [4]Pharmacological and Drug Safety Research, Gedeon Richter Plc, Hungary

Advancements in Glycine Transporter-1 Research: New Horizons in Pain Relief

14:20-15:20 **T-KB6** **GUT-DERIVED HORMONES – FROM BENCH TO BEDSIDE**
(at the end 20′ joint discussion)
Chair: Béla Juhász, Péter Bay

T-KB6-1 **[1]Gilberto De Nucci (10′)**

[1]Department of Pharmacology, School of Medical Sciences, University of Campinas, Brazil

Basal release of 6-nitrodopamine from rat gastric fundus and its role in smooth muscle relaxation

T-KB6-2 [1]Emese Tóth, [1]Patrik Kovács, [2]Péter Szűcs, [3]Tamás Csonka, [1]Dóra Pál, [1]István Révész, [1]Csaba Hegedűs, [1]Tibor Docsa, [1]Luca Anna Varga, [4]Éva Sebő, [5]Judit Tóth, [5]Péter Árkosy, [6]Dezső Tóth, [5]Andrea Bakó, [1]Éva Kerekes, [1]Petra Nyerges, [1]Edit Mikó, [7]Róbert Pórszász, [8]Kálmán Rácz, [8]Gergely Péter, [3]Gábor Méhes, [1]Uray Karen, [1, 9, 10, 11]**Peter Bai (10′)**

[1]Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Hungary, [2]Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Debrecen, Hungary; HUN-REN Neuroscience Research Group, Hungary, [3]Department of Pathology, Faculty of Medicine, University of Debrecen, Hungary, [4]Breast Center, Kenézy Hospital, University of Debrecen, Hungary, [5]Department of Oncology, Faculty of Medicine, University of Debrecen, Hungary, [6]Department of Surgery, Faculty of Medicine, University of Debrecen, Hungary, [7]Department of Pharmacology, Faculty of Medicine, University of Debrecen, Hungary, [8]Department of Forensic Medicine, Faculty of Medicine, University of Debrecen, Hungary, [9]HUN-REN Cell Biology and Signaling Research Group, Hungary, [10]MTA-DE Lendület Laboratory of Cellular Metabolism, Hungary, [11]Research Center for Molecular Medicine, Faculty of Medicine, University of Debrecen, Hungary

Catecholamine overproduction in breast cancer patients and its sequelae

T-KB6-3 [1]Beata Pelles-Tasko, [1,3]Daniel Varga, [1]Reka Szekeres, [1]Chun Sungmin, [1]Rita Kiss, [1]Vera Tarjanyi, [1]Daniel Priksz, [1]Mariann Bombicz, [1]Rudolf Gesztelyi, [1]Balazs Varga, [2]Sandor Somodi, [2]Zoltan Szabo, [1]Zoltan Szilvassy, [1,2]**Bela Juhasz (10′)**

[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Debrecen, Hungary, [2]Deparment of Emergency Medicine, University of Debrecen Clinical Centre, University of Debrecen, Hungary, [3]Department of Urology, University of Debrecen Clinical Centre, University of Debrecen, Hungary

Targeting cognitive decline in aging with anti-diabetic agents

T-KB6-4 **[1]Sándor Somodi (10′)**

[1]Department of Emergency Medicine, Faculty of Medicine, University of Debrecen, Hungary

Clinical experiences with agents acting on the incretin axis

15:20-15:50 **T-KB7** **KEYNOTE 3 – Lea Ann Dailey**

[1]Lee Ann Dailey

[1]Dept. of Pharmaceutical Sciences Division of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Austria

The drug delivery technologies behind RNA therapeutics

15:50-16:20		**Coffee break – Industrial Exhibition**
16:20-17:40	**T-KA5**	**ATMP DEVELOPMENTS AND REGULATIONS** Chair: András Dinnyés, Péter Ferdinandy
	T-KA5-1	[1,2]**András Dinnyés**, [1,2]Laura Colar Zanjko, [1]Andrea Balogh, [1]Kornél Kistamás, [1]Melinda Zana **(13'+3')** [1]BioTalentum Ltd., Hungary, [2]Department of Physiology and Animal Health, Institute of Physiology and Animal Nutrition, MATE, Hungary ***Regulatory progress towards novel cell and gene therapy ATMPs***
	T-KA5-2	[1,2]**Joel C. Glover (13'+3')** [1]Dept of Molecular Medicine, University of Oslo, Norway, [2]Norwegian Center for Stem Cell Research, Oslo University Hospital, Norway ***Best practice in Norway on dynamic consent and regulatory requirements for hipsc banks***
	T-KA5-3	[1]**Melinda Zana**, [3]Jo Huiqing Zhou, [1,2]András Dinnyés **(13'+3')** [1]BioTalentum Ltd, Hungary, [2]Department of Physiology and Animal Health, Institute of Physiology and Animal Nutrition, Hungarian University of Agriculture and Life Sciences, Hungary, [3]Molecular Developmental Biology, Faculty of Science, Radboud University, The Netherlands ***Stem cell therapy development for corneal epithelium regeneration: the stem-core horizon europe project***
	T-KA5-4	[1]**Zoltán Jakus (13'+3')** [1]Department of Physiology, Semmelweis University, Hungary ***Lymphatic vasculature-dependent and innate immune mechanisms in organ-specific responses to nucleoside-modified mRNA-LNP platforms***
	T-KA5-5	[1]**Péter Ferdinandy (13'+3')** [1]Center for Pharmacology and Drug Development, Semmelweis University, Hungary, [1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [1]Pharmahungary Group, Hungary ***Do we need multi-target drugs to modulate the gene expression profile of complex diseases? Development of miRNA therapeutics for cardioprotection***

16:20-17:40	**T-KB8**	**BRIDGING TRADITION AND INNOVATION - A SNAPSHOT OF PHARMACOLOGY TEACHING IN THE DIGITAL AND AI ERA – ROUNDTABLE DISCUSSION** Moderator: Zsófia Onódi **Topics**: Learning objectives and competencies Digitalization and AI in pharmacology education Assessments and evaluations Future of educators **Panelists:** **Pál Riba** – Semmelweis University, Hungary **István Baczkó** – University of Szeged, Hungary **Béla Juhász** – University of Debrecen, Hungary **Rudolf Gesztelyi** – University of Debrecen, Hungary **Erika Pintér** – University of Pécs, Hungary **Gábor Pethő** – University of Pécs, Hungary **Zsófia Onódi** - Semmelweis University, Hungary
17:40-19:40		**Poster Session - Galya Room and Foyer of the Galya Room on the 1st floor**
20:00-22:00		**Gala Dinner – Mátra Restaurant**
22:00-24:00		**Dance – Mátra Restaurant**

June 5, 2026 (Friday)

8:00-16:00 **Registration**

9:00-9:40 **F-KB1** **PLENARY LECTURE 3 – Maria José Diógenes**

[1]Maria José Diógenes

[1]Faculdade de Medicina, Universidade de Lisboa, Lisboa

Targeting TrkB-FL Cleavage: A Novel Therapeutic Strategy in Alzheimer's Disease and Epilepsy

9:40-10:40 **F-KA1** **NON-MAMMALIAN MODELS IN PHARMACOLOGICAL RESEARCH**
Chair: Zsolt Pirger, István Fodor

F-KA1-1

[1]Zoltán K. Varga, [1]Diána Pejtsik, [2]Dominika Csáki, [2]Dávid Czimer, [1]Tímea Csorvási, [1]Éva Mikics, [3]Ádám Miklósi, [2]**Máté Varga** **(15'+5')**

[1]Translational Behavioral Neuroscience Research Group, HUN-REN Institute of Experimental Medicine, Hungary, [2]Department of Genetics, ELTE Eötvös Loránd University, Hungary, [3]Department of Genetics, ELTE Eötvös Loránd University, Hungary

The use of complementary fish models to assess neuropharmacological agents

F-KA1-2

[1]**István Fodor**, [2]Veronica Rivi, [3]János Schmidt, [4]Anuradha Batabyal, [5]Ken Lukowiak, [2]Johanna M. C. Blom, [2]Cristina Benatti, [1]Zsolt Pirger **(15'+5')**

[1]HUN-REN Balaton Limnological Research Institute, Hungary, [2]University of Modena and Reggio Emilia, Italy, [3]University of Pécs, Hungary, [4]FLAME University, India, [5]University of Calgary, Canada

First evidence of an anxiety-like behavior and its pharmacological modulation in a widely used molluscan model

F-KA1-3

[1,2]**Luis Alfonso Yañez-Guerra**, [3]Tatiana D. Mayorova, [1]Li Guan, [4,5,6]Gáspár Jékely, [1,2]Herman Wijnen, [3]Adriano Senatore **(15'+5')**

[1]School of Biological Sciences, University of Southampton, UK, [2]Institute for Life Sciences, University of Southampton, UK, [3]University of Toronto Mississauga, Canada, [4]Heidelberg University, Germany, [5]Living Systems Institute, University of Exeter, UK, [6]Biosciences, Faculty of Health and Life Sciences, University of Exeter, UK

Large-scale pharmacological characterization of placozoan GPCRS reveals the prebilaterian origin of monoaminergic signalling

9:40-10:40 **F-KB2** **FOCUSING ON INFLAMMATION, A KEY DRIVER OF GASTROINTESTINAL AND OTHER DISEASES**
Chairs: Zoltán Zádori, Viktória Venglovecz

F-KB2-1 [1]Gergely Koszta, [1]Attila Ébert, [2]Tamás Gajdos, [2]Miklós Erdélyi, [2]Réka Ormos, [1]Mingjuan Li, [3,4,5,6]Péter Hegyi, [1,3,4]**Viktória Venglovecz** **(10'+5')**

[1]Department of Pharmacology and Pharmacotherapy, University of Szeged, Hungary, [2]Department of Optics and Quantum Electronics, University of Szeged, Hungary, [3]Translational Pancreatology Research Group, Interdisciplinary Center of Excellence for Research Development and Innovation, University of Szeged, Hungary, [4]Institute for Translational Medicine, Medical School, University of Pécs, Hungary, [5]Centre for Translational Medicine, Semmelweis University, Hungary, [6]Institute for Pancreatic Disorders, Semmelweis University, Hungary

Endocrine alterations in a mouse model of chronic pancreatitis

F-KB2-2 [1]**Dóra Zelena**, [1]Csilla Lea Fazekas, [1] Krisztina Bánrévi, [1]Bibána Török, [1]Adrienn Szabó, [2]Anita Bufa, [1]Kitti Mintál, [3]Peter Karaliev, [3]Daniela Jezova **(10'+5')**

[1]University of Pécs, Medical School, Institute of Physiology, Szentágothai Research Centre, Centre for Neuroscience, Hungary, [2]Institute of Bioanalysis, Medical School, University of Pécs, Hungary, [3]Biomedical Research Center, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Slovakia

Gut–brain axis alterations as a vulnerability substrate in a rat model of PTSD

F-KB2-3 [1]**Shie-Liang Hsieh (10'+5')**

[1]Immunology Research Center, National Health Research Institutes, Taiwan

The glycan–lectin axis in viral immunity: mechanisms of immune activation and immune suppression from dengue virus to HBV

F-KB2-4 [1]**Zoltán Zádori**, [1]Gerda Wachtl, [1]Arezoo Haghighi, [1]Zsuzsanna Demeter, [1]Anna Zsidai, [1]Klára Gyires **(10'+5')**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary

Toll-like receptor 5 engagement by bacterial flagellin mitigates tissue inflammation in nsaid enteropathy

10:40-11:00 **Coffee break – Industrial Exhibiton**

11:00-12:30 F-KA2 **NEW MODALITIES IN DRUG DISCOVERY AND DEVELOPMENT**
(at the beginning 5' introduction, at the and 5' joint discussion)
Chair: György M. Keserű

F-KA2-1 [1]**György. M Keserű (20')**

[1]Drug Innovation Center, HUN-REN Research Center for Natural Sciences, Hungary

Covalent approaches in drug discovery

F-KA2-2 [1]**András Kotschy (20')**

[1]Servier Research Institute of Medicinal Chemistry, Hungary

Protein degradation as a new therapeutic approach: from molecular glues to bifunctional degraders

F-KA2-3 [1]**Akos Tarcsay**, [1]Daniel Veres **(20')**

[1]Turbine Simulated Cell Technologies, Hungary

Antibody-drug conjugates: Emerging challenges and strategic opportunities

F-KA2-4 [1]**Judit Moldvay (20')**

[1]1st Department of Pulmonology, National Korányi Institute of Pulmonology, Hungary, [1]Pulmonology Clinic, University of Szeged, Albert Szent-Gyorgyi Medical School, Hungary, [1]Translational Oncopulmonology Research Group, Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Hungary

Development of mRNA vaccines against lung cancer

11:00-12:30 F-KB3 **EXPERIMENTAL PHARMACOLOGY AND NANOMEDICINE: A 60-YEAR PERSPECTIVE FROM THE BRAZILIAN SOCIETY OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS (SBFTE)**
Chair: Soraia K. P. Costa

F-KB3-1 [1,2]**Julia Sapienza Passos**, Giovanna B de Melo [1]Giovanna Salata, [1]João Augustinho Machado-Neto, [2]Alyssa Panitch, [1]Luciana B Lopes **(15'+5')**

[1]Department of Pharmacology, Institute of Biomedical Sciences, University of Sao Paulo, Brazil, [2]Georgia Institute of Technology, Department of Biomedical Engineering, USA

Cytotoxicity, molecular effects and tissue localization of multifunctional nanoparticles: influence of nanocarrier type

F-KB3-2 [1]**Rodrigo dos Anjos Miguel**, [1]Giovanna Barros de Melo, [1]João A Machado-Neto, [1]Glaucia Machado-Santelli, [1]Ana Paula Lepique A, [1]Luciana B Lopes, [1]Leticia V Costa-Lotufo **(15'+5')**

[1]Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, Brazil

Polymeric nanoparticles as tools to enhance seriniquinone translational potential

F-KB3-3 [1]**Giovanna Melo**, [1,2]Rodrigo Kawassaki, [2]Robson Guimarães, [1]Jessica Nunes, [2]Koiti Araki, [1]Luciana Lopes **(15'+5')**

[1]Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, Brazil, [2]Department of Fundamental Chemistry, Institute of Chemistry, University of São Paulo, Brazil

Unravelling the cytotoxic and molecular effects of a theranostic co-delivery nanosystem of methotrexate and iron decorated nanoparticles on breast cancer cell models as a novel strategy in breast cancer treatment

F-KB3-4 [1]Larissa Gonzaga Santos, [1]Jorge Dallazen, [1]Simone A Teixeira, [2]Mathew Whiteman, [3]Mariana Oliveira, [4]Fabiola T Mónica, [1]Marcelo N Muscará, [4]Edson Antunes, [1]**Soraia K P Costa (25'+5')**

[1]Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, Brazil, [2]Exeter Medical School, University of Exeter, United Kingdom, [3]Laboratory of Pharmacology, São Francisco University, Brazil, [4]Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, Brazil

Sex-independent role of hydrogen sulfide in bladder pain syndrome

12:30-13:50 **F-KA3** **MOLECULAR TARGETS IN CARDIOVASCULAR PHARMACOLOGY**
Chair: Ján Kyselovič, Rainer Schulz

F-KA3-1 [1]**Ľubica Janovičová**, [1]Michal Pastorek, [1]Peter Celec **(10'+5')**

[1]Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University, Slovakia

Cleavage of extracellular DNA - a treatment target

F-KA3-2 [1]**Andrea Gažová**, [1]Orsolya Hrubá, [1]Lucia Žigová, [1]Simona Valášková, [2,3]Nikola Chomaničová, [3]Peter Jackuliak, [3]Juraj Payer, [2,3]Ján Kyselovič **(10'+5')**

[1]Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Comenius University, Slovakia, [2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Slovakia, [3]5th Department of Internal Medicine, Faculty of Medicine, Comenius University, Slovakia

Pharmacotherapeutic and systemic factors influencing hospital mortality: a 13-year follow-up

F-KA3-3 [1,2]**Ján Kyselovič**, [3]Nikola Chomaničová, [1]Adriana Adamičková, [1]Zdenko Červenák, [2]Andrea Gažová **(10'+5')**

[1]5th Department of Internal Medicine, Faculty of Medicine, Comenius University, Slovakia, [2]Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Comenius University, Slovakia, [3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Slovakia

Pleiotropic antifibrotic actions of atorvastatin: Inhibiting fibroblast activation and enhancing cell therapy via dual modulation of GATA4/MEF2C and the microRNA axis

F-KA3-4 [1]**Rolf Schreckenberg**, [1]Nadja Itani, [1]Rainer Schulz, [2,3]Péter Bencsik, [2,4,5]Péter Ferdinandy, [1]Klaus-Dieter Schlüter **(13'+4')**

[1]Institute of Physiology, Faculty of Medicine, Justus-Liebig University Gießen, Germany, [2]Pharmahungary Group, Hungary, [3]Department of Pharmacology and Pharmacotherapy, University of Szeged, Hungary, [4]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [5]Center for Pharmacology and Drug Research & Development, Semmelweis University, Hungary

Roquin-dependent regulation of microRNA stability in cardiac remodeling after ischemic injury

F-KA3-5 [1]**Klaus-Dieter Schlüter (13'+4')**

[1]JLU Giessen, Physiologisches Institut, Germany

The impact of the mitochondrial uncoupling protein 2 on post-infarct remodeling

12:30-13:50	**F-KB4**	**SOFT TISSUE CALCIFICATION AS AN UNMET MEDICAL NEED** Chair: Flóra Szeri
	F-KB4-1	**[1,2,3]O.M. Vanakker MD, PhD (15'+5')** [1]Center for Medical Genetics, Ghent University Hospital, Belgium, [2]Department of Biomolecular Medicine, Ghent University, Belgium, [3]Ghent Ectopic Mineralization Research Group, Belgium ***From tank to treatment: how zebrafish drive innovation in ectopic mineralization therapeutic research***
	F-KB4-2	[1]Andrea Tóth, [1]Enikő Balogh, [1]Dávid Máté Csiki, [2]Árpád Szöőr, [3]Béla Nagy, [1]**Viktória Jeney (15'+5')** [1]Vascular Pathophysiology Research Group, Research Centre for Molecular Medicine, Faculty of Medicine, a, [2]Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Hungary, [3]Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Hungary ***HIF activation: an ethiopathogenic factor in cardiovascular calcification***
	F-KB4-3	**[1,2]Magnus Bäck (15'+5')** [1]Center for Molecular Medicine, Department of Medicine Solna, Karolinska Institutet; Department of Cardiology, Karolinska University Hospital, Sweden, [2]Department of Chronic and Acute Cardiovascular disease (DCAC), Université de Lorraine, Inserm; Department of Research and Innovation, Centre Hospitalier Régional Universitaire de Nancy, France ***Calcific aortic valve stenosis: Mechanistic insights enabling pharmacological therapeutic discovery***
	F-KB4-4	**[1]Flora Szeri (15'+5')** [1]Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Hungary ***Understanding pathomechanism and advancing anti-calcification therapy through rodent models***
13:50-14:30		**Lunch – Mátra Restaurant**
14:30-15:30	**F-KA4**	**TRENDS IN CLINICAL TRIALS: SHAPING THE FUTURE OF MEDICINE** Moderator: Renáta Papp, Péter Ferdinandy
	F-KA4-1	**Edit Szepessy (15')** ***EU and global trends in clinical research***
	F-KA4-2	**Ágnes Zita Hajdú (15')** ***Clinical trials in Hungary: The national regulatory perspective***
	F-KA4-3	**Kriszt Rókus(15')** ***Innovative management approaches in clinical research***
	F-KA4-4	**Zsolt Bagyura (15')** ***From data to practice: Real-world evidence at Semmelweis University***
15:30-16:00		**ROUNDTABLE DISCUSSION**

Topics:

Future of clinical research

Opportunities and challenges of Investigator Initiated Clinical Trials

Panelists:

Edit Szepessy - European Commission, Belgium

Kriszt Rókus - Sanofi Plc., Hungary

Ágnes Zita Hajdú - National Centre for Public Health and Pharmacy, Hungary

Zsolt Bagyura - Semmelweis University, Hungary

Viola Bardóczy - National Centre for Public Health and Pharmacy, Hungary

Ágnes Zotter - National Centre for Public Health and Pharmacy, Hungary

Anna Katalin Baráné Gilicze - National Centre for Public Health and Pharmacy, Hungary

Renáta Papp - Semmelweis University, Hungary

Péter Ferdinandy - Semmelweis University, Hungary

14:30-15:00 **F-KB5** **KEYNOTE 5 – Ramesh K. Goyal**

[1]Ramesh K. Goyal, Ph.D.

[1]Professor Emeritus, FRIGE-Institute of Human Genetic, Ahmedabad, Former Vice Chancellor, the Maharaja Sayajirao University of Baroda & Delhi Pharmaceutical Sciences and Research University, India

Pharmacology in next-gensequencing to next gen therapeutics

15:00-16:30 **F-KB6** **STRUCTURE-BASED DRUG DISCOVERY FOCUSING ON CRYO-EM**
Chair: Zsuzsanna Helyes, András Perczel

F-KB6-1 **[1,2]András Perczel (15'+5')**

[1]Laboratory of Structural Chemistry and Biology, Institute of Chemistry, ELTE Eötvös Loránd University, Hungary, [2] HUN-REN–ELTE Protein Modelling Research Group, Hungary

From dynamics to drug design: NMR reveals hidden, mutation-specific sites in KRas

F-KB6-2 **[1,2]Anna Júlia Kiss-Szemán (10'+5')**

[1]Laboratory of Structural Chemistry and Biology, Institute of Chemistry, ELTE Eötvös Loránd University, Hungary, [2]HUN-REN–ELTE Protein Modelling Research Group, Hungary

Cryo-EM as a tool to understand physiological drug-drug interactions

F-KB6-3 **[1,2]Dóra Karancsiné Menyhárd (10'+5')**

[1]*Laboratory of Structural Chemistry and Biology, Institute of Chemistry, ELTE Eötvös* Loránd University, Hungary, [2]HUN-REN–ELTE Protein Modelling Research Group, Hungary

Cryo-EM in optimizing diagnosis

F-KB6-4 **[1,2]Dániel Horváth (10'+5')**

[1]*Laboratory of Structural Chemistry and Biology, Institute of Chemistry, ELTE Eötvös* Loránd University, Hungary, [2]HUN-REN–ELTE Protein Modelling Research Group, Hungary

From risk to opportunity: Harnessing amyloid formation in GLP-1–related peptide drugs

F-KB6-5 **[1]Anass Jawhari (15'+5')**

[1]Thermo Fisher Scientific, Hungary

De-risking drug programs with cryoEM: faster answers, better decisions

16:00-16:30 **F-KA5** **KEYNOTE 5 – Bianca Rocca**

[1]Bianca Rocca

[1]Department of Medicine and Surgery, LUM University Casamassima, Italy

Surrogate Endpoints in Clinical Trials vs. Reality: Lessons from the Development and Withdrawal of Andexanet Alfa

16:30-16:50 **Closing of the Conference**

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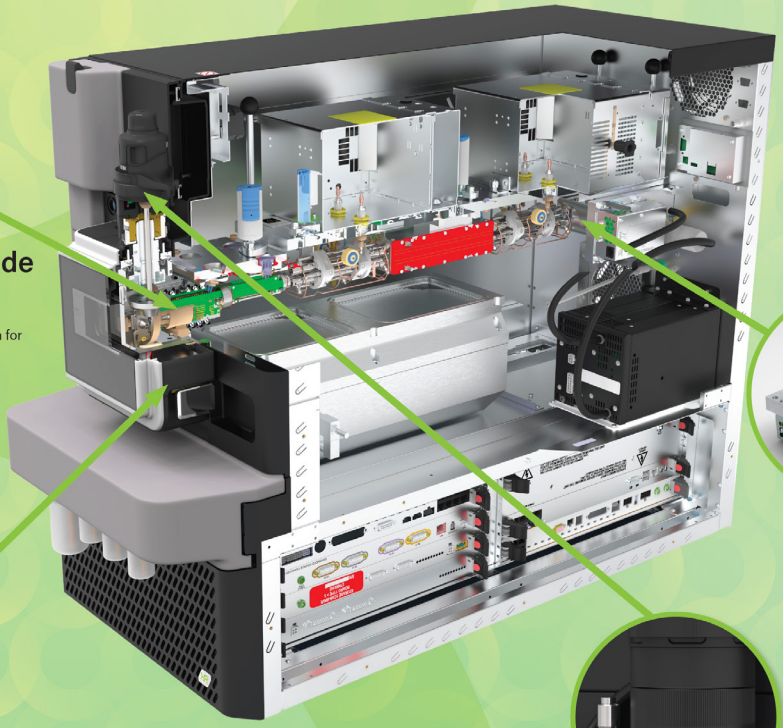

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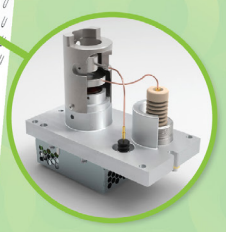

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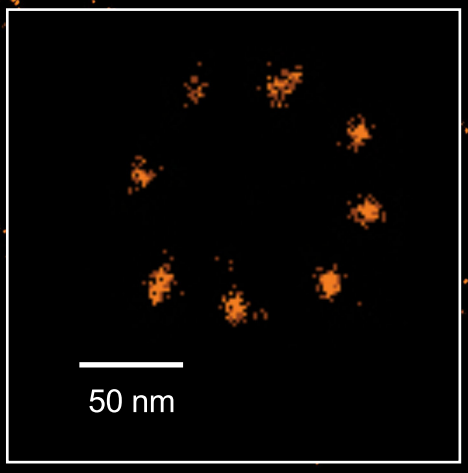
(a)



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A stabil U2OS NUP96 SNAP sejtvonalat Prof. Dr. Jan Ellenberg és Dr. Jonas Ries, EMBL Heidelberg biztosította

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ORAL ABSTRACTS

International Conference of Pharmacology, Drug Research and Development

HUPHAR 2026

MATRAHAZA **3-5, JUNE**

WEDNESDAY
3 JUNE 2026

W-KB1 CLINICAL RELEVANCE OF THE CYP2C8 ENZYME

[1]Janne Backman

[1]University of Helsinki, Finland

Over the past two decades, cytochrome P450 (CYP) 2C8 has become firmly established as a clinically important drug-metabolizing enzyme. Highly expressed in the human liver, CYP2C8 contributes to the metabolism of well over 100 drugs, including amodiaquine, cerivastatin, dabrafenib, daprodustat, dasabuvir, enzalutamide, imatinib, montelukast, paclitaxel, pioglitazone, repaglinide, rosiglitazone, and selexipag. A growing number of drugs and metabolites have also been characterized as CYP2C8 inhibitors or inducers. Both gemfibrozil and clopidogrel form major acyl-β-glucuronide metabolites that act as mechanism-based inhibitors of CYP2C8, rendering the parent drugs strong CYP2C8 inhibitors that interact with multiple CYP2C8 substrates and underlie several clinically significant drug–drug interactions. Several other glucuronide metabolites have likewise been shown to interact with CYP2C8 as substrates or inhibitors. Of note, daprodustat has recently been proposed as a selective and sensitive index substrate for CYP2C8.

A persistent challenge in the field has been the lack of fully selective and safe probe substrates, inhibitors, and inducers, which complicates the design and interpretation of drug–drug interaction studies. Beyond drug interactions, CYP2C8*2, *3, and *4 are associated with altered enzyme activity and display marked interethnic frequency differences, with clinically relevant consequences for selected substrates.

This talk will summarize current knowledge on the substrates, inhibitors, inducers, and pharmacogenetics of CYP2C8, and discuss its role in clinically relevant drug interactions, including practical implications for the selection of marker and perpetrator drugs in preclinical and clinical studies.

W-KB2 RESEARCH AND DEVELOPMENT POTENTİAL OF EXTRACELLULAR VESİCLES

[1,2]Zoltán Giricz

[1]Semmelweis University, Hungary, [2]Pharmahungary Group, Hungary

Extracellular vesicles (EVs) have emerged as a rapidly evolving field in medicine, offering novel opportunities for both diagnostics and therapeutics. EVs are membrane-enclosed nanoparticles released by virtually all cell types and participate in intercellular and interorgan communication through the transfer of bioactive cargo, including proteins, lipids, and nucleic acids. Advances in EV biology and technologies have substantially accelerated translational research efforts; however, significant scientific and developmental barriers still limit clinical implementation and market opportunities of EV-based solutions.

In this presentation achievements, challenges and promises will be summarized in EV research, as well as in diagnostic- and therapeutic use of EVs. Financial and regulatory aspects, technological possibilities, and avenues for future progress will also be highlighted, with special attention to the pharmacological applications.

W-KA1-1 SCİENTİFİC NETWORKS AROUND RİCHTER: COLLABORATİVE EFFORTS BETWEEN BASİC AND İNDUSTRİAL SCİENCE

[1]Zsolt Némethy

[1]Department of Pharmacology and Drug Safety Research, Laboratory of Systems Biology, Gedeon Richter Plc., Hungary

As an innovation-driven pharmaceutical company, Richter continuously evolves and aligns its R&D strategy with global trends, with a particular focus on CNS disorders. Over recent decades, fundamental research has increasingly underscored the importance of neuroinflammation - namely, the brain's inflammatory and immune responses - in a wide spectrum of neurological and psychiatric conditions. Although gaining a comprehensive understanding of neuroinflammation has become a strategic priority for Richter, the underlying mechanisms remain insufficiently elucidated. Advancing this field requires both the seamless integration of cutting-edge basic research findings into the drug discovery pipeline and access to a continuously updated, state-of-the-art technological toolkit.

Beyond scientific and technological progress, there is a pronounced need for effective integration between industry and academia through shared platforms that enable cross-disciplinary expertise to converge, fostering novel scientific breakthroughs with clear societal impact. Consequently, it has become critically important for Richter to establish an open laboratory and research framework that addresses these challenges while providing a unique competitive advantage for both the company and the broader Hungarian scientific community.

The newly developed scientific network and research facility - equipped with the most advanced technologies currently available - enable comprehensive neuroinflammation research by facilitating close collaboration between in-house experts and specialized external laboratories. At the same time, this framework ensures strategic oversight of discovery efforts and continuous assessment of emerging results.

W-KA1-2 EXPLORING CHECKPOINTS OF NEUROINFLAMMATORY CASCADES IN CNS DISEASE STATES

[1]Adam Denes

[1]HUN-REN Institute of Experimental Medicine, Hungary

Inflammation contributes to diverse brain disorders, but the exact mechanisms remain improperly understood. Microglia, the main inflammatory cells in the CNS show altered phenotypes in common neurological disorders, but how microglial modulation of neuronal- glial- and neurovascular processes changes in diverse disease states, is not well understood. We have identified novel forms of purinergic microglia-neuron interactions, through which microglia sense neuronal activity and injury to modulate neuronal function. Microglia also shape vascular responses via purinergic, compartment-specific actions, through which microglia modulate cerebral blood flow, neurovascular coupling and cerebral hypoperfusion. In the inflamed brain, altered microglia-neurovascular interactions are associated with perfusion changes and modulation of central leukocyte recruitment. By using advanced tools from in vivo imaging to spatial transcriptomics and molecular anatomy, we systematically assess the key molecules that contribute to neurovascular modulation by microglia in the mouse and the human brain, to understand how different neurological conditions affect microglial states and what functional consequences this may have on disease outcomes. Understanding the molecular mechanisms of microglial inflammatory transformation and interactions with other cells is likely to help the identification of novel therapeutic targets in common neurological disorders.

W-KA1-3 IN VITRO MACROPHAGE MODELS FOR DRUG RESEARCH IN NEUROINFLAMMATION

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Nod-like receptors (NLRs) are intracellular pattern recognition receptors (PRRs) that form protein complexes upon recognition of pathogen- or danger-associated patterns. They may form multiprotein complexes (inflammasomes) to regulate IL-1beta / IL-18 cytokines production and pyroptosis; or function as regulatory NLRs to modulate signal transduction pathways, metabolism, differentiation, cell division or cell death, which makes them ideal targets for pharmaceutical manipulations in various diseases. The main mediators of inflammatory processes are macrophages, which develop to various phenotypes depending on their origin, tissue localization or activation. While NLRs are intensively studied in the various populations of peripheral macrophages, the contribution of microglial NLRs to neuroinflammatory mechanisms in the brain is purely understood. We carried out comparative analysis of NLR/inflammasome function in *in vitro* differentiated macrophages modelling peripheral- and central cells. Our results show dynamically changing, cell-specific expression and function of NLRs, that highlight their potential usage in therapeutic interventions.

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W-KA1-4 SUPPRESSION OF NEUROINFLAMMATION BY MESENCHYMAL STEM CELLS

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Neuroinflammation can be a causative or accompanying process in most neurologic/psychiatric diseases. Suppressing neuroinflammation – especially within the central nervous system (CNS) – is challenging due to the CNS's unique immune cell composition and the blood-brain barrier. Although peripheral and neuroinflammation differ in many respects, there is substantial evidence that robust peripheral immunosuppression may stabilize BBB function and, consequently, reduce neuroinflammation. However, this requires a multitargeted anti-inflammatory therapy that does not leave the patient unprotected from pathogenic microbes.

One of these possibilities is the utilization of mesenchymal stem cells (MSCs). MSCs, in addition to their regenerative capacity, are potent anti-inflammatory cells that modulate T cells, NK cells, macrophages, dendritic cells, B cells, and other inflammatory cell types. Moreover, they can respond to different types of inflammatory conditions with unique, specific anti-inflammatory actions and can produce a broad range of antimicrobial compounds. Therefore, it is not surprising that, in addition to the many ongoing clinical studies using MSCs in neuroinflammatory conditions, MSC therapy has been approved by the South Korean drug agency for the treatment of amyotrophic lateral sclerosis.

As participants in the Richter Neuroinflammatory Network program, our aim is to establish highly standardized production and characterization of umbilical cord MSCs and to assess the molecular and cellular mechanisms by which MSCs suppress neuroinflammation.

We isolate MSCs from healthy umbilical cord Wharton jelly, propagate and characterize them *in vitro* according to the International Society for Cellular Therapy criteria. We have isolated and cryopreserved more than 100 individual MSC lines, providing a statistically sufficient basis for comparing their anti-inflammatory capacity. Immunosuppression is assessed using several functional and "omics" strategies, and the establishment of *in vivo* models has begun. Richter Neuroinflammatory Network program links us with neurology/neuroinflammation research groups, and MSCs or supernatant of MSCs can be tested using *in vitro* neuroinflammation models.

The use of MSC therapy has great potential. By standard production and characterization, and by precisely revealing anti-inflammatory mechanisms, MSC therapy may be applied to many other neuropsychiatric diseases.

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W-KA1-5 AUTİSM İN PETRİ-DİSH: İNVESTİGATİONS İN 2D AND 3D MODELS

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Kleefstra syndrome (KS) is a neurodevelopmental disorder associated with autism spectrum disorder (ASD), intellectual disability and hypotonia. The syndrome is caused by mutations in the EHMT1 gene, which plays a crucial role in gene expression. However, the impact of the functional loss of EHMT1 on the development of neuronal networks in humans remains unclear.

In this study, we modelled neuronal maturation and network formation using human iPSC-derived neurons from Kleefstra syndrome (KS) patients and neurotypical (NT) controls in both 2D and 3D cultures. Over a 9-week period, we performed longitudinal functional and structural characterization using patch-clamp recordings, Ca^{2+} imaging, multielectrode arrays (MEA) and immunocytochemistry. Our analyses revealed an accelerated maturation in KS-derived neurons. Specifically, KS cultures showed spontaneous excitatory postsynaptic currents (sEPSCs) from week 1 of development, whereas in NT-derived cultures, these appeared only from week 3. This functional acceleration was mirrored by structural changes; Ankyrin G immunostaining showed that KS neurons reached a stable, proximal axonal initial segment (AIS) localization two weeks earlier than NT controls. In line with patch clamp studies, using Ca^{2+} imaging, KS cultures demonstrated elevated network activity as early as the first week; in contrast, the NT cultures reached a comparable level only by the fourth week. Multielectrode array (MEA) measurements also showed a significant rise in network activity in the KS arrays also from the first week of induction. Notably, the activity in the KS cultures exhibited a decline towards the end of the observation period. To investigate developmental disparities in KS-affected nervous tissue, we established 3D spheroid cultures. KS spheroids exhibited accelerated initial growth but eventually lagged behind NT controls during later stages of development.

Together, our findings indicate that Kleefstra syndrome significantly influences network formation properties. This accelerated maturation and activity may represent a core mechanism where aberrant timing underlies the circuit dysfunction observed in Kleefstra syndrome and associated ASD.

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W-KB3-1 EXTRACELLULAR VESICLES FOR NUCLEIC ACID DELIVERY: FROM MECHANISTIC INSIGHTS TOWARDS THERAPEUTIC APPLICATIONS

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Extracellular vesicles (EVs) play a pivotal role in intercellular communication through various mechanisms, including transfer of bioactive cargo. Despite increasing interest in EVs as drug delivery vehicles, understanding of the pathways and mechanisms regulating EV uptake, targeting and cargo delivery is limited.
This talk will highlight some of recent insights into the efficiency of EV uptake and cargo transfer into target cells, and the molecular players involved in this process. In addition, it will show how EVs can be engineered for delivery of therapeutic nucleic acid cargo.

SUPRAMOLECULAR ANTIBIOTICS WITH VESICLE MANIPULATING ACTION MECHANISMS

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Recently it has become clear that bacteria have an affinity to release various extracellular vesicles as part of their regular function in colonies. However, should this occur at an increased pace, that could rapidly lead to destroy individual cells. We have recently designed a beta-peptide (named 3K) (1), which here we shot that it can be triggered into extensive nanonet formation by extracellular ATP, a molecular signal of hostile microbial attacks. 3K and ATP co-assembles into highly entangled 3D architectures of infinite fibrils, with high antibacterial activity against *E. coli*. The mechanism of antibacterial action was tracked by cryo-EM which has revealed that these fibrils not only entrap microbes by entangling them, but these fibrils also enforce various extracellular vesicles to be released from the bacteria, leading eventually to membrane disruption. An additional set of self-assembled tubular morphology, derived from a short membrane anchor motif, shows that membrane perturbation on the bacterial surface can cause release, and detachment of extracellular vesicles from the outer membrane of *E. coli*. These mechanistic insights could in turn provide better understanding of the natural molecular processes underlying e.g. the toxic behaviour of natural peptide nanonets, but these also suggest that enforcing increased amount of bacterial extracellular vesicles could be a viable strategy towards the development of conceptually new antimicrobial therapies.

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W-KB3-3 EXTRACELLULAR VESICLE-BASED DIAGNOSTICS AND PROGNOSTICS: CURRENTLY APPLIED TECHNOLOGIES AND UNMET NEEDS – INSIGHTS FROM A SCOPING REVIEW ON HEART FAILURE

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Heart failure (HF) is one of the leading causes of deaths worldwide, despite improving therapeutic and diagnostic options. Extracellular vesicles (EVs) are cell-secreted membrane vesicles, carrying a wide variety of molecular cargo, which is highly regulated in diseased conditions. Therefore, EVs are intensively studied as biomarkers in various diseases, including HF. This scoping review aimed to identify current evidence and limitations about potential EV-based diagnostics and prognostics in HF.

Records presenting data on blood-derived EVs from HF patients were collected from PubMed, Embase, Scopus and Web of Science databases. Applied EV-related technologies, technological and reporting rigor, EV-related outcomes and their potential benefit over canonical HF diagnostics were analyzed.

Sixty-one reports were included in the final data synthesis of which 49 cross-sectional, 15 interventional, 2 case-control and 6 cohort studies were identified. Technologies showed great variance, in which most commonly flow cytometry with- or without EV enrichment were applied. Most of the studies did not follow guidelines related to the analysis of EVs. Results suggest differences between HF and non-HF populations, indicating possible feasibility of EV-based analytical tools for HF, however, no superiority over established HF measures has been demonstrated so far.

In conclusion, results strengthen the hypothesis that EVs may serve as the basis for diagnostics or prognostics in HF, however, additional studies designed to provide stronger clinical evidence, including rigorous application of EV technologies are needed.

W-KB3-4 SEPARATION OF PLASMA EXTRACELLULAR VESICLES FROM LIPOPROTEINS USING DENSITY GRADIENT ULTRACENTRIFUGATION AND SIZE EXCLUSION CHROMATOGRAPHY FOR DIAGNOSTIC APPLICATIONS

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Background: Biomarkers in circulating extracellular vesicles (EVs) hold great potential for diagnostic applications. However, liporoteins (LPs) share overlapping physical properties and molecular cargo with EVs, which can obscure EV-related signals. Therefore, efficient separation of EVs and LPs is needed to increase the sensitivity and specificity of EV-based diagnostics.

Aim: Our aim was to develop a method which separates EVs from LPs from human blood samples with optimal purity, yield and time efficiency, and to apply it in samples of patients suffering from acute myocardial infarction (AMI).

Methods: EVs were isolated from 1 mL human platelet-free plasma using iodixanol density gradient ultracentrifugation (DGUC) followed by size exclusion chromatography (SEC) with Sepharose 4 Fast Flow (4FF) columns. Isolates were analysed using nanoparticle tracking analysis (NTA), Qubit protein assay and Western blot (WB).

Results: WB analysis of iodixanol DGUC fractions revealed significant overlap between LP and EV markers, demonstrating the need of further purification steps. After 4FF SEC of the EV-rich fractions, WB analysis revealed the presence of EV markers, while mild LP contamination also presented. Application of the total workflow needed two experimental days for 6 samples. NTA revealed the presence of EV-sized nanoparticles in the isolates, with a significantly larger size than LP samples. NTA and Qubit protein assay revealed no significant difference in EV sample concentration between AMI and non-AMI patients.

Conclusion: Combined DGUC followed by 4FF SEC demonstrated acceptable EV-LP separation and processing time for plasma EV isolation. No significant difference was observed in plasma EV numbers of AMI and non-AMI patients. Molecular analysis of the EV samples may highlight EV-related differences, that can be used for the development of future diagnostic-prognostic applications.

W-KB4 -1 LIPID RAFT DISRUPTION AS A NOVEL ANALGESIC STRATEGY: MULTILEVEL EVIDENCE FROM NEUROPATHIC PAIN, CELLULAR CHOLESTEROL DYNAMICS AND IN SILICO MODELLING

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Cyclodextrins (CDs) have recently gained attention as modulators of membrane architecture through their capacity to extract cholesterol from lipid raft microdomains. These domains contribute to the stabilization of several nociceptive signaling pathways. We previously showed that cholesterol depletion by CDs disrupts the ordered microenvironment of nociceptive receptors, reducing TRPA1 and TRPV1mediated acute pain. Here we investigated whether this membranetargeted mechanism also applies to chronic neuropathic pain by integrating behavioural data with cellular cholesterol assays and modelling.

In this study, we focused on three complementary approaches. First, we tested topically applied CD derivatives - random methyl β-cyclodextrin (RAMEB), (2-hydroxypropyl)-β-cyclodextrin (HPBCD) and sulfobutylether-β-cyclodextrin (SBECD) - in the Seltzer model of mononeuropathy in mice. We also examined cholesterol dynamics using filipin III labeling in fluorescence microscopy in two paradigms assessing the robustness and reversibility of CDmediated cholesterol extraction: in the overload condition, cells were preloaded with cholesterol before CD exposure, while in the restorative condition cholesterol was replenished afterwards. Finally, we incorporated *in silico* modelling to relate these findings to membrane structural changes.

Partial sciatic ligation induced sustained mechanical hypersensitivity (50–65% reduction in withdrawal thresholds). After topical treatment with RAMEB (3 mM), HPBCD (10 mM) or SBECD (10 mM) animals showed rapid mechanical improvement (20–60 min), while thermal sensitivity remained unchanged. In the overload condition, CDs produced a strong reduction in filipin signal even in cholesterolenriched cells, confirming potent extraction capacity. In the restorative paradigm, filipin fluorescence returned close to baseline, supporting reversibility. *In silico* analyses further revealed distinct cholesterolbinding modes: while HPBCD and SBECD accommodated cholesterol deeply within their cavities, RAMEB's more closed, methylsubstituted conformation enabled shallower binding and favored formation of a more stable 2:1 host–guest complex. These structural and energetic differences offer a plausible explanation for the superior cholesteroldepleting efficacy—and accompanying cytotoxicity—of methylated derivatives such as RAMEB.

Our results indicate that targeted modulation of membrane lipid environments preferentially influences the mechanical components of neuropathic pain. The combined behavioral, cellular, and modelling data support that CDs alleviate pain through selective disruption of cholesterolrich membrane microdomains, underscoring their potential as promising peripheral, nonopioid analgesic candidates.

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W-KB4 -2 PLASMİNOGEN ACTİVATOR İNHİBİTOR-1 AS A THERAPEUTİC TARGET İN CHRONİC PANCREATİTİS

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Background. Chronic pancreatitis (CP) is a progressive inflammatory disease characterized by pancreatic destruction and fibrotic tissue transformation. The incidence of the disease is steadily increasing, but no specific therapy is currently available, making the identification of new therapeutic targets a priority. Plasminogen activator inhibitor-1 (SerpinE1/PAI-1) has been implicated in various pathological processes, including fibrosis and inflammation. However, its specific involvement in the pathogenesis of CP is not yet fully understood.

We therefore aimed to explore the role of PAI-1 in the development of CP and assess the therapeutic potential of PAI-1 inhibitors as antifibrotic agents to prevent the progression of CP.

Method. PAI-1 expression was determined in human pancreatic tissue with immunohistochemistry, and blood serum with ELISA. CP was induced in FVB/N mice with cerulein injection, and PAI-1 activity was inhibited with a specific oral inhibitor at a dose of 10 mg/kgbw. Inflammation and fibrosis were assessed by histological staining and biochemical assay measured hydroxyproline concentration. SerpinE1 gene expression was quantified in pancreatic tissue and in isolated pancreatic cell types by RT-qPCR, PAI-1 protein levels and localization were assessed via immunohistochemistry and immunofluorescence co-staining. In vitro, fibroblasts derived from CP mice were treated with 10 µM PAI-1 inhibitor. Cell migration, cell number and cluster formation were quantified, and the gene expression levels of SerpinE1, Tgfb1, Fibronectin, and Acta2 were analysed by qPCR.

Results. PAI-1 expression was elevated in pancreatic tissue and blood samples obtained from CP patients. In CP-induced mice, the body weight and the pancreatic weight/body weight ratio significantly reduced, while histopathology showed significant pancreatic atrophy and fibrosis. This was also confirmed by the increased hydroxyproline concentration of the pancreatic tissue. SerpinE1 gene expression was upregulated in pancreatic tissue and fibroblast cultures in the CP group, while PAI-1 protein levels were elevated in pancreatic tissue. The elevated PAI-1 signal localization and the high overlap with vimentin suggests a possible expression role for activated pancreatic stellate cells (PSC). Administration of a PAI-1 inhibitor reduced body weight and pancreatic weight loss rates, along with significant decreases in pancreatic atrophy, fibrosis, hydroxyproline concentrations, and SerpinE1 gene expression compared to the CP group. In vitro, PAI-1 inhibition reduced cell migration, decreased cell number and number of cell clusters and lowered SerpinE1 and profibrotic gene expression in PSCs derived from CP mice.

Conclusion. Our results indicate an elevation in PAI-1 expression in CP, with the potential for fibrosis and tissue atrophy reduction by the inhibition of PAI-1. These findings propose PAI-1 as a promising therapeutic target for the treatment of chronic pancreatitis.

W-KB4 -3 NEW CLASS OF URIDINE- AND ADENOSINE-BASED MORPHOLINO-NUCLEOSIDES AS POTENTIAL ANTI-SARS-COV-2 AGENTS

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Over the past few decades, nucleoside analogs have been at the forefront of the search for novel therapeutic agents. One significant class of these analogs is morpholino-nucleosides, which have seen huge success in antisense-based treatment, with 4 FDA-approved drugs. Nevertheless, much less effort has been employed to explore the potential biological activities of substituted morpholino-nucleoside monomers. On the other hand, conformational restriction has been proven to be a highly effective method for enhancing the binding affinity of nucleos(t)ides to a specific target, thereby increasing biological activity. However, synthesizing such analogs is challenging due to the complex, multi-step synthesis and the need for precise control over regio- and stereochemistry. Herein, we present a novel reductive aminocyclization strategy that utilizes bifunctional amines with nucleoside secodialdehydes. This approach enables the single-step synthesis of a novel fused bicyclic, as well as a new type of *N*-substituted monocyclic morpholino-nucleosides (Fig. 1).

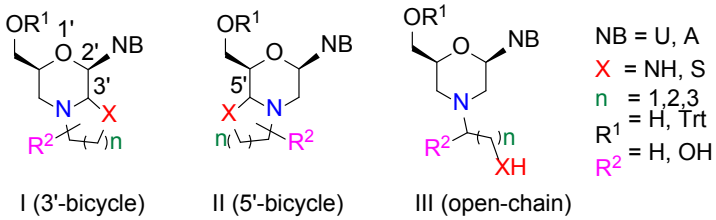

Fig.1 General Structure of novel morpholino-nucleosides

As a result of our thorough optimization of the reaction conditions, two major routes were proven to be effective - the $ZnCl_2$-mediated method and the glacial acetic acid strategy - affording a diverse range of 3'- and 5'-bicyclic morpholino-uridine and adenosine (General structures I and II, respectively), as well as open-chain products (General structure III). A total of 44 novel morpholino derivatives were synthesized and subsequently evaluated for their anti–SARS-CoV-2 activity. Among these, six bicyclic derivatives exhibited significant antiviral effects, with three compounds belonging to general structure I identified as the most potent. To elucidate their mode of action, multiple stages of the SARS-CoV-2 replication cycle were examined. The results indicate that all tested compounds act at the later stages of viral replication. Furthermore, molecular docking studies of these analogs with SARS-CoV-2 target proteins were performed, providing additional insight into their potential mechanisms of action.

W-KB4 -4 UCP2 DELETION EXACERBATES MECHANICAL BUT NOT COLD HYPERSENSITIVITY IN A CHRONIC RESTRAINT STRESS RAT MODEL

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Background & Aims: Chronic stress contributes to the development and maintenance of conditions associated with chronic pain—including fibromyalgia-, although the underlying mechanisms remain unclear. Uncoupling protein 2 (UCP) lowers mitochondrial membrane potential by uncoupling oxidative phosphorylation from ATP production and reducing reactive oxygen species (ROS). It is widely expressed in brain regions involved in stress and pain processing and in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis. Therefore, the aim of the present study is to investigate the role of UCP2 in chronic restraint stress (CRS)-induced pain and anxiety-like behaviour using gene deleted rats.

Methods: Chronic restraint stress (CRS; 4 hours/day for 14 days) was performed on UCP2 gene-deficient ($UCP2^{-/-}$) rats of both sexes compared to their wild-type (WT) controls (250-350 g, 10-14 weeks). Touch sensitivity was measured by using dynamic plantar aestesiometry, while cold sensitivity was detected by measuring the paw withdrawal latency from -1°C cold plate. Open field test (OFT) and light-dark box (LDB) were performed after the stress, and the weights of the thymus and adrenal glands were detected at the end of the experiment.

Results: CRS induced significant drops of the mechanonociceptive thresholds (between 15-30%) in both sexes of WT rats compared to their baselines, but this decrease was bigger in $UCP2^{-/-}$, mainly in females (40-50%). Significant stress-evoked cold hypersensitivity (27-30%) was observed without any sex- or genotype-dependent differences. Stressed $UCP2^{-/-}$ rats spent significantly more time in the center area (OFT) and in the light (LDB) compared to the stressed WT animals in both sexes. CRS increased the relative weights of the adrenal gland and reduced thymus weights.

Conclusion: Lacking UCP2, together with the presence of chronic stress, leads to mechanical but not cold hypersensitivity mainly in female rats, suggesting the possible relevant role of UCP2 in stress-induced pain conditions. Further analyses of the mitochondrial functions, ROS, and UCP2 gene/protein expressions might help to identify novel therapeutic targets in fibromyalgia.

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W-KB4 -5 2D AND 3D MICROCARRIER GROWN MESENCHYMAL STEM CELL DERIVED EXTRACELLULAR VESICLES COMPARISON, FUNCTIONAL STUDY AND THEIR UTILIZATION FOR THE REDUCTION OF CHEMOTHERAPEUTICS-CAUSED CARDIOTOXICITY

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Chemotherapeutic agents are widely used in the treatment of various cancers. However, their cardiotoxic side effects limit their clinical efficacy and compromise patient health. These drugs induce oxidative stress, inflammation, and cellular apoptosis in cardiac tissues, leading to cardiomyopathy, arrhythmias, and heart failure. As a result, strategies to mitigate these adverse effects are urgently needed to improve the quality of life and survival rates of cancer patients.

Mesenchymal stem cells (MSCs) have gained significant attention for their regenerative potential, partly attributed to their secretion of extracellular vesicles (EVs). MSC-derived EVs carry bioactive molecules, such as proteins, lipids, and RNAs, that can modulate cellular behaviour, promote tissue repair, and reduce inflammation. Recent studies have demonstrated the cardioprotective properties of MSC EVs, suggesting their potential to alleviate the cardiotoxic effects of a wide range of chemotherapeutics.

Mesenchymal stem cells are exclusively adherent; they can proliferate only once attached to a surface. For this reason, they can grow in 2D flask-based environments, 3D microcarrier-based spinner flasks, or bioreactors. 3D microcarrier systems allow for increased cell proliferation and improved EV release while still potentially maintaining the quality and functionality of the 2D systems.

We aim to validate and understand the therapeutic potential and differences between 2D and 3D MSC EVs in counteracting the cardiotoxicity induced by doxorubicin, 5-fluorouracil, trastuzumab, and lapatinib. Specifically, we explore the molecular mechanisms by which MSC EVs exert their protective effects, including modulation of oxidative stress, inhibition of apoptosis, changes in mitophagy and autophagy, and promotion of cardiac tissue regeneration while also measuring and comparing the health of the MSCs, their proliferation, EV production and the quality of their EVs (TP, TL, NTA, WB).

In conclusion, MSC-derived EVs represent a promising therapeutic strategy for reducing the cardiotoxic side effects of chemotherapeutics. Utilising the advantages of the 3D microcarrier-based systems, we can improve the proliferation of our MSC cells, increase their EV production, while the EVs still maintain their quality and functionality. By harnessing the natural regenerative properties of MSC EVs, it may be possible to enhance the safety and efficacy of cancer treatments, providing a vital improvement in patient outcomes.

W-KB4 -6 CARDIOVASCULAR SAFETY OF VISTA IMMUNE CHECKPOINT INHIBITION: EVIDENCE FROM HUMAN HEART FAILURE AND MURINE ISCHEMIA MODELS

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Introduction: V-domain immunoglobulin suppressor of T cell activation (VISTA) is a next-generation immune checkpoint currently undergoing clinical testing. However, cardiovascular safety data remains limited. Beyond T cells, VISTA influences innate immune responses and shows maximum inhibitory activity in acidic environments, coinciding with key characteristics of acutely ischemic myocardium, highlighting the importance of evaluating cardiovascular safety.

Aim: To characterize myocardial VISTA expression in human heart failure and to evaluate the cardiovascular safety of VISTA inhibition in healthy mice and in a model of acute ischemic cardiac injury.

Methods: Myocardial tissue was obtained at heart transplantation from patients with ischemic and non-ischemic heart failure. In a screening cohort, $n = 7$ samples per group were analyzed, followed by validation in 80 failing hearts. Non-failing control myocardium ($n = 6$) was obtained from organ donors whose hearts were not transplanted for technical reasons. Myocardial expression of selected co-inhibitory immune checkpoints, including VISTA, was quantified by Western blot and correlated with available clinical and functional parameters. In vivo safety was assessed in aged C57BL/6 mice and in an isoprenaline-induced catecholamine-mediated myocardial injury model. Healthy mice received anti-VISTA or isotype control for 4 weeks. In the ischemia model, injury was induced by isoprenaline, with anti-VISTA or isotype control administered before, during, and after injury. Cardiac function was assessed by echocardiography, strain analysis, and electrocardiography, and myocardial injury by histology.

Results: In failing human myocardium, PD-L1 expression was increased, and VISTA expression was uniformly reduced across both ischemic and non-ischemic heart failure. Myocardial PD-L1 correlated with LVEF ($r = -0.403$, $p = 0.002$), whereas VISTA expression showed no association with LVEF ($r = 0.016$, $p = 0.904$), cardiac structure, or hemodynamic parameters. In aged healthy mice, VISTA inhibition did not alter cardiac structure or systolic function compared with controls (LVEF: $61.52 \pm 10.04\%$ vs $61.14 \pm 8.28\%$, $p = 0.994$). Following pharmacological cardiac ischemia, VISTA inhibition did not exacerbate myocardial injury or functional impairment. Histological analysis demonstrated no increase in infarct extent or inflammatory cell infiltration.

Conclusion: Despite reduced myocardial VISTA expression in heart failure, this was not associated with cardiac dysfunction. VISTA inhibition did not induce adverse cardiac effects in healthy or acutely injured hearts in vivo. These findings address an important gap in cardio-oncology and support further clinical evaluation of VISTA as a next-generation immune checkpoint target.

THURSDAY 4 JUNE 2026

T-KB1 GPR55 IN CARDIOMETABOLIC CONTROL: THE GOOD, THE BAD AND THE UGLY

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GPR55 is an orphan receptor, originally considered to be a third cannabinoid receptor, however lysophosphatidyl inositol (LPI) is now regarded to be its endogenous ligand. The GPR55/LPI system signals through multiple pathways, including RhoA/ROCK and ERK, and is present in multiple tissue, including those involved in cardiovascular and metabolic control. Mice with a gene deletion for GPR55 exhibit an obese phenotype, are hypertensive and develop ventricular remodelling and associated reduced diasolic function and contractile reserve, possibly through interference with adrenoceptor signalling, indicating that GPR55 plays an integral role in maintaining normal cardiac function. However while acute activation of GPR55 does not modify cardiac performance in normal wild type mice, chronic stimulation administration of a GPR55 agonist results in increased end systolic and diastolic volumes, elevated stroke volume and augmented stroke work in WT, alongside reductions in the 18:0 and 18:2 isoforms of LPI.

In a pathophysiological setting, while a lack of GPR55 *per se* has no effect on cardiac injury resulting from ischaemia/reperfusion (I/R), acivation of GPR55 by LPI significantly increases myocardial infarct size through activation of ROCK, suggesting that under ischaemic conditions GPR55 activation is detrimental. However, exposure of GPR55 KO mice to a high fat diet (HFD) abrogates the protective effects of HFD I/R induced myocardial injury seen in wild type mice (obesity paradox), an effect associated with significant alterations in the composition of LPI isoforms present in both cardiac tissue and plasma. This presentation will provide a summary of the data showing that the GPR55/LPI system has contrasting effects in cardiometabolic control and explore the potential mechanisms underlying these complex findings.

T-KB2 FLOW INDUCED DİSPERSİON ANALYSİS (FIDA): A CORE TECHNOLOGY FOR PROTEİN CHARACTERİZATİON AND KİNETİCS IN-SOLUTİON

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Over the years FIDA has become one of the most versatile technology capable of characterizing practically all possible options in protein research. Mainly QC of the sample, kinetics, oligomeric state, yes/no binding. The technology also found its space in liposome, lipid nanoparticle and extracellular vesicles research. The most interesting feature is complete characterization of kinetic constants like affinity, association constant and dissociation constant which is not possible with any other in solution techniques.

T-KA1-1 A 3D HUMAN CNS ORGANOİD PLATFORM FOR TRANSLATİONAL AND THERAPEUTİC DİSCOVERİES İN NEURODEGENERATİON

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Animal models and two-dimensional human cell culture platforms do not faithfully recapitulate the human-specific aspects of cell diversity or complex cell interactions relevant to neurodegenerative pathobiology. This issue has hampered therapeutic advances, which is an urgent unmet need, especially for amyotrophic lateral sclerosis (ALS), a fatal disease affecting the corticospinal motor system. To overcome this problem, we have developed a novel human multicellular, multiregional, translational in vitro model.

Here, we generated a three-dimensional functionally connected human corticospinal organoid platform. This can be grown from ALS patient-derived pluripotent stem cells harbouring the C9ORF72 mutation or its mutation-corrected variant allele. Leveraging a combination of cutting-edge long- and short-read single-cell multiomics alongside a comprehensive suite of biological assays, we investigated early glia- and neuron-specific vulnerabilities.

We demonstrate that this complex in vitro model consistently reproduces cortical and spinal cord tissue architecture, captures broad cell-type diversity, and recapitulates early ALS pathological hallmarks. Specifically, we identified distinct stress responses in astrocytes and corticospinal neurons marked by impaired proteostasis, including TDP-43 mislocalisation and downstream disruptions to transcription, RNA processing, and DNA repair — collectively increasing susceptibility to secondary somatic mitochondrial DNA mutations and driving cell loss. As a proof-of-principle, we further demonstrate that drug-based targeting of the unfolded protein response can mitigate these early pathological processes.

Altogether, our findings highlight initial steps in both cell-autonomous and previously uncharacterized non-cell-autonomous disease processes, establishing a unique human translational precision platform for ALS research. We propose that our corticospinal organoid system offers an unprecedented opportunity for novel therapeutic target identification and pre-clinical drug testing, which could inform ongoing and future clinical trials.

T-KA1-2 GASTROINTESTINAL ORGANOID CULTURE IN BASIC AND TRANSLATIONAL STUDIES

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Organoids are three-dimensional cell culture systems derived from pluripotent stem cells or tissue-specific adult stem cells, that self-organize into miniaturized, organ-like structures recapitulating key features of the native tissues. These advanced models preserve cellular diversity, architecture, and function absent in traditional two-dimensional cultures, bridging the gap between *in vitro* experiments and patient physiology. The presentation is intended to provide a comprehensive overview of the rapidly evolving field of organoids with a special focus on the application of organoids in gastrointestinal research. We will cover foundational concepts such as organoid definitions and types, and summarize the derivation of organoid models from gastrointestinal tissues (such as intestine, stomach, liver and pancreas) as well as from other tissues. The emerging role of organoids in basic research and clinical applications will be discussed in broad terms, including their use in studying cell physiology, predicting therapeutic responses (personalized medicine) and modeling gastrointestinal diseases *in vitro* for research and diagnostics. Organoid cultures are also suitable for establishing organoid biobanks that will be also introduced in the presentation.

T-KA1-3 ADVANCES IN 3D BLOOD-BRAIN BARRIER MODELS AND BRAIN ORGANOIDS IN DRUG TESTING ACROSS THE BLOOD-BRAIN BARRIER

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Microfluidic lab-on-a-chip devices allow complex and physiological modelling of the central nervous system (CNS) and the blood-brain barrier (BBB). The use of *in vitro* cell culture models is essential in drug development to study the penetration of potential drug compounds across biological barriers. The use of cell cultures derived from human-induced pluripotent stem cells (iPSCs) and the creation of human brain spheroids and organoids from these cells is a novel trend in the non-animal method field. Brain organoids replicate key aspects of brain architecture and function, therefore they have a fundamental role in studying toxicity and effects of drug candidates, and in modeling neurodevelopmental and neurodegenerative disorders. Innovative microphysiological systems can mimic the fluidic environment of brain vessels, allowing more accurate analysis of drug transport across the BBB. With the use of brain organoids, we tested the passage of nanocarriers across the BBB and the entry of guest molecules to the tissue (Veszelka et al, 2021; Mészáros et al, 2023). We also have developed a versatile microfluidic lab-on-a-chip device to model biological barriers (Walter et al, 2016). We have advanced this microelectronic system by combining a human stem cell-based dynamic blood-brain barrier model with iPSC-derived brain organoids. With this we have built a unique platform to study the penetration of molecules across the blood-brain barrier together with tracking the entry of the agents into brain organoids. Recently a hydrogel-based micro device was optimized to our laboratory conditions to study neuroinflammation. We are convinced that lab-on-a-chip systems, brain organoids and their combination are revolutionizing neuroscience and drug development by providing more accurate, human-relevant testing platforms and they also pave the way to personalized medicine.

Funding: The project was supported by the National Research, Development and Innovation Office (NKFIH), NNE-129617, OTKA-K 143766 and ERA-NET-Neuron, B3Phrenia (to M.A.D.), OTKA-PD 138930 (to M.M.), and the NKKP Advanced Grant-153360 (to F.R.W.). Also by the Secretariat of the Hungarian Research Network (former ELKH, SA-111/2021 to F.R.W.) and the Hungarian Academy of Sciences Lendület „Momentum" Program (to F.R.W., LP2025-22/2025). The National Council for Scientific and Technological Development (CNPq), process no. 200562/2025-8 provided the support for the research stay of two guest PhD students.

T-KA1-4 CEREBELLAR ORGANOIDS IN LIVE-CELL SCREENING APPLICATIONS

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Given how different the structure, size and composition of the human cerebellum is compared to other species, the establishment and application of human model systems are essential for studying the molecular mechanisms of neurodegenerative diseases, such as spinocerebellar ataxias (SCAs), specifically affecting this brain region. Human induced pluripotent stem cell (hiPSC)-derived cerebellar organoid systems contain disease relevant cell types and therefore can provide a platform for investigating pathomechanisms when spinocerebellar ataxia-like neurodegeneration is initiated.

First, we developed a simplified protocol for the generation of hiPSC-derived cerebellar organoids (CerO). The cell types found in the CerOs were characterized at different time points by immunocytochemical methods, using markers specific to the developmental stages of the cerebellum. The CerOs show tissue like organization, comprise Kirrel2-positive Purkinje progenitors which later give rise to Calbindin-positive Purkinje neurons. After 50 days of differentiation, astroglial cells emerge. To prove that the model is also suitable for detecting pathological changes, we treated the organoids with IL-1β, capable of inducing ataxia in mice and shown to be secreated at higher level in SCA-1, -2 and -7 patients compared to healthy individuals. IL-1β treatment evoked ER stress and increased the expression level of the ubiquitin-binding protein P62. CRISPR/Cas9 gene targeting was used to establish a reporter iPSC line allowing live cell detection of changes in P62 expression levels upon the administration of IL-1β alone or in combination with various molecules known to reduce different types of ER stress. A JNK inhibitor was capable to rescue the Purkinje neuron-specific pathology, while the activation of astroglial cells was not affected, suggesting the need for combinational therapeutic approaches.

In summary, we have created an organoid model of the human cerebellum, which is suitable for examining cell-specific pathological changes in live cell applications and can serve as a platform for the development of therapies targeting them.

T-KB3-1 MİCROFLUİDİC MANUFACTURİNG OF NANOPARTİCLES FOR DRUG DELİVERY

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Microfluidic technologies have emerged as powerful platforms for the controlled and scalable manufacturing of nanoparticles for drug delivery applications. By enabling precise manipulation of fluids at the microscale, microfluidic systems offer unparalleled control over mixing, reaction kinetics, and particle self-assembly. This results in nanoparticles with highly uniform size distributions, improved encapsulation efficiency, and reproducible physicochemical properties. Compared to conventional batch methods, microfluidic approaches enhance formulation tunability, reduce material waste, and facilitate rapid optimization of therapeutic payloads. This presentation highlights key principles, recent technological innovations, and future opportunities for microfluidic manufacturing as an enabling platform for next-generation drug delivery systems. The talk will cover lipid based and polymeric nanoparticles, and mRNA delivery carriers. Additionally, it will highlight benefits such as precise size control, reproducibility, and scale-up towards GMP.

T-KB3-2 MİCRONEEDLE PLATFORMS FOR TOPİCAL ANTİFUNGAL DRUG DELİVERY USİNG 3D PRİNTİNG AND VACUUM COMPRESSİON MOLDİNG

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Introduction: Topical therapy is first-line for many cutaneous mycoses, however clinical outcomes are often limited by poor drug penetration through the stratum corneum and insufficient drug levels at the infected site. Microneedle (MN) platforms can transiently disrupt the barrier and enhance localized delivery while potentially reducing the need for systemic antifungals administration. The aim is to present two clinically oriented MN approaches enabled by additive manufacturing: i. 3D-printed solid MN arrays coated with clotrimazole (CLO) and ii. voriconazole (VRC)-loaded dissolving MNs (DMNs) fabricated using 3D printing and vacuum compression molding (VCM).

Materials and methods: Solid MN masters were produced by LCD-based 3D printing and optimized for geometry and sharpness; arrays were drug-loaded by coating with 1% CLO hydrogels (ethanolic solution vs. suspension). Separately, DMN masters (3D-printed) and PDMS molds were used to form Soluplus®-based DMNs containing 1% VRC by VCM. Systems were characterized for dimensions, mechanical/insertion performance (Parafilm®M, agarose, and ex vivo human skin with histology), drug content and in vitro release. Clinically focused endpoints included antifungal activity against clinical Candida albicans isolates and preliminary safety screening (Microtox®).

Results: For CLO-coated solid MNs, coating enabled drug loading in the range ~0.17-0.24 mg per array depending on geometry/coating type, with rapid early release up to ~66–91% within 60 min and sustained release up to 12 h. Insertion testing confirmed penetration through skin-mimicking barriers and deep perforation in gel models, supporting the ability to bypass the stratum corneum. Microbiological assays demonstrated growth inhibition versus C. albicans.

For VRC-loaded DMNs, sharp tips and effective insertion were confirmed. Histology showed reproducible microchannels in human skin. DMNs dissolved completely within ~12.5 min and released drug rapidly. *Ex vivo* studies showed localized deposition in epidermis and dermis. Antifungal activity was pronounced against both clinical and fluconazole-resistant *C. albicans*.

Conclusions: 3D printing enables precise, customizable MN geometries that can be translated into clinically relevant antifungal platforms either via surface coating (solid MNs) or VCM-fabricated dissolving MNs. Together, these findings - human skin microchannel formation, rapid drug release from DMNs, strong activity against clinical and azole-resistant *Candida* and localized skin deposition with minimal transdermal permeation - support the translational potential of these microneedle systems for improving topical antifungal therapy.

T-KB3-3 OF HORMONE-CONTAINING VAGINAL FILM AND APPLICATOR USING QBD APPROACH

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The cause of menopause is the disruption of hormonal homeostasis, which is accompanied by a decrease in oestrogen levels. These changes may lead to symptoms and diseases, including cardiovascular alterations, osteoporosis, hot flashes, vaginal dryness, and decreased libido [1]. Although various forms of hormone therapy are available, none provide a comprehensive therapeutic solution, therefore, the demand for innovative medicinal products is continuously increasing.

The aim of the present research is to develop an oestrogen-releasing vaginal film and applicator that ensure appropriate bioavailability of the active pharmaceutical ingredient through a cyclodextrin–polymer complex, while also locally alleviating vaginal dryness. The formulation development was carried out based on the Quality by Design (QbD) approach, supported by a questionnaire-based survey. Critical quality attributes (pH, tensile strength, and moisture content) were optimized using factorial experimental design. The pH range of the prepared film corresponds to the physiological values of the vaginal environment, its low moisture content ensures stability, and its high tensile strength allows application using an applicator. The applicator was manufactured by 3D printing, taking user preferences into account and improving the functionality of previous devices. In conclusion, the developed oestrogen-containing vaginal film and applicator may represent an innovative therapeutic alternative that effectively alleviates the most common symptoms of menopause, thereby contributing to an improvement in women's quality of life.

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T-KB3-4 A LİTTLE CYCLODEXTRİN HERE, A LİTTLE CYCLODEXTRİN THERE

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Cyclodextrins (CDs) are everywhere. This presentation offers an outlook of currently marketed CD-containing drug products, with a specific emphasis on APIs that are commercialized in more than one dosage form or administered via different routes. A key focus is placed on identifying and comparing those APIs that exist in multiple CD-based formulations, and different types of CDs are employed depending on the formulation needs.
By reviewing data from scientific publications, review articles, and patent documents, we aim to uncover formulation strategies that use the versatility of CDs across various pharmaceutical platforms. The presentation discusses the choice of CD types in each dosage form, as well as how their physicochemical and regulatory properties influence product development. Trends in dosage forms, routes of administration, therapeutic indications, and the role of CDs in overcoming formulation challenges are also explored in detail.

Finally, we will share exciting new areas for the use of cyclodextrins in formulations of vaccines, antibodies and innovative delivery technologies.
This analysis aims to inform and inspire formulation scientists and developers by showing the formulation flexibility of CDs and pointing toward new opportunities for innovation in drug delivery.

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T-KB3-5 THE REGULATORY LANDSCAPE OF PHAGE THERAPIES

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The increasing prevalence of multi drug resistant organisms requires the search for innovative non-antibiotic therapeutic methods and strategies. The World Health Organization (WHO) estimates that these infections will be the leading cause of 10 million deaths worldwide by 2050. Phage therapies present a promising approach for future therapies, but their regulatory framework is still in its adaptive stage, with discussions ongoing regarding their classification, manufacturing standards, and clinical trial design. Last year, EMA published Draft guideline on quality aspects of phage therapy medicinal products for quality documentation of bacteriophage active substances and finished products for human use.

T-KA2-1 INFLAMMATORY MECHANİSMS UNDERLYİNG LONG-TERM COGNİTİVE DEFİCİTS İNDUCED BY PERİNATAL İNSULTS

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Perinatal asphyxia (ASX) and preceding intrauterine inflammation are major contributors to long-term neurodevelopmental morbidity, yet the mechanisms underlying their synergistic effects on cognitive and affective outcomes remain poorly understood. Generating translationally relevant preclinical insight requires behavioural tools that capture the full complexity of cognitive and affective phenotypes with high throughput and ecological validity. To this end, besides conventional tests of emotional and cognitive function, our studies employ the IntelliCage (IC), an automated home-cage-based monitoring system enabling continuous, simultaneous assessment of multiple behavioural domains – including spatial learning, cognitive flexibility, working memory, impulsivity, and affective traits – in group-housed mice with minimal experimenter interference.

Applying this platform, we investigated the long-term consequences of a "double-hit" perinatal insult, combining systemic IL-1β administration during the early postnatal period with a subsequent non-invasive ASX insult. IC-based testing revealed pronounced sex-specific phenotypes: males exhibited impaired spatial learning, cognitive flexibility, and impulse control, while females showed predominantly disrupted emotional processing and reduced exploratory motivation. Several deficits emerged exclusively in the double-hit group, supporting an additive interaction between prior systemic inflammation and hypoxia. Microglial density and morphology were subsequently quantified across approximately 500 brain regions. The double-hit condition produced lasting increases in microglial density and hyper-ramification in behaviourally relevant circuits – including the basolateral amygdala, mediodorsal thalamus, and prefrontal cortex – with regional patterns closely mirroring behavioural deficits and differing markedly between sexes.

These findings identify IL-1β pathway activation as a key mediator of the long-term neuropsychiatric consequences of perinatal insults and reveal sex-specific neuroinflammatory vulnerabilities. They further illustrate how automated home-cage behavioural monitoring can substantially enhance the translational resolution of preclinical pharmacological research.

T-KA2-2 INVESTIGATING MOTIVATION IN AN AUTOMATED HOME CAGE SYSTEM IN RATS

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IntelliCage is an innovative, fully automated home-cage system designed to assess spontaneous activity, spatial learning, memory, and cognitive abilities in up to 8 rats simultaneously. It has many advantages such as enabling animals to live in a social environment, continuously recording their behavior, and requiring minimal human intervention. Additionally, the system supports a wide range of behavioral paradigms, such as different learning tasks and motivation assessment — the latter of which can be evaluated by using the progressive ratio test (PR). This task is of particular interest due to its strong clinical relevance, as it detects motivational impairments characteristic of psychiatric disorders such as schizophrenia or depression.

During our recent experiments in the rat IntelliCage, the PR paradigm was successfully implemented to measure the animals' effort-based motivation. The PR task requires participants to perform an increasingly demanding series of effortful actions to obtain a fixed reward, ending when they judge that the required effort no longer matches the reward's value and cease responding—thus reaching their breakpoint. After the rats mastered the task, subjects had to perform an increasing number of nose-pokes to receive a saccharin water reward (the modules became progressively more challenging for the animals).

In a recent study, we successfully tested different agents to validate the test system. These compounds were chosen based on human findings to ensure translational relevance. In the first experiment, tetrabenazine treatment significantly reduced the breaking point levels, while bupropion partially reversed the impairment. In another experiment, we aimed to examine the potential enhancing effects of different substances, including amphetamine, and ketamine. These experiments revealed that amphetamine significantly increased breaking point levels compared to vehicle group, whereas ketamine did not produce any motivational enhancement.

Overall, the IntelliCage system is a highly valuable tool for pharmacological measurements and the PR test provides a quantitative and sensitive measure of motivation. Because the increasing effort requirement taxes both willingness and persistence, it is considered a translationally relevant method for studying motivational processes in rodents. The advantages of this test include its stress-free home-cage environment and its high ecological validity, as group-housed rodents exhibit behavioral patterns that more closely reflect their natural behavior. To the best of our knowledge, this study is the first to employ a progressive ratio paradigm within the IntelliCage apparatus in rats. Our findings confirm its sensitivity to both impairing and enhancing pharmacological effects, underscoring its usefulness for studying motivation and cognition in rodents.

T-KA2-3 AUTOMATED MOUSE TRAINING FOR BEHAVIORAL PHARMACOLOGY

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Training animals to perform complex behavioral tasks is essential for studying neural mechanisms of cognition and for testing pharmacological interventions. However, traditional behavioral training protocols require substantial human involvement, including handling animals, transferring them between cages and training chambers, and manually initiating sessions. These procedures limit training throughput, introduce experimenter-dependent variability, and increase stress in animals. Here we present an automated rodent training system that enables efficient behavioral training with minimal experimenter intervention.

Water-restricted mice were trained in the automated system on cognitive tasks. In this setup, a central training chamber is connected directly to the animals' home cages, allowing animals to voluntarily enter the training chamber and initiate behavioral sessions. Training occurs in repeated short sessions available throughout the day, enabling animals to engage in tasks according to their natural activity patterns. Mice were trained on the 5-choice serial reaction time task (5CSRTT), a widely used rodent paradigm in which animals detect brief visual stimuli presented randomly in one of five ports and report detection by nose-poking into the correct port for a water reward. In addition to the 5CSRTT, the system can also be adapted to train animals in other behavioral paradigms, including probabilistic two-armed bandit tasks and sequential learning tasks.

Automated training enabled rapid acquisition of behavioral tasks and substantially increased the number of trials performed per day compared with traditional manual training. Animals trained in the automated system progressed through training stages significantly faster and maintained stable performance across the day despite variations in activity levels. Automated training also reduced physiological stress, as indicated by lower corticosterone levels compared with manually trained animals. To validate the system for behavioral pharmacology, we tested the effects of the muscarinic antagonist scopolamine during the 5CSRTT. Consistent with previous studies, scopolamine impaired task performance by reducing accuracy and increasing premature responses.

Together, these results demonstrate that automated behavioral training provides an efficient and low-stress platform for studying cognitive processes and pharmacological modulation of behavior. By reducing experimenter intervention while increasing training throughput, automated systems offer a scalable tool for behavioral pharmacology and systems neuroscience.

T-KA2-4 ENHANCİNG BEHAVİOURAL PHARMACOLOGY İN NON-HUMAN PRİMATES: LONGİTUDİNAL PROFİLİNG AND MULTİDİMENSİONAL TESTİNG

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Understanding drug effects on cognition and behaviour requires experimental settings capable of capturing multiple functional domains within the same subjects over time. In non-human primates (NHPs), behavioural pharmacology has traditionally relied on individual paradigms, which limits the ability to generate comprehensive cognitive profiles and to track pharmacologically induced changes longitudinally. To address this limitation, we established an integrated behavioural assessment framework combining complementary paradigms that together enable multidimensional and longitudinal profiling of behavioural and cognitive functions in macaque monkeys, with particular emphasis on translational relevance to human neurocognitive testing.

Altogether 34 macaques were trained across seven behavioural paradigms encompassing measures of spontaneous activity, motivation, and several cognitive domains. The framework included a large arena monkey open field (mOF), a progressive ratio operant schedule (PR), and five touchscreen-based tasks assessing visual short-term working memory (delayed matching to sample, DMTS), associative object–location learning (paired associates learning, PAL) or visual discrimination, executive function (intra–extra dimensional set shifting, IDED), sequential spatial memory (self-ordered spatial search, SOSS) and motivation (self-ordered motivation measurement, SOMM). Task acquisition and performance were facilitated through systematic manipulation of task parameters, while pharmacological interventions were used to evaluate task sensitivity.

Monkeys achieved stable and accurate performance across multiple paradigms and were able to complete several tasks within a single experimental session. This enabled multidimensional behavioural profiling within individual animals and supported repeated measurements suitable for longitudinal experimental designs. Notably, animals demonstrated robust learning capacity across tasks and flexible switching between paradigms, highlighting the feasibility of integrated cognitive testing in NHPs.

Our findings indicate that multidimensional cognitive test batteries provide a powerful framework for behavioural pharmacology studies in NHPs and may also offer a valuable platform for investigating the effects of functional neuromodulation on cognitive processes. Such approaches allow longitudinal characterization of cognitive function and improve the translational alignment between preclinical primate models and human neurocognitive research.

T-KB4-1 ADVANCES IN HUMAN-RELEVANT MODELS FOR DRUG AND DEVICE SAFETY TESTING

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The continuous demand for innovation in science and industry drives the development of novel compounds and medical devices. This process operates within a tightly regulated framework that is itself subject to constant adaptation. In Europe, the introduction of the Medical Device Regulation (MDR) has transformed the device landscape, with initiatives such as the breakthrough medical device (BtMD) designation further redefining regulatory pathways. Similar dynamics apply to the development and testing of new drugs and in vitro diagnostics (IVD)'s.

This presentation will outline the regulatory framework required to bring either a new drug or device to the testing stage. It will then explore human-relevant models beyond traditional rodent and canine studies, with a particular focus on *in silico* and *in vitro* approaches, including high-throughput iPSC-based organoid systems. For medical devices, we will examine available small and large animal models used to evaluate biocompatibility and functional performance of implantable technologies. Finally, a case study will illustrate the journey of a Class III medical device—from initial concept through patenting to prototype development.

By the conclusion of this presentation, participants will possess a clear understanding of the key steps and models that underpin the development and evaluation of innovative medical products within the current EU regulatory framework.

T-KB4-2 HIGHER SENSITIVITY TO OUABAIN-INDUCED TOXICITY IN HIPSC-CARDIOMYOCYTES THAN HUMAN ADULT HEART TISSUE DESPITE SIMILAR NA$^+$/K$^+$-ATPASE PUMP CURRENT AMPLITUDES

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) have gained a huge interest as a pharmacological/pathophysiological model. However, ongoing concern exists about the immaturity of hiPSC-CM, complicating translation of results obtained in hiPSC-CM to adult human heart. Therefore, we studied the properties of the key player in maintaining ionic homeostasis, the Na$^+$/K$^+$-ATPase (NKA), in hiPSC-CM.

Atrial and ventricular engineered heart tissues (EHTs) were produced from hiPSC-CMs obtained from healthy probands. For comparison we used adult atrial and ventricular tissues obtained from patients undergoing open heart surgery. We measured NKA gene expression, NKA pump currents (I_{NKA}) and effects of the NKA blocker ouabain on action potentials and contractility. Computational modelling was used to further investigate the direct and indirect impacts of NKA inhibition.

The mRNA abundance of the major NKA isoforms was higher in ventricular than atrial CM (both adult tissue and hiPSC-CM-based EHT). Accordingly, I_{NKA} was also higher in ventricular than in atrial hiPSC-CM (1.32±0.1 pA/pF *vs.* 0.92±0.09 pA/pF) and higher in ventricular than in atrial adult CM (1.44±0.1 pA/pF *vs.* 1.04±0.07 pA/pF). The potency of ouabain to block I_{NKA} did not differ between hiPSC-derived and adult CM. Ouabain shortened plateau phase in both EHT and adult tissue. However, lower ouabain concentrations than in adult human tissue depolarized diastolic potential and depressed force in EHTs, demonstrating a higher integrated sensitivity to NKA inhibition. Computational modelling indicated that lower /K1 (as typically seen in hiPSC-CM) increases the susceptibility to depolarization by NKA.

Our findings demonstrate that hiPSC-EHTs express NKA with biophysical characteristics and ouabain-sensitivity that are not different from adult human tissue. As seen in human heart APD responses in EHT recapitulate chamber-specific response pattern. The much higher sensitivity of EHTs to depolarize under ouabain-induced block of NKA needs to be considered when hiPSC-CM will be employed in drug research.

T-KB4-3 INVESTİGATİNG THE ARRHYTHMOGENİC EFFECT OF ACTİON POTENTİAL ALTERNANS İN HUMAN HEART FAİLURE

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Heart failure (HF) is a complex, progressive disease characterized by structural and electrical remodeling of the heart, significantly increasing the risk of ventricular fibrillation (VF). However, prior to the onset of VF, a potentially reversible phenomenon known as alternans may develop. Action potential (AP) alternans refers to beat-to-beat oscillations in AP duration (long–short), leading to repolarization inhomogeneity. As no specific pharmacological intervention currently exists to prevent alternans, this study investigated the effects of the selective Na^{+}/Ca^{2+} exchanger (NCX) inhibitor ORM-10962 on alternans formation in both failing and non-failing human hearts.

Tissue samples from failing and non-failing human hearts were used. HF samples were obtained from the Városmajor Heart and Vascular Center, while non-failing tissues were collected from donor hearts. APs were recorded using conventional microelectrode techniques in left and right ventricular preparations.

In HF samples, AP duration (APD) alternans was significantly larger than in non-failing hearts. In several cases, pronounced amplitude alternans were observed in left ventricular HF preparations, predominantly at higher pacing rates. These further increased APD alternans magnitude and ultimately led to 2:1 conduction block. Preparations exhibiting amplitude alternans also showed slower AP amplitude restitution kinetics. Treatment with 1 μM ORM-10962 shortened APD in HF samples, reduced APD alternans magnitude, and shifted the onset of amplitude alternans and conduction block to higher pacing rates.

Electrical remodeling in HF markedly increases APD alternans, which appears to worsen with age, possibly due to progressive SERCA downregulation. Notably, failing hearts may develop amplitude alternans at higher heart rates, further amplifying APD alternans. The underlying mechanism may involve impaired recovery of the sodium current. Selective NCX inhibition attenuates alternans in the failing human heart, likely through APD shortening and its effects on calcium handling.

T-KA3 TRANSİENT RECEPTOR POTENTİAL ANKYRİN 1 (TRPA1) AS A FACTOR İN İNFLAMMATİON

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Transient receptor potential ankyrin 1 (TRPA1) is a non-selective cation channel primarily known for its role in sensory neurons, where it mediates itch, pain and cough. TRPA1 is activated by pungent exogenous compounds and physical stimuli but also by endogenous inflammatory mediators such as reactive oxygen and nitrogen species (ROS and RNS). Beyond its sensory functions, we and others have shown that TRPA1 is also expressed in many non-neuronal cells and modulates inflammatory responses. Activation of TRPA1 promotes the release of proinflammatory factors, including cytokines and chemokines and contributes to the development of neurogenic inflammation. Notably, TRPA1 has been implicated in both acute and chronic inflammatory conditions, such as allergies, asthma and arthritis. Our recent studies further suggest that TRPA1 influences inflammation phenotype skewing, affecting the balance between type 1 and 2 responses and shaping the functional outcome of inflammatory reactions. Understanding TRPA1's dual role as a sensory and immunomodulatory channel holds promise for the development of targeted therapies in inflammatory diseases.

T-KB5 DİVERSE FUNCTİONS OF CALCİUM-CALMODULİN DEPENDENT PROTEİN SERİNE KİNASE (CASK) İN HEALTH AND DİSEASES

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Calcium/calmodulin-dependent serine protein kinase (CASK) is a multidomain scaffolding protein that has attracted considerable attention for its pivotal roles in neural development, synaptic organization, and brain function. Despite harboring a CaM kinase domain, the kinase activity of CASK and its downstream targets remain incompletely characterized, leaving many of its signaling mechanisms unresolved. Historically, research has focused primarily on its neuronal functions, yet emerging evidence suggests that CASK exerts diverse biological effects beyond the nervous system. Its roles in non-neuronal tissues, and the molecular mechanisms arising from both its scaffolding properties and potential kinase activity, remain underexplored.

Our investigations have expanded the functional landscape of CASK into several critical physiological and pathological contexts, including myogenesis, adipogenesis, tumorigenesis, and retinopathy. These studies reveal that CASK is a versatile modulator of cellular processes across multiple tissue types. Depending on the cellular context, CASK influences cell growth and differentiation, orchestrates oxidative stress responses, and regulates mitochondrial respiration. Furthermore, it participates in the fine-tuning of receptor-mediated signaling pathways, notably those involving the EGFR, thereby linking CASK to metabolic regulation and oncogenic signaling. Taken together, these findings highlight the multifaceted nature of CASK as both a structural scaffold and a potential signaling kinase. Its ability to integrate diverse molecular cues positions CASK as a critical regulator of cellular homeostasis and disease progression. A deeper understanding of CASK's non-neuronal functions and its context-dependent mechanisms may uncover novel therapeutic opportunities in metabolic disorders, cancer biology, and degenerative diseases.

T-KA4-1 DHEA(S) AND BDNF İN NEURODEGENERATİON: NEUROBİOLOGY AND THERAPEUTİC POTENTİAL

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Alzheimer´ s disease (AD) is a serious neurodegenerative disorder and most common form of dementia, characterized by progressive cognitive decline and behavioural changes, as well as accumulation of amyloid beta plaques and neurofibrillary tangles in the brain, leading to neuronal damage and death. Despite substantial progress in understanding AD, the factors driving its development remain unclear, and available treatments only manage symptoms without halting disease progression, emphasizing the ongoing need for further research. Neurosteroids, dehydroepiandrosterone (DHEA) and its sulfate (DHEAS), as well as neurotrophin brain-derived neurotrophic factor (BDNF), are potent modulators of neurogenesis, neuronal growth and survival, brain plasticity, cognition, and behaviour, and have emerged as promising targets for AD prevention and treatment. Previous studies suggest that DHEA(S) and BDNF might both act via shared neuroprotective pathways. In our study we have investigated potential neuroprotective actions of DHEA(S) and BDNF in cellular and animal models of AD, as well as in AD patients.

To investigate neuroprotective effects of DHEA(S) and BDNF on cellular survival and viability in an in vitro model of AD, we have exposed primary mouse neurons to toxic Aβ oligomers. In a genetic AD model, triple-transgenic AD (3xTg-AD) mice were chronically treated with DHEAS using subcutaneously implanted osmotic pumps. Pharmacologically induced AD model was established by intracerebroventricularly injecting the C57BL/6 mice with Aβ oligomers and chronically administered with DHEA via intraperitoneal injection. Cognitive and behavioural testing of mice was conducted. Changes in the expression of proteins involved in PI3K-Akt signalling have been investigated in both cellular and animal models of AD. Cognitive symptoms of individuals with AD and mild cognitive impairment (MCI) were evaluated by MMSE (Mini mental state examination) and CDT (Clock Drawing test). DHEA(S) and BDNF plasma concentrations were determined by ELISA, whereas genotyping for polymorphisms in BDNF as well as SULT2A1 gene, coding for enzyme catalysing DHEAS formation, was conducted using qPCR.

Findings obtained using in vitro model of AD indicated potential neuroprotective effects of DHEA(S) and BDNF probably via their antiapoptotic actions on cell survival. Behavioral analysis in mouse models of AD demonstrated some beneficial effects of DHEA(S) on cognition and behavior. Results also suggested that DHEA(S) and BDNF might modulate the expression of key apoptosis regulators via PI3K-Akt signalling pathway. In human patients, plasma BDNF and DHEAS levels were lower in AD patients, compared to individuals with MCI; however, no significant associations of BDNF and SULT2A1 polymorphisms with AD or plasma BDNF and DHEAS levels, respectively, were observed. Findings suggest potential of DHEA(S) and BDNF in AD prevention and treatment, which should be further investigated.

T-KA4-2 PRECLİNİCAL BRAİN İMAGİNG İN NEUROSCİENCE AND TRANSLATİONAL DRUG DİSCOVERY

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The BRAIN (Biomarkers Research and Imaging for Neuroscience) Centre at King's College London is a preclinical neuroimaging facility dedicated to collaborative research with both academic and industry partners. Researchers at the BRAIN Centre are committed to advancing forward- and back-translational studies by applying advanced brain imaging techniques to investigate the mechanisms and treatments of various neurological and psychiatric conditions, including dementia, schizophrenia, unhealthy ageing, autism, Parkinson's disease, stroke, and more.

Dr Cash lecture will provide an overview of the Centre's core activities and highlight selected examples from their work in experimental neuroscience, focusing on neurodegeneration and demyelination. Using a mouse model of inflammatory demyelination, we demonstrate how multimodal in vivo MR imaging and spectroscopy can deliver detailed pathological characterization, complementing neurochemical and histological findings. This approach also enables the assessment of treatment efficacy and contributes toward elucidation of mechanisms of action—key factors in clinical translation.

Another case study will illustrate the monitoring of anti-Alzheimer's drug efficacy in rodent models through integrated MR imaging, histology, and behavioural analyses. Employing a transgenic mouse model, we have developed a sensitive method to detect amyloid plaques in vivo, allowing for longitudinal measurement of drug effects in the same animal.

T-KA4-3 NK3 RECEPTOR AND TERMOREGULATİON İN DEMENTİA

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Dementia, including Alzheimer's disease (AD), is increasingly recognized as a systemic disorder accompanied not only by cognitive decline but also by physiological disturbances such as impaired thermoregulation. Emerging evidence suggests that neurokinin 3 receptor (NK3R) signaling, a pathway implicated in central thermoregulatory control, may be disrupted in neurodegenerative conditions. Given its therapeutic potential, it is important to characterize NK3R-related thermoregulatory alterations during disease progression.

To address this, core body temperature (cBT) was continuously monitored using implantable telemetry in female 3xTg-AD mice and wild-type (WT) controls during cold and warm exposure tests, as well as following acute administration of senktide, a selective NK3R agonist. Ovariectomy (OVX) was used to model menopause and to accelerate disease progression.

Cold exposure elicited comparable cBT responses across genotypes and hormonal conditions, indicating preserved cold-defense mechanisms. Although warm exposure induced comparable changes in all groups, but intact 3xTg-AD females exhibited an exaggerated compansatory cBT decline, suggesting altered heat-dissipation mechanisms. This genotype-dependent difference was abolished following OVX, highlighting a modulatory role of ovarian hormones. Senktide administration induced a significant reduction in cBT in WT mice, consistent with NK3R-mediated peripheral vasodilatory responses. However, this effect was markedly attenuated in 3xTg-AD animals, indicating impaired NK3R signaling in the dementia model. OVX further modified senktide-induced temperature responses and accentuated genotype-specific differences. Gene expression analysis in brown adipose tissue revealed no significant differences in thermogenic markers, including uncoupling protein 1 (UCP1) and iodothyronine deiodinase 2 (Dio2), although a modest increase in β3-adrenergic receptor (Adrb3) expression was observed, suggesting subtle alterations in sympathetic regulation.

Together, these findings demonstrate that thermoregulatory dysfunction in dementia involves impaired NK3R-mediated pathways and is influenced by hormonal status. Targeting NK3R signaling may therefore represent a novel approach to mitigate systemic physiological disturbances associated with neurodegenerative diseases.

T-KA4-4 CHALLENGES AND ALTERNATIVE METHODS IN DETECTION OF TRPA1 ION CHANNEL, A POTENTIAL DRUG TARGET IN NEURODEGENERATIVE DISORDERS

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The transient receptor potential ankyrin 1 (TRPA1) ion channel is a potential therapeutic target in neurodegenerative disorders. However, the reliable detection of TRPA1 expression in the central nervous system remains challenging. A major limitation is the lack of highly specific antibodies suitable for immunohistochemical detection. Although PCR-based approaches are more reliable, these measurements are typically performed on tissue homogenates, which prevents precise anatomical localization of the channels with spatial information.

We aimed to identify and functionally verify TRPA1 expression in the central nervous system. Trpa1 mRNA expression was investigated using RNAscope in situ hybridization allowing single molecule detection in histological context at the cellular level. To assess functional activity, electrophysiological recordings were performed using the patch clamp technique on acute brain slices while applying a potent and selective TRPA1 agonist (JT010). In addition, a fluorescence-labelled TRPA1 ligand was used to assess the presence of the channel.

Using these approaches, we consistently detected Trpa1 mRNA and functional activity in neurons of the mouse Edinger-Westphal nucleus. RNAscope analysis revealed clear punctate signals corresponding to TRPA1 transcripts within this brain region. Patch clamp recordings demonstrated TRPA1 agonist-induced currents, confirming that the detected transcripts are localized to neurons that carry the functional ion channels. Fluorescent ligand labelling further supported the presence of TRPA1-expressing cells in this brain area.

Together, these findings provide molecular, anatomical, and functional evidence for TRPA1 expression in the mouse Edinger-Westphal nucleus. These data contribute to a more precise understanding of TRPA1 distribution in the brain and may support future investigations into its potential role as a therapeutic target in neurodegenerative disease.

T-KA4-5 ADVANCEMENTS İN GLYCİNE TRANSPORTER-1 RESEARCH: NEW HORİZONS İN PAİN RELİEF

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Introduction: Glycine transporter-1 (GlyT1) has recently emerged as a therapeutic target for diseases associated with N-methyl-D-aspartate receptors' (NMDARs) hypofunction like cognition impairment associated with schizophrenia. Importantly, hyperfunctioning of NMDARs, particularly within the spinal dorsal horn involved in pain modulation, has been implicated in the development of neuropathic pain (NP) and opioid analgesic tolerance (OAT). Central sensitization is a well-established mechanism in NP and OAT, with NMDAR activation identified as a pivotal step in initiating and maintaining this state. Extrasynaptic NMDARs containing GluN2B subunits have been reported to be involved in the development of NP and OAT. Glycine and D-serine are co-agonists of NMDARs; thus, changes in their levels could influence NMDAR activity. Glycine levels in the synaptic and extrasynaptic spaces are controlled by GlyT1 and GlyT2, respectively. Thus, these transporters are promising targets for the management of NP and OAT. Aims: To investigate the effect of GlyT1 and 2 inhibitors against NP and morphine antinociceptive tolerance (MAT). Methods: Male Wistar rats underwent partial sciatic nerve ligation (pSNL) to induce mononeuropathic pain. At day 14 after the operation, rats that developed tactile allodynia, indicated by low paw pressure thresholds (PWT) measured by the dynamic plantar aesthesiometer (DPA), were acutely and chronically treated with NFPS (GlyT1 inhibitor) or Org-25543 (GlyT2 inhibitor). In the second set of experiments, rats received a combination of both inhibitors. In the third set of experiments, rats were chronically treated with morphine combined with NFPS or Org-25543 for ten days and thermal tail-flick assay was used to determine morphine analgesic tolerance (MAT). The rats weighed 170–260 g at the time of measurement. The cerebrospinal fluid (CSF) levels of glycine and glutamate were measured by capillary electrophoresis with laser-induced fluorescence detection method. All test compounds and vehicles were administered subcutaneously. Results: Acute systemic NFPS or Org-25543 produced a significant antiallodynic effect only at a dose of 4 mg/kg. Upon chronic treatments with small doses, only NFPS (1 mg/kg) produced an effect. However, the combination of NFPS (1 mg/kg) and Org-25543 (2 mg/kg) produced a significant acute antiallodynic effect alongside an increase in the glycine and glutamate levels in the CSF. Chronic treatment with a low dose of NFPS, but not Org-25543 at 0.6 mg/kg, delayed MAT that was accompanied by an increase in CSF glycine levels. Conclusions: Combining GlyT1 and 2 inhibitors may halt NP by promoting GluN2B-containing NMDAR internalization. This mechanism of action may also prevent the development of MAT. This interpretation is largely supported by our previous work showing that high glycine concentration inhibits NMDAR currents evoked by glutamate in patch-clamp experiments. Fund: FK_138389

T-KB6-1 BASAL RELEASE OF 6-NITRODOPAMINE FROM RAT GASTRIC FUNDUS AND ITS ROLE IN SMOOTH MUSCLE RELAXATION

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The endogenous catecholamine 6-nitrodopamine (6-ND) has recently been identified in various human and animal tissues, originating primarily from epithelial and endothelial sources. Current research suggests that 6-ND exhibits tissue-specific physiological actions. For instance, it acts as a robust relaxant in the mouse urinary bladder (Oliveira et al., 2024), yet triggers smooth muscle contraction in the human vas deferens (Britto-Júnior et al., 2022) and rat seminal vesicle (Fuguhara et al., 2025). Given that its role in gastric motility remains unexplored, this study aimed to quantify the release of 6-ND from the rat gastric fundus (RGF) and evaluate its functional impact on tissue contractility.

Gastric fundus samples were obtained from 40 male Wistar rats (70 days old) following euthanasia via isoflurane overdose. Tissues were maintained in 3 mL organ chambers containing Krebs–Henseleit solution at 37°C, aerated with 95% O_2 and 5% CO_2, and treated with ascorbic acid (3 mM) to inhibit catecholamine oxidation. To isolate basal release, preparations were incubated for 30 minutes with tetrodotoxin (TTX, 1 μM) and the nitric oxide synthase inhibitor L-NAME (100 μM). The resulting medium was analyzed using LC–MS/MS to quantify levels of 6-ND, dopamine, noradrenaline, and adrenaline.

For functional evaluation, RGF strips were mounted vertically in a glass tissue bath containing 10 mL KHS at 37°C with one end attached to a metal hook in the bottom of the chamber and the other end tied to an isometric force transducer. Tissues were allowed to equilibrate for 60 minutes under a resting tension of 10 mN, and isometric tension was recorded using a PowerLab system (ADInstruments). Strips were pre-contracted with carbachol (1 μM), and cumulative concentration–response curves to 6-nitrodopamine, dopamine, adrenaline and noradrenaline (100 pM–1 mM) was performed. Detection of tyrosine hydroxylase was carried out using immunohistochemistry assay. All the experimental protocols were approved by the UNICAMP animal ethics committee.

6-ND was the major catecholamine released from RGF. Dopamine and noradrenaline were detected at a concentration of 0.4±0.1 and 0.4±0.3 ng/mL, n=8, respectively. 6-ND induced relaxation with pEC50=5.4±0.2 and Emax=74.6±1.4% (n=4. Potency rank: Adrenaline (pEC50=7.2±0.1, n=3) > Noradrenaline (6.7±0.1, n=4) > 6-ND (5.4±0.2, n=4) = Dopamine (4.9±0.2, n=4). Tyrosine hydroxylase was positive in the epithelium of rat gastric fundus (n=3).

The rat gastric fundus releases 6-ND as its predominant catecholamine, where it functions as a significant relaxant of the smooth muscle. These findings suggest that 6-ND may serve as a key endogenous modulator in the regulation of gastric motor activity.

T-KB6-2 CATECHOLAMİNE OVERPRODUCTİON İN BREAST CANCER PATİENTS AND İTS SEQUELAE

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Previous studies showed that neoplastic diseases, but not breast cancer, are associated with local intestinal catecholamine production and subsequent damage to the small intestine. Furthermore, adrenergic and dopamine receptor antagonist consumption was linked to changes in breast cancer behavior and incidence. These findings prompted us to assess whether breast cancer is also characterized by changes to catecholamine homeostasis. We observed increases in urinary in urinary adrenalin in breast cancer patients, similar changes were found in mice. We identified the duodenum and the tumor as sources of excess catecholamines. Tyrosine hydroxylase knockdown in breast cancer cells showed that the commitment enzyme of catecholamine biosynthesis supports cell viability and movement. In humans, the overexpression of participants in catecholamine biosynthesis and catecholamine signaling in tumors was associated with shorter survival. Furthermore, signs of enteropathy were characterized by intestinal edema, slower passage, and lower intestinal motility, including contraction tone and amplitude. At the ultrastructure level, lateral cell-cell and cell-basal lamina connections of the enterocytes were dysfunctional. These changes were more pronounced in the duodenum and the proximal jejunum compared with the terminal ileum. Reduction in the number of Paneth cells was observed in the ileal intestinal glands. These changes to the structure and function of the intestines lead to absorption disorders. Altogether, the catecholamine system represents a drug repurposing opportunity in breast cancer.

T-KB6-3 TARGETING COGNITIVE DECLINE IN AGING WITH ANTI-DIABETIC AGENTS

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Age-related structural and functional brain changes contribute to a progressive decline in cognitive performance, posing increasing challenges for independence and quality of life in elderly people. This study evaluated whether three commonly used anti-diabetic agents could mitigate such a decline in an aging rat model. Eighteen-month-old female Wistar rats were randomized into the control group or into one of three treatment groups receiving metformin, semaglutide, or empagliflozin over a 12-week period. Cognitive performance was assessed using the Morris Water Maze, focusing on escape latency and time spent in the target quadrant, as indicators of spatial memory.

Treatment with metformin and semaglutide significantly improved cognitive performance compared to the untreated controls, reflected by reduced escape latency and increased retention of spatial memory. In contrast, empagliflozin did not produce comparable effects. These findings indicate that metformin and semaglutide, two widely used anti-diabetic drugs, may exert neuroprotective effects and hold potential for mitigating age-associated cognitive decline, warranting further investigations of their underlying mechanisms and translational relevance.

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T-KB6-4 CLINICAL EXPERIENCES WITH AGENTS ACTING ON THE INCRETIN AXIS

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Incretin-based therapies are a class of antidiabetic treatments that target the physiological actions of incretin hormones, primarily glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). The major classes are dipeptidyl-peptidase-4 inhibitors (DPP4 inhibitors), GLP-1 receptor agonists (GLP-1 RA) and the recently developed dual incretin receptor agonists, such as tirzepatide. While DPP4 inhibitors cause only moderate HbA1C reduction and are weight neutral, GLP1 RAs improve effectively the glycemic control and promote weight loss. Liraglutide and semaglutide demonstrate significant benefits in body weight reduction, therefore these compounds were approved for treatment of obesity. Large cardiovascular outcome trials (SELECT, FLOW) have also shown that several GLP-1 RAs reduce major cardiovascular outcomes and clinically important kidney outcomes in high-risk patients, expanding their role in cardiovascular risk management. Dual receptor agonist tirzepatide shows greater effects on glycemic control and weight reduction.

Incretin-based therapies have become indispensable medications in the management of type 2 diabetes mellitus and obesity particularly in patients with high cardiovascular risk. The glucose-dependent mechanism of action prevents hypoglycemia while providing metabolic and cardiovascular benefits.

T-KB7 HE DRUG DELİVERY TECHNOLOGİES BEHİND RNA THERAPEUTİCS

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The development of RNA therapeutics has experienced an explosive increase following the success of COVID-19 vaccines, with the industry now shifting focus toward oncology, infectious diseases, and rare genetic disorders. Delivery strategy options for RNA therapeutics are also expanding. This presentation will take you on a tour of the different delivery strategies for RNA therapeutics, explaining their active/inactive components and how they contribute to therapeutic success. We will look at current technologies enabling the targeting of specific cell populations and discuss how these systems should be optimized for different administration routes.

T-KA5-1 REGULATORY PROGRESS TOWARDS NOVEL CELL AND GENE THERAPY ATMPs

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International clinical developments in the field of gene and cell therapies for regenerative medicine and genetic mutation corrections are progressing at an increasing speed. Recent regulatory approvals and streamlining of preclinical and clinical requirements are dramatically shaping the future of the field, creating a new landscape for a maturing industrialised medical field with transformative potential.

In the last five years FDA and EMA demonstrated asynchronous but converging advanced therapy medicinal product (ATMP) regulatory activity, with distinct patterns emerging across cell and gene therapy modalities. The FDA has maintained numerical leadership, approving a total of 43 cell and gene therapy products through early 2026, with notable acceleration waves in 2022–2024. The agency's 2024 peak delivered seven approvals spanning diverse platforms: CAR-T therapies (Aucatzyl for B-cell ALL), tumor-infiltrating lymphocyte therapy (Amtagvi for melanoma), TCR-T cell therapy (Tecelra for synovial sarcoma), mesenchymal stem cells (Ryoncil for graft-versus-host disease), tissue-engineered vascular conduits (Symvess), and in vivo AAV gene therapies for hemophilia B (Beqvez) and AADC deficiency (Kebilidi).

The EMA's trajectory shows a more conservative volume but strategic focus on transformative therapies. With 31 active ATMPs, the agency experienced slower 2024 activity (only Casgevy and Beqvez), but executed a significant 2025 rebound authorizing four products: Vyjuvek (topical HSV-based gene therapy for dystrophic epidermolysis bullosa), Aucatzyl (CAR-T mirroring FDA approval), Zemcelpro (allogeneic cord blood stem cell therapy addressing unmet transplant needs), and Waskyra (lentiviral ex vivo gene therapy for Wiskott-Aldrich syndrome). Both agencies prioritize orphan/rare disease indications, with 74% of EMA ATMPs carrying orphan designation. The 2025 EMA surge versus FDA's limited new approvals suggests regulatory cycle timing and reorganizational differences rather than fundamental strategic divergence, with both agencies maintaining rigorous standards while accelerating access to life-saving advanced therapies for previously untreatable genetic and oncologic conditions. FDA and EMA both tailor data demands to product risk, but FDA is more flexible via expedited, adaptive pathways, while EMA embeds flexibility in stricter, guidelinedriven, documented risk management.

In January 2026, FDA introduced a more flexible CMC and trial framework for cell and gene therapies, easing early-phase GMP expectations and enabling lifecycle, riskbased validation, which should accelerate trial starts, derisk scaleup, and shorten time to BLA for highneed indications. EMA, in parallel, is implementing its July 2025 guideline on quality, nonclinical and clinical requirements for investigational ATMPs, emphasizing riskbased design, geneediting, paediatric use and platform technologies, which should streamline EU trial approvals while keeping stringent quality and safety standards. The FDA's 2026 changes are likely to yield tangible sevenfigurescale savings per CGT program and faster time to pivotal trials. Indeed, FDA preferred by ATMP developers for the larger US market/higher pricing; faster approvals via RMAT/accelerated paths; CMC flexibility (2026 guidances cut early costs); surrogate endpoints/RWE acceptance. EMA offers EU access but slower reviews, stricter data demands. Among ATMP developers parallel strategies are common with US leading first launches.

In the mean time Asian ATMP developments are surging due to regulatory reforms, expedited paths, oncology/rare disease focus, and an ATMP pipeline boom (~2961 global) and increasing regional harmonisation. China (NMPA) for the ATMP field eased restrictions in the 2025 ATMP regulations and a fast Priority Review with 130-day timelines, allowing surrogate endpoints and approved 6 new CAR-T products. Japan/PMDA offers conditional approval using Phase II data plus post-marketing studies, rare/orphan disease (Sakigake) designation with six-month priority review, and approved 10 new gene therapies. Korea/MFDS provides conditional approval after Phase II for orphan drugs and anti-cancer therapies, accepting small patient cohorts, approved 18 CGTs. Overall, Asian regulators offer ATMP development advantages by accepting smaller pivotal trials for rare diseases and accelerating market access compared to larger randomised controlled trials typically required by FDA and EMA.

The talk will review our own progress in the development of human induced pluripotent stem cell (hiPSC)-derived therapeutic and model systems, including cardiomyocytes, beta-cell organoids and neuronal models. Several of the current European initiatives with an active Hungarian contribution and the first Health IPCEI project (Med4Cure) aiming at the clinical translation of advanced therapy medicinal products (ATMPs) will be presented. Overview of complexity of the challenges to resolve, some of the potential roadblocks and best practices will be presented.

The presented projects received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 953138 (EMAPS-Cardio), from EU Horizon Europe Marie Sklodowska-Curie Action ITN No. 101120256 (MMM), COST Action CA21113 (GenE-Humdi),nCOST Action CA21151 (HAPLO-iPS), COST Action CA 21117 (IMMUPARKNET) and 2020-1.1.5.-GYORSÍTÓ SÁV-2021-00016 from the National Research, Development and Innovation Fund. The Med4Cure/CARDIABETTER IPCEI Health project will receive Hungarian and EU funding.

T-KA5-2 BEST PRACTICE IN NORWAY ON DYNAMIC CONSENT AND REGULATORY REQUIREMENTS FOR HIPSC BANKS

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In connection with the establishment in Norway of a national biobank of induced pluripotent stem cells (iPSCs) to be used for clinical applications (Norwegian iPSC Biobank for Clinical Applications, or NIBCA), we have created an informed consent form and an associated information document based on the principle of dynamic consent. The principal aims are to ensure that donors providing somatic cell biopsies for reprogramming to iPSCs have the opportunity to 1) request, at any future time, information about the use of their cells, 2) consent to be contacted should the future use of cells encompass uses not foreseen at the time of original consent, 3) consent to be contacted should analyses of their cells reveal genetic conditions with clear medical consequences that should be addressed, and 4) retract their consent at any future time.

This platform goes far beyond the normal static consent forms used for cell donations in Norway (for example in connection with bone marrow donations), which is important given that it is impossible to predict the scope of future applications using the iPSCs to be generated. A number of conditions are implicit, in keeping with current Norwegian laws and regulations governing cell therapeutics and GDPR. These laws and regulations may also change in the future, and the platform also provides a way to re-assess the situation should that occur, since donors can contact a designated office affiliated with the biobank at any time, and vice versa.

GDPR is ensured by setting up a two-tier system in which all donor contact is handled by the designated office, which establishes a pseudonymized coding of the donated cells, and stores the coded personal information separately (with no physical or net-based connection) from the biobank itself and its managers, thus restricting access to non-pseudonymized information to the designated donor handling office, separate from the biobank.

The consent form and information document have already been used in connection with recruiting adult donors to NIBCA.

This dynamic consent platform is a step towards developing the principle of dynamic governance, which is attaining increasing traction internationally, especially within the EU (Isasi et al 2024).

Reference:
Isasi R, Bentzen HB, Fabbri M, Fuhr A, Glover JC, Mah N, Mascalzoni D, Mueller S, Seltmann S, Kurtz A (2024) Dynamic governance: A new era for consent for stem cell research. Stem Cell Reports. 19(9):1233-1241.

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T-KA5-3 STEM CELL THERAPY DEVELOPMENT FOR CORNEAL EPITHELIUM REGENERATION: THE STEM-CORE HORIZON EUROPE PROJECT

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Corneal disease, the fourth leading cause of blindness worldwide, affects over 10 million patients; current therapies rely on donor tissue or pharmaceutical palliatives, neither addressing the underlying stem cell deficit in limbal stem cell deficiency (LSCD). Corneal transplantation faces critical bottlenecks: 50% of the world lacks donor access, graft durability is 5-10 years, and immunological rejection occurs in 10-20% of cases. This review examines the evolving landscape of advanced therapy medicinal products (ATMPs) and cell-based approaches transforming corneal regeneration.

This work reviews recent advances in the development of stem cell therapies for corneal epithelium regeneration, with a focus on limbal epithelial stem cells, mesenchymal stem cells, and induced pluripotent stem cells. Key aspects of cell sourcing, in vitro expansion, differentiation protocols, and scaffold-based delivery systems are discussed. Particular attention is given to strategies that enhance cell survival, promote epithelial stratification, and support long-term tissue homeostasis following transplantation.

ATMP-class therapies now leverage human induced pluripotent stem cell (hiPSC)-derived corneal epithelium (iCEPS), addressing scalability and immunogenicity. Japan's Osaka study transplanted allogeneic iCEPS (hiPSC-derived) in 4 LSCD patients; no immunological rejection occurred without HLA-matching or systemic immunosuppression at 18-month follow-up, marking the first successful hiPSC-corneal ATMP in humans.

Regulatory pathways have accelerated: the EMA's Committee for Advanced Therapies (CAT) assesses ATMP classification, quality, safety, and efficacy under Regulation 1394/2007. FDA designates qualifying therapies as Regenerative Medicine Advanced Therapies (RMAT), enabling expedited review for unmet needs. Tissue-engineering innovations—biodegradable scaffolds, hydrogels, and 3D bioprinting—complement cellular therapies, reducing manufacturing timelines and costs.

Current limitations include standardization of hiPSC-derived cell products, long-term stability data and cost-of-goods for GMP manufacturing. Future directions integrate CRISPR potency enhancement, AI-driven quality assays, and off-the-shelf allogeneic products to democratize access. Corneal disease ATMPs represent a paradigm shift from transplant dependency to regenerative, donor-independent therapies, poised for broader clinical adoption and regulatory approval within 2-3 years.

Acknowledgements: This work acknowledges the EU-funded STEM-CORE Marie Skłodowska-Curie Doctoral Network (HORIZON 101226474) for pioneering affordable hiPSC-based corneal therapies through interdisciplinary training across Europe, and the EU COST Action HAPLO-iPS (CA21151) for advancing haploid-selected hiPSC generation from cord blood to reduce production costs and enable homozygous HLA-matched allogeneic therapies for future ocular ATMP development.

T-KA5-4 LYMPHATİC VASCULATURE-DEPENDENT AND INNATE IMMUNE MECHANİSMS İN ORGAN-SPECİFİC RESPONSES TO NUCLEOSİDE-MODİFİED MRNA-LNP PLATFORMS

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Nucleoside-modified, lipid nanoparticle-encapsulated mRNA (mRNA-LNP) vaccines have emerged as a highly effective platform for inducing robust humoral immune responses. Despite their success, the cellular and molecular mechanisms governing antigen delivery, processing, and immune activation remain incompletely understood. In particular, the coordinated roles of the lymphatic vasculature and innate immunity in shaping vaccine efficacy represent critical yet underexplored aspects of this technology.

In this study, we investigate the lymphatic-dependent processes and neutrophil activity that contribute to the initiation and amplification of mRNA-LNP-induced immune responses. Using fluorescently labeled LNP formulations and transgenic mouse models with selective lymphatic deficiency or neutropenia, we characterized the uptake, trafficking, and cellular expression of mRNA-LNPs at the injection site and in draining lymph nodes. Furthermore, antigen-specific humoral responses were assessed following immunization with influenza hemagglutinin-encoding mRNA-LNPs.

Our findings identify the lymphatic vasculature as a central organizer of mRNA-LNP distribution and antigen presentation, and reveal a previously unrecognized role for neutrophils in facilitating effective adaptive immune responses. We show that multiple spatially and functionally distinct mechanisms involving several cell types participate in LNP transport and antigen expression, ultimately influencing the magnitude of the antibody response.

These results provide important mechanistic insights into the early events following mRNA-LNP administration and highlight key determinants of vaccine performance. A deeper understanding of these processes is essential for the rational optimization of mRNA vaccine platforms, with the potential to enhance their efficacy, precision, and translational applicability in infectious diseases and beyond.

T-KA5-5 DO WE NEED MULTI-TARGET DRUGS TO MODULATE THE GENE EXPRESSION PROFILE OF COMPLEX DISEASES? DEVELOPMENT OF MIRNA THERAPEUTICS FOR CARDIOPROTECTION.

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Despite intensive research in the last almost 4 decades, drug treatment of myocardial infarction and the consequent post-infarction heart failure is still an unmet clinical need, possibly due to its complex molecular mechanism. Therefore, targeting a molecular network rather than a single molecular drug target may be the adequate strategy for cardioprotective therapy. Unbiased, comprehensive analysis of gene expression pattern in normal and protected ischemic myocardium may lead to exploration of the transcriptomic and proteomic molecular network to identify novel molecular targets for cardioprotection. The non-coding oligonucleotides microRNAs (miRNA) targeting multiple mRNAs form a transcriptomic dynamic molecular network. Targeted perturbation of the molecular network by miRNA oligonucleotide compounds is a promising approach for treatment of diseases of complex molecular mechanisms.

By a systematic unbiased analysis of the transcriptomic network of healthy, infarcted, as well as protected by ischemic conditioning heart samples, we have discovered endogenous cardioprotective miRNAs (e.g. miR125b-3p, miR-450a-3p) and validated them in vitro and in vivo and patented the nucleotide sequences of around these miRNAs. We termed these miRNAs protectomiRs. We are developing a miRNA discovery platform consists of bioinformatic software packages (see miRNAtarget.com) to build the transcriptomic and proteomic molecular network and identify potential oligonucleotide drug candidates to treat myocardial infarction and other diseases with unmet clinical need. We improve the performance of these algorithms by machine learning to reach an effective drug discovery enabling platform.

In conclusion, miRNA therapy may represent a breakthrough in the treatment of diseases with complex molecular mechanisms such as myocardial infarction. Here we developed a miRNA discovery enabling platform and currently developing miRNA compound pipeline towards clinical phases for several indications.

FRIDAY
5 JUNE 2026

F-KB1 TARGETİNG TRKB-FL CLEAVAGE: A NOVEL THERAPEUTİC STRATEGY İN ALZHEİMER'S DİSEASE AND EPİLEPSY

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Brain-derived neurotrophic factor (BDNF) signaling through its full-length receptor, tropomyosin receptor kinase B (TrkB-FL), is essential for neuronal survival, synaptic plasticity, and cognitive function. Disruption of this pathway has been implicated in multiple neurological disorders.

We identify TrkB-FL cleavage as a convergent pathological mechanism occurring in both Alzheimer's disease (AD) and mesial temporal lobe epilepsy (mTLE).

Across both conditions, TrkB-FL cleavage is triggered under pathological stimuli, amyloid-β (Aβ) in AD and excitotoxicity in epilepsy, leading to reduced receptor levels and the generation of an intracellular fragment (TrkB-ICD). This process occurs early and persists throughout disease progression, being associated with increased pathological severity. In AD, TrkB-FL cleavage correlates with disease progression, while in epilepsy it correlates with seizure severity and duration, and is also observed in hippocampal tissue from patients with refractory epilepsy.

Functionally, this disruption of TrkB signaling contributes to synaptic deficits and cognitive impairment. TrkB-ICD formation was shown to be biologically active, with its overexpression inducing long-term memory deficits and altering gene and protein expression profiles associated with neuronal function and disease.

Importantly, targeting TrkB-FL cleavage using a TAT-fused peptide (TAT-TrkB) revealed therapeutic potential across models. In AD, this peptide prevented receptor cleavage, rescued synaptic and cognitive deficits, and reduced Tau pathology without detectable toxicity. In epilepsy models, preventive administration reduced the incidence of status epilepticus, while therapeutic administration decreased seizure development in a subset of animals and consistently prevented long-term memory impairment.

Together, these findings highlight TrkB-FL cleavage as a shared mechanism underlying neuronal dysfunction in distinct neurological disorders. Targeting this process represents a promising disease-modifying strategy, particularly for preserving cognitive function across both neurodegenerative and epileptic conditions.

F-KA1-1 THE USE OF COMPLEMENTARY FİSH MODELS TO ASSESS NEUROPHARMACOLOGİCAL AGENTS

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The growing demand for reliable vertebrate models in neuropharmacological research has highlighted both the promise and the limitations of zebrafish as a screening tool. While zebrafish offer well-established advantages in physiological and genetic research, their inherently social nature complicates the interpretation of individual-based behavioural assays — a critical requirement in drug discovery and neurobiology. To address this, we propose the paradise fish as a complementary solitary model, enabling a broader and more robust neuropharmacological screening framework.

We systematically compared late larval zebrafish and paradise fish across a battery of behavioural paradigms, including social and non-social exploration, anxiety assessments, and working memory tasks. Paradise fish demonstrated independence from conspecific influence, greater intra- and intertest consistency, and robust spontaneous alternation in Y-maze trials — indicative of reliable working memory and suitability for pharmacological modulation studies. Zebrafish, by contrast, proved more appropriate for socially mediated endpoints.

To enhance the objectivity and throughput of behavioural quantification, we integrated deep learning-based tracking and pose-estimation algorithms into our analysis pipeline. These approaches enabled the automated extraction of fine-grained kinematic and postural features, reducing observer bias.

Together, these findings advocate for a dual-species strategy in neuropharmacological screening: leveraging zebrafish for social behavioural endpoints and paradise fish for individual-based assays. This complementary framework, supported by automated behavioural analysis, offers a more comprehensive and translationally relevant platform for evaluating neuropharmacological compounds.

F-KA1-2 FIRST EVIDENCE OF AN ANXIETY-LIKE BEHAVIOR AND ITS PHARMACOLOGICAL MODULATION IN A WIDELY USED MOLLUSCAN MODEL

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Anxiety is a state that occurs even in the absence of a real threat and enables prediction and preparedness for uncertainty, thereby increasing the chances of survival in dynamically changing environments. However, when anxiety becomes excessive, it can be maladaptive and lead to anxiety-related disorders, which may interfere with the ability to cope with stressful events and affect cognitive functions. Anxiety-related disorders are among the most prevalent psychiatric conditions, impacting 7–10% of the global population. Despite their widespread nature, the precise etiopathogenetic mechanisms underlying these disorders remain unclear. Animal models are essential for unraveling the pathogenic mechanisms of anxiety disorders, offering critical insights that drive the development of safe and effective therapies. Anxiety-like behavior has been characterized in some vertebrates but remains largely unexplored in invertebrates.

We developed and validated a behavioral paradigm to investigate, for the first time in a molluscan model, the anxiogenic effects of predator exposure on behavior. We demonstrated that after being exposed to fish water, which simulates the presence of predators, the great pond snails (*Lymnaea stagnalis*) exhibit a series of sustained fear responses. These include increased aerial respiration, changes in righting behavior, and reduced escape responses. Notably, these behaviors persist even after the stressor (fish water) is removed, indicating that they likely represent an anxiety-like state rather than a simple conditioned reflex. Additionally, exposure to fish water enhances long-term memory formation for the operant conditioning of aerial respiration, suggesting that the predator scent potentially induces a state of heightened alertness, which enhances memory consolidation processes. Furthermore, when snails experience fish water alongside an appetitive stimulus, they form configural learning – a higher form of learning – where the appetitive stimulus now triggers a fear response instead of eliciting feeding. Through dose-response experiments, we found that alprazolam at a concentration of 0.1 µM for 15 min effectively counteracts predator-induced anxiety-like responses without causing sedation. This treatment also prevents the effects of predator cues on learning and memory. However, consistent with data from vertebrates, alprazolam induced temporary anterograde amnesia, impairing the formation of new memories for up to 3 h after treatment.

Overall, this is the first study showing that a molluscan model organism exhibits anxiety-like behaviors similar to those seen in vertebrates, and these behaviors can be mitigated by an anti-anxiety drug. Our results suggest that fundamental anxiety mechanisms are evolutionarily conserved across species. These findings pave the way for future research aimed at dissecting the components of anxiety-like behavior and evaluating anxiolytic drugs in non-vertebrate organisms.

This research was supported by the National Brain Project (#NAP2022-I-10/2022), the Hungarian Scientific Research Fund (#138039, #146787), the COST Action AFFECT-EVO (#CA23106), and the FAR2023_Ricerca diffusa and FAR2024_Ricerca diffusa of University of Modena and Reggio Emilia.

F-KA1-3 LARGE-SCALE PHARMACOLOGICAL CHARACTERIZATION OF PLACOZOAN GPCRS REVEALS THE PREBILATERIAN ORIGIN OF MONOAMINERGIC SIGNALLING

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Monoamine neurotransmitters, derived from aromatic amino acids, play crucial roles in modulating behaviour and physiology in bilaterians. Their presence and function in non-bilaterians have been less clear, leading to the hypothesis that monoaminergic signalling is a bilaterian innovation. This study challenges that view by identifying and characterising monoamine receptors in the non-bilaterian *Trichoplax adhaerens* through comprehensive phylogenetic analyses and large-scale combinatorial deorphanisation assays.

These *Trichoplax* receptors exhibit high sensitivity to simple monoamines such as tyramine, tryptamine, and phenethylamine, consistent with the limited monoamine biosynthesis capacity in *Trichoplax* and other non-bilaterians, which is restricted to aromatic amino acid decarboxylase (AADC). Additionally, one of these receptors, previously misidentified as a "placopsin" (Tadh173), is now identified as a tryptamine receptor and appears to be homologous to a group of orphan human receptors.

These findings demonstrate that monoamine signalling predates the bilaterian lineage and highlight the evolutionary flexibility of receptor-ligand interactions. This study redefines our understanding of receptor evolution and ligand specificity in early metazoans.

F-KB2-1 ENDOCRINE ALTERATIONS IN A MOUSE MODEL OF CHRONIC PANCREATITIS

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Introduction: Chronic pancreatitis (CP) is associated with an increased risk of diabetes, yet the mechanisms underlying endocrine dysfunction are not fully understood. The present study examined endocrine pancreatic function in a mouse model of CP, with particular emphasis on the expression of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel, which may play a role in insulin secretion in β-cells.

Methods: CP was induced in FVB/N mice through repeated intraperitoneal injections of cerulein. Animals were allocated to control, CP, and regeneration groups (REG1–REG4). Endocrine function was evaluated using glucose tolerance tests and insulin ELISA measurements. Pancreatic tissues were either processed for histological analysis or used for islet isolation. Isolated islet cells were immunostained for CFTR, insulin, and glucagon, and subsequently analyzed using dSTORM microscopy.

Results: Transmission electron microscopy revealed mitochondrial damage in the CP group and pronounced cytoplasmic vacuolization during the regenerative phase. CP impaired glucose tolerance, and only partial recovery was observed during regeneration. The insulin response to glucose remained reduced both after CP induction and throughout the regeneration period. Immunostaining demonstrated an early decline in β-cell numbers, which approached near-normal levels by the fourth week of regeneration. Despite this recovery in cell numbers, endocrine cells appeared smaller and displayed an increased nuclear-to-cytoplasmic ratio. CFTR expression in β-cells decreased initially following CP induction but showed a marked increase in later stages of regeneration.

Conclusion: Chronic pancreatitis induces long-lasting structural and functional changes in the endocrine pancreas. Although partial β-cell regeneration occurs during recovery, glucose tolerance and insulin secretion remain compromised. These findings suggest that regenerative processes may restore cell numbers but fail to fully re-establish normal endocrine function, potentially contributing to diabetes development in CP.

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F-KB2-2 GUT–BRAİN AXİS ALTERATİONS AS A VULNERABİLİTY SUBSTRATE İN A RAT MODEL OF PTSD

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Background: Posttraumatic stress disorder (PTSD) develops only in a subset of trauma-exposed individuals, highlighting the importance of identifying biological mechanisms that confer vulnerability. We therefore investigated whether trauma-induced behavioral vulnerability is associated with coordinated alterations in gut intestinal structure and barrier function, and parallel molecular changes in the prefrontal cortex (PFC), a key region implicated in PTSD pathophysiology.

Methods: Male Long–Evans rats were exposed to electric footshock as a traumatic stressor. Based on subsequent behavioral performance, animals were classified as control, resilient, or vulnerable. Post-mortem analyses included ileal histology, quantification of short-chain fatty acids (SCFAs), and gene expression analysis of microbiome-related and barrier-associated molecules. Expression of tight junction and barrier proteins was assessed in both intestinal tissue and the PFC to evaluate gut–brain barrier interactions.

Results: Vulnerable rats exhibited significantly thinner intestinal villi compared to control and resilient animals, while classical tight junction gene expression (ZO-1, occludin, claudins) remained unchanged. Mucosal adaptations were evident: vulnerable animals displayed elevated Muc2 mRNA expression. SCFA levels in plasma and feces were unchanged, suggesting that alternative microbiota-derived mediators may be involved. In the PFC, expression of barrier-related proteins was enhanced in vulnerable rats, with ZO-1 and claudin-11 levels positively correlating with behavioral vulnerability.

Conclusion: PTSD-like vulnerability is associated with coordinated alterations along the gut–brain axis, characterized by mucosal remodeling, and parallel changes in brain barrier-related gene expression. Targeting gut–brain axis mechanisms may therefore offer novel preventive strategies for individuals at risk of developing PTSD.

F-KB2-3 THE GLYCAN–LECTIN AXIS IN VIRAL IMMUNITY: MECHANISMS OF IMMUNE ACTIVATION AND IMMUNE SUPPRESSION FROM DENGUE VIRUS TO HBV

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Viral pathogens engage distinct lectin receptors to drive divergent immune outcomes—from protective immunity to lethal pathology or chronic immune evasion. In dengue virus infection, TLR-mediated interferon responses provide host protection, while CLEC5A—a C-type lectin receptor recognizing dengue envelope glycans—triggers cytokine storm and vascular leakage that culminate in hemorrhagic shock. CLEC5A thus mediates immunopathology rather than antiviral defense, distinguishing pathogenic from protective innate responses. Conversely, hepatitis B virus exploits CD33, a sialic acid-binding lectin, to suppress adaptive immunity. CD33 engagement by HBV surface glycoproteins inhibits anti-HBsAg antibody production, enabling viral persistence through active immune silencing. Targeted disruption of CLEC5A and CD33 pathways attenuates disease in preclinical models, positioning glycan–lectin interactions as rational therapeutic targets to restore protective immunity without triggering immunopathology.

F-KB2-4 TOLL-LIKE RECEPTOR 5 ENGAGEMENT BY BACTERIAL FLAGELLIN MITIGATES TISSUE INFLAMMATION IN NSAID ENTEROPATHY

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Non-steroidal anti-inflammatory drugs (NSAIDs) can cause injury and inflammation to the small intestine, which largely depends on the presence of gut bacteria and the immune responses triggered by them. Previous studies have provided evidence of the crucial role of Toll-like receptor 4 (TLR4) in inflammation associated with enteropathy. Interestingly, activation of TLR5, a receptor for bacterial flagellin, has been shown to reduce intestinal inflammation in various models of intestinal injury. In this study, we aimed to assess whether activation of TLR5 also provides protection against NSAID-induced enteropathy.

Enteropathy was induced by gastric gavage with a high dose of indomethacin (IND, 40 mg/kg) in C57BL/6 mice. Control groups received the vehicle (1% hydroxyethylcellulose). Salmonella-derived flagellin and the TLR5 antagonist TH1020 were given intraperitonelly. The severity of intestinal injury and inflammation was assessed at macroscopic, histological, and molecular levels. Intestinal bacterial counts were assessed using qPCR.

NSAID enteropathy was associated with downregulation of TLR5, intestinal inflammation and apoptosis, mucosal histological damage and higher bacterial counts in the intestine. IND-induced changes, except the fall in TLR5 expression, were reduced or completely prevented in animals that had been pretreated with flagellin. In contrast, treatment with the potent and selective TLR5 antagonist TH1020 exacerbated the intestinal inflammation caused by IND.

Our findings show that TLR5 activation protects against NSAID-induced enteropathy and may therefore be a new therapeutic avenue.

The work was supported by the National Research, Development and Innovation Office of Hungary (NKFI FK 138842).

F-KA2-1 COVALENT APPROACHES İN DRUG DİSCOVERY

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Compounds that form covalent bonds with the nucleophilic amino acid side chains of proteins were long considered undesirable in drug discovery programs. Although compounds with particularly high reactivity should still be avoided due to expected toxicity and immunogenicity problems, the use of covalent mechanism of action has many advantages. This approach may also be suitable for protein targets that cannot be attacked in the traditional way and provides high biochemical efficiency even against targets containing resistant mutations. Our primary focus were target identification and validation that was achieved by covalent fragment approaches developed in our lab.

Covalent fragments, which can be characterized as small molecules containing polar and electrophilic groups, combine the advantages of fragment-based approaches and covalent mechanisms of action. Fragments effectively sample the chemical space relevant to binding sites, allowing them to identify hard-to-target binding sites (PPIs, transcription factors, RNA, allosteric and buried pockets). However, due to their small size, fragments have weak binding affinity, which often makes them difficult to detect. An electrophilic group that forms a covalent interaction with the amino acids at the binding site helps in this regard, as the resulting covalent complex can be easily identified by mass spectrometry.

In my presentation, I summarize our methodological developments targeting cysteine modifications and give a brief overview of the results achieved in drug discovery programs targeting challenging targets including KRAS, STAT3 and STAT5, HDAC8, and viral targets.

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F-KA2-2 PROTEİN DEGRADATİON AS A NEW THERAPEUTİC APPROACH: FROM MOLECULAR GLUES TO BİFUNCTİONAL DEGRADERS

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The field of chemically induced protein proximity, particularly in connection with targeted protein degradation, has emerged as a major focus in medicinal chemistry[1]. Although drugs acting as molecular glues have been in use for decades, their mode of action has only been deciphered recently[2]. Bringig and E3 ligase together with a protein of interest by a small molecule (molecular glue) or by chemically linked individual binders of each protein (Proteolysis Targeting Chimera, PROTAC) in a stable ternary complex can open the way for the degradation of the protein of our interest. The process is not without difficulties. In case of molecular glues the typical challenge is the identification of a suitable starting point, while for PROTACs the size of the bifunctional molecule can significantly impair its drug-likeness.

The presentation showcases some of our recent work in Targeted Protein Degradation including both the molecular glue and the PROTAC approaches[3]. Challenges and opportunities associated with the different modalities will be highlighted from the design of drug candidates to the evaluation of their biological activity.

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F-KA2-3 ANTİBODY-DRUG CONJUGATES: EMERGİNG CHALLENGES AND STRATEGİC OPPORTUNİTİES

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Antibody-drug conjugates (ADCs) represent a transformative modality in oncology by combining the target specificity of antibodies with the high potency of diverse cytotoxic payloads. The field is advancing rapidly, with 17 ADCs approved to date, including 15 by the U.S. FDA, and more than 300 candidates in clinical development. Among these, dual-payload conjugates and other next-generation designs are emerging to address limitations of current ADCs.

However, the complexity of ADC biology continues to hinder rational optimization. Key challenges include understanding the influence of antigen expression (heterogeneity), mechanisms of resistance, and the systematic design of payloads or synergistic combinations. All components of the conjugates (antibody, linker and payload) are to be precisely optimized to reach the desired therapeutic window by reducing systemic exposure and off-target effects.

To address these challenges, we developed a virtual cell–based screening framework for understanding payload mechanism and identifying synergistic payload partners, integrated with automated in vitro validation. Experimental data generated from validated hits are incorporated into subsequent training cycles, forming a lab-in-the-loop learning workflow comprising predict, measure, learn, and train steps. By integrating large-scale simulations with automated validation, this workflow enables the rapid and systematic discovery of payload synergies that can inform next-generation dual-payload ADC design.

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F-KA2-4 DEVELOPMENT OF MRNA VACCİNES AGAİNST LUNG CANCER

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The development of mRNA vaccines represents a promising frontier in the fight against lung cancer, a leading cause of cancer-related mortality worldwide. Traditional treatments, such as chemotherapy and immunotherapy, often face limitations due to resistance and side effects. In contrast, mRNA vaccines offer a more targeted and personalized approach by encoding tumor-specific antigens that stimulate the immune system to recognize and attack cancer cells. Recent advancements in mRNA vaccine technology have demonstrated its potential in preclinical models, showing promising results in eliciting strong immune responses against lung cancer. Studies have also explored the combination of mRNA vaccines with immune checkpoint inhibitors to enhance efficacy and overcome immune evasion mechanisms. Despite the success of mRNA-based COVID-19 vaccines, significant challenges remain, including optimizing delivery systems and ensuring long-term immunity. Ongoing clinical trials are crucial in determining the safety, feasibility, and clinical effectiveness of mRNA vaccines in lung cancer patients. If successful, mRNA vaccines could revolutionize lung cancer treatment by offering a highly specific, adaptable, and less toxic alternative to current therapies.

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F-KB3-1 CYTOTOXICITY, MOLECULAR EFFECTS AND TISSUE LOCALIZATION OF MULTIFUNCTIONAL NANOPARTICLES: INFLUENCE OF NANOCARRIER TYPE

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Breast cancer remains a major clinical challenge due to its heterogeneity, drug resistance, and the adverse effects associated with conventional therapies. Local strategies, such as intraductal administration, have emerged as promising alternatives for managing early-stage lesions, enabling high local drug concentrations while minimizing systemic toxicity. In this context, nanostructured systems may enhance tissue retention, selectivity, and cytotoxicity, thereby maximizing the therapeutic potential of this route. Here, nanostructured lipid carriers (NLC) were developed for paclitaxel (PTX) delivery and systematically evaluated regarding their physicochemical properties, cellular responses, and *in vivo* retention. NLCs displayed sizes below 600 nm and high encapsulation efficiency (>90%). To improve local retention and introduce thermosensitive behavior, two strategies were explored: dispersion of NLCs into Poloxamer 407 to form *in situ* gelling systems, and surface coating with poly(N-isopropylacrylamide) (PNIPAM) to obtain hybrid nanoparticles functionalized with the collagen-binding peptide SILY, which targets a component overexpressed in the breast tumor microenvironment. Incorporating NLCs into the gel enhanced intraductal and tissue retention *in vivo* of hydrophilic and lipophilic probes by approximately 2.1–3.0-fold compared to unmodified NLCs or probe solutions. PNIPAM coating prolonged PTX release for up to 120 h in response to biological temperature and increased nanoparticle interaction with collagen secreted by MCF-7 and T-47D breast cancer cells. In 3D spheroids, nanoencapsulation improved PTX cytotoxicity relative to the drug in solution, although values varied according to the nanoparticle type; NLC (59.0–80.3 μM) > NP-H (90.6–116.5 μM). SILY-functionalized H-NPs showed reduced cytotoxicity toward non-tumorigenic MCF-10A cells, indicating enhanced selectivity. At the molecular level, both NLCs and H-NPs loaded with PTX induced apoptosis in MCF-7 and MDA-MB-231 cells, as demonstrated by elevated BAX levels and PARP-1 cleavage. However, additional carrier-dependent effects were identified. Unloaded nanocarriers increased α-tubulin acetylation, suggesting an inherent ability to modulate cytoskeletal organization. PTX-loaded NLCs induced stronger α-tubulin acetylation and markedly enhanced drug-induced DNA damage (reflected by a 13.4-fold increase in γH2AX) compared to both H-NPs and PTX solution, indicating that nanocarrier composition modulates the molecular response to therapy. In the chicken chorioallantoic membrane model, nanoencapsulation reduced PTX-induced irritation from moderately irritant to non-irritant while preserving its antiangiogenic activity, resulting in a 6.1–7.8-fold inhibition of vessel growth at subcytotoxic doses. These results support nanoencapsulation as a promising approach to enhance PTX efficacy while improving safety in local breast cancer therapy. The distinct molecular responses of lipid and hybrid systems show that nanocarrier composition and structure shape biological outcomes, highlighting the importance of rational nanoparticle design to address current therapeutic challenges.

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F-KB3-2 POLYMERIC NANOPARTICLES AS TOOLS TO ENHANCE SERINIQUINONE TRANSLATIONAL POTENTIAL

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Seriniquinone (SQ) is a selective cytotoxic drug candidate against melanoma cells that uniquely targets dermcidin, a cell survival- and migration-related protein, triggering endoplasmic reticulum stress, loss of cell adhesion, autophagy, and apoptosis. However, SQ is extremely poor water-soluble, which has so far hampered *in vivo* studies, and previous efforts, such as chemical optimization and incorporation into lipid-based nanocarriers, have not succeeded. In this work, we encapsulated SQ into poly(lactic-co-glycolic) acid (PLGA) nanoparticles (NPs) and evaluated their biological activity against melanoma *in vitro* models. NPs were produced by single emulsion-solvent evaporation and characterized via electron microscopy. Encapsulation efficiency and *in vitro* release at plasmatic and lysosomal pH values were quantified by high performance liquid chromatography. Cytotoxicity and uptake were evaluated in SK-MEL-28 and SK-MEL-147 melanoma monolayers by clonogenic assay, flow cytometry, and confocal microscopy. Homotypic and heterotypic spheroids (co-cultured with THP-1-derived macrophages) were used to assess cell viability by ATP quantification, and NP-mediated drug penetration with rhodamine B as a fluorescent probe. Spherical NPs (260–280 nm) with ~83% encapsulation efficiency were obtained, with no visible SQ precipitate under the microscope. SQ release was slow at pH 7.4 (~16% over 96 h) but markedly accelerated under acidic conditions (up to 70% at pH 4.5). In monolayers, NPs increased SQ cell association by clathrin-mediated endocytosis, and showed a reservoir-like behaviour, enhancing long-term cytotoxicity. In homotypic spheroids, IC_{50} values increased by 3.7 to 14.7-fold relative to monolayers, reflecting their higher complexity compared to monolayers. However, in SK-MEL-28 spheroids, encapsulated SQ showed a 2-fold lower IC_{50} than free SQ, and fluorescent probes confirmed NP penetration and broader drug distribution. In SK-MEL-147 spheroids, NPs induced an additional ~25% reduction in viability at the highest concentrations tested, likely due to solubility improvement. In heterotypic spheroids, macrophages were located both peripherally and centrally within non-viable tumour regions. When compared to homotypic spheroids, IC_{50} values increased by 2.2–2.7-fold, although NPs still reduced IC_{50} by up to 50% across models. PLGA nanoparticles successfully addressed the key pharmaceutical barrier of SQ: its poor water solubility. More than that, the nanoparticle system effectively unlocked SQ therapeutic potential by heightening cellular uptake and tumour penetration, while potentiating its antimelanoma activity in *in vitro* models of increasing complexity. The formulation therefore provides a practical and translational route for the long-standing challenge of SQ *in vivo* delivery, now advancing to evaluation in syngeneic, immunocompetent melanoma mice models.

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F-KB3-3 UNRAVELLING THE CYTOTOXIC AND MOLECULAR EFFECTS OF A THERANOSTIC CO-DELIVERY NANOSYSTEM OF METHOTREXATE AND IRON DECORATED NANOPARTICLES ON BREAST CANCER CELL MODELS AS A NOVEL STRATEGY IN BREAST CANCER TREATMENT

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In breast cancer treatment, achieving targeted drug delivery, reducing systemic toxicity, and enabling real-time tumor monitoring remain defiant challenges. Nanomedicine offers solutions through theranostic nanosystems that combine therapeutic interventions and diagnostic procedures, offering a multifaceted approach. In this context, a platform was developed for targeted intraductal delivery using ethosomes coencapsulating methotrexate (MTX) and iron-decorated titanium dioxide nanoparticles (UPNs) for enhanced MRI imaging [3], aiming to improve disease management beyond current limitations. Ethosomes (ETs) were produced via ethanol injection. They measured 100 nm in diameter and demonstrated a round-shaped morphology as demonstrated by transmission electron microscopy. To investigate cytotoxicity, MCF-7 breast cancer cells were employed both in monolayer and as spheroids (outlining a more complex and reliable model); cytotoxicity was assessed via MTT and Resazurin assays. MTT assay showed similar IC_{50} values for ET-MTX and ET-MTX+UPN treatments (19.4 µL/mL and 21.2 µL/mL, respectively). Interestingly, in spheroids UPNs seems to boost ET MTX cytotoxicity, as Resazurin assay reported a lower IC_{50} value for ET-MTX+UPN treatment (4.1 µL/mL) compared to ET-MTX (6.8 µL/mL).

To assess whether MTX encapsulation affects its molecular effects, the expression of Bax (proapoptotic protein) and acetyl-α-tubulin (death marker) was analyzed through Western blot assay. ET-MTX+UPN increased Bax expression in monolayer and spheroids (4.8- and 5-fold, respectively, $p<0.05$), while ETs loaded with UPNs only led to the highest acetyl-α-tubulin expression (2-fold, $p<0.001$) in monolayers. These results suggest that ET-MTX+UPN influences intracellular signaling. The penetration mediated with Ethosomes into spheroids was evaluated using fluorescent probes of phosphatidylcholine (PC, which composes the ET lipid bilayer) and fluorescent methotrexate. Enhanced penetration of fluorescent PC and MTX in the spheroids was observed after 48 h of treatment with of ET-MTX compared to a MTX solution, suggesting that the increase in cytotoxicity may be associated with a deeper and more pronounced drug delivery mediated by ETs.

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F-KB3-4 SEX-INDEPENDENT ROLE OF HYDROGEN SULFIDE IN BLADDER PAIN SYNDROME

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Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a chronic inflammatory condition characterized by pelvic pain, bladder dysfunction, and reduced quality of life, with limited therapeutic options and unclear pathophysiology. This study investigated, for the first time, the endogenous hydrogen sulfide (H_2S) pathway in a cyclophosphamide (CYP)-induced mouse model of IC/BPS, focusing on functional, molecular and oxidative alterations in both sexes.

CYP administration induced mechanical allodynia, urinary dysfunction, and impaired bladder contractility in male and female mice. Mechanical hypersensitivity was similarly increased in both sexes. Voiding frequency increased and urine volume decreased, with females exhibiting more pronounced urinary frequency. Bladder hypocontractility was observed through reduced cholinergic (carbachol-induced) and depolarization-induced (KCl) contractions, indicating both receptor-dependent and receptor-independent contractile impairment. Endogenous H_2S synthesis was significantly reduced 4 hours after CYP administration, accompanied by decreased expression of cystathionine-γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST). At 24 hours, H_2S levels partially recovered despite persistent downregulation of CSE and 3-MST, coinciding with increased cystathionine-β-synthase (CBS) expression in the urothelium and detrusor muscle, suggesting a compensatory enzymatic response. Treatment with the slow-releasing H_2S donor GYY-4137 produced marked therapeutic effects. GYY-4137 reduced mechanical allodynia in both male and female mice, demonstrating, for the first time, its antinociceptive effect in a model of visceral pain associated with experimental cystitis. Additionally, GYY-4137 improved voiding function by reducing urinary frequency. Restoration of bladder contractility was observed in males at all tested doses and in females at intermediate doses. CYP-induced cystitis significantly reduced antioxidant enzyme activity in bladder tissue, including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST). GYY-4137 treatment restored antioxidant defenses in both sexes, although GST activity was not significantly increased in females. These findings indicate that oxidative stress contributes to bladder dysfunction in this model and that H_2S supplementation exerts protective antioxidant effects.

In conclusion, CYP-induced cystitis disrupts the endogenous H_2S pathway, characterized by early reduction in H_2S synthesis and differential regulation of its biosynthetic enzymes. Exogenous H_2S donation with GYY-4137 alleviates pain, improves bladder function, restores contractility and enhances antioxidant defenses in a largely sex-independent manner. These findings highlight the therapeutic potential of H_2S donors for the treatment of IC/BPS-associated bladder dysfunction and pain.

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F-KA3-1 CLEAVAGE OF EXTRACELLULAR DNA - A TREATMENT TARGET

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While intracellular DNA stores genetic information, extracellular DNA (ecDNA) is a damage-associated molecular pattern (DAMP). In a healthy state, endogenous deoxyribonucleases, specifically DNase 1 and DNase 1L3, maintain low ecDNA by continuously hydrolyzing DNA fragments released during cell death. This enzymatic clearance prevents the accumulation of DNA-protein complexes that might otherwise activate innate immune sensors such as the Toll-like receptor 9. In various diseases, the equilibrium between ecDNA release and its enzymatic degradation is disrupted. Insufficient activity of endogenous DNases allows for the persistence of high-molecular-weight ecDNA, which can facilitate the formation of thrombi or promote inflammatory cascades, especially in combination with other DAMPs. The therapeutic application of exogenous DNases aims to restore this balance. By administering recombinant nucleases, it is possible to cleave ecDNA and decrease inflammation in sepsis, hepatorenal injury or other ecDNA-associated inflammatory diseases. The causes and consequences of endogenous DNase variability are not yet clear.

F-KA3-2 PHARMACOTHERAPEUTIC AND SYSTEMIC FACTORS INFLUENCING HOSPITAL MORTALITY: A 13-YEAR FOLLOW-UP

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Objectives: This study aims to analyze the intersection between systemic healthcare factors and pharmacological risks by evaluating the „weekend effect" on in-hospital mortality and the safety profile of oral anticoagulants (OACs) in a high-risk patient population.
Methods: We conducted a 13-year retrospective observational study (2010–2022) analyzing 45,955 hospitalizations at a major internal medicine department in Bratislava, Slovakia. Multivariable logistic regression was used to identify predictors of mortality, focusing on the day of admission. Additionally, a detailed pharmacological sub-analysis was performed on 461 deceased patients to compare the safety of warfarin versus direct oral anticoagulants (DOACs), focusing on INR levels, bleeding complications, and the need for reversal agents.

Results: Patients admitted during weekends faced a significantly higher risk of mortality compared to weekday admissions (AOR = 1.31; 95% CI: 1.22–1.41). Cardiovascular diseases were the leading cause of death (70.4%). Anticoagulation Safety: Among the deceased, warfarin treatment was associated with high INR variability (reaching values up to 23.37) and a significantly higher rate of complications (40.2%) compared to DOACs (6.6%). Clinical Intervention: Therapeutic intervention (Vitamin K, fresh frozen plasma, or prothrombin complex concentrate) was required in 67% of warfarin-related complications and 50% of DOAC-related cases. Prescribing Trends: A significant shift in clinical practice was observed, with DOAC prescriptions increasing from 0% to 80% over the study period.
Conclusions: Our findings confirm that weekend admission is a robust independent predictor of in-hospital mortality in Slovakia. This systemic risk is further compounded by pharmacotherapeutic challenges, where warfarin remains a higher-risk option compared to the superior safety profile of DOACs. Optimizing weekend access to diagnostic services and clinical pharmacological expertise is essential to mitigate these risks and improve patient outcomes.

Keywords: weekend effect, in-hospital mortality, warfarin, DOACs, INR, clinical pharmacology.

F-KA3-3 PLEİOTROPİC ANTİFİBROTİC ACTİONS OF ATORVASTATİN: INHİBİTİNG FİBROBLAST ACTİVATİON AND ENHANCİNG CELL THERAPY VİA DUAL MODULATİON OF GATA4/MEF2C AND THE MİCRORNA AXİS

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Background: Atorvastatin is recognized for cardioprotective effects beyond lipid-lowering; however, the molecular mechanisms governing its impact on cardiac remodelling and its synergy with regenerative cell therapies require further elucidation. This study synthesizes findings on atorvastatin's role in modulating fibroblastic activity and its clinical utility in autologous bone marrow cell (BMC) transplantation.

Methods: The research utilized a dual-model approach: (1) an *in vitro* analysis of human cardiac fibroblasts to evaluate the expression of fibrosis-related transcription factors (GATA4, MEF2C), markers (alpha-SMA), and microRNAs; and (2) a clinical review of 33 patients with "no-option" critical limb ischaemia (CLI) treated with intramuscular BMCs, focusing on prior pharmacotherapy as a predictor of therapeutic response.

Results: Molecular Insights: Atorvastatin significantly reduced the expression of GATA4, MEF2C, and alpha-SMA in cardiac fibroblasts. It also upregulated antifibrotic microRNAs (miR-24, miR-26a, and miR-133a), directly mitigating the drivers of cardiac fibrosis.

Clinical Outcomes: In CLI patients, prior atorvastatin treatment was associated with a significantly higher concentration of bone marrow-derived mononuclear cells (BM-MNCs). These patients showed improved transcutaneous oxygen pressure ($TcPO_2$) and reduced pain scales post-transplant compared to non-statin users.

Predictive Factors: Low baseline C-reactive protein (CRP < 8.1 mg/L) and high BM-MNC concentration were identified as key predictors for "super-responders." Synergy was also observed in patients concurrently treated with atorvastatin and renin-angiotensin system (RAS) inhibitors.

Conclusion: Atorvastatin exerts potent pleiotropic effects by suppressing profibrotic genetic pathways and enhancing the quality of bone marrow-derived cells for regenerative therapy. These findings support its use as a critical adjunct in both managing cardiac remodelling and improving the efficacy of cell-based interventions in ischemic diseases.

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F-KA3-4 ROQUİN-DEPENDENT REGULATİON OF MİCRORNA STABİLİTY İN CARDİAC REMODELİNG AFTER ISCHEMİC INJURY

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Cardiac ischemia and subsequent reperfusion are major contributors to myocardial injury and adverse post-infarct remodeling. While microRNAs are recognized as key regulators of these processes, the mechanisms controlling microRNA stability in the cardiac context remain incompletely understood. The RNA-binding protein Roquin has emerged as an important post-transcriptional regulator, yet its role in the heart has not been fully elucidated.

In this study, we investigated the expression and functional relevance of Roquin in cardiac cells and its involvement in post-ischemic remodeling. Roquin expression was detected in cardiomyocytes and non-myocyte cell populations and was modulated under hypoxic conditions, indicating a role in stress adaptation. Mechanistically, Roquin was found to regulate the stability of specific microRNAs, thereby influencing downstream target gene expression.

In particular, the Roquin-2–miR-23b-5p–ZBTB20 axis was identified as a key regulatory pathway contributing to cardiac remodeling processes. Functional analyses demonstrated that modulation of this axis affects cellular responses associated with survival and remodeling. These findings establish Roquin as a central regulator of microRNA-mediated signaling in the post-ischemic myocardium.

Collectively, this work provides new insights into the post-transcriptional control of cardioprotective pathways and highlights Roquin as a potential therapeutic target for limiting adverse cardiac remodeling following ischemic injury.

F-KA3-5 THE IMPACT OF THE MITOCHONDRIAL UNCOUPLING PROTEIN 2 ON POST-INFARCT REMODELING

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Background: Lack of uncoupling protein 2 (UCP2) expression was identified in pressure overloaded hearts as a molecule determining the shift from fatty acid metabolism to carbohydrate metabolism of the heart. Its role in post-infarct remodeling, however, has still to be defined.

Materials and Methods: This study used UCP2 deficient rats to establish the relevance of UCP2 expression on the level of mitochondria, cardiomyocytes, the heart and the body.

Results: Lack of UCP2 increased the ability of isolated mitochondria to store calcium and therefore protect mitochondria from apoptosis induction. On the single cells level, lack of UCP2 improved the post-hypoxic functional recovery. On the level of the isolated organ, lack of UCP2 improved post-ischemic recovery and increased glucose metabolism and decreased fatty acid metabolism. In vivo, lack of UCP2 expression improved post-infarct function and reduced mortality from 40% to 20%.

Conclusion: A high expression of UCP2 as it occurs in older hearts decreases the ability of the heart to withstand myocardial infarction and post-ischemic remodeling.

F-KB4-1 FROM TANK TO TREATMENT: HOW ZEBRAFISH DRIVE INNOVATION IN ECTOPIC MINERALIZATION THERAPEUTIC RESEARCH

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Ectopic calcification, the pathological deposition of mineral in soft tissues, underlies a wide range of disorders including vascular calcification, chronic kidney disease, and rare genetic conditions such as pseudoxanthoma elasticum (PXE) or generalized arterial calcification of infancy (GACI). Despite its clinical significance, effective therapies remain limited, in part due to incomplete understanding of the molecular and cellular mechanisms underlying aberrant mineralization. This talk will highlight the emerging role of zebrafish as a powerful and versatile model system to address these challenges and accelerate therapeutic discovery.

Zebrafish offer unique advantages for studying ectopic calcification, including optical transparency during early development, rapid growth, and high genetic and physiological conservation of ectopic calciifcation-related genes with humans. These features enable real-time visualization of mineral deposition and facilitate high-throughput genetic and chemical screening approaches. We will discuss how zebrafish models have been engineered to recapitulate key aspects of human calcification disorders, providing insight into disease pathogenesis and identifying novel regulatory pathways.

Furthermore, this talk will explore how zebrafish-based screening platforms have been leveraged to identify candidate compounds that modulate mineralization processes *in vivo*. These efforts bridge the gap between basic biology and translational research and can offer a cost-effective and scalable strategy for early-stage drug discovery. Nonetheless, there are also limitations to be considered, which will be discussed.

By integrating genetic tools, live imaging, and pharmacological screening, zebrafish research provides additional approaches to help understand and treat ectopic calcification. Ultimately, this will contribute to improved outcomes for patients affected by these debilitating conditions.

F-KB4-2 HIF ACTİVATİON: AN ETHİOPATHOGENİC FACTOR İN CARDİOVASCULAR CALCİFİCATİON

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Cardiovascular calcification is characterized by the deposition of calcium–phosphate crystals within the vessel wall and on aortic valves. Rather than being a passive process, it is an actively regulated, cell-driven phenomenon that significantly contributes to morbidity and mortality in chronic kidney disease, atherosclerosis, and calcific aortic valve disease. Our work has focused on elucidating the molecular mechanisms underlying osteogenic reprogramming of vascular smooth muscle cells (VSMCs) and valve interstitial cells (VICs), with particular emphasis on hypoxia signaling.

Using in vitro calcification models and in vivo disease settings, we identified hypoxia as a potent inducer of calcification and as a critical micro-environmental factor that amplifies phosphate-induced mineralization. The pro-calcific effects of hypoxia are mediated through activation of the hypoxia-inducible factor (HIF) pathway. Exposure to hypoxic conditions or pharmacological stabilization of HIF enhances osteo/chondrogenic differentiation, as evidenced by increased expression of RUNX2, SOX9, alkaline phosphatase, and extracellular matrix remodeling enzymes, ultimately accelerating calcium-phosphate deposition.

Importantly, our studies demonstrate that hypoxia-induced metabolic reprogramming, particularly enhanced glycolysis, plays a pivotal role in promoting VSMC calcification. Inhibition of the glycolytic pathway significantly attenuates both hypoxia- and phosphate-induced mineralization, indicating that metabolic adaptation is an essential component of the pro-calcific response. This HIF-driven metabolic shift promotes phenotypic switching from a contractile or quiescent fibroblast-like state toward an osteoblast-like phenotype, thereby linking micro-environmental hypoxia to pathological mineral deposition in vascular tissues.

Collectively, our findings position hypoxia signaling as a central regulatory axis in cardiovascular calcification and identify the HIF–glycolytic pathway interplay as a key mechanistic node integrating stress adaptation with osteogenic reprogramming. Targeting this pathway may represent a novel therapeutic strategy to limit vascular and valve calcification.

F-KB4-3 CALCİFİC AORTİC VALVE STENOSİS: MECHANİSTİC İNSİGHTS ENABLİNG PHARMACOLOGİCAL THERAPEUTİC DİSCOVERY

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Calcific aortic valve stenosis (CAVS) is a progressive fibro-calcific disease for which no pharmacological therapy exists, leaving surgical or transcatheter interventions as the only treatment options. To enable therapeutic discovery, we investigated the cellular and molecular mechanisms driving calcification using primary human aortic valve interstitial cells (VICs) and complementary human tissue analyses.

Our mechanistic studies reveal that inflammation is a major upstream driver of VIC osteogenic reprogramming, with lipid mediators emerging as key modulators of this process. In particular, bioactive lipid pathways and omega-3 polyunsaturated fatty acids appear to influence inflammation and its resolution and may represent promising therapeutic targets. In addition, evidence for intraleaflet hemorrhage implicates erythrophagoctosis and iron-driven oxidative stress as contributors to calcific progression.

Finally, single-cell RNA sequencing of human stenotic valves highlights distinct inflammatory and osteogenic VIC phenotypes, offering a high-resolution roadmap for drug target identification.

Together, these findings provide new mechanistic insights into CAVS pathobiology and outline emerging pharmacological targets for the development of novel innovative medical therapies for this highly prevalent disease.

F-KB4-4 UNDERSTANDİNG PATHOMECHANİSM AND ADVANCİNG ANTİ-CALCİFİCATİON THERAPY THROUGH RODENT MODELS

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Ectopic calcification is the pathological deposition of calcium–phosphate crystals in tissues that normally remain non-mineralized. It represents a central pathological feature of both rare monogenic and common multifactorial disorders (e.g., cardiovascular disease, chronic kidney disease, and diabetes) and is strongly associated with tissue dysfunction, disease progression, and increased mortality. Despite its broad clinical relevance, ectopic calcification remains largely untreatable, primarily due to an incomplete understanding of its underlying molecular mechanisms. Progress in the field has been hindered by the typically late onset and slow progression of calcification in experimental systems, which obscures causal mechanisms and limits mechanistic interrogation.

As in physiological bone mineralization, ectopic calcification is largely governed by the balance between inorganic phosphate, which promotes hydroxyapatite formation, and inorganic pyrophosphate (PPi), a potent endogenous inhibitor of crystal growth. Circulating PPi is generated exclusively from extracellular nucleotide triphosphates, primarily ATP, exported by the transmembrane transporters ABCC6 and ANK(H) and subsequently hydrolyzed by ENPP1. PPi is rapidly degraded by alkaline phosphatase, which removes the inhibitory PPi coating from nascent mineral deposits while simultaneously generating phosphate, thereby promoting calcification.

Monogenic disorders that disrupt PPi homeostasis provide powerful models to dissect the molecular mechanisms underlying ectopic calcification.

Here, we employed genetically modified mouse strains to define the role of extracellular PPi homeostasis in both pathological and physiological settings, and to translate these insights to human rare and common multifactorial diseases. In addition, we established a robust and rapidly progressive preclinical mouse model that overcomes key limitations of existing systems, enabling precise temporal analysis of calcification onset and progression. Using this platform, we can identify critical elements within the phosphate/PPi axis that govern mineral deposition, and demonstrate their therapeutic tractability. Importantly, our model provides a sensitive and reproducible framework for evaluating novel pharmacological interventions, facilitating the preclinical development of targeted therapies aimed at restoring PPi balance and preventing ectopic mineralization.

F-KB5 PHARMACOLOGY İN NEXT-GENSEQUENCİNG TO NEXT GEN THERAPEUTİCS

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The subject of pharmacology after its birth in 1847 has transitioned from identifying plant-based compounds to understanding molecular mechanisms, receptor theory, and genomics, enabling modern personalized medicine and targeted drug development. The drug receptors theories extended to hormones and transduction mechanisms, and identification of biologically active small molecules for optimization into candidate drugs and development of clinical pharmacology for the regulation of efficacy and safety have been the great achievements for the subject during 20th century. However, while the success was challenged with ethnic variations, the success Human Genome Project and the NextGene Sequencing and emergence of Covid-19 brought significant revolution in pharmacology within the first quarter of the 21st Century.

Biomarkers commonly utilized earlier in drug development and drug discovery included biochemical surrogate markers, enzymes, receptors and genes. Of late, application of technologies like antisense oligonucleotides, stem cell research, novel biomarkers like microRNAs, extracellular RNAs, circulating tumour cells have emerged not only as non-invasive diagnostics, but also targets for drug discovery. Today we have many Next-generation therapeutics like gene editing (CRISPR), nucleic acid therapies (mRNA, siRNA), cell therapies (CAR-T), and regenerative medicines. Although, the pharmaceutical industry is benefitted, pharmacology stands at a cross road in the current situation. These NextGene Therapies, beyond doubt, target the molecular root of diseases such as rare genetic disorders and cancers and provide personalized, long-lasting, or one-time cures. However, the unmet need for common acute as well as chronic diseases still remains. The new drug discovery pipeline still remains dry. In many parts of the world, pharmacology is revisiting herbal based drug discovery. There are concerns on the dearth of funding from pharmaceutical companies to the researchers in pharmacology, in spite of the significant role of pharmacology in the new drug discovery. Further to it are the emerging Artificial Intelligence and its application in newer drug discovery and pharmacovigilance.

We have developed concepts of 'Reverse Engineering' in the newer drug discovery utilizing traditional knowledge and 'Reverse Translation' through pharmacovigilance. These strategies are expected to provide required therapeutic opportunities to patients' diseases other than rare genetic disorders, cancer of some common chronic diseases like metabolic syndrome and cardiac diseases, being taken care of by Next-Gene Therapeutics.

F-KB6-1 FROM DYNAMİCS TO DRUG DESİGN: NMR REVEALS HİDDEN, MUTATİON-SPECİFİC SİTES İN KRAS

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Modern structural biology relies on high-resolution methods, such as NMR spectroscopy, X-ray crystallography, and cryo-electron microscopy to achieve atomic-level characterization of biomolecules. While these techniques provide detailed structural information, biological function cannot be understood from static snapshots alone. Proteins are inherently dynamic systems, with motions spanning timescales from femtoseconds to seconds and beyond. Capturing this dynamic behavior is essential for mechanistic insight and for rational drug design.

Here we investigate KRas, a key regulator of the EGF signaling pathway and one of the most prominent oncogenic drivers in colorectal and lung cancers (>80%)[1,2]. Using advanced NMR methods, we characterized the backbone dynamics of wild-type KRas and its major oncogenic mutants (G12C, G12D, G12V).[3,4] Our results demonstrate that both GDP- and GTP-bound forms exist in a dynamic equilibrium of multiple conformational states, including low-population ("minor") states. Remarkably, the Mg^2 -free form closely mimics this minor state and represents an on-pathway intermediate of nucleotide exchange.

This transient conformation reveals mutation-specific "hidden" binding sites that are undetectable in the dominant 3D-structures, offering a route toward selective targeting of oncogenic variants while preserving the function of the wild-type protein. To exploit these findings, we apply complementary drug design strategies: *i)* development of covalent inhibitors with optimized scaffold and warhead interactions (notably for KRasG12C), *ii)* use of photoswitchable ligands to probe weak and dynamic binding regions[5], and *iii)* design of foldamers[6], conformationally constrained non-canonical polypeptide mimetics incorporating β-amino acids, to target shallow and transient surfaces inaccessible to classical small molecules.

These results demonstrate that mapping protein dynamics across multiple timescales, an area where NMR provides uniquely powerful insight, enables the identification of mutation-specific binding sites and supports the development of selective therapeutic strategies against oncogenic KRas.

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F-KB6-2 CRYO-EM AS A TOOL TO UNDERSTAND PHYSİOLOGİCAL DRUG-DRUG İNTERACTİONS

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Meropenem (MEPM) is a broad-spectrum carbapenem antibiotic that unexpectedly interacts with the physiological enzyme acylpeptide-hydrolase (APEH), a large tetrameric serine hydrolase with deeply buried active sites that regulate protein metabolism. Carbapenems uniquely fit into APEH's small substrate pocket because they carry a small functional group on the β-lactam ring, explaining why this interaction occurs with carbapenems, but no other β-lactam antibiotics. Structural rearrangement in the active site leads to covalent binding of MEPM and leaves the enzyme irreversibly inhibited. This binding blocks the enzyme's interaction with valproic acid glucuronide (VPA-G), a broadly used anticonvulsant, activated to function by APEH, causing a drug-drug interaction, which renders valproate-containing medications ineffective.

F-KB6-3 CRYO-EM İN OPTİMİZİNG DİAGNOSİS

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Transthyretin (TTR) is a homotetrameric extracellular protein responsible for the distribution of the hormone Thyroxine (T4), and vitamin A. The stability of its tetrameric functional form is challenged by mutations but also by the processes of aging, resulting in dissociation, unfolding and amyloid deposition. The appearance of aggregated amyloid fibrils of TTR leads to the emergence of amyloid diseases (transthyretin amyloidosis, ATTRs), such as cardiomyopathy (CM) or polyneuropathy (PN). The most widespread diagnostic tool for the detection of ATTR fibrils is 99mTechnetium-based scintigraphy, however, neither the structure nor the binding mode of the radioactive tracers to ATTR amyloid fibrils is known to date. This hinders prompt and accurate diagnosis and formulating effective treatment plans. A combination of theoretical methods and cryo-EM structure determination of the TTR fibrils allows for the atomic characterization of the binding and creates the basis of the design of more specific and effective diagnostic tools and specific interaction partners that may delay or even disrupt amyloidic build-up.

F-KB6-4 FROM RİSK TO OPPORTUNİTY: HARNESSİNG AMYLOİD FORMATİON İN GLP-1–RELATED PEPTİDE DRUGS

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Glucagon-like peptide-1 (GLP-1) analogues and related GPCR agonists (such as semaglutide) have become key therapeutics in the management of widespread diseases, including diabetes, obesity, and cardiovascular complications. Despite their success, large-scale production and long-term stabilization of chemically modified peptide drugs remain major industrial challenges, often constraining supply and limiting the ability to meet market demand. Here, we highlight that the strong aggregation propensity of these peptides is not merely a formulation obstacle, but an evolutionarily encoded feature. Although uncontrolled aggregation can compromise drug safety, harnessing and controlling peptide self-assembly opens new opportunities. In vivo, these hormones are stored as condensed amyloid nanofibers and are released as active monomers upon receiving physiological signals. By mapping the molecular-level structural basis of this reversible self-assembly by Cryo-EM, we aim to translate a natural hormone storage strategy into a rational design principle. This approach may enable the development of a general formulation platform for peptide therapeutics, where the amyloid state serves as a tunable nanofibrous drug reservoir.

F-KB6-5 DE-RİSKİNG DRUG PROGRAMS WİTH CRYOEM: FASTER ANSWERS, BETTER DECİSİONS

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[1]Thermo Fisher Scientific

Structural insights are critical to reducing uncertainty in drug development, yet traditional approaches often struggle to keep pace with the speed and complexity of modern programs. Cryo-electron microscopy (cryo-EM) has emerged as a transformative technology, enabling high-resolution visualization of biomolecular structures without the need for crystallization and across a wide range of target classes.

In this presentation, we explore how cryo-EM can de-risk drug discovery and development by providing faster, actionable insights into target structure, ligand binding, and mechanism of action. By accelerating structure determination timelines and enabling the study of dynamic and challenging systems, cryo-EM supports more informed decision-making at key inflection points—from hit validation to lead optimization and beyond.

We will highlight case studies demonstrating how cryo-EM data has improved confidence in target engagement, guided structure-based design, and helped prioritize programs with the highest likelihood of success. Additionally, we will discuss recent technological advancements that are expanding accessibility and throughput, making cryo-EM an increasingly practical tool across the pharmaceutical pipeline.

Ultimately, integrating cryo-EM into drug discovery workflows enables teams to reduce risk, save time and resources, and make better decisions earlier—driving more efficient and successful therapeutic development.

F-KA5 SURROGATE ENDPOINTS IN CLINICAL TRIALS VS. REALITY: LESSONS FROM THE DEVELOPMENT AND WITHDRAWAL OF ANDEXANET ALFA

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Andexanet alfa was developed as a targeted reversal agent for factor Xa inhibitors (FXaIs) to address the unmet clinical need for rapid anticoagulation reversal in patients with major bleeding or requiring urgent procedures. Acting as a recombinant decoy factor Xa, it neutralizes circulating FXa inhibitors and restores thrombin generation, but also inhibits tissue factor pathway inhibitor, potentially creating a transient procoagulant state. Early studies demonstrated effective laboratory reversal, leading to accelerated regulatory approval in 2018 based primarily on surrogate pharmacodynamic endpoints and single-arm clinical data.

Subsequent evidence raised important concerns. In the ANNEXA4 study, while haemostatic efficacy was judged favourable, thrombotic events occurred in approximately 10% of patients. More definitive data from the randomized ANNEXAI trial showed only modest improvement in haemostatic efficacy compared with usual care, with no reduction in mortality or disability, but a higher incidence of thrombotic complications. Additional safety signals emerged from reports of severe heparin resistance and thrombotic events in cardiac surgery settings.

These limitations, combined with high cost, short duration of action, and logistical complexity, reduced its clinical utility relative to more widely available strategies such as prothrombin complex concentrates. Ultimately, andexanet alfa was withdrawn from the US market in December 2025. This experience highlights the risks of approving therapies based on surrogate endpoints without robust outcome data and underscores the importance of integrating reversal strategies within a broader, multidisciplinary haemostatic management approach

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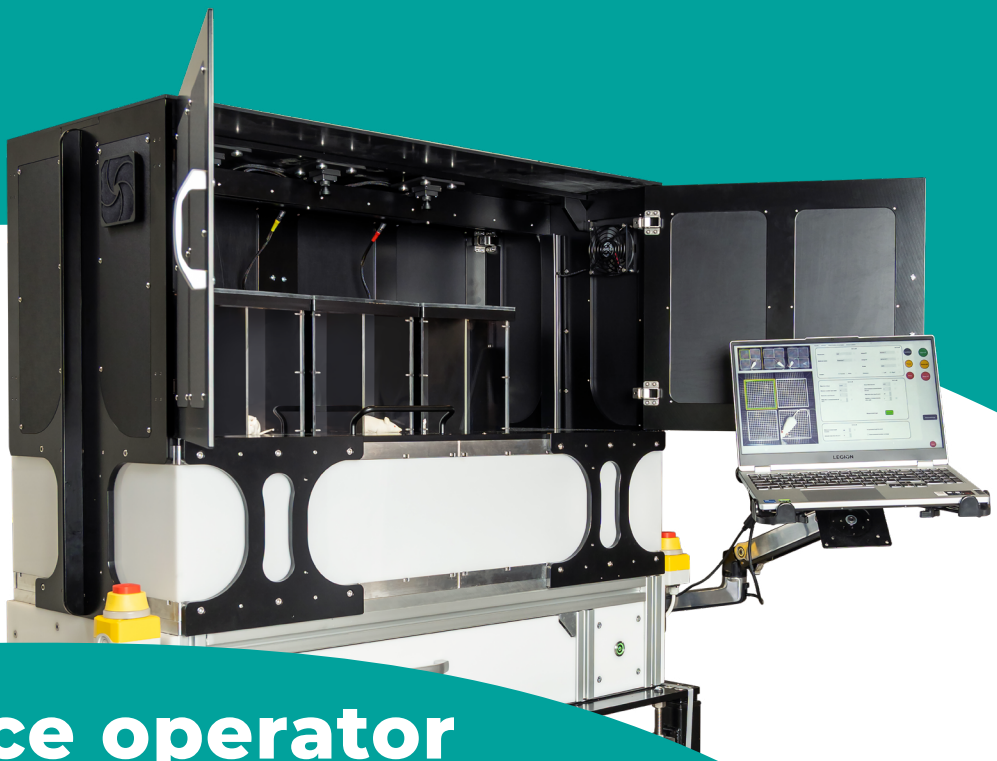

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POSTER ABSTRACTS

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1. MECHANİCAL MODULATİON OF TİTİN ELASTİCİTY BY HEAT SHOCK PROTEİNS

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Heart failure with preserved ejection fraction (HFpEF) accounts for over half of heart failure cases and is strongly linked to comorbidities such as obesity, hypertension, diabetes, and aging. Cardiomyocyte stiffness function emerges from titin, the principal determinant of passive stiffness and diastolic compliance, represents another major biomechanical property of cardiomyocytes and a hallmark in HFpEF, also known as diastolic dysfunction, partly driven by oxidative stress and inflammation. Titin is the main determinant of Fpassive, which is either driven by isoform shift or posttranslational modifications such as phosphorylation, oxidation, and methylation. Heat shock proteins (HSPs) play a central role in protein quality control and protection against redox stress, yet their regulation in HFpEF and impact on titin-based stiffness remain poorly understood (Figure 1-2). The protein quality control system (PQS) maintains myocardial and mitochondrial protein homeostasis by regulating protein synthesis, folding, assembly, trafficking, and clearance of misfolded/damaged proteins. The main cellular signalling pathways through which PQS maintains a healthy proteostasis are HSPs. Here, we performed mechanical, mass spectrometry, immunohistochemistry, and proteomic analyses of titin, titin post-translational modifications, and titin-based stiffness in cardiac and cardiomyocytes from HFpEF patients to investigate the role of titin and titin-dependent mechanical modulation in HFpEF and HFrEF male and female patients. HSPs and redox balance are disrupted in HFpEF and differ from HFrEF and across sex. This leads to impaired titin regulation and diastolic dysfunction. Sex-specific patterns highlight the need for personalized treatment strategies. Targeting protein quality control and redox pathways may offer new therapies.

Keywords: HFpEF, Cardiovascular diseases, HSPs, Titin, Phosphorylation, Oxidation

2. DAMAGES TO THE SARCO/ENDOPLASMIC RETICULUM-MITOCHONDRIA INTERACTION IN A TYPE-2 MYOCARDIAL INFARCTION MODEL

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Myocardial infarction is a leading cause of death worldwide. Type-2 myocardial infarction is caused by non-atherothrombotic events leading to critical imbalance between oxygen supply and demand. Despite available treatments, mortality from the disease is much higher than in atherothrombosis-associated type-1 myocardial infarction. Therefore, better understanding of the underlying pathomechanism and identification of novel therapeutic targets are heavily needed. Many cellular functions damaged in the disease are regulated by signalling between the sarco/endoplasmic reticulum (SR/ER) and mitochondria. SR/ER-mitochondria communication requires close physical contacts between the two organelles which is mediated by interaction between two molecular tethering proteins, the ER membrane protein vesicle-associated membrane protein-associated protein B (VAPB) and the outer mitochondrial membrane protein, protein tyrosine phosphatase interacting protein 51 (PTPIP51). However, despite the importance of SR/ER-mitochondria signalling, the exact local disease-associated changes in organelle tethering in the heart are not characterized. Here, we show, using a mouse isoprenaline induced type-2 myocardial infarction model and in situ proximity ligation assay that VAPB-PTPIP51 tethering is significantly increased in the heart of isoprenaline treated animals compared to the age matched control group. A key function of VAPB-PTPIP51 tethering is to promote calcium delivery from the SR/ER stores to mitochondria. This calcium communication involves in some extent the inositol 1,4,5-trisphosphate (IP3) receptor in the SR/ER and the voltage-dependent anion-selective channel (VDAC) in mitochondria. IP3 receptor and VDAC are closely coordinated in the opposing membranes, and we quantified their juxtaposition. We found that significantly more IP3 receptor is localised near VDAC in the isoprenaline treated than in the control group. Damages to SR/ER-mitochondria interaction may therefore contribute to pathological events in type-2 myocardial infarction and represent a potential new therapeutic target for the disease.

3. IDENTIFICATION AND DEVELOPMENT OR MIR-450A FOR CARDIOPROTECTION

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Acute myocardial infarction (AMI) remains one of the leading causes of death worldwide, however effective pharmacotherapy is not available to protect the heart against ischaemia-reperfusion (I/R) injury. MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression at the post-transcriptional level and have emerged as promising multitarget therapeutic candidates in ischaemic heart disease. Using a translational porcine model of AMI, we previously identified cardioprotective miRNA candidates termed ProtectomiRs. Among these, miR-450a was selected for further development. The present study aimed to validate the cardioprotective effect of miR-450a across species and to explore the potential molecular pathways underlying its action.

ProtectomiR candidates were identified in a clinically relevant porcine AMI model and subsequently validated in neonatal rat cardiomyocytes (NRCM) and AC16 human cardiomyocyte cells exposed to simulated I/R conditions. The effect of miR-450a mimic was assessed over a concentration range of 0.75-100 nM using cell viability measurements. To investigate the underlying molecular mechanisms, predicted target genes of miR-450a were identified using miRNAtarget™ software, followed by Gene Ontology (GO) and KEGG pathway enrichment analyses.

miR-450a demonstrated a dose-dependent cardioprotective effect in both NRCM and AC16 cells under simulated I/R. A concentration of 25 nM significantly increased cell viability, and protective effect was also observed at lower concentrations. Bioinformatic analysis identified SMAD2, DAPK2 and SOD2 among the strongest predicted targets, linking miR-450a to TGF-β signalling, apoptosis and redox regulation. Pathway enrichment analysis further highlighted mTOR and phosphatidylinositol signalling pathways, suggesting modulation of key survival networks.

In conclusion, miR-450a induces cardioprotective effects in both rat and human cardiomyocytes through the regulation of key cardioprotective signalling pathways. These findings support the further development of miR-450a as a potential cardioprotective therapeutic candidate.

4. THE IMPACT OF AGING BONE MARROW ON CARDIAC DYSFUNCTION IN A MOUSE MODEL OF HFPEF

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Heart failure with preserved ejection fraction (HFpEF) is responsible for more than half of all heart failure cases and continues to rise, particularly with aging. In addition to cardiac remodelling, fibrosis, and systemic inflammation seen in HFpEF, recent studies suggest an important role of the bone marrow-heart axis in inflammatory and immune responses. However, the underlying mechanisms driving these changes, especially with aging, are still not well understood.

We aim to investigate age-associated immunomodulatory and structural changes in the heart and bone marrow, providing insights into their roles in HFpEF disease progression.

Ten-week-old male C57Bl/6J mice were randomized to receive a control diet (CON) or a high-fat diet (HFD) + L-NAME for 17 weeks (n=10/group). Cardiac function was assessed by echocardiography. At termination, organs were collected for histological examination (Picrosirius Red, WGA, Isolectin B4), molecular analysis (qPCR), and RNA sequencing. To study the effect of aging, findings were compared with 17-month-old mice (n=8/group) subjected to the same protocol.

Our echocardiographic results confirmed the development of the HFpEF phenotype in treated mice, characterized by preserved ejection fraction and signs of diastolic dysfunction (elevated E/E' ratio), as well as Structural changes (LV wall thickening and increased LV mass). Histology revealed increased fibrosis, cardiomyocyte hypertrophy, and reduced microvascular density in the treated mice compared to the control. Molecular and sequencing analyses of the heart showed elevated inflammatory and fibrotic markers, as well as cardiac remodeling, in treated mice. Importantly, aged mice exhibited more pronounced functional and structural cardiac changes than young mice. Bone marrow studies using molecular and RNA sequencing revealed aging-driven inflammatory, fibrotic, and remodeling changes, associated with CHIP (clonal hematopoiesis of indeterminate potential; *DNMT3A* mutation), and promoting stress hematopoiesis.

Our study successfully established an HFpEF mouse model with preserved ejection fraction and diastolic dysfunction. The model reproduced key features of HFpEF, including fibrosis, hypertrophy, microvascular rarefaction, and inflammation. These changes were exacerbated by the aged bone marrow, suggesting a link between aging, bone marrow alteration, and heart dysfunction. Moving forward, we aim to investigate different inflammatory pathways within the bone marrow-heart axis, providing novel therapeutic targets for the treatment of HFpEF.

5. OPTİMİZİNG A DRUG SCREENİNG PLATFORM TO MİMİC İRON-LADEN MACROPHAGES WİTH PRO-İNFLAMMATORY PHENOTYPE

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Introduction: Cardiovascular diseases, such as ischemic heart disease or chronic heart failure (HF), are among the leading causes of death worldwide. Following cardiac injury, in the hemorrhagic microenvironment, recruited macrophages take up iron and predominantly differentiate into a pro-inflammatory phenotype. Macrophages are crucial in maintaining iron homeostasis; however, in multiple aetiologies, the balance between their activation states tilts toward chronic inflammation. The iron stays trapped within the macrophages, further aggravating functional iron-deficiency and energy loss in cardiomyocytes. Anemia and iron deficiency are common among HF patients. We aim to establish a cell platform of iron-laden macrophages to investigate whether the currently approved HF medications have any effect on them.

Methods: To model macrophages, we differentiated THP-1 human monocyte cells with 100 ng/mL PMA, then primed the M0 macrophages toward a pro-inflammatory phenotype with 100 ng/mL LPS. To simulate iron-overload, we tested freshly isolated red blood cells (RBC), senescent RBCs, or 200 µM ferric ammonium citrate (FAC) supplementation for 6 hours. Then, to induce NLRP3 inflammasome activation, we added nigericin for 20 minutes to the FBS-free media. We conducted qRT-PCR analysis on our samples to assess major changes in macrophage phenotype and iron homeostasis.

Results: Our preliminary findings showed that RBCs induced a significant elevation of *Cxcl10* (an M1 macrophage marker) expression in all conditions. Senescent RBC treatment induced significant *Nlrp3* expression in the pro-inflammatory macrophages. Interestingly, *Hmox1* showed the most prominent elevation by FAC treatment. Transferrin-dependent iron uptake was significantly higher in the RBC and LPS-treated group. However, iron export via ferroportin was elevated in all treatment groups, indicating that the 6-hour treatment might not be sufficient to induce iron-overload. The marked elevations in *Ncoa4* (a ferritinophagy marker) by RBC and senescent RBC treatment suggest that iron is accumulating in the labile iron pool (LIP), which is a step toward iron-overload and might sensitize cells toward ferroptosis.

Conclusion: We developed an inflammatory, iron-laden phenotype, but our model needs further optimization. Iron-laden macrophages might prove to be an important drug target in the future. The expression change in *Ncoa4* is in line with previous observations that it might have a detrimental role indicating elevated ferritinophagy in heart failure. To further elucidate the mechanisms behind the disturbances of iron homeostasis, we plan to investigate the non-transferrin-bound iron uptake of macrophages and their co-culture with cardiomyocytes to detect ferroptosis.

6. THE EFFECT OF FSCPX, AN İRREVERSİBLE ANTAGONİST, ON THE MAXİMUM OF THE DİRECT NEGATİVE İNOTROPİC EFFECT MEDİATED BY THE A_1 ADENOSİNE RECEPTOR İN RAT AND GUİNEA PİG ATRİA

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In this study, concentration-effect (E/c) curves were constructed with CPA, a selective A_1 adenosine receptor full agonist, in isolated, paced, rat and guinea pig left atria after a pretreatment with one of three concentrations (0, 10 or 50 µmol/L) of FSCPX, an irreversible A_1 adenosine receptor antagonist, via measuring the contractile force.

To evaluate the E/c curves, the operational model of agonism and the Signal Amplification, Binding affinity and Receptor-activation Efficacy (SABRE) model were applied. The equilibrium dissociation constant of the agonist-receptor complex (K_d) was determined with both models, whereas the fraction of the operable receptors after partial irreversible receptor inactivation (q) was assessed with the SABRE model. K_d, when determined from E/c curve data, is characteristic of the so-called macroscopic affinity, while q can be used to compute a simple measure of receptor reserve, i.e. the percentage of receptors not required to produce (the practically) maximal response: (1-q)·100%.

Our findings were as follows: 1) the FSCPX pretreatment, although implemented with an optimized protocol, was unable to significantly reduce the maximal direct negative inotropic effect of CPA, indicating a huge receptor reserve in this regard; 2) K_d values estimated with the two models were very similar, representing the similar capabilities of these models to determine K_d; 3) q obtained with the SABRE model was very small, supporting the presence of a huge A_1 adenosine receptor reserve for the direct negative inotropic effect of CPA in both the rat and guinea pig atrial myocardium (99.3% for both species).

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7. THE INTERACTION BETWEEN AB-680 (A CD73 INHIBITOR) AND NBTI (A NUCLEOSIDE TRANSPORTER INHIBITOR) ON THE A_1 ADENOSINE RECEPTOR-MEDIATED DIRECT NEGATIVE INOTROPIC EFFECT IN THE GUINEA PIG ATRIUM

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We investigated the action of AB-680, a potent CD73 inhibitor, on the effect of NBTI, a nucleoside transport inhibitor, on concentration-effect (E/c) curves constructed with CPA, a selective, full agonist of the A_1 adenosine receptor, in the isolated, paced guinea pig left atrium.

Modifications of the CPA E/c curves, in the absence and presence of AB-680 and NBTI, were used to evaluate the alterations in the interstitial level of adenosine. These changes were determined with the receptorial responsiveness method (RRM), a procedure yielding the CPA concentration equieffective with the increase in the interstitial adenosine concentration produced by NBTI.

The major findings of the present study were that AB-680, when administered alone, did not affect the response to CPA. However, when co-administered with NBTI, AB-680 could reverse the elevating effect of NBTI on the interstitial adenosine level, but only to a moderate extent. AB-680 reduced the increase in the interstitial adenosine concentration caused by NBTI by at least half, when co-administered with NBTI.

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8. DAMAGES TO THE ENDOPLASMIC RETICULUM-MITOCHONDRIA INTERACTION AND STEROID HORMONE SYNTHESIS IN MYOCARDIAL INFARCTION

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Myocardial infarction and associated heart failure are leading cause of death worldwide. Excessive steroid hormone production in the adrenal gland and inflammatory responses all contribute to the deterioration of the patients' condition. Despite available treatments, mortality from the disease is still high. Therefore, better understanding of the underlying pathomechanism and identification of novel therapeutic targets are heavily needed. Signalling between the endoplasmic reticulum (ER) and mitochondria is involved in steroid hormone synthesis and inflammatory processes. ER-mitochondria communication requires close physical contacts between the two organelles which is mediated by interaction between two molecular tethering proteins, the ER membrane protein vesicle-associated membrane protein-associated protein B (VAPB) and the outer mitochondrial membrane protein, protein tyrosine phosphatase interacting protein 51 (PTPIP51). However, despite the importance of ER-mitochondria signalling, the exact local disease-associated changes in organelle tethering in the adrenal gland is not characterized. Here, we show, using a rat myocardial infarction and heart failure model and in situ proximity ligation assay that VAPB-PTPIP51 tethering is significantly increased in the adrenal gland of infarcted animals compared to the age matched control group. To dissect the extent by which steroidogenic and inflammatory signals are involved in this defect we utilised human adrenocortical carcinoma 15 (HAC15) cells. We demonstrate, again using in situ proximity ligation assay, that treating HAC15 cells with steroidogenic or pro-inflammatory stimulant both significantly increase VAPB-PTPIP51 tethering. Moreover, they also affect inositol 1,4,5-trisphosphate receptor and voltage-dependent anion-selective channel proximity implying that calcium signalling between the ER and mitochondria is also potentially perturbed. Damages to the ER-mitochondria interaction may therefore contribute to pathological events in myocardial infarction and associated heart failure, and represent a potential new therapeutic target for the disease.

9. CHRONIC BEMPEDOIC ACID TREATMENT INDUCES SLIGHT TRANSCRIPTOMIC AND PROTEOMIC ALTERATIONS IN THE RAT MYOCARDIUM

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While conventional lipid-lowering therapies like statins provide cardioprotection, they might be associated with the risk of hidden cardiotoxicity. We have demonstrated that chronic treatment with bempedoic acid (BA), a novel ATP-citrate lyase inhibitor, had no effect on infarct size in a rat model of acute myocardial infarction, however, reduced reperfusion-induced arrhythmias. The molecular mechanism of the effect of the BA is not known.

Therefore, here we aimed to explore the transcriptomic and proteomic changes of the left ventricle (LV), liver, and adipose tissue following chronic in vivo BA treatment.

Male Wistar rats received BA (30 mg/kg/day) or vehicle for 28 days. The rats were subdivided into sham-operated and ischemia/reperfusion (I/R) groups. Ischemia was induced by a 30-minute ligation of the left anterior descending coronary artery, followed by 120 minute of reperfusion. RNA sequencing was performed on LV, liver, and adipose tissues. In the LV, the proteomic and phosphoproteomic profiles were also analyzed, followed by Gene Ontology (GO) enrichment analysis to characterize differentially expressed genes (DEGs).

In sham-operated rats, BA treatment significantly modulated 63 genes in the LV (50 upregulated, 13 downregulated), primarily associated with cellular metabolism. Integration with proteomic data confirmed significant expression shifts in Mpc2, Pfdn2, Tpm, and Mlycd. Furthermore, BA induced dephosphorylation of key cardiac proteins, including Gys1, Ctnna1, and Ttn. Under I/R conditions, BA altered the expression of Lrpprc, Cfd, and Lsm10 in the LV. The most extensive transcriptomic changes occurred in the liver (432 DEGs), where GO analysis identified enrichment in cholesterol and lipid metabolic pathways. Adipose tissue exhibited modest changes (30 DEGs) related to stress response pathways.

In conclusion, chronic bempedoic acid leads to transcriptomic changes mainly in the liver, affecting several pathways including lipid-metabolism. The effect of BA on gene expression profile of cardiac and adipose tissue are less pronounced, suggesting that the direct impact of BA on the myocardial transcriptome and proteome is minimal compared to its effects on the liver.

10. EXTRACELLULAR VESİCLES DERİVED FROM UMBİLİCAL CORD-MESENCHYMAL STEM CELLS PROTECT CARDİOMYOCYTES AGAİNST CARDİOTOXİCİTY İNDUCED BY CHEMOTHERAPEUTİC DRUGS BY REGULATİNG APOPTOSİS AND AUTOPHAGY

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Cardiotoxicity is often a side effect of treatments used to treat cancer. Its severity ranges from asymptomatic to life-threatening, and there is currently no effective specific treatment. At present, discontinuation of cancer therapy and symptom management are the most common approaches to treating chemotherapy-induced cardiac side effects. Mesenchymal stem cells (MSCs) are multipotent stem cells found predominantly umbilical cord (UC), bone marrow, and adipose tissue, that are being thoroughly studied for their regenerative properties. The secretome of MSCs, and especially MSC-derived extracellular vesicles (EVs), exert numerous protective effects, mainly through bioactive molecules such as miRNAs found in the cargo. MSC-EVs are promising treatment options for chemotherapy-induced heart damage, but so far only a few studies showed feasibility.

To understand the cardioprotective potential of UC-MSC-derived EVs against cardiotoxicity causing chemotherapeutics we utilized in vitro cell-based functional assays and transcriptomics. EVs were isolated from UC-MSC cells by ultracentrifugation, and their effect on reducing chemotherapy-induced cardiotoxicity was studied using in vitro cell-based methods and transcriptomic analyses.

Our treatment reduced the degree of chemotherapeutics-induced cardiotoxicity. Our transcriptomic analysis highlighted that the treatment affected several cell survival signaling pathways, two of which, apoptosis and autophagy, were experimentally confirmed. UC-MSC-EVs derived from immortalized cells were able to significantly reduce chemotherapy-induced apoptosis and autophagy. In both cases, a variability was observed in mechanistic efficacy, highlighting the importance quality control of products and manufacturing.

In conclusion, UC-MSC-derived EVs have cardioprotective potential and reduce the cardiotoxicity caused by commonly used chemotherapeutic agents.

11. CARDIOPROTECTIVE MIR-125B*: STEPS TOWARDS CLINICAL TRANSLATION

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MicroRNAs (miRNA) have the potential to be therapeutic agents and to become multitarget drugs for complex diseases like ischemia-reperfusion (I/R) injury. Previously, we have identified miR-125b* ProtectomiR, i.e. cardioprotective miRNA showing protection against I/R injury in vitro and in vivo rat preclinical models. Here, we aimed to test miR-125b* in a human-derived cardiomyocyte cell line, AC16, under sI/R conditions as a next step to its clinical translation.

We transfected AC16 cells with miR-125b* at 6.25, 12.5, 25, 50, and 100 nM concentrations. After 24 hours, we subjected the cells to 16 hours of simulated ischemia followed by 2 hours of reperfusion and measured cell viability using CellTiter-Glo assay. We predicted the targets of miR-125b* in humans and rats using miRNAtarget.com, analyzed their expression in AC16 cells based on our previous RNA sequencing data, and assessed their function by gene ontology (GO) enrichment analysis. We determined the overlapping predicted target genes in rats and humans and performed GO analysis to investigate the function of the overlapping and non-overlapping target genes.

miR-125b* did not influence significantly cell viability after sI/R in the tested concentration range in AC16 cells. There were 6020 predicted target genes in humans and 2549 in rats. Of these, 78% and 72% of human and rat target genes are expressed in AC16 cells, respectively. 21.5% and 50.8% of human and rat predicted target genes were overlapping, respectively. We found those target genes that are involved in biological processes, such as cell death, angiogenesis, and stress response could contribute to the cardioprotective effect of miR-125b* in neonatal rat cardiomyocytes and potentially in human cardiomyocytes.

In summary, miR-125b* was not cardioprotective in AC16 cardiomyocytes within the observed concentration range. Although the AC16 cell line is suitable model for in vitro screening experiments, it might be less optimal for translational models for cardioprotection. Therefore, to move forward the clinical translation of miR-125b*, we continue the cardioprotection testing in human induced pluripotent stem cell-derived cardiomyocytes.

12. CARDIOVASCULAR EFFECTS OF ANTI-GOUT DRUGS: EVALUATION OF ECHOCARDIOGRAPHIC DIFFERENCES OF FEBUXOSTAT AND ALLOPURINOL IN A MOUSE MYOCARDIAL INJURY MODEL

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Introduction: In the last decade, many trials have been published about cardiovascular safety of two anti-gout medications. Results are ambiguous, given that CARES trial have revealed elevated mortality in patients with febuxostat in comparison with allopurinol. Other trials have shown no alteration in cardiovascular events. Additionally, females with gout are more vulnerable to cardiovascular diseases. However, little data are in literature of sex differences of anti-gout medications.

Purpose: Our objective was to evaluate the echocardiographic and fibrotic differences of febuxostat and allopurinol in a myocardial injury mouse model.

Methods: C57BL/6 mice from both sexes were used in present study. After an acclimatization period, baseline echocardiographic measurements were carried out, followed by the randomization into the following five treatment groups: vehicle control, isoprenaline (ISOP)-only, febuxostat-only, febuxostat plus isoprenaline and allopurinol plus isoprenaline. In the first two weeks, animals were only received per os daily treatment with 10 mg/ body weight kg (BWkg) febuxostat and 20 mg/BWkg allopurinol. Then, echocardiographic data were acquired, followed by myocardial injury induction with 200 mg/BW kg ISOP, and followed by another ultrasonographic imaging. In the next three weeks, the animals were treated two times weekly with ISOP 100 mg/BW kg for myocardial injury maintenance, alongside the daily per os regimen. After a total of five weeks of treatment, following the last echocardiography, mice were sacrificed for histological analysis. Long and short axis M-modes were acquired for ultrasonographic assessment. Fibrosis was assessed by picrosirius red staining and image analysis software.

Results: Diminished relative wall thickness (RWT) was observed in febuxostat plus ISOP treatment group (RWT= 0.493±0.053), in comparison with ISOP-only group (RWT=0.662±0.026) in females. Febuxostat also resulted in higher end-systolic and end-diastolic values. Additionally, significant decline in fractional shortening was observed in febuxostat plus ISOP group (FS before/after induction: 27.244±3.666 / 19.512±5.047), meanwhile such alteration was not present in the allopurinol plus ISOP group (FS: 24.887±5.735 / 25.295±7.346). On the other hand, males with febuxostat plus ISOP were presented with enhanced wall thickening (RWT=0.557±0.119) in comparison with febuxostat-only group (RWT=0.403±0.059). Febuxostat treatment in comparion with ISOP-only group in females did not alter fibrosis, meanwhile it was enchanced in males.

Conclusion: Our study revealed key differences of febuxostat effects on diametric data: in females it diminishes wall thickening, resulting in dilated cardiomyopathy, meanwhile in male more pronounced wall thickness was observed with more pronounced fibrosis. In future studies, mechanism of cardiovascular effects of febuxostat on females will be investigated.

13. MODULATION OF APOPTOTIC SIGNALING IN HUMAN CARDIAC FIBROBLASTS BY ACETYLSALICYLIC ACID AND IBUPROFEN UNDER ACUTE INFLAMMATORY STRESS

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Introduction: Non-steroidal anti-inflammatory drugs (NSAIDs), such as acetylsalicylic acid (ASA) and ibuprofen (IBU), exert pleiotropic effects beyond cyclooxygenase inhibition, including modulation of cell survival and apoptosis. In the cardiovascular system, these pleiotropic effects on cardiac fibroblasts may be critical, as dysregulation of their survival–apoptosis balance can contribute to pathological myocardial fibrosis and heart failure. Aim: This study aimed to evaluate the effects of ASA and IBU on apoptosis under tumor necrosis factor alpha (TNF-α)–induced pro-inflammatory conditions in human cardiac fibroblasts (HCFs). HCFs were treated with therapeutic and supratherapeutic doses of ASA (3,12 µM, 30 µM) and IBU (150 µM, 300 µM) for 48 h following TNF-α stimulation (5 ng/mL). Results: High-dose IBU, combined with TNF-α, significantly reduced cell viability (MTT assay), increased Annexin V positivity, and activated the intrinsic apoptotic pathway, as indicated by an elevated Bax/Bcl-2 ratio. Low-dose IBU caused only marginal viability loss with minimal changes in p-p53, Bax, or Bcl-2. While low-dose ASA maintained HCF homeostasis, high-dose ASA was associated with apoptosis induction via p-p53 and Bax upregulation. Conclusion: These findings suggest that high-dose NSAID therapy may inadvertently impair cardiac tissue repair, emphasizing the need for dose-optimized anti-inflammatory strategies in cardiovascular patients.

Keywords: human cardiac fibroblasts, apoptosis, acetylsalicylic acid, ibuprofen, TNF-α

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14. FEBUXOSTAT EXACERBATES DOXORUBİCİN-INDUCED CARDİAC REMODELİNG İN MİCE

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Introduction: Clinical trials evaluating the cardiovascular risk of the anti-gout medication febuxostat (FEBU) show contradictory mortality results. As FEBU is often prescribed alongside cardiotoxic chemotherapeutics like doxorubicin (DOXO), assessing their combined cardiac risk is clinically essential.

Purpose: This study investigated whether FEBU exacerbates DOXO-induced cardiotoxicity in a murine model of cardiomyopathy.

Methods: Ten-week-old female BALB/c mice were randomised into four groups: control (n=12), FEBU (n=12), DOXO (n=13), and DOXO+FEBU (n=13). To induce subacute cardiomyopathy, mice received DOXO (5 mg/kg) or saline intraperitoneally twice weekly to reach a cumulative dose of 20 mg/kg. Concurrent FEBU (20 mg/kg) or vehicle was administered via oral gavage. Body and tibia-normalised organ weights were recorded, while cardiac structure and function was assessed via echocardiography and histology.

Results: Both DOXO and DOXO+FEBU groups exhibited significant weight loss compared to controls, however, the combination therapy caused significantly greater weight loss than DOXO alone (-2.23 ± 0.34 g vs. -3.39 ± 0.32 g, p=0.017). In both DOXO and DOXO+FEBU groups the ex vivo heart weights were significantly reduced. While both treatments reduced cardiac output, only the DOXO+FEBU group showed significantly elevated left ventricular end-diastolic and systolic volume indices (0.79 ± 0.03 vs. 0.94 ± 0.03, p=0.007 and 0.36 ± 0.02 vs. 0.47 ± 0.03, p=0.03). Furthermore, co-administration significantly reduced key structural parameters, namely diastolic and systolic left ventricular posterior wall thickness, as well as the relative wall thickness of the heart (0.31 ± 0.01 vs. 0.27 ± 0.01, p=0.03). Picrosirius red staining confirmed increased myocardial fibrosis in both DOXO and DOXO+FEBU groups compared to controls ($p<0.01$). Fluorescent immunohistochemistry for isolectin B4 confirmed significantly lower capillary count in the DOXO+FEBU group compared to DOXO only ($p<0.0001$).

Conclusions: FEBU co-administration exacerbates adverse cardiac remodelling and functional impairment in DOXO-induced cardiomyopathy. These findings suggest a potential drug interaction that warrants further mechanistic investigation to ensure patient safety in oncologic settings.

15. STATIN TREATMENT AND SARCOPENIA: A SYSTEMATIC REVIEW AND METANALYSIS

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Statin-associated muscle symptoms are frequent side-effects of statins, ranging from mild discomfort to severe weakness. A significant proportion of statin-treated patients are at increased risk for sarcopenia, questioning safety of statins in this category. Therefore, we aimed to investigate whether statin treatment is associated with physical traits recognized in sarcopenia through systematic review and metanalysis.

A systematic search of MEDLINE, Embase, and Cochrane databases was conducted on 19/11/2025, adhering to our Cochrane protocol. 75 articles comparing statin against not-statin treated subjects were included, regardless of dominant comorbidity. Primary outcomes, the most often encountered across modern definitions of sarcopenia, were hand grip strength, walking speed and chair stand test. Secondary ones were timed up-and-go test, stair climb test, peak knee force and Short Physical Performance Battery. A random-effects model was employed to calculate difference of means (MD) with 95% confidence intervals (CI), accounting for inter-study heterogeneity.

None of the main outcomes were statistically or clinically significant differences: hand grip strength, (MD) 0.10 kg [95% CI: −0.90, 1.09], walking speed (MD) 0.00 m/s [−0.05, 0.04], and chair stand test (SMD: −0.01 [−0.41, 0.39]) showed virtually no change with statin therapy. Post-hoc analysis was performed for 6-minute walking test; in a MD plot consisting of 6373 patients, a statistically significant increase in walking performance (MD: 20.72 [6.32, 35.12] m) was reported for the statin-treated group. Subgrouping by comorbidities, chronic heart failure patients displayed highest benefit with intervention (MD: 53.52 [21.37, 85.68] m). All but one study showed risk of bias ranging from low to high, and level of evidence for most outcomes was low.

We conclude statin treatment is not associated with sarcopenia. Moreover, chronic heart failure patients appear to have some benefit with respect to 6-minute walk test. According to current evidence, prescribing statins for patients susceptible to sarcopenia appears to be safe.

16. ATORVASTATIN ATTENUATES TGF-B-INDUCED FIBROTIC RESPONSES IN HUMAN CARDIAC FIBROBLASTS

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Activation of cardiac fibroblasts into α-smooth muscle actin (α-SMA)-expressing myofibroblasts is a key step in cardiac fibrosis. While atorvastatin is known for its pleiotropic cardioprotective effects, its ability to modulate the transforming growth factor beta (TGF-β) signaling pathway and specific transcriptional regulators remains incompletely characterized.
Primary human cardiac fibroblasts (HCFs) were stimulated with TGF-β (10 ng/mL) for 48 h in the presence or absence of atorvastatin (10 μM). Atorvastatin partially reduced TGF-β-induced α-SMA expression and decreased extracellular matrix proteins COL1A1 and COL3A1. This was accompanied by lower levels of the profibrotic transcription factors GATA4 and MEF2C, and a selective upregulation of antifibrotic microRNAs (miR-24, miR-26a, miR-133a). These results indicate that atorvastatin may modulate fibrotic responses in HCFs via transcriptional and post-transcriptional mechanisms, suggesting a potential role in mitigating pathological remodeling.

Keywords: human cardiac fibroblasts, cardiac fibrosis, atorvastatin, GATA4, MEF2C, microRNAs

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17. REPURPOSING SGLT2 INHIBITORS TO MITIGATE DOXORUBICIN-INDUCED CARDIAC INJURY: FOCUS ON DAPAGLIFLOZIN AND EMPAGLIFLOZIN

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Doxorubicin (DOX) is an effective anthracycline anticancer agent, but its dose-dependent cardiotoxicity severely limits its clinical use and compromises patient outcomes. Sodium–glucose cotransporter 2 (SGLT2) inhibitors such as Dapagliflozin (DAPA) and Empagliflozin (EMPA) have demonstrated notable cardiovascular benefits, including reduced heart failure risk and cardiovascular mortality. Although multiple mechanisms contribute to DOX-induced cardiomyopathy, the molecular pathways through which DAPA and EMPA exert cardioprotective effects remain largely unresolved.

The aim of this study was to investigate the cardioprotective effects of DAPA and EMPA in a model of DOX-induced cardiotoxicity. Sprague–Dawley rats were randomly divided into four experimental groups. Animals in the DOX group received intraperitoneal injections of DOX at a dose of 2.5 mg/kg, administered six times. Rats in the DAPA- or EMPA-treated groups were given DAPA (10 mg/kg/day) or EMPA (25 mg/kg/day), respectively, by oral gavage prior to and throughout the DOX administration period. At the end of the treatment protocol, ECG recordings were obtained, after which the hearts were isolated for ex vivo assessment of cardiac function. Histological evaluation was performed on cardiac tissue sections using H&E staining to assess general morphology and Masson's trichrome staining to determine the extent of fibrosis. Lipid peroxidation was quantified by measuring cardiac malondialdehyde (MDA) levels. Western blot analyses were conducted to examine key molecular pathways: autophagy-related proteins including p-AMPK/total AMPK, Beclin-1, LC3B, and p62; the FEM1B–FNIP1 axis; and ferroptosis-associated markers, including SLC7A11 and GPx4. Additionally, apoptosis was evaluated in cardiac sections using the TUNEL assay. Together, these methodologies were employed to elucidate the mechanisms underlying the potential cardioprotective actions of DAPA and EMPA in the setting of DOX-induced cardiac injury.

Pretreatment with both DAPA and EMPA markedly ameliorated doxorubicin-induced cardiac injury. Several key parameters of cardiac function showed significant improvement compared with the DOX group, and histological analyses revealed reduced structural damage and fibrosis. In addition, DAPA and EMPA substantially decreased the extent of lipid peroxidation, as reflected by lower MDA levels, and significantly reduced the number of TUNEL-positive apoptotic cells. The SGLT2 inhibitors also modulated the autophagic pathway, as demonstrated by alterations in the expression of autophagy-related proteins. Moreover, our findings indicate that DAPA attenuated DOX-induced ferroptosis, supported by changes in ferroptosis-associated biomarkers. Nevertheless, further studies are required to clarify the mechanisms underlying the observed group-specific differences.

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18. A NOVEL H_2S-RELEASING IBUPROFEN DERIVATIVE (EV-34) MITIGATES DOXORUBICIN-INDUCED CARDIAC INJURY

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Although doxorubicin (DOX) is a fundamental chemotherapeutic drug used against various solid and hematologic cancers, its clinical application is significantly restricted by dose-dependent, cumulative heart damage. Because the molecular basis of this cardiac damage is still poorly understood, the development of effective cardioprotective strategies remains a critical unmet need. [1]. Accumulating research suggests that the disruption of endogenous hydrogen sulfide (H_2S) production is an important factor in DOX-mediated cardiomyocyte injury. [2]. In the present study, we used EV-34, a newly synthesized H_2S-releasing ibuprofen derivative, which was chemically designed for this study according to the procedure described by Gyöngyösi et al. [3]. We demonstrate that EV-34 markedly attenuates DOX-induced cardiotoxicity by reducing oxidative stress, apoptosis, and mitochondrial dysfunction. Mechanistically, EV-34 enhances intracellular H_2S levels both in the presence and absence of DOX through dual mechanisms: direct H_2S supplementation and enhancement of endogenous H_2S biosynthesis. In addition, EV-34 suppresses DOX-induced reactive oxygen species (ROS) generation, reduces mitochondrial superoxide production, and increases mitochondrial superoxide dismutase levels. Furthermore, EV-34 exerts its cardioprotective effects through activation of the PI3K/AKT/FoxO3a and AMPK signaling pathways, alongside inhibition of apoptosis via suppression of caspase-3 activation. Taken together, these results highlight EV-34 as a viable therapeutic agent for mitigating or preventing DOX-induced cardiac damage. To benchmark its efficacy, the pharmacological effects of EV-34 were assessed alongside GYY4137, an established slow-release H_2S donor.This work was supported by grant from NKFIH-143360. Project no.TKP2021-EGA-18has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund.

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19. DEVELOPMENT OF NOVEL RHODANINE-BASED INHIBITORS OF MATRIX METALLOPROTEINASE-2 FOR CARDIOPROTECTION

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We have previously shown newly developed imidazol carboxylic acid- and thiazol carboxylic acid-based drug candidates to moderately inhibit matrix metalloproteinase-2 (MMP-2) and thereby we have achieved cardioprotection in a rat model of acute myocardial infarction (AMI). However, those drug candidates lost their efficacy when hyperlipidemia was present as a co-morbidity for AMI. Therefore, now we tested rhodanine-based chemical compounds to inhibit MMP-2 selectively and moderately.

Newly synthetized and developed rhodanine-based structures have been subjected to in silico molecular docking to 8H78 (the newest available catalytic centre domain of MMP-2). Twenty drug candidates possessing the best docking scores to 8H78 have been selected for further measurements. Gelatin zymography has been performed to check the in vitro MMP-2 inhibitory potency of the compounds. Heart sample homogenates from young adult control rats were loaded to a 8%-SDS-polyacrylamide gel containing 2 mg/mL gelatin as a substrate to obtain biologically active MMP-2 enzyme activities. For positive control, ilomastat was used as one of the most potent hydroxamate type, non-selective MMP inhibitor. Gels were cut into pieces and were incubated at 37°C for 40h in the presence or absence of the drug candidates at 10 micromolar or that of ilomastat at 10 nanomolar concentration. Dimethyl sulfoxide served as vehicle.

Docking scores of the drug candidates varied between -5.32 to -7.23. MMP-2 activities appeared in all rat myocardial samples at 568±34 arbitrary units (AU). Ilomastat at 10 nM achieved over 90% inhibition of MMP-2 activities (23.3±4.2 AU, $p<0.001$ vs vehicle). Three from the 20 drug candidates at 10 microM achieved a moderate but significant reduction in MMP-2 activities (19.3%, 20.4%, and 26.7%, respectively, $p<0.05$ vs. vehicle), which have been found the most potent rhodanine-based compounds.

This is the first demonstration that rhodanine-based new chemical entities possess MMP-2 inhibitory potential and may be promising drug candidates for anti-ischemic cardioprotection. The next steps of development include the determination of IC50 values and selectivity measurements.

20. PHARMACOKINETIC DRUG-DRUG INTERACTIONS BETWEEN ANTIARRHYTHMICS AND DIRECT ORAL ANTICOAGULANTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Concomitant use of direct oral anticoagulants (DOACs) and antiarrhythmic agents, particularly class III and IV drugs such as amiodarone, is common in patients with atrial fibrillation (AF). This combination raises concerns about pharmacokinetic drug–drug interactions that may elevate DOAC plasma concentrations and increase the risk of major bleeding. Previous meta-analyses and observational studies reported conflicting findings, and the clinical relevance of these interactions remains uncertain.

This systematic review and meta-analysis evaluates whether concomitant use of class III/IV antiarrhythmics with direct oral anticoagulants (DOACs) increases the risk of bleeding in patients with atrial fibrillation (AF). Eligible studies include adult AF populations receiving DOAC therapy and reporting direct comparisons between regimens, including class III/IV antiarrhythmics and DOAC monotherapy or DOACs combined with class I/II antiarrhythmic agents. Outcomes of interest include major, gastrointestinal, and intracranial bleeding, pharmacokinetic parameters such as peak plasma concentration (Cmax) and trough levels where available, and mortality. Studies evaluating only vitamin K antagonists or lacking appropriate comparator groups will be excluded. Data will be synthesized using a random-effects meta-analysis, with heterogeneity assessed across studies. Risk of bias will be independently evaluated by two reviewers using the ROB-2 tool for randomized trials and the ROBINS-I tool for non-randomized studies, and the overall certainty of the evidence will be assessed using the GRADE framework.

A systematic search of PubMed, Embase, and the Cochrane Library, conducted using a predefined search strategy that combined terms for class III/IV antiarrhythmics and DOACs, yielded 10,791 records. After duplicate removal in EndNote, 9398 unique articles remained. Title and abstract screening and full-text screening were performed in duplicate by two independent reviewers using Rayyan, resulting in 54 studies included for qualitative and quantitative synthesis. Data extraction and analysis are currently ongoing, and results will be available at the time of presentation. This systematic review and meta-analysis is registered in PROSPERO (CRD420251050211).

21. DIFFERENTIAL ROLES OF ICOS AND ICOSL SIGNALING IN CARDIAC FIBROSIS AND REMODELING

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Introduction: Cardiac fibroblasts are essential for myocardial repair after injury, yet their overactivation drives maladaptive fibrosis and heart failure. While the Inducible T-cell Co-stimulator (ICOS) and its Ligand (ICOSL) have emerging roles beyond adaptive immunity, their specific contributions to cardiac remodeling remain unclear.

Aims: To investigate the role of ICOS-ICOSL signaling in the progression of cardiac remodeling and fibrosis using human samples and a murine model of angiotensin II (AngII)-induced cardiac injury.

Methods: Human myocardial tissue was obtained from patients with advanced heart failure of non-ischemic (DCM, n=5) or ischemic origin (ISC, n=5), and from non-failing donor hearts (n=5) to analyze ICOS and ICOSL expression by Western Blot. In vivo, 6-month-old male Balb/C mice received Angiotensin II (Ang-II; 1.5 mg/kg/day) or saline via osmotic mini-pumps for 15 days. ICOS or ICOSL signaling was inhibited using monoclonal antibodies (150 µg/dose i.p., every 3 days). Cardiac structure and function were assessed by echocardiography, and myocardial remodeling was evaluated using histology, RNAscope, and qPCR.

Results: In human hearts, ICOS protein expression was significantly elevated in both ISC and DCM groups, while ICOSL increased only in the ISC group. In the mouse model, combining Ang-II with either ICOS or ICOSL inhibition significantly increased mortality to 40-50%, compared to 13% with Ang-II alone. Both treatments aggravated systolic dysfunction; ejection fraction (EF) was reduced (ICOS: 39.0±5.6%, ICOSL: 33.8±8.1%) compared with controls. Notably, ICOSL blockade specifically worsened adverse remodeling, increasing relative wall thickness (0.54±0.11) and the left ventricular remodeling index (2.63±0.77). Myocardial fibrosis was further exacerbated by ICOSL blockade, accompanied by elevated Ctgf expression. ICOS inhibition increased Col3a1 expression, whereas ICOSL blockade elevated Col1a1 and Tgfb1 expression and increased Col1a1/Col3a1 ratio, indicating a shift toward a stiffer extracellular matrix phenotype. These findings were supported by COL1A1 and fibroblast activation protein (FAP) immunohistochemistry. Icosl and Col1a1 co-expression was increased in fibrotic myocardium but not in perivascular regions. The AngII-induced reduction in Myh6/Myh7 ratio was attenuated by ICOS blockade but worsened with ICOSL inhibition. Consistently, Nppb expression and circulating NT-proBNP levels normalized with ICOS inhibition but remained elevated with ICOSL blockade.

Conclusions: ICOS-ICOSL signaling critically modulates the cardiac stress response. While ICOS inhibition has minor effects on hypertrophy and fibrotic responses, ICOSL blockade accelerates adverse remodeling, matrix stiffening, and functional decline. These findings identify ICOSL-driven co-stimulatory signaling as a key regulator of fibrotic pathology and suggest distinct therapeutic risks in targeting this pathway.

22. TAAR1 AS A TARGET FOR MONOAMINERGIC ACTIVITY ENHANCERS: CAMP PROFILING OF (–)-IPAP VS B-PEA

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Trace amine–associated receptor 1 (TAAR1) is an intracellular, pleiotropic G protein–coupled receptor (GPCR) activated by endogenous trace amines such as βphenethylamine (β-PEA) and tryptamine (TRP), and it is also engaged by monoamine-releasing agents (MRAs) including amphetamine and methamphetamine. At low concentrations, β-PEA and TRP can act as monoaminergic activity enhancers (MAEs), preferentially facilitating vesicular neurotransmitter release, whereas MRAs trigger both vesicular release and non-vesicular, transporter-mediated monoamine efflux. 1-(indol-3-yl)-2-propylaminopentane ((–)-IPAP) is a synthetic, tryptamine-derived MAE candidate designed to avoid MRA-like releasing properties.

We aimed to investigate the MOA of the synthetic MAE compound (–)-IPAP at TAAR1 by measuring receptor-mediated cAMP accumulation in rat-TAAR1-expressing Expi293F suspension cells.

Dose–response curves (10^{-10}–10^{-4} M) were generated for β-phenethylamine (β-PEA; Sigma-Aldrich) and 1-(indol-3-yl)-2-propylaminopentane ((–)-IPAP; Fujimoto Pharmaceutical Corp, Japan) using seven-point serial dilutions prepared in assay buffer and applied to rTAAR1-expressing Expi293F cells to quantify TAAR1-mediated cAMP accumulation.

rTAAR1 cDNA (rTAAR1; GeneWiz) was cloned into the pcDNA3 mammalian expression vector with a CMV promoter. The plasmid was transformed into DH5α and grown using ampicillin selection. Plasmids were isolated and purified using a plasmid preparation kit, and sequenced prior to use. Expi293F suspension cells were transiently transfected using polyethyleneimine. After 48–72 h, receptor expression was confirmed by PCR, and cells were harvested immediately before the assay. cAMP accumulation was measured using the Revvity cAMP-Gs Dynamic homogeneous time-resolved fluorescence (HTRF) assay, a competitive immunoassay based on HTRF. Fluorescence ratios (665 nm/620 nm) were plotted against a compound concentration, signals were inversely proportional to intracellular cAMP levels. EC_{50} values were determined using 4-parameter logistic (4PL) non-linear regression.

In rTAAR1-expressing Expi293F cells, cAMP responses were quantified across seven-point concentration–response curves and normalised to the maximal response evoked by β-phenethylamine (β-PEA; 100%). (–)-IPAP produced a robust, concentration-dependent increase in cAMP with slightly reduced maximal efficacy, reaching ~90% of the β-PEA Emax. This profile supports (–)-IPAP as a potent TAAR1 agonist consistent with an MAE-like pharmacological signature rather than an MRA-like pattern.

23. PHARMACOTHERAPEUTIC AND SYSTEMIC FACTORS INFLUENCING HOSPITAL MORTALITY: A 13-YEAR FOLLOW-UP

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Objectives: This study aims to analyze the intersection between systemic healthcare factors and pharmacological risks by evaluating the „weekend effect" on in-hospital mortality and the safety profile of oral anticoagulants (OACs) in a high-risk patient population.
Methods: We conducted a 13-year retrospective observational study (2010–2022) analyzing 45,955 hospitalizations at a major internal medicine department in Bratislava, Slovakia. Multivariable logistic regression was used to identify predictors of mortality, focusing on the day of admission. Additionally, a detailed pharmacological sub-analysis was performed on 461 deceased patients to compare the safety of warfarin versus direct oral anticoagulants (DOACs), focusing on INR levels, bleeding complications, and the need for reversal agents.

Results: Patients admitted during weekends faced a significantly higher risk of mortality compared to weekday admissions (AOR = 1.31; 95% CI: 1.22–1.41). Cardiovascular diseases were the leading cause of death (70.4%). Anticoagulation Safety: Among the deceased, warfarin treatment was associated with high INR variability (reaching values up to 23.37) and a significantly higher rate of complications (40.2%) compared to DOACs (6.6%). Clinical Intervention: Therapeutic intervention (Vitamin K, fresh frozen plasma, or prothrombin complex concentrate) was required in 67% of warfarin-related complications and 50% of DOAC-related cases. Prescribing Trends: A significant shift in clinical practice was observed, with DOAC prescriptions increasing from 0% to 80% over the study period.
Conclusions: Our findings confirm that weekend admission is a robust independent predictor of in-hospital mortality in Slovakia. This systemic risk is further compounded by pharmacotherapeutic challenges, where warfarin remains a higher-risk option compared to the superior safety profile of DOACs. Optimizing weekend access to diagnostic services and clinical pharmacological expertise is essential to mitigate these risks and improve patient outcomes.

Keywords: weekend effect, in-hospital mortality, warfarin, DOACs, INR, clinical pharmacology.

24. RESTORING ANTIVIRAL DEFENSE IN HEART FAILU4RE: PHARMACOLOGICAL REGULATION OF IFITM3 IN HUMAN CARDIOMYOCYTES

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Viral infections are major risk factors for heart failure exacerbations and frequently contribute to mortality in affected patients. A key host defense against viral infections is the interferon (IFN)-mediated antiviral pathway, which is regulated by IFN regulatory factors (IRFs) and viral restriction factors such as IFITM3. IFITM3 plays a crucial role in inhibiting viral replication, and its deficiency has been linked to increased susceptibility to severe outcomes from infections like influenza and COVID-19. Moreover, IFITM3 has been reported to have protective effects in cardiac tissue during viral infections. In our study, we analyzed the expression of various antiviral factors in cardiac tissues from patients with heart failure, and found that the level of IFITM3 was significantly reduced compared to controls, which can contribute to increased susceptibility to infections and a higher risk of mortality. Based on these findings, we investigated whether the protective IFITM3 signaling could be restored through pharmacological intervention.

Human cardiomyocytes (AC16 cells) were treated with drugs commonly used in the management of heart failure and related comorbidities, including ischemic heart disease, diabetes mellitus, gout, and hypercholesterinaemia, at different concentrations. Following treatment, the expression of antiviral pathway components was assessed. Total RNA was isolated from treated and control cells, and the mRNA expression of antiviral factors was analyzed using RT-qPCR. Protein expression levels were evaluated by Western blot.

Our findings suggest that pharmacological agents commonly used in cardiovascular and metabolic diseases may influence antiviral defense pathways in cardiomyocytes. Restoration or enhancement of IFITM3 expression through pharmacological intervention may represent a potential strategy to improve antiviral resilience in patients with heart failure.

25. MODULATİON OF IMMUNE CHECKPOİNT GENE EXPRESSİON BY IMMUNOSUPPRESSİVE AGENTS İN HUMAN PERİPHERAL BLOOD MONONUCLEAR CELLS

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Long-term immunosuppressive therapy after transplantation is associated with a higher risk of developing malignancies. Immune checkpoints play a key role in regulating T-cell activity, however tumor cells can also express co-inhibitory molecules, enabling them to evade immune surveillance. By blocking these pathways, immune checkpoint inhibitor (ICI) therapy has become a widely used and highly effective form of anticancer treatment. In transplant recipients, however, the use of ICIs is generally contraindicated, as it significantly increases the risk of graft rejection.

In this study, our aim was to investigate whether long-term immunosuppressive therapy directly modulates immune checkpoint expression, potentially contributing to tumor development. Furthermore, we aim to identify immune checkpoints that could later be targeted as effective antitumor agents without increasing the risk of graft rejection.

In our pilot study, human peripheral blood mononuclear cells (PBMCs) from healthy individuals were isolated using Ficoll density gradient centrifugation. Various treatments were then applied to the PBMCs. First, control conditions were established, including both a non-activated control and an activation control to assess gene expression under T-cell-activated conditions. In addition, the cells were treated with four different immunosuppressive agents (tacrolimus, cyclosporin-A, methylprednisolone, and mycophenolate), both individually and in triple combinations that included one of the two calcineurin inhibitors. Samples were collected after 24 and 72 hours of incubation, modeling the effects of long-term immunosuppression. Following RNA isolation and cDNA synthesis, gene expression levels were analyzed using a chip-based, high-throughput qPCR platform.

Our preliminary results show that, as expected, the expression of activation markers such as IL-2, TNFα, and CD25 increases following T-cell activation. In addition, several immune checkpoints reported in the literature to change upon activation showed similar patterns in our model: CD40L was strongly upregulated, while CTLA-4 was also increased, acting as an early negative feedback marker. Among the immunosuppressive treatments, the triple-drug combination caused the most pronounced changes in gene expression. Methylprednisolone alone induced notable alterations, and in some cases calcineurin inhibitors also affected the expression of immune checkpoint molecules.

These preliminary results indicate that immunosuppressive agents can alter immune checkpoint expression, potentially influencing tumor development under long-term immunosuppression.

26. INVESTIGATING THE EFFECT OF GLP-1 RECEPTOR AGONISTS ON SKELETAL MUSCLE AND CARDIAC CACHEXIA IN MICE

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Obesity and type 2 diabetes mellitus represent major public health challenges, significantly increasing the risk of developing a range of comorbid conditions, including atherosclerosis, nephropathy, neuropathy, and sarcopenia. In recent years, glucagon-like peptide-1 receptor (GLP-1R) agonists (such as semaglutide and liraglutide (Lira)), as well as the novel dual GLP-1R/glucose-dependent insulinotropic polypeptide (GIP) receptor co-agonist tirzepatide (TZP), have emerged as widely used and highly effective therapeutic options. These agents provide substantial improvements in glycaemic control and promote clinically meaningful weight loss, thereby contributing to the prevention or delay of obesity- and diabetes-related complications. However, whether the weight-reducing effects of these agents are achieved through selective adipose tissue loss or concomitant reduction of skeletal muscle mass remains insufficiently characterised.

The aim of the present study was to evaluate the effects of GLP-1R agonists on skeletal muscle mass, strength, and the associated molecular regulators of muscle atrophy and growth in a murine model. We also examine the potentially cardiac cachexic effect.

In our study, 12–14-week-old male Balb/c mice were used. Animals were randomised based on body weight and cardiac function and subsequently treated with Lira (*i.p.* 300 µg/kg/day) or TZP *(i.p.* 144 µg/kg/3 days) for 28 days. Skeletal muscle strength was measured using a grip strength meter, and echocardiography was performed to monitor cardiac function and structure. After 28 days, the animals were terminated, and heart and muscle tissues were collected for further *in vitro* examination.

Both treatments resulted in weight loss, which was more significant in the TZP group; however, no measurable change in grip strength was observed in either treated group. Skeletal muscle mass, as assessed by wet tissue weights of *m. tibialis anterior, m. extensor digitorum longus*, and *m. gastrocnemius*, was reduced by TZP treatment, whereas Lira selectively reduced the mass of *m. gastrocnemius* alone. Gene expression analysis of the atrophy regulator Atrogin-1 and the myokines myostatin and irisin in *m. tibialis anterior* and *m. gastrocnemius* revealed no significant treatment-induced transcriptional changes. Echocardiographic assessment demonstrated that Lira increased fractional shortening compared with both the TZP and vehicle groups, whereas no other cardiac functional or structural parameters differed between the groups. Both drugs also reduced heart weight.

Our results indicate that GLP-1R agonist–induced weight loss also reduces skeletal and cardiac muscle mass, more prominently with TZP, preceding detectable functional loss in healthy animals. In aged or diseased individuals, such reductions may lead to more overt functional decline, warranting further investigation on long term myotoxicities of these agents.

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27. INTENSİVE PHYSİCAL TRAİNİNG İNCREASES THE RİSK OF VENTRİCULAR ALTERNANS İN A CANİNE ATHLETE'S HEART MODEL

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Regular physical training provides undeniable benefits and exerts positive effects on the cardiovascular system and overall quality of life. However, multiple cases of sudden cardiac death (SCD) among elite athletes have been reported, and the underlying mechanisms remain incompletely understood. In this study, we tested the hypothesis that intensive exercise-induced electrical remodeling facilitates the development of cardiac alternans, which may serve as a substrate for arrhythmogenesis.

A total of 24 Beagle dogs of both sexes were randomly assigned to sedentary or trained groups and underwent a 16-week vigorous treadmill running protocol. Following the training period, in vivo arrhythmia provocation, action potential measurements in left ventricular tissue slices, patch-clamp recordings from isolated left ventricular cells, and Western blot analyses were performed.

Trained animals exhibited enhanced in vivo arrhythmia susceptibility, prolonged action potentials, greater action potential and Ca^{2+} transient alternans, and increased Purkinje–ventricle action potential dispersion. Additionally, sarcoplasmic reticulum Ca^{2+} content was reduced, while Ca^{2+} buffering capacity was elevated. Western blot analysis revealed downregulation of CACNA1C (L-type Ca^{2+} channel) expression.

These findings suggest that intensive physical training promotes the development of cardiac alternans through electrical and Ca^{2+}-handling remodeling, which may contribute to the increased arrhythmia susceptibility. Overall, these results provide new insights into the mechanisms underlying sudden cardiac death in competitive athletes.

28. PROTEGAN: A GENERATIVE, SELF-SUPERVISED MODEL WITH EDGE-LEVEL ATTENTION FOR NETWORK-BASED PROTEIN–PROTEIN INTERACTION PREDICTION

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The exploration of possible protein–protein interactions (PPIs) remains a central yet challenging task of drug target discovery due to the time and cost restrains of existing experimental methods. Consequently, numerous *in silico* prediction tools have been proposed to accelerate the selection of the most likely candidate protein pairs. In our prior work (Balogh et al., 2022, DOI: 10.1186/s12859-022-04598-x), we introduced a conditional generative adversarial network model for the efficient prediction of PPIs, relying solely on the topology of PPI networks (interactomes). This model combined the principles of systems biology and the neural architectures used for image-to-image translation in computer vision, handling the adjacency matrix of the interactome similar to an image.

Here, our aim was to expand our model architecture with transformer-inspired encoder blocks that use the attention mechanism of language models from natural language processing, in order to further improve the performance of our model.

ProteGAN, the next iteration of our model now includes several encoder blocks before the convolutional u-net part, providing context-aware information about the input subgraphs through the use of edge-level attention. Furthermore, reconstruction loss was added and the network preprocessing Python code was streamlined, adding support for node2vec embedding and network topology analytics. Version 12 of the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database was used as the primary source of interactomes for the fine-tuning of ProteGAN, with other sources such as BioGRID and HuRI utilized only for evaluations. As for performance metrics, we focused on improving the area under the precision-recall curve (AUPRC), and the precision in the top 500 predictions (P@500), an important metric for real-life applicability.

Our evaluations on the available PPI networks reveal the importance of utilizing intuitive and simple image preprocessing techniques from the field of computer vision along with powerful, state of the art neural architectures from the field of natural language processing. On the *H. sapiens* interactome of the STRING database, utilizing the common 0.7 confidence threshold, our old model achieved AUPRC: 0.092 and P@500: 0.260, while utilizing image preprocessing techniques and adding encoder blocks improved them to AUPRC: 0.415 and P@500: 0.966.

In conclusion, here we introduce ProteGAN, a generative adversarial network outfitted with transformer-inspired encoder blocks for the prediction of PPIs based on the topology of the interactome. ProteGAN shows considerable performance improvements compared to our previous model and the importance of utilizing simple yet effective preprocessing techniques instead of fully relying on machine learning. As such, ProteGAN could be a valuable tool for the high throughput prediction of candidate PPIs, contributing to a more efficient drug target identification process.

29. ESTIMATION OF TARGET-LIGAND BINDING AFFINITY BY IN SILICO APPROACH

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Reliable thermodynamic data of target-ligand binding are essential for successful drug design and molecular modelling projects, especially in the early (screening) stages of drug design for identifying effective ligands. The binding affinity between the target and ligand is expressed by the binding free energy (ΔG_b), which is composed of the binding enthalpy (ΔH_b) and entropy (ΔS) based on the equation $\Delta G_b = \Delta H_b - T\Delta S$ (where T is the thermodynamic temperature). These parameters can be determined experimentally or predicted computationally.

Our research group aimed to develop a simple, fast, and cost-effective computational method for estimating binding enthalpy (ΔH_b) and binding free energy (ΔG_b), using a combination of semi-empirical PM7/1SCF calculations, implicit COSMO, and predicted explicit interfacial water structures, and ligand-based descriptors. This method was validated using a diverse dataset comprising 43 systems, including small molecules and peptides as ligands.

As a case study, our validated protocol was applied to predict the binding data of somatostatin receptor complexes. Somatostatin receptors are drug targets in Alzheimer's disease, Cushing's disease, type 2 diabetes, neuroendocrine tumours, acromegaly, pain-related conditions, and depression. Our predicted binding affinities correlated well with the experimentally determined data.

In summary, this method can significantly support the identification of effective ligands and accelerate the early stages of drug design.

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30. INVESTIGATION OF GLUTATHION REACTIVITY OF CYCLIC C5-CURCUMINOID DERIVATIVES

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The extracted curcumin from *Curcuma* sp. shows a promising target for the development of anti-cancer drugs in the pharmaceutical field. However, its limited pharmacokinetic properties present a significant challenge. In the Institute of Pharmaceutical Chemistry, at the University of Pécs, numerous curcumin analogues, particularly cyclic C5-curcuminoids, have been synthesized which exhibit improved bioavailability. These newly developed compounds were partially evaluated for their antiproliferative activity against different human adherent cancer cell lines, demonstrating encouraging biological effects. Many of the compounds tested have significant antioxidant capacity. Due to their structure as α,β-unsaturated carbonyl compounds, curcuminoids are presumed to undergo conjugation reactions with thiol-containing glutathione, and they may also influence the levels of reduced and oxidized glutathione. This can be of great importance in living cells, since glutathione plays a significant role in maintaining the redox homeostasis of the human body.

Our aim was to investigate the glutathione reactivity of some curcuminoid derivatives. The formation of conjugates was preliminarily confirmed using spectrophotometric and HPLC–MS methods. At the same time, it is important to investigate the effect of the compounds with antioxidant and possible prooxidant effects on the GSH/GSSG system.

Since in the body the oxidation of GSH to GSSG is catalyzed by the enzyme glutathione peroxidase, the effects of the compounds on glutathione peroxidase activity were investigated using a spectrophotometric method. The assay involves a coupled reaction in which glutathione peroxidase reduces hydrogen peroxide while oxidizing glutathione; then the glutathione is regenerated by glutathione reductase at the expense of NADPH oxidation.

Our experimental results demonstrate that the investigated compounds undergo conjugation reactions with glutathione spontaneously and also promote GSH oxidation.

31. SEPARATION OF PLASMA EXTRACELLULAR VESICLES FROM LIPOPROTEINS USING DENSITY GRADIENT ULTRACENTRIFUGATION AND SIZE EXCLUSION CHROMATOGRAPHY FOR DIAGNOSTIC APPLICATIONS

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Background: Biomarkers in circulating extracellular vesicles (EVs) hold great potential for diagnostic applications. However, liporoteins (LPs) share overlapping physical properties and molecular cargo with EVs, which can obscure EV-related signals. Therefore, efficient separation of EVs and LPs is needed to increase the sensitivity and specificity of EV-based diagnostics.

Aim: Our aim was to develop a method which separates EVs from LPs from human blood samples with optimal purity, yield and time efficiency, and to apply it in samples of patients suffering from acute myocardial infarction (AMI).

Methods: EVs were isolated from 1 mL human platelet-free plasma using iodixanol density gradient ultracentrifugation (DGUC) followed by size exclusion chromatography (SEC) with Sepharose 4 Fast Flow (4FF) columns. Isolates were analysed using nanoparticle tracking analysis (NTA), Qubit protein assay and Western blot (WB).

Results: WB analysis of iodixanol DGUC fractions revealed significant overlap between LP and EV markers, demonstrating the need of further purification steps. After 4FF SEC of the EV-rich fractions, WB analysis revealed the presence of EV markers, while mild LP contamination also presented. Application of the total workflow needed two experimental days for 6 samples. NTA revealed the presence of EV-sized nanoparticles in the isolates, with a significantly larger size than LP samples. NTA and Qubit protein assay revealed no significant difference in EV sample concentration between AMI and non-AMI patients.

Conclusion: Combined DGUC followed by 4FF SEC demonstrated acceptable EV-LP separation and processing time for plasma EV isolation. No significant difference was observed in plasma EV numbers of AMI and non-AMI patients. Molecular analysis of the EV samples may highlight EV-related differences, that can be used for the development of future diagnostic-prognostic applications.

32. STANDARDIZING PLASMA-DERIVED EXTRACELLULAR VESICLE ISOLATION ACROSS SPECIES FOR TRANSLATIONAL AND CLINICAL PHARMACOLOGY STUDIES

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Extracellular vesicles (EVs) are cell-secreted nanoscale membrane particles, which transport proteins, lipids, and nucleic acids. EV composition reflect the phenotypic state of their donor cell, therefore, they are intensively studied as biomarkers in various diseases. However, for the translation of preclinical experimental results to the clinics, the cross-species transition of the applied EV isolation workflow from experimental animals to human samples is need to be investigated.

The aim of this study was to investigate cross-species characteristics of combined density gradient ultracentrifugation (DGUC) and size-exclusion chromatography (SEC) for plasma-derived EV isolation using human, rat, and mouse samples.

Platelet-free plasma samples were prepared from human, rat, and mouse blood using serial centrifugation. EVs were isolated from the plasma samples with iodixanol DGUC, followed by SEC with 4 fast flow columns. DGUC fractions were characterized by density measurement, Western blot analysis and multi-angle light scattering (MALS). EV characterization was performed using nanoparticle tracking analysis (NTA) and Western blot.

Density profiling revealed comparable fractionation patterns with DGUC between human and mouse samples. Western blot analysis revealed similar distribution of EV and lipoprotein (LP) markers in mouse and rat samples, however, in human samples both EV and LP markers had a wider distribution, with their presence in later fractions as well. NTA confirmed the presence of nano-sized particles in DGUC+SEC EV samples. EV markers were apparent on Western Blot with minor LP contamination. NTA revealed a significantly lower particle number in rat samples as compared to human samples.

In conclusion, human samples showed similar, but not identical fractionation by DGUC as compared to rodent samples. Further isolation with SEC reduced, but not diminished LP contamination in the samples. NTA highlighted significant differences in particle yield among the species. These differences highlight that DGUC+SEC workflow possibly cannot be directly translated from preclinical experiments to clinical investigations.

33. INNOVATIVE STEM CELL-BASED BLOOD-BRAIN BARRIER-ON-A-CHIP FOR MODELING NEUROINFLAMMATION

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The blood-brain barrier (BBB) is a dynamic interface comprising brain microvascular endothelial cells, pericytes, and astrocytic endfeet, collectively responsible for maintaining cerebral homeostasis through tightly regulated paracellular and transcellular molecular transport. Despite its critical neuroprotective role, BBB integrity is frequently compromised under neuroinflammatory conditions — a pathological hallmark shared across neurodegenerative disorders including Alzheimer's and Parkinson's disease. Sustained exposure to pro-inflammatory mediators disrupts tight junction complexes, promotes endothelial dysfunction, and initiates a cascade of vascular injury that accelerates disease progression. Although considerable therapeutic effort has been directed at preserving endothelial function, the limited clinical translatability of conventional animal models underscores a critical demand for physiologically relevant, human-specific in vitro systems capable of recapitulating BBB pathology with greater fidelity.

To address this, we developed a human stem cell-based BBB-on-a-chip system incorporating a thrombin-fibrinogen hydrogel matrix within a microfluidic architecture. This configuration facilitates the three-dimensional self-organization of brain microvascular endothelial cells and pericytes into anatomically coherent vascular networks. We performed structural and cellular characterization via immunofluorescence staining for PECAM-1 as an endothelial marker and α-smooth muscle actin for pericyte identification. Barrier integrity and inflammatory injury were assessed following cytokine-induced neuroinflammation, with quantitative viability analysis revealing a significant reduction in Calcein AM fluorescence and a concurrent increase in Propidium Iodide uptake, indicative of progressive endothelial cell death. To restore compromised barrier function, we applied the cARLA cocktail (Porkoláb et al, 2024) — a multi-pathway therapeutic formulation targeting key molecular axes in brain endothelial cells. cARLA re-establishes the molecular and structural integrity of the BBB acting through the concurrent activation of cAMP signaling and the Wnt/β-catenin pathway, alongside inhibition of TGF-β signaling.

Our microphysiological platform provides an innovative framework for evaluating the neuroprotective efficacy of candidate therapeutics under defined neuroinflammatory conditions, thereby supporting more informed preclinical decision-making prior to in vivo or clinical investigation.

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34. PHARMACOGENETIC INSIGHTS INTO CYP ENZYME VARIABILITY IN HUNGARY

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Adverse drug reactions (ADRs) are increasingly reported in Hungary and globally, often resulting from inappropriate dosing, lack of therapeutic effect, or increased side effects.

The liver is the primary organ responsible for drug metabolism, with cytochrome P450 (CYP) enzymes playing a key role in catalyzing oxidative drug-metabolizing reactions. Significant inter-individual differences in hepatic CYP activities arise from genetic polymorphisms. DNA-based pharmacogenomic profiling enables the identification of clinically relevant CYP polymorphisms that result in increased or decreased metabolism of CYP substrate drugs, thereby aiding in the prevention of drug-induced toxicity or therapeutic failure through appropriate dose adjustment. The Metabolic Drug Interaction Research Group at the HUN-REN Research Centre for Natural Sciences analyzed 2,001 individuals in Hungary, and found that the frequency of function-altering CYP mutations closely mirrored that of the broader Caucasian population. The aggregated data also confirmed that there are significant inter-population differences in the prevalence of these variants across major ethnic groups (Caucasian, Asian, African/Afro-american). These findings support the use of population-based pharmacogenomic data to guide personalized therapy. Among the CYP variants, CYP2C9*2 and *3 polymorphisms were shown to significantly reduce CYP2C9 activity in Hungarian subjects. A documented case of a 30-year old patient with severe ibuprofen-induced hepatotoxicity was directly linked to the CYP2C9*2/*3 genotype, highlighting the clinical relevance of genotyping in prevention of adverse reactions.

These results underscore the importance of incorporating genetic factors into clinical decision-making and personalized drug therapy.

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35. EVALUATION OF PERIPHERALLY RESTRICTED FUNCTIONALLY SELECTIVE CB1 RECEPTOR ANTAGONISTS FOR OBESITY AND METABOLIC DISORDERS

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Cannabinoid receptor 1 (CB1R) antagonism represents a promising therapeutic strategy for obesity and metabolic disorders; however, centrally acting CB1R antagonists are associated with adverse neuropsychiatric effects. Therefore, peripherally restricted CB1R antagonists that preserve metabolic efficacy while minimizing brain exposure are of high interest. Accordingly, we evaluated and pharmacologically characterized a series of amino acid–functionalized tetrahydropyridazine analogs as functionally selective CB1R antagonists with limited central penetration and improved safety profiles.

Structure–activity relationships were explored using in vitro pharmacological assays, including CB1R radioligand binding, functional characterization, and pathway selectivity assessed by [^{35}S]GTPγS binding and β-arrestin-2 recruitment assays. Pharmacokinetics and tissue distribution were determined by LC–MS/MS following oral and intraperitoneal administration in mice. In vivo CB1R antagonism was evaluated using upper gastrointestinal (GI) motility assays, while central behavioral effects were assessed using the catalepsy assay. Anti-obesity efficacy was determined in a diet-induced obesity (DIO) mouse model by measuring body weight, food intake, and glucose tolerance.

In vitro radioligand binding studies identified potent CB1R antagonists with high affinity and selectivity in the low nanomolar range. Based on *in vitro* efficacy, compound 13 was selected for further studies and retained strong antagonistic activity in [^{35}S]GTPγS binding assays. Pharmacokinetic analysis revealed good systemic exposure following oral administration (C_{max} ~1800 nM) with rapid clearance. The compound exhibited limited brain penetration (brain-to-plasma ratio of 12%), indicating peripheral restriction. At 10 mg/kg, maximal in vivo CB1R antagonism was observed in the upper gastrointestinal (GI) motility assay. In diet-induced obesity (DIO) mice, oral administration (10 mg/kg) significantly reduced body weight gain and food intake, and improved glucose tolerance. In the catalepsy assay, the compound 13 did not reverse agonist-induced catalepsy even at higher doses, indicating limited brain penetration and minimal central CB1R engagement.

These findings support the concept that selective blockade of peripheral CB1R produces beneficial metabolic effects while avoiding central side effects, highlighting the therapeutic potential of CNS-sparing CB1R antagonists as a safer strategy for the treatment of obesity and related metabolic disorders.

36. INVESTIGATION OF THE INTESTINAL ABSORPTION, METABOLISM AND STABILITY OF P-AMINOPHENOL USING A UV HPLC METHOD

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P-aminophenol is a major impurity in paracetamol formulations. It may remain from synthesis or form during storage and environmental exposure. In an aqueous medium the paracetamol is converted significantly and quickly into p-aminophenol, which as an intermediate product further decomposes into hydroquinone and benzoquinone. During metabolism, p-aminophenol is primarily acetylated but conjugated metabolites are also formed such as p-aminophenol-glutathione, -glucuronide, -sulphate and acetylated conjugates. Studies have shown that renal damage is mainly due to the glutathione conjugate produced via a γ-glutamyl transpeptidase-dependent pathway.

In this study, we investigated the intestinal absorption, metabolism and stability of p-aminophenol. A UV-HPLC method was developed to detect p-aminophenol and its metabolites. We also examined the effect of N-acetyltransferase (NAT) inhibition on the metabolic profile. Concurrently with our investigations, we performed stability studies of p-aminophenol under several conditions that are of interest to our research.

Small intestinal perfusion experiments were performed in male Wistar rats (250-270g). A segment of the small intestine was perfused with p-aminophenol solution for 90 minutes, and perfusate samples were collected at defined time points. For the detection a C18 Atlantis T3, (5µm, 4.6 x 250 mm) column was used on an Agilent 1100 workstation.

As to stability test, in vitro studies were performed under perfusion-mimicking conditions, and additional samples were sored in and ultra-low temperature freezer and analysed over time. A reversed-phase C18 Symmetry Waters column (250x4.6 mm; 5 µm) was used on an Agilent 1100 workstation for the determination.

To inhibit NAT, rats were treated with apocynin (100 mg/kg/day) for 2 weeks. Enzyme activity was measured in tissue homogenates using p-anisidine as substrate, followed by spectrophotometric detection after derivatization with dimethyl-aminobenzaldehyde. We also performed an in vitro experiment to verify apocynin inhibition.

P-aminophenol was extensively absorbed and acetylated in the small intestine. The main metabolites detected in the perfusates were paracetamol and paracetamol glucuronide. In NAT inhibited animals, lower levels of acetylated metabolites were observed. Enzyme activity measurements showed no significant inhibition in the small intestine, while a slight decrease was observed in the liver. In the in vitro experiment, apocynin significantly inhibited the enzyme.

During in vitro perfusion, 26.52% of p-aminophenol degraded and small amounts of hydroquinone and benzoquinone were detected. Gradual changes were also observed during storage.

37. BIOLOGICAL STUDIES OF HETEROBIMETALLIC COMPLEXES AS HYPOXIA-ACTIVATED ANTI-CANCER PRODRUGS

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Cancer is a leading cause of global mortality. Pt(II) complexes, such as cisplatin, are widely used for the treatment of solid tumors. However, their clinical application is hampered by severe side effects caused by a lack of selectivity. Hence, other metal complexes with selective targeting properties have been investigated. Co(III) complexes can act as hypoxia-activated prodrugs. By incorporating a cytotoxic ligand into a Co(III) center, the ligand can be rendered inactive under the normoxic conditions of healthy cells. Under the hypoxic conditions of tumors, Co(III) is reduced to unstable Co(II) complexes, thereby releasing the cytotoxic ligand. (1)

The aim of this work was the biological characterization of newly synthesized Co(III), Co(III)/Ru(II), and Co(III)/Ir(III) complexes and their corresponding ligands. Cytotoxicity assays and RT-qPCR were performed under normoxic and hypoxic conditions to study the hypoxia-activation of the complexes. For both normoxic (approx. 20% O_2) and hypoxic (1% O_2) conditions, MCF-7 human breast cancer cells were seeded, treated with different concentrations of the substances, and incubated for 24 hours. An MTT assay was then performed to determine cell viability. For the RT-qPCR, MCF-7 cells were treated with the ligands and the Co(III) complexes and incubated for 72 hours. The expression of TfR1 and p21 genes was quantified by qPCR.

MTT assays confirmed that the Co(III) complex exhibits significant hypoxia-activation with moderate potency. The incorporation of Co(III) rendered the ligand less toxic under normoxia, while it was activated under hypoxia. RT-qPCR showed that the ligand behaved as a typical iron chelator, indicated by the induction of TfR1 and p21 genes under hypoxic conditions. This study demonstrates the potential of using Co(III) complexes as bioreductive carriers for cytotoxins. Iron chelators also show promise as effective anticancer agents.

Literature: (1) Tran, T. B., Sipos, É., Bényei, A., Nagy, S., Lekli, I., Buglyó, P.: Synthesis and Characterization of Novel Co(III)/Ru(II) Heterobimetallic Complexes as Hypoxia Activated Iron-Sequestering Anticancer Prodrugs. Molecules. 29 (24), 1-14, 2024.

38. ADME SAMPLES: METHOD DEVELOPMENT AND MEASUREMENT WİTH (U)HPLC

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My presentation focuses on the HPLC-based analysis of pharmacological samples generated during in vitro ADME studies. The method is designed for the quantification of small molecules with diverse physicochemical properties from biologically derived matrices, in line with high-throughput screening requirements. Particular challenges include low analyte concentrations and significant matrix effects. The talk will discuss sample preparation and chromatographic strategies applied to ensure robustness and reproducibility.

39. ANXIOLYTIC AND ANTIDEPRESSANT EFFECTS OF ORGANIC POLYSULFIDE, DIMETHYL TRISULFIDE ARE PARTLY MEDIATED BY THE TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 ION CHANNEL IN MICE

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Depression and anxiety disorders represent a global health burden, yet the efficacy of current therapeutic options is often limited. Dimethyl trisulphide (DMTS) is a naturally occurring organic polysulphide found in garlic, possessing excellent blood-brain barrier permeability and antioxidant properties. Previous research has confirmed that DMTS is an agonist of the Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel. The aim of this study was to investigate the effect of DMTS on chronic stress-induced depression-like behaviour and anxiety, and to clarify the extent to which these effects are mediated via the TRPA1 ion channel. Experiments were conducted on Trpa1 wild-type (WT) and knockout (KO) male mice using a 21-day chronic unpredictable mild stress (CUMS) model. DMTS treatment (30 mg/kg, i.p.) was applied on days 16 and 20 of the protocol. Behavioural changes were evaluated using the open field test (OFT), marble burying test (MBT), sucrose preference test (SPT), forced swim test (FST), and tail suspension test (TST). To examine the physiological background of stress reactions, adrenal and thymus weights, serum corticosterone and ACTH levels were measured, and chronic neuronal activation was examined via FOSB immunohistochemistry in brain areas involved in stress processing. CUMS induced significant depression-like behaviour (anhedonia, increased immobility) and anxiety in WT mice, accompanied by HPA-axis dysregulation and thymic involution. DMTS treatment significantly mitigated depressive symptoms and anxiety in WT animals and inhibited thymus weight loss. Mechanistic investigations revealed that the lack of Trpa1 modified the stress response: KO mice showed higher baseline depression-like behaviour. The antidepressant effect of DMTS-particularly the reduction of anhedonia (SPT)-proved to be TRPA1-dependent, as this effect was absent in KO mice. In contrast, the anxiolytic effect (OFT) was partially independent of TRPA1. During the study of neuronal activation, DMTS reduced stress-induced FOSB expression in the examined brain areas of WT mice. Notably, in the paraventricular nucleus (PVN) of the hypothalamus, the inhibitory effect of DMTS was specifically dependent on the presence of TRPA1. Our results prove that DMTS is capable of alleviating chronic stress-induced depressive and anxious symptoms and normalising stress-adaptation processes. The mechanism of action is twofold: the reduction of anhedonia and PVN neuronal activation is a TRPA1-dependent process, while anxiolytic effects may be realised through other pathways (e.g., modulation of serotonergic systems). DMTS may thus be a promising candidate for the adjunct therapy of mood disorders or as a dietary supplement.

40. THE ROLE OF THE TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 ION CHANNEL IN MEMORY AND MOTOR COORDINATION DURING AGING IN FEMALE MICE

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Transient Receptor Potential (TRP) ion channels are non-selective cation channels expressed on peripheral sensory nerve endings and sensory neurons. Previous studies have shown that TRP Ankyrin 1 (TRPA1) receptor has a prominent role in pain sensation. Furthermore, our research group has previously demonstrated the involvement of the TRPA1 ion channel in age-related cognitive decline in male mice, manifested as a slower rate of memory loss in knockout animals. In our current study, we aimed to investigate the agedependent changes in cognitive abilities and motor functions in TRPA1 knockout and wild-type female mice.

In our *in vivo* experiments, motor coordination and muscle strength were assessed using the Double horizontal bars test (DHB), the Static Rod test (SR) and the Rotarod test. Memory performance was evaluated with the Ymaze and Novel Object Recognition tests (NOR). In parallel with the behavioral assessments, we monitored the estrous cycle of the animals by performing vaginal smears at the same time each day.

We found that aging did not result in differences in muscle strength or motor coordination between wild-type (WT) and knockout (KO) animals (DHB, SR, Rotarod). We also demonstrated that spontaneous alternation in 12monthold mice was significantly lower compared to young animals (Ymaze). Furthermore, in NOR shortterm memory performance wild-type mice showed agerelated decline, whereas the KO group performed better than the agematched WT controls. We observed that at 3 months of age there was no difference in the estrous cycle between WT and KO groups, however, by 12 months of age most WT mice had become acyclic, while the KO mice continued to display regular estrous cycles. These findings suggest that the TRPA1 ion channel contributes to the development of dementia, the deterioration of motor coordination, and the decline in muscle strength during aging. Therfore, its inhibition may be beneficial in alleviating these agerelated impairments.

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41. EEG THETA-ENHANCING EFFECTS OF (R)-KETAMINE IN CONTRAST TO (S)-KETAMINE

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Ketamine and (S)-ketamine are proven rapid-acting antidepressants. While (R)-ketamine was also considered a potential candidate, clinical trials could not confirm its effectiveness for major depressive disorder. Similarly, our own preclinical studies in rats demonstrated that (R)-ketamine does not have antidepressant-like properties at the dose tested. However, (R)-ketamine has gained interest for other indications in recent years. For example, it has shown significant therapeutic potential in animal models of cognitive impairment, Parkinson's disease, and multiple sclerosis. Our goal was to identify a brain parameter independent of antidepressant action that could confirm the in vivo effects of (R)-ketamine in rats.

Male Wistar rats were implanted with electroencephalography (EEG) and neck muscle electromyography (EMG) electrodes. Following a recovery period, animals received an intraperitoneal injection of either (S)-ketamine, (R)-ketamine (7.5, 15, or 30 mg/kg), or vehicle at the start of the passive phase. Post-injection, EEG, EMG, and motility were recorded for 23 hours. Quantitative EEG (qEEG) analysis was performed to evaluate the effects on various frequency bands.

(R)-ketamine, but not (S)-ketamine, dose-dependently increased EEG theta power (5-9 Hz) during both wakefulness and rapid eye movement (REM) sleep throughout the whole 23-hour recording period. The most pronounced effects were observed at the 15 mg/kg dose, with the peak increases occurring specifically in the 7-8 Hz frequency range.

Theta oscillations are generated predominantly in the hippocampus, and they play a fundamental role in processes ranging from episodic memory to complex cognitive-affective functions. Our findings demonstrate that (R)-ketamine, unlike (S)-ketamine, exerts sustained effects on EEG theta power in rats. This distinct neurophysiological profile may serve as a biomarker for its unique hippocampal actions, particularly in models of cognitive impairment. Future research should further investigate the therapeutic potential and underlying mechanisms of (R)-ketamine in these indications.

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42. HEMOKININ-1 AS A MODULATOR OF THERMOREGULATION

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Hemokinin-1 (HK1) is a neuropeptide of the tachykinin family known to exert several functions in the central nervous system[1]; however, its role in thermoregulation has not been previously described. Based on its stable expression in the hypothalamus[2]—a key region in temperature regulation—HK1 represents a strong candidate for a regulatory role in thermal homeostasis. Therefore, our study aimed to elucidate its potential involvement in thermoregulatory mechanisms.

Experiments were conducted on adult male HK1 gene-deficient (KO) and C57Bl/6 (wildtype, WT) mice. We compared core temperature responses following cold and heat exposure, as well as after intraperitoneal administration of low (120 µg/kg) and high (5 mg/kg) doses of lipopolysaccharide (LPS). This experimental process was based on a well-known model, our research group used in previous studies[3].

Our findings show that under physiological conditions, there was no difference between KO and WT groups in response to either cold or heat exposure. However, under pathophysiological stress, i.e., systemic inflammation, we found significant differences. In the late phase of the multiphasic febrile response following low-dose LPS, the temperature rise was significantly attenuated in the KO group. Furthermore, in response to high-dose LPS, HK1 deficiency prevented the development of hypothermia.

These results demonstrate that HK1 may have both pyrogenic and cryogenic properties. Further studies are needed to elucidate the precise mechanisms underlying its role in thermoregulatory responses.

43. EFFORTFUL CHOICE IN RATS: A PRECLINICAL TOOL FOR UNDERSTANDING ALTERED MOTIVATION IN PHARMACOLOGICAL ANHEDONIA AND APPETITE SUPPRESSION

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Anhedonia and reduced motivation are negative psychiatric symptoms, which are assessed in rodent behavioural essays with usually low predictive validity on the human therapeutic responses to drugs. Here we developed and validated a modified Effort Expenditure for Reward Task (EEfRT) for rats, a new translational paradigm originally designed to quantify motivational states and assess pharmacological effects on them. In this task, animals choose between a high-effort/high-reward (HEHR) option and a low-effort/low-reward (LELR) alternative, allowing detailed evaluation of effort-based decision making. To validate the paradigm, first, we divided rats into high-preference (HP) and low-preference (LP) groups based on their baseline performance in the EEfRT. Then, in HP rats, pharmacological anhedonia was induced using the dopamine-depleting agent tetrabenazine, and this deficit was reversed by the dopamine reuptake inhibitor bupropion. In LP rats, natural low motivation was improved following bupropion treatment alone. Cross-validation analyses showed no correlation between EEfRT performance and anxiety measures or food preference, suggesting that the EEfRT paradigm selectively captures motivational dimensions of behaviour. In a subsequent drug-repurposing study, we examined how commonly used GLP-1 receptor agonists influence EEfRT performance. All GLP-1 receptor agonists consistently reduced food intake, confirming their expected appetite-suppressing effects. Exenatide decreased the effort animals exerted for food rewards, while liraglutide and semaglutide did not alter effort expenditure, indicating distinct effects among GLP-1 agonists on motivation. Findings highlight that GLP-1 agonists differ in their impact on reward-related behaviours, beyond appetite suppression. Importantly, the EEfRT proved sensitive to GLP-1–induced reductions in motivational drive, validating the task as a translational tool for detecting drug-related changes in effort-based decision making. The differential effects of individual GLP-1 agents further suggest that these compounds may modulate effortful food choice through distinct motivational pathways, rather than solely via appetite suppression. These insights open the possibility that GLP-1 receptor agonists could be strategically tested in psychiatry to target motivational alterations in comorbid conditions frequently accompanying obesity, such as depression or negative symptoms in schizophrenia. Our next goal is to use this paradigm across various psychiatric disease models to determine how behavioural disturbances associated in those models may respond to pharmacological treatments and result in overlapping or distinct patterns of motivational perturbations.

44. NEUROIMMUNE CROSSTALK AND PHARMACOLOGICAL MODULATION OF SEGMENT REGENERATION

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Regeneration in annelids is critically mediated by the central nervous system and by free-floating coelomocytes, analogues of mammalian leukocytes, which migrate to and accumulate in a high amount to the amputation site. However, the exact cellular and molecular mechanisms underlying this process have remained largely unexplored. In vertebrates, immune cells are known to play a key role in neuro-immune crosstalk by synthesizing and releasing neurotransmitters and by responding to neurotransmitter signals. We investigated whether coelomocytes contain classical neurotransmitters and, if so, whether neurotransmitter signals integrated by coelomocytes contribute to segment regeneration.

Mass spectrometry and (immune)histochemistry revealed pronounced levels of glutamate (Glut), γ-aminobutyric acid (GABA), serotonin (5-HT), and dopamine (DA) in coelomocytes of the earthworm model, *Eisenia andrei*. The vesicular storage of 5-HT and GABA was confirmed by immunogold electron microscopy. Using CLANS and phylogenetics, we mapped sequences related to synaptic transmission of classical neurotransmitters in the *E. andrei* genome. Transcriptomic analysis of coelomocytes for the obtained sequences revealed both evolutionarily conserved and lineage-specific features: transcripts encoding Glut decarboxylase, tyrosine hydroxylase, phenylalanine hydroxylase, aromatic L-amino acid decarboxylase, GABA transporter (GAT1), $GABA_A$ β subunit, as well as $5\text{-}HT_{1/5}$, $5\text{-}HT_4/D_1$, $5\text{-}HT_7$, D_1 (DopR1), and D_2 receptors were detected, whereas transcripts for tryptophane hydroxylase, 5-HT transporter, DA transporter, $GABA_A$ α/γ subunits, $GABA_B$, and $5\text{-}HT_2$ were absent. These results provide the first direct evidence that protostome coelomocytes contain and utilize classical neurotransmitters, emphasizing both conserved and divergent features of neuroimmune communication across Bilateria.

Subsequent mass spectrometry analysis of coelomocytes isolated at different time points (0, 3, 7, 14 days) during regeneration showed that the concentration of Glut, 5-HT, and DA significantly increased, while the concentration of GABA significantly decreased on day 3 and 7, indicating a potential role of neurotransmitters in early stages of regeneration. To test the functional relevance of this potential neurochemical regulation, pharmacological experiments were performed using haloperidol (D_1/D_2 antagonist), ondansetron ($5\text{-}HT_3$ antagonist), GABA, and their mixtures during the regeneration process. All treatments significantly reduced the number of regenerated segments, confirming the key role of classical neurotransmitters in proper segment regeneration.

Our results 1) reinforce the concept that neuroimmune communication is an ancient and evolutionarily conserved feature of animal physiology and 2) demonstrate the key role of coelomocyte-mediated neurotransmitter homeostasis in wound healing and tissue regeneration. Our findings may inform new strategies in regenerative medicine and immune modulation in higher organisms.

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45. ARTIFICIAL INTELLIGENCE-ASSİSTED EXTRACTİON OF BEHAVİORAL DYNAMİCS İN LABORATORY RODENTS

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Recent advances in certain types of artificial intelligence (AI) methods have begun to influence multiple areas in life science research. Inclusion of AI-based tools into research pipelines could boost efficiency via automation of laborious processes or aid in gaining further insight into the research topic of interest. In vivo pharmacology research has also adopted strategies involving AI-assisted components. Among the possible application domains, state-of-the-art computer vision techniques that aim to assist in the evaluation of animal behavior have received a particularly large adoption rate. This concept is built upon the high versatility of recent deep neural networks that can identify and track fine details of laboratory animals resulting in a representation of animal body keypoints within acquired video data suitable for downstream AI-based processing.

To exemplify the utility of such tools, selected use cases are presented related to in vivo pharmacology experiments on laboratory rodents. The overall aim of the introduced analysis pipeline is to facilitate identification of behavioral alterations upon pharmacological manipulation of tracked, freely-moving animals. The presented proof-of-concept studies demonstrate reliable detection of well-defined behaviors, such as grooming, with high accuracy using AI-based approaches. In addition, results are presented on quantifying expected behavioral patterns induced by reference compounds with well-characterized effects, including drugs administered via targeted microinjection into defined brain regions. The poster includes detailed descriptions of the experimental schemes and the analysis steps relying on AI tools. Adoption of such techniques is proposed to significantly increase throughput of experiments while decreasing the large inter-laboratory variability in evaluation. Employing AI tools also inherently facilitates data-driven analysis, potentially enabling identification of otherwise overlooked, unexpected behavioral categories. Finally, subtle behavioral differences and transitions across behaviors (behavioral dynamics) might also become more tractable than what can be achieved via traditional manual scoring.

46. PHARMACOLOGICAL TARGETING OF TRPM3 BY CLINICALLY USED ANTIEPILEPTIC DRUGS

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The transient receptor potential melastatin 3 (TRPM3) channel is a calcium-permeable nonselective cation channel expressed in sensory neurons and various brain regions, where it contributes to neuronal excitability and intracellular Ca^2 signaling. Gain-of-function mutations in the TRPM3 gene have been identified as a cause of severe neurodevelopmental disorders, including developmental and epileptic encephalopathies. Pharmacological evidence further supports its pathogenic relevance, as the antiepileptic drug primidone is a potent TRPM3 inhibitor.

Based on these findings, we aimed to determine whether TRPM3 inhibition may also contribute to the mechanism of action of other antiepileptic drugs. Experiments were performed on HEK293T cells expressing recombinant TRPM3 channels. Channel activity was monitored using the fluorescent Ca^2 indicator Fluo-4 AM by measuring changes in intracellular Ca^2 concentration. TRPM3 was activated with its established agonist, the neurosteroid pregnenolone sulfate (PS).

17 antiepileptic drugs widely used in clinical practice were investigated at concentrations approaching the upper limit of their therapeutic range (except everolimus, which was applied at 50 M concentration): acetazolamide, eslicarbazepine, etosuximide, everolimus, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, phenytoin, piracetam, primidone, rufinamide, stiripentol, tiagabine, topiramate, valproic acid, and zonisamide.

Consistent with previous reports, primidone significantly inhibited PS-induced TRPM3 activity. In addition, partial but significant inhibition was observed with everolimus (28%; $p < 0.05$), eslicarbazepine (60%; $p < 0.01$) and stiripentol (92%; $p < 0.01$). Importantly, the inhibitory effects of eslicarbazepine and stiripentol were concentration dependent. These findings were independently confirmed by electrophysiological recordings.

Our findings expand the current understanding of antiepileptic drug mechanisms and identify additional TRPM3 inhibitors. Targeting TRPM3 may represent a promising therapeutic strategy in central nervous system disorders associated with epilepsy, particularly in developmental and epileptic encephalopathies. Further studies based on these results may support the development of more selective therapeutic approaches.

47. TRPA1 MODULATES UROCORTIN 1 TURNOVER IN THE CENTRALLY PROJECTING EDINGER-WESTPHAL NUCLEUS IN A CGRP-INDUCED MIGRAINE MODEL

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The urocortin 1 (UCN1)-expressing neurons of the centrally projecting Edinger-Westphal nucleus (EWcp) regulate the function of migraine-related brain areas *via* direct urocortinergic connections. In the central nervous system, EWcp/UCN1 neurons uniquely co-expresses transient receptor potential ankyrin 1 (TRPA1) cation channel, which has also been linked to migraine. Here we aimed to investigate whether central TRPA1 receptors regulate the EWcp/UCN1 neurons' response to migraine.

The intraperitoneal calcitonin gene related peptide (CGRP) injection model of migraine was implemented and validated using light-dark box and von Frey assays in wild-type (WT) and TRPA1 knockout (KO) male mice. RNAscope *in situ* hybridization and immunofluorescence were used to examine the *Ucn1*, *Trpa1* mRNA expression and UCN1 peptide content in the EWcp. FOS immunohistochemistry was performed to assess acute neuronal activation in the EWcp and the antinociceptive lateral periaqueductal gray matter (lPAG).

CGRP administration induced light aversion, periorbital hyperalgesia and increased FOS immunoreactivity in the lPAG in both genotypes supporting the model validity. Additionally, *Trpa1* deficient mice exhibited reduced sensitivity to light, regardless of the treatment conditions. In the EWcp, CGRP treatment increased FOS immunosignal and *Ucn1* mRNA expression in both genotypes. Moreover, in WT mice, the treatment increased the EWcp UCN1 peptide and *Trpa1* mRNA levels, with no such changes observed in *Trpa1* KO animals. These findings suggest a possible role of central TRPA1 in migraine by regulating UCN1 dynamics in the EWcp. Targeting TRPA1 ion channels through pharmacological interventions may offer a new strategy for migraine treatment.

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48. PLASMA METABOLIC ALTERATIONS IN MIGRAINEURS DURING AND BETWEEN MIGRAINE ATTACKS

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Migraine is a widespread and disabling neurological condition affecting a large portion of the population. The precise pathophysiology of migraine remains poorly understood. Despite progress in understanding the disorder, treatment options still prove unsatisfactory for many patients. We aimed to analyse changes in plasma metabolite levels in migraineurs in a self-controlled manner, both during and between attacks, to better understand the underlying metabolomic shifts. Migraineurs without co-morbidities participated in the study, with five plasma samples collected during headache episodes (ictal) and fourteen during headache-free periods (interictal). Eight control samples were obtained from age- and gender-matched healthy volunteers. Plasma samples were subjected to untargeted fingerprinting via mass spectrometry. Additionally, the MxP® Quant 500 kit, designed for targeted assays, was used to achieve quantitative measurements and to validate the untargeted analysis results.

During the ictal phase, metabolite concentrations, such as methionine sulfoxide (Met-SO) and tauromurocholic acid, were elevated. Conversely, during the interictal phase, levels of arachidonic acid (AA), TG (22:6_34:1), TG (22:5_34:1), PC aa C36:0, PC aa C36:5, and PC aa C36:6 were higher. Targeted measurements showed that deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) levels decreased in migraineurs' plasma during the interictal phase compared with those of healthy controls. Untargeted measurements confirmed several metabolites—such as PC 32:1, PC 34:2, PC 34:3, PC 36:1, and FA 18:2—exhibiting consistent trends across both analytical methods. AA and other polyunsaturated fatty acids (FAs) serve as precursors in the production of prostaglandins and leukotrienes, which are closely linked to pain signalling. An imbalance in Met/Met-SO reflects cellular stress or compromised repair ability. Changes in bile acids such as DCA, CDCA, and TMCA suggest interactions among the gut, immune system, and metabolism. Overall, our results suggest that disruptions in lipid metabolism and mitochondrial function may significantly contribute to increased migraine susceptibility.

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49. IMPAIRED LOCOMOTION AND ASSOCIATIVE LEARNING INDUCED BY CHRONIC EXPOSURE TO CARBAMAZEPINE IN A WIDELY USED MOLLUSCAN MODEL

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Carbamazepine (CBZ) is a widely used anticonvulsant prescribed for epilepsy, bipolar disorder, and neuropathic pain since the 1960s. In mammals, CBZ binds to voltage-gated sodium channels and reduces both transient (INaT) and persistent (INaP) currents, resulting in the stabilization of hyperexcitable neuronal membranes and a reduction in high-frequency firing during focal and tonic-clonic seizures. CBZ is a persistent aquatic contaminant due to its high global consumption and poor removal by wastewater treatment plants. Although numerous studies have documented its adverse effects on aquatic organisms, knowledge about its impact on learning and the underlying neuronal mechanisms in invertebrates remains limited.

We investigated the behavioral and cellular effects of chronic exposure to an environmentally relevant concentration of CBZ (1 µg/L) in the great pond snail (*Lymnaea stagnalis*), a well-established model in neuroscience and ecotoxicology. Adult snails were exposed for 21 days, followed by assessments of feeding and locomotion activity, as well as food-reward classical conditioning. According to our results, the exposure did not affect feeding behavior but caused a significant reduction in locomotor activity and a pronounced impairment of associative learning and long-term memory. Locomotor deficits recovered after a 21-day depuration period, whereas learning impairment persisted, identifying cognition as a particularly vulnerable and long-lasting target of CBZ exposure. Electrophysiological recordings on the Cerebral Giant Cell (CGC), a key serotonergic interneuron involved in food-reward classical conditioning, revealed that CBZ selectively blocked the INaP, but not the INaT, component of voltage-gated sodium currents, providing a mechanistic link between CBZ exposure and long-lasting cognitive deficits.

From a neuroscientific perspective, our findings indicate that CBZ interferes with evolutionarily conserved neuronal targets in invertebrates, yet with species- and circuit-specific consequences that differ from those observed in vertebrate (disease) models. From an ecotoxicological perspective, our results pinpoint cognition as a sensitive and persistent endpoint of CBZ pollution and highlight that CBZ may severely compromise individual fitness, with potential consequences for survival and adaptation in natural ecosystems.

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50. TRPM3 REGULATES FEAR-MEMORY ENCODING AND SEIZURE SUSCEPTIBILITY IN THE LATERAL AMYGDALA

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Transient receptor potential melastatin 3 (TRPM3) is a member of the transient receptor potential (TRP) family of ion channels and is best known for its role as a peripheral sensor of pain and heat in sensory neurons. Recent studies reporting gain-of-function *TRPM3* mutations in patients with neurodevelopmental and epilepsy-associated disorders suggest a previously unrecognized role of this channel in the central nervous system. However, the expression pattern and function of TRPM3 in the brain remain largely unexplored.

Here, we identify for the first time TRPM3 expression in excitatory neurons of the lateral nucleus of the amygdala (LA), a brain region critical for fear learning and implicated in neuropsychiatric and epileptic disorders. We show that TRPM3 is functionally active in LA neurons and regulates their intrinsic electrophysiological properties. Consistent with this role, genetic ablation of TRPM3 in mice impairs fear memory formation without affecting basal anxiety-like behavior. Moreover, in a kainic acid–induced epilepsy model, loss of TRPM3 reduces seizure severity, whereas pharmacological activation of the channel exacerbates seizure phenotypes. Finally, we demonstrate the presence of TRPM3 transcripts in neurons of the human LA.

Together, these findings uncover a central role for TRPM3 in amygdala function and identify this ion channel as a modulator of fear memory and seizure susceptibility.

51. POOR SLEEP DISRUPTS COMMUNICATION BETWEEN BRAIN ACTIVITY AND BLOOD FLOW: EVIDENCE FROM LASER DOPPLER AND LASER SPECKLE IMAGING

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Growing evidence indicates that insufficient sleep and chronic sleep disturbances contribute to the development of several neurological and psychiatric disorders, including depression and neurodegenerative diseases such as Alzheimer's disease. Sleep deficiency has been associated with impaired synaptic plasticity, neuroinflammation, and altered clearance of metabolic waste products, including amyloid-β from the brain. Chronic sleep fragmentation has also been linked to dysregulation of cerebral blood flow and impaired neurovascular coupling, which may further contribute to cognitive decline and mood disorders. These findings highlight the importance of sleep integrity for maintaining normal brain function and vascular regulation.

In the present study, we investigated the effects of four weeks of sleep fragmentation on neurovascular function in rats. Sleep fragmentation was induced using the single platform technic. In this method, animals are placed on a small platform surrounded by water. When the animal enters deeper stages of sleep, muscle relaxation causes it to lose balance and slip from the platform into the water, leading to immediate awakening and thus repeated interruption of sleep.

Following the four-week treatment period, neurovascular unit function was assessed. Animals were anesthetized, and the skull above the somatosensory cortex was thinned until it became sufficiently transparent for laser-based measurements. Changes in cortical blood flow evoked by electrical stimulation of the contralateral forepaw were recorded using Laser Doppler flowmetry and Laser Speckle contrast imaging.

Rats exposed to sleep fragmentation showed a significantly reduced stimulation-induced increase in cerebral blood flow compared with control animals. This attenuation of the hemodynamic response was consistently detected with both Laser Doppler and Laser Speckle techniques.

These findings indicate that chronic sleep fragmentation impairs neurovascular coupling in the cortex. Moreover, the combination of the single platform sleep-fragmentation model with Laser Doppler and Laser Speckle measurements provides a suitable experimental approach for investigating dysfunction of the neurovascular unit and for evaluating potential therapeutic compounds targeting neurovascular uncoupling.

52. NAD+/SIRT1 PATHWAY IN HUMAN DEPRESSION

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Preclinical evidence links early-life stress (ELS) to alterations in adipose tissue composition and depressive-like behavior in rodents, with reduced SIRT1 expression in the nucleus accumbens (NAc) identified as a central molecular mechanism. Notably, these metabolic and behavioral consequences were sex-specific, being prominent in males but largely absent in females.

We sought to translate these preclinical observations to humans by leveraging population-scale genetic data and neuroimaging resources from the UK Biobank, complemented by a smaller Hungarian imaging cohort.

Polygenic risk scores (PRS) targeting 20 genes within the NAD^+/SIRT1 signaling pathway were derived using LDpred2 for the full UK Biobank sample (application no. 1602) and for sex-stratified subgroups (N_males = 46,581; N_females = 59,073). Linear regression models implemented in R (v4.1.2) were used to assess PRS × ELS interaction effects on current depressive symptom severity. Mediation analyses via lavaan (v0.6-12) examined whether body fat percentage accounted for the observed interaction. In a separate Hungarian cohort (N = 102), we evaluated PRS × ELS effects on NAc functional connectivity using resting-state neuroimaging.

A significant PRS × ELS interaction on depressive symptoms was detected exclusively in males ($\beta = 2.93$, $p = 0.0002$). This interaction was further associated with sex-dependent differences in NAc functional connectivity to the middle frontal gyrus and the triangular portion of the inferior frontal gyrus ($p = 0.014$). Mediation analysis indicated that the depressogenic interaction effect operates independently of adult body fat percentage, suggesting that adiposity does not mediate this genetic vulnerability.

Across both cohorts, males carrying elevated polygenic burden in the NAD^+/SIRT1 pathway who were also exposed to ELS showed greater susceptibility to depressive symptoms and altered reward-related NAc connectivity. These results provide a human translation of prior animal models and underscore the NAD^+/SIRT1 axis as a sexually dimorphic mechanism linking early adversity to depression. From a pharmacological perspective, these findings position the NAD^+/SIRT1 pathway, including NAD^+precursor supplementation and SIRT1-activating compounds, as a promising, biologically grounded therapeutic target for stress-related depression, particularly in males, warranting future interventional studies.

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53. ENANTIOMER-SELECTIVE EFFECTS OF KETAMINE ON THE GAMMA-DELTA SHIFT PHENOMENON AND DEPRESSION-LIKE BEHAVIOUR IN WISTAR RATS

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Subanaesthetic racemic ketamine produces rapid antidepressant effects that have been linked to characteristic quantitative electroencephalography (EEG) alterations, including a temporal shift between gamma and delta activity, which we have termed the gamma-delta shift phenomenon. However, the contribution of individual enantiomers to this phenomenon remains unclear. Therefore, we investigated the dose-dependent effects of (S)-ketamine and (R)-ketamine on the gamma-delta shift and also evaluated the antidepressant-like activity of the enantiomers in a chronic restraint stress model of depression in Wistar rats.

Fronto-parietal EEG recordings were performed following intraperitoneal administration of (S)-ketamine (7.5, 15, and 30 mg/kg), or (R)-ketamine (7.5, 15, and 30 mg/kg), or vehicle (saline 1 mL/kg) at the beginning of the passive phase. The gamma-delta shift was defined as an increase in gamma activity during wakefulness followed by enhanced delta power during NREM sleep. In a separate group of chronically stressed rats, the antidepressant-like activity of the two enantiomers was compared in the forced swimming test after administration of 15 mg/kg (S)- or (R)-ketamine or vehicle.

(S)-ketamine produced dose-dependent effects on the gamma-delta shift. At 7.5 mg/kg, no shift was observed, whereas at 15 mg/kg, increased gamma activity during wakefulness was followed by a rebound elevation of delta power during NREM sleep, indicating a pronounced gamma-delta shift. A similar but more robust effect was observed at 30 mg/kg, with marked gamma enhancement followed by a rebound increase in delta power. In contrast, (R)-ketamine failed to induce a gamma-delta shift at 7.5, 15 or 30 mg/kg despite moderate gamma increases at higher doses. In chronically stressed rats (S)-ketamine (15 mg/kg) significantly reduced immobility time in the forced swimming test, whereas (R)-ketamine at the same dose produced no antidepressant-like effect.

These findings demonstrate that the gamma-delta shift is specific to (S)-ketamine and parallels its antidepressant-like efficacy, supporting the hypothesis that this EEG biomarker may reflect mechanisms underlying rapid antidepressant action. Nevertheless, further investigations are needed to validate the gamma-delta shift phenomenon as a potential biomarker of antidepressant effects.

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54. EFFECT OF THERAPY ON THE EXPRESSION OF MICRORNAS IN PLASMA SAMPLES FROM PATIENTS WITH PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the degeneration of dopaminergic neurons and the presence of motor and non-motor symptoms. As the disease progresses, therapeutic management becomes increasingly complex and often requires individualized treatment strategies and continuous adjustment of therapy. Continuous dopaminergic delivery systems have been developed to improve symptom control, particularly intestinal gel infusion pumps such as Duodopa® (levodopa/carbidopa) and Lecigon® (levodopa/carbidopa/entacapone). However, reliable and minimally invasive biomarkers reflecting disease progression and treatment response are still lacking. MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression at the post-transcriptional level and are involved in many physiological and pathological processes, including neurodegeneration. Increasing evidence suggests that altered miRNA expression is associated with Parkinson's disease and may reflect underlying molecular mechanisms.

The aim of this study was to evaluate the expression of selected circulating miRNAs (miRNA-1, miRNA-133a, miRNA-133b, miRNA-29a, and miRNA-29b) in plasma samples from patients with Parkinson's disease undergoing different therapeutic regimens and to assess the potential impact of therapy on miRNA expression profiles.

The study included 42 patients with PD and 10 control subjects. Patients were stratified by type of treatment, including continuous intestinal infusion therapies (Duodopa® and Lecigon®) and standard pharmacotherapy. Blood samples were collected at baseline and after 6 months of treatment. Plasma was isolated, RNA extracted, and miRNA expression was analyzed by RT-qPCR. Our preliminary findings suggest that circulating miRNA expression may be influenced by the type and duration of treatment. The differences observed between treatment groups indicate that miRNAs could reflect treatment-related molecular changes and may serve as accessible biomarkers for monitoring disease progression and therapeutic response.

In conclusion, circulating miRNAs represent a promising tool for improving the understanding and treatment of Parkinson's disease. Further studies with larger cohorts and longer follow-up are needed to confirm these findings and clarify the role of miRNAs in the pathophysiology and treatment of PD.

Keywords: Parkinson's disease, circulating microRNAs, plasma biomarkers, therapy response

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55. INVESTIGATION OF THE MEDIATING ROLE OF THE ENDOCANNABINOID SYSTEM IN THE ANXIOLYTIC AND ANTIDEPRESSANT-LIKE MECHANISMS OF DIMETHYL TRISULFIDE IN A CHRONIC STRESS MODEL

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Psychiatric disorders associated with stress, such as anxiety and depression, represent a significant public health burden. Dimethyl trisulfide (DMTS) is an organic polysulfide previously shown to possess significant antioxidant and TRPA1 agonist properties. Literature data suggests that organic polysulfides can interact with the metabolic enzymes of the endocannabinoid system (ECS), such as monoacylglycerol lipase (MAGL), thereby increasing endocannabinoid tone. The aim of this study was to explore the role of the ECS, specifically CB_1 receptors, in the behavioral and physiological effects of DMTS under chronic stress, and to compare the pharmacological profile of DMTS with that of selective endocannabinoid-degrading enzyme inhibitors.

Experiments were conducted on male C57BL/6J mice using a 21-day chronic unpredictable mild stress (CUMS) model. The effects of DMTS (30 mg/kg) were investigated alone and in combination with the selective CB_1 receptor antagonist AM251 (1 mg/kg). The MAGL inhibitor JZL184 and the FAAH inhibitor URB597 were used as reference compounds. Behavior was assessed using Open Field Test (OFT), Marble Burying Test (MBT), Forced Swim Test (FST), Tail Suspension Test (TST) and Sucrose Preference Test (SPT). Additionally, the weights of stress-sensitive organs (adrenal gland, thymus) were measured.

While the effects of DMTS did not show CB_1 dependence in acute stress situations, the role of the ECS proved to be determinant in alleviating depression-like symptoms in the chronic stress model. DMTS significantly reduced "despair" behavior (FST, TST) and anhedonia (SPT) induced by CUMS. This protective effect was completely abolished by the co-administration of the CB_1 receptor antagonist AM251. Conversely, the anxiolytic effect (OFT) persisted even in the presence of the CB_1 antagonist, suggesting a different mechanism. Comparative studies revealed that the antidepressant profile of DMTS (FST, SPT) most closely resembled the effect pattern of the MAGL inhibitor JZL184, while its efficacy in anxiety tests (MBT) fell short of that of the FAAH inhibitor URB597. At the somatic level, DMTS prevented stress-induced thymus involution through CB_1 receptors, appearing more effective in this regard than classical enzyme inhibitors.

Our results demonstrate that the antidepressant and anti-anhedonic effects of DMTS observed in chronic stress, as well as its immunomodulatory (thymus-protective) properties, are mediated through CB_1 receptors. The pharmacological profile of the compound shows partial overlap with MAGL inhibitors that increase 2-arachidonylglycerol levels. Consequently, DMTS may be a novel candidate with a dual mechanism of action (TRPA1 and ECS modulator) for the treatment of stress-associated disorders.

56. FOLATE-DECORATED LIPOSOMAL CO-ENCAPSULATION OF PIPLARTINE AND FENRETINIDE: A NOVEL STRATEGY FOR LOCALIZED NON-INVASIVE BREAST CANCER THERAPY

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Approximately 25% of breast cancer cases corresponds to a non-invasive form of the disease, the Ductal Carcinoma *in Situ* (DCIS), which holds defiant challenges regarding its treatment. Nanomedicine stands as a potential approach to surpass limitations that include lack of selectivity, invasiveness of available treatments and systemic toxic effects. In this study, liposomes (LP) were developed for intraductal co-delivery of associations of antitumoral drugs aiming local treatment of non-invasive formas of breast cancer. In the first part of the study, piplartine, an alkaloid with antitumor properties, was combined at various molar ratios with fenretinide or ceramide C6 to identify synergism against breast cancer cells. The Chou-Talalay method was employed to determine the combination index (CI). Synergism was detected with the combination of piplartine (PL) and fenretinide (FE) at 1:1 and 2:1 ratios (PL:FE). In the second part of the study, the production of liposomes was studied with various proportions of soy phosphatidylcholine (PC), propylene glycol (PG) and the drugs, using the film hydration method associated with sonication (for size reduction). Liposomes containing PC and PG at 10%, PL at 0.25% and FE at 0.12% (2:1 PL:FE) sonicated for 3 cycles, 50 s ON and 30 s OFF, generated liposomes of 150 nm (polydispersity index of 0.25), with round-shaped morphology identified by transmission electron microscopy and over 99% of drug entrapment. Afterward, LPs were tested for their cytotoxicity in the breast cancer cell lines to investigate the impact of piplartine encapsulation on cytotoxicity. Piplartine-loaded liposomes demonstrated an enhanced cytotoxicity compared to the drug solution in MCF-7 cells, displaying an IC_{50} of 7.5 µM against 9.5 µM for the solution; more interesting results was seen for liposomes entrapping piplartine+fenretinide, which demonstrated an IC_{50} reduction of ~2-fold compared to PL solution. These results reveal a nanosystem with improved cytotoxicity compared to piplartine solution that can potentially enable local delivery and contribute to the management of non-invasive breast cancer.

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57. ALLYL-İSOTHİOCYANATE DECREASES THE VİABİLİTY OF HUMAN PROSTATE ADENOCARCİNOMA CELL LİNES İNDEPENDENTLY OF TRANSİENT RECEPTOR POTENTİAL ANKYRİN 1 RECEPTOR ACTİVATİON

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The Transient Receptor Potential Ankyrin 1 (TRPA1) agonist allyl isothiocyanate (AITC) has been demonstrated to reduce the proliferation and progression of various malignancies including glioblastoma, osteosarcoma, small and non-small cell lung cancer, but its actions on prostate adenocarcinoma (PAC) remain unknown. Therefore, here we investigated the effects of AITC on PAC cell line viability and proliferation along with its functional expression.

The viability of 22Rv1 and LNCaP PAC cell lines following AITC treatments was assessed by ATP-based luminescence assay at concentrations of 1, 2, 10, 50, 100, 200 µM. Cell proliferation was evaluated after 48 hours of AITC treatment at 1, 10, 50 µM using Luna II automated cell counter. TRPA1 mRNA expression was determined by RNAscope *in situ* hybridization on benign prostate hyperplasia, PAC tissues and cell lines, and validated by qPCR. The functionality of the ion channel was measured by fluorescent Ca^{2+}-influx assay after 100 µM AITC administration.

AITC concentration-dependently reduced the viability of 22Rv1 and LNCaP with the IC50 values of 91.6 µM and 32.8 µM, respectively. This viability decreasing effect was not reversed by the TRPA1 antagonist HC-030031 (50 µM) and A967079 (1 µM) suggesting non-TRPA1-mediated cytotoxic effect. 48 h incubation with 1 µM AITC significantly inhibited the proliferation of both cell lines.

Although TRPA1 transcripts were detected in benign prostate hyperplasia, PAC tissues and both cell lines, AITC did not induce intracellular Ca^2 increase demonstrating the lack of functionally active ion channel proteins. Interestingly, the non-selective TRPA1 agonists cinnamaldehyde, thymol and formaldehyde induced Ca^2 influx, but it was not inhibited by A967079.

In conclusion, although AITC reduces PAC cell viability and proliferation, these actions are not mediated by TRPA1 activation. Despite their mRNA expressions, no TRPA1 channel is expressed functionally on these cell lines. Future studies will investigate the Ca^{2+}-influx-inducing mechanisms and effects of cinnamaldehyde, thymol and formaldehyde in PAC cells.

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58. ANTIPROLIFERATIVE EFFECTS OF NOVEL CANNABIGEROL DERIVATIVES IN RENAL CELL CARCINOMA: TARGETING THE PI3K/AKT/MTOR PATHWAY

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Renal cell carcinoma (RCC) is often diagnosed at advanced stages, and although targeted therapies such as sunitinib and temsirolimus are effective, drug resistance frequently develops. Cannabigerol (CBG) is a compound derived from *Cannabis sativa*, has demonstrated antitumor properties, but its clinical application is limited by low solubility and bioavailability. To overcome these limitations, eight CBG derivatives were synthesized and tested on human RCC cell lines (CAKI-2 and A498). Cell proliferation was assessed using a Cell Titer-Blue assay. Among the tested compounds, three showed significant antiproliferative effects, with PFD45/I being the most potent at 30 µM.

Importantly, PFD45/I exhibited low cytotoxicity in non-malignant NIH-3T3 fibroblast cells while maintaining strong activity against RCC cells, indicating favorable selectivity. Mechanistically, the compound may act through CB1 and CB2 receptor-associated pathways and influence apoptosis and proliferation. Western blot results suggest involvement of the PI3K/Akt/mTOR signaling pathway via modulation of PI3K, Akt, and p-Akt expression.

These findings highlight PFD45/I as a promising candidate for RCC treatment, although further studies are required to fully elucidate its mechanism of action and therapeutic potential.

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59. THE THYMUS-HEART AXİS: ACTİVE THYMİC OUTPUT İS A PREREQUİSİTE FOR IMMUNE CHECKPOİNT INHİBİTOR-ASSOCİATED CARDİOTOXİCİTY

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Introduction: While immune checkpoint inhibitors (ICIs) have redefined cancer management, however their clinical utility is severely capped by fatal cardiovascular complications. The prevailing consensus attributes this cardiotoxicity exclusively to the dysregulation of peripheral T-cells. We challenge this view by proposing an upstream etiology: the thymus. We propose that anti-PD-1 therapy compromises central tolerance mechanisms, effectively priming the immune system for myocardial injury.

Purpose: We have investigated whether a functional thymus is required for the development of ICI-mediated cardiac dysfunction. Furthermore, we evaluated whether inducing thymic involution through either naturally with biological aging or pharmacologically via 5-Azacytidine (AZA), confers cardioprotection against immunotherapy-induced injury.

Methods: We employed a comparative murine model involving young (12-week) and aged (16-month) C57BL/6J cohorts subjected to anti-PD-1 regimens. To isolate the role of thymic output, we induced pharmacological atrophy in young mice using AZA. Cardiac performance was assessed via high-resolution echocardiography, complemented by myocardial gene expression analysis and deep analysis of thymic and splenic microenvironments via RNAseq, flow cytometry and RNAscope.

Results: Administration of anti-PD-1 precipitated significant systolic impairment in young mice, a cardiotoxic effect that was notably absent in the aged cohort. Mechanistically, young thymuses exhibited profound transcriptional alterations indicative of impaired T-cell selection and regulatory T-cell reduction. In the heart, this resulted in a pro-inflammatory milieu characterized by upregulated *Cxcl9*, *Cxcl10*, and *Ifng*, occurring even in the absence of fulminant myocarditis. Moreover, pharmacological suppression of thymic activity with AZA successfully preserved systolic function and dampened myocardial inflammatory signaling, effectively replicating the protective phenotype observed in aging.

Conclusions: These findings expand the peripheral-centric model of immunotherapy complications, identifying the thymus as a key driver of ICI-induced cardiotoxicity. We demonstrate that anti-PD-1 lowers tolerance thresholds centrally, fueling a heart-specific inflammatory loop. Consequently, thymic activity represents a novel biomarker for cardiovascular risk stratification, and its therapeutic modulation offers a promising new way to decouple potent anti-tumor immunity from life-threatening cardiotoxicity.

60. TRANSCRIPTOMIC AND MIRNA PROFILING REVEAL MECHANISMS OF MSC-EV-MEDIATED CARDIOPROTECTION AGAINST CHEMOTHERAPY-INDUCED TOXICITY

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Chemotherapy-induced cardiotoxicity remains a major limitation in cancer treatment, particularly with anthracyclines and other commonly used agents, which can cause irreversible damage to cardiac myocytes. Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) have emerged as a promising cell-free therapeutic approach, but the molecular mechanisms of action remain incompletely understood. In particular, the contribution of EV-derived microRNAs (miRNAs) and downstream transcriptomic reprogramming to cardioprotection has not yet been fully clarified.

This study aimed to characterize the miRNA cargo of MSC-EVs derived from primary and immortalized umbilical cord MSCs and to investigate mRNA transcriptomic changes in human AC16 cardiomyocytes. EVs were isolated from both MSC sources and subjected to miRNA profiling. AC16 cardiomyocytes were treated with Chemotherapy A in the presence or absence of immortalized MSC-EVs, followed by RNA sequencing. Differential gene expression and KEGG pathway enrichment analyses were performed to identify key biological processes associated with cell survival and stress responses.

miRNA profiling revealed that the top 10 most abundant miRNAs were conserved between primary and immortalized MSC-EVs, with predicted functions related to anti-apoptotic, anti-inflammatory, and anti-hypertrophic processes. mRNA analysis identified 591 significantly differentially expressed genes in AC16 cells treated with Chemotherapy A with versus without MSC-EVs, with excellent dataset quality. GO enrichment and KEGG analysis demonstrated involvement of cell survival, cell cycle progression, cell proliferation, apoptosis and autophagy pathways.

The previous experiment finds primary MSC-EVs exhibited strong cardioprotective effects against doxorubicin, while immortalized MSC-EVs showed enhanced protection against Chemotherapy A. Notably, despite similar miRNA cargo profiles, the protective effects differed between EV sources, suggesting a complex mechanism of action in which shared miRNAs contribute to cardioprotection but additional factors modulate treatment-specific responses. These findings provide novel insight into complex MSC-EV-mediated cardioprotection and support their therapeutic potential in mitigating chemotherapy-induced cardiac injury.

61. SYNTHETIC CANNABINOID DERIVATIVES INDUCE ER-STRESS-ASSOCIATED AUTOPHAGY AND INHIBIT SURVIVAL SIGNALLING IN HUMAN CUTANEOUS MELANOMA CELLS

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Cutaneous melanoma is an aggressive form of skin cancer with a poor prognosis in advanced stages, highlighting the need for novel therapeutic strategies. Non-psychoactive cannabinoids derived from *Cannabis sativa*, such as cannabidiol (CBD) and cannabigerol (CBG), have shown promising antitumor activity.

In this study, we investigated the antiproliferative effects and underlying molecular mechanisms of two new water-soluble synthetic derivatives, PFD 35/II and PFD 11/A, in two human melanoma cell lines, WM 35 and WM 3000. HaCaT keratinocytes and 3T3 fibroblasts were used as control cell lines to evaluate cytotoxicity in normal non-malignant cells. For the cell viability assay, the compounds were applied at concentrations ranging from 1.25 to 80 µM, and cell proliferation was assessed using the CellTiter-Blue assay.

Both PFD derivatives reduced melanoma cell viability while showing lower cytotoxicity in non-tumorous cells. Western blot analysis of proteins revealed the modulation of multiple signaling pathways involved in cell survival and death. Treatment with the PFD compounds decreased PI3K, mTOR, and NF-κB levels, while PTEN and FOXO1 expression increased. AKT phosphorylation showed a transient increase followed by a reduction, and ERK phosphorylation was elevated. Apoptosis-related markers were also altered, including decreased caspase-3 and increased Bax expression, alongside modulation of Bcl-2. In addition, ER-stress-related proteins such as calnexin and PERK were affected, suggesting the involvement of ER-stress signaling in melanoma cell death.

These findings indicate that the examined synthetic water-soluble cannabinoid derivatives exert selective antiproliferative effects in melanoma cells through the inhibition of survival signaling pathways and the modulation of autophagy. Our results also suggest that these novel cannabinoid derivatives selectively inhibit melanoma cell growth by modulating PI3K/AKT/mTOR signaling and inducing ER-stress-associated cell death.

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62. ANTITUMOUR ACTIVITY OF THIOUREAS IN BREAST CANCER

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Cancer has emerged as one of the leading global health challenges, driving extensive research into new therapeutic strategies and drug development. Thiourea derivatives have attracted significant attention for their potential anti-cancer properties. Studies have indicated that thiourea and its derivatives may possess anti-cancer activity, supported by their biological properties such as antioxidant, anti-inflammatory, and antimicrobial effects. These properties may play a critical role in enhancing their anti-cancer efficacy and positioning them as promising candidates for future oncological therapies.

Here, we aimed to investigate the anti-cancer activity of newly designed thiourea derivatives in breast cancer cell lines.

The effects of 22 different thioureas compounds were investigated at five different concentrations (100 nM, 1 µM, 10 µM, 50 µM, and 100 µM) using cell viability and cell proliferation methods *in vitro* on two breast cancer cell lines, i.e. CAL-85-1 and MCF-7. The compounds were freshly dissolved prior to each experiment to ensure stability and reproducibility of the results.

According to our results, compounds TU-1118, TU-1119, TU-1121, and TU-1122 significantly reduced the viability of CAL-85-1 cells, as measured by the CellTiter-Glo assay. In the MCF7 cell line, compounds TU-1118, TU-1119, TU-1131, and TU-1095 significantly decreased cell viability. In addition, compounds TU-1094, TU-1095, TU-1101, TU-1102, and TU-1107 significantly inhibited cell proliferation in MCF7 cells.

In conclusion, amino-acid-containing thioureas were the most effective group in reducing CAL-85-1 cell viability, whereas N-benzoyl-thiourea derivatives demonstrated the strongest antiproliferative activity. In MCF7 cells, amino-acid-containing compounds as well as selected N-benzoyl-thioureas significantly decreased cell viability. These findings highlight specific amino-acid-containing and N-benzoyl-thiourea derivatives, particularly 1118, 1119, and 1095, as promising candidates for further preclinical anticancer development.

63. IMMUNE CHECKPOİNT BLOCKADE RELATED CARDİAC DYSFUNCTİON VARİES BY MELANOMA MODEL: IMPLİCATİONS FOR CARDİAC ANTİGEN CROSS-REACTİVİTY

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Immune checkpoint inhibitors (ICIs) improve cancer outcomes but may lead to severe cardiovascular immune-related adverse events, including cardiotoxicity by currently still unclear mechanism. One of the proposed mechanisms are that T cell recognition of antigens shared between tumor and myocardium contributes to cardiac injury during PD-1 blockade. To test this shared antigen hypothesis, first we collected publicly available human RNA sequencing pre- and post-treatment data from patients receiving pembrolizumab or nivolumab to skin cancer. After batch normalization of data, the upregulated cardiac genes (e.g. MYBPC3) were identified in these cohorts.

Since no cardiac phenotype data are available for these patients, we conducted an *in vivo* proof of concept study using B16F10 or YUMM1.7 ($Braf^{V600E/wt}$; $Cdkn2a^{-/-}$; $Pten^{-/-}$) murine melanoma cells, which were implanted into C57BL/6J male mice, followed by anti-PD-1 or isotype treatment. Echocardiography revealed distinct, tumor-dependent cardiac phenotypes. ICI-treatment reduced left ventricular ejection fraction in YUMM1.7-bearing mice, whereas B16F10-bearing mice developed increased left ventricular mass without systolic impairment. Cardiac-specific gene expression, similar to human data was detected in tumors grown *in vivo*.

These findings indicate that melanoma subtype and their differential expression of cardiac-related genes might determine the side-effect profile of PD-1 blockade and qPCR-based routine screening from tumor biopsies could predict incidence of severe side effects.

Keywords: cancer immunotherapy, cardio-oncology, cardiotoxicity, heart failure, molecular mimicry, shared antigen hypothesis, cardiac autoantibodies

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64. TARGETED ACTIVATION OF SOMATOSTATIN RECEPTOR 4 ALLEVIATES PAIN AND TUMOR PROGRESSION IN A MURINE OSTEOSARCOMA MODEL

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Background: Osteosarcoma (OS) is the most prevalent painful primary bone cancer in adolescents. Despite surgical and chemotherapeutic advances, mortality remains high and managing cancer-induced bone pain (CIBP) is often hindered by the limited efficacy and severe side effects of conventional antinociceptive medication. The somatostatin 4 receptor (SSTR4) has emerged as a promising non-endocrine target for analgesia and inflammation. Our working group demonstrated the expression of the SSTR4 in human OS tissue samples and in the K2M7 OS cell line, where the selective agonist J-2156 and the multi-target compound TT-232 reduced cell viability in vitro. Based on these findings, we investigated the potential therapeutic effect of the compounds on tumor progression and cancer-induced pain in an in vivo osteosarcoma mouse model.

Methods: Male BALB/c mice (9-10 weeks old) were used for the OS model, where K2M7 cells were injected into the right tibial epiphysis. To ensure stable and constant plasma levels and minimize animal stress, TT-232 and J-2156 (0,6 mg/kg/day) were administered via subcutaneously implanted ALZET osmotic minipumps.

Results: Both SSTR4 agonists attenuated the tumor-induced mechanical hypersensitivity, albeit with different efficacy profiles. While J-2156 caused only a significant improvement in the meantime (days 19-22), TT-232 significantly elevated the mechanonociceptive threshold from day 11 until the termination. Furthermore, TT-232 significantly reduced the frequency and duration of spontaneous pain behaviors, while J-2156 showed only a non-significant positive trend. Notably, TT-232 significantly inhibited tumor progression – as measured by mediolateral and anteroposterior limb diameters - whereas J-2156 had no impact on tumor growth.

Conclusion: Our findings demonstrate that SSTR4 agonists effectively alleviate CIBP. The enhanced efficacy of TT-232, the highly significant levels of antinociception in different pain parameters, combined with the inhibition of the tumor progression, can be attributed to its complex, pleiotropic mechanism of action. Targeted SSTR4 activation represents a novel, promising strategy for combined oncological treatment of osteosarcoma.

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65. A PRECLINICAL PLATFORM FOR IMMUNE CHECKPOINT INHIBITOR-INDUCED ELECTRICAL DYSFUNCTION

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Immune checkpoint inhibitors (ICIs) can cause rare but potentially fatal arrhythmias. The mechanisms underlying these electrical abnormalities remain poorly defined, in part due to the absence of preclinical models designed to study ICI-induced conduction disturbances. Considering roughly one-third of patients with ICI-related arrhythmias have prior hypertension, we hypothesized that hypertensive stress sensitises the heart to ICI-induced conduction abnormalities. Adult male C57BL/6J mice received continuous infusion of Ang II (1.0 mg/kg/day) or saline via osmotic minipumps for 14 days. After 7 days of infusion, mice were treated with two doses of combined anti-PD-1 and anti-CTLA-4 antibodies (25 mg/kg each, intraperitoneally, every 3 days). Ang II pre-treatment preceded ICI administration to minimise confounding early inflammatory effects, and ICI exposure was restricted to one week (2 injections) to avoid overt myocarditis. At study end, echocardiogram and surface electrocardiogram (ECG) were acquired under isoflurane anaesthesia. Electrical vulnerability was further evaluated using a bolus of epinephrine (2 mg/kg) and caffeine (120 mg/kg) during ECG recording. Surface ECG revealed that ICI treatment induced QRS prolongation and increased P-wave duration exclusively in Ang II-infused mice. High-grade atrioventricular block (AVB) and ventricular tachycardia also occurred exclusively in Ang II-treated mice. Echocardiogram revealed preserved left ventricular ejection fraction and fractional shortening in both groups. Ang II-treated mice exhibited modest increases in left ventricular mass and heart weight, consistent with hypertensive stress-induced cardiac hypertrophy. Histological analysis showed mild infiltrative lesions (<20% of longitudinal section area) in 3 of 8 Ang II-treated hearts. Overall, angiotensin II unmasked ICI-induced conduction abnormalities and ventricular arrhythmias in the absence of myocardial dysfunction or overt myocarditis. These data provide proof-of-principle that hypertensive remodelling lowers the threshold for ICI-induced electrical toxicities within the cardiac conduction system. Our model enables interrogation of early conduction system-specific mechanisms and provides a platform for defining causal and temporal relationships between ICIs and arrhythmias.

66. ANTITUMOR POTENTIAL OF CYCLIC C5-CURCUMINOID IN LUNG CANCER CELLS

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In many cases, natural compounds serve as a primary source of chemical diversity for starting materials in drug discovery, and over the past century they have substantially advanced pharmaceutical research. A notable example in this regard is the history of curcumin, illustrating how a spice-derived compound evolved into a pharmaceutical lead molecule. However, the clinical applicability of curcumin is limited due to its unfavorable pharmacokinetic properties such as poor bioavailability and rapid metabolism. In the Institute of Pharmaceutical Chemistry, at University of Pécs, more potent and more stable curcumin derivatives were synthesized with structural modifications for enhancing their bioactivities. Therefore, in the research presented here, we aimed to evaluate the bioactivity of cyclic C5-curcuminoid compounds using both cancerous and healthy lung cell lines with characterization of their mechanisms of action within cellular systems.

During our work we investigated the cytotoxic effects of the test compound as well as determining IC_{50} values and potential tumor selectivity. As tubulin polymerization is an essential process during cell cycle, we further investigated the direct effect of the compound on the microtubule system by tubulin polymerization assay. Finally, we analyzed how individual phases of the cell cycle change upon treatment with cyclic C5-curcuminoid.

In these biological studies we were able to demonstrate selective cytotoxic effects on A549 human lung adenocarcinoma cell line. However, the compound did not show cytotoxic effects on PC-9 lung adenocarcinoma cells and primary, healthy NHLF lung fibroblast cells in the concentration range we tested. In the tubulin polymerization assay the compound was found to interact with tubulin and alter characteristic phases of polymerization. Cell cycle analysis showed that the compound induced a G2/M phase arrest with a marked increase in the portion of the cells in this phase.

Taken together, this result indicates the tumor cell selectivity of the compound, which can be considered a particularly favorable property for a potential antitumor agent. Furthermore, the antitumor activity is partially mediated by its ability to disrupt tubulin polymerization and the cell cycle. These findings collectively support the compound as a promising candidate for further investigation.

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67. TRANSLATIONAL MULTIOMICS PHARMACOLOGY: AN INNOVATIVE FRAMEWORK FOR INTEGRATING PHARMACOGENOMICS AND DYNAMIC METABOLIC MODULATION IN COMPLEX POLYPHARMACY

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Although pharmacogenomics (PGx) has transformed precision medicine, its clinical impact is limited by its reliance on static genetic data, which fail to account for dynamic, multifactorial determinants of drug metabolism. This gap is particularly critical in the context of polypharmacy, where phenoconversion—driven by drug–drug interactions, comorbidities, and environmental exposures—often leads to discordance between genotype and observed drug response. No standardized, clinically actionable framework currently exists to synergistically assess germline and dynamic metabolic factors for individualized drug safety in complex therapeutic regimens.

Currently, there is no standardized, clinically applicable framework for integrating germline genetics and dynamic metabolic factors for personalized drug safety in complex therapeutic regimens.

Clinical observations in pediatric hematology indicate that dynamic phenotypic variability results from a complex interplay among modifiable factors, including drug-drug interactions, inherited pharmacogenetic variants, disease-related changes, and environmental exposures. This multifactorial dynamics results in significant inter- and intraindividual variability in drug response and toxicity risk, exceeding predictions based on static pharmacogenomic data. The Dynamic-Phenotype Framework addresses this variability by quantifying the real-time impact of these factors, enabling more accurate and individualized risk assessment in the context of complex polypharmacy.

Vincristine is primarily metabolized by CYP3A enzymes, and the CYP3A5 genotype significantly influences drug clearance and risk of neurotoxicity. Individuals with the CYP3A5*3/*3 genotype (non-expressors) have significantly increased vincristine exposure and increased susceptibility to peripheral neuropathy. Coadministration of potent CYP3A inhibitors, such as azole antifungals (e.g., voriconazole, itraconazole, posaconazole), further inhibits vincristine metabolism, exacerbating the risk of toxicity regardless of genotype. This clinically significant phenoconversion illustrates how drug-drug interactions can override genotype-based predictions, highlighting the importance of dynamic, integrative risk assessment in polypharmacy.

A similarly significant genotype-environment interaction is observed in thiopurine therapy. The metabolism of 6-mercaptopurine is primarily regulated by the TPMT and NUDT15 genotypes, which influence the production of cytotoxic thioguanine nucleotides and the risk of severe myelosuppression. Patients with reduced TPMT or NUDT15 activity require significant dose adjustments to prevent toxicity. Concomitant use of allopurinol, a xanthine oxidase inhibitor, significantly disrupts thiopurine metabolism by inhibiting the degradation of mercaptopurine and directing metabolism toward active, cytotoxic metabolites. This interaction amplifies the genotype-dependent variability in metabolite accumulation and toxicity, underscoring the limitations of static genetic risk assessment and the need for dynamic metabolic monitoring.

Our clinically significant, innovation-defining examples highlight the importance of strong genotype-environment interactions, the inadequacy of traditional risk assessment, and emphasize the need for dynamic, multi-omic monitoring in precision pharmacotherapy.

68. LYSOPHOSPHATİDİC ACİD TYPE 5 RECEPTOR (LPAR5) PROTECTS AGAİNST NONSTEROİDAL ANTİ-İNFLAMMATORY DRUG-İNDUCED ENTEROPATHY İN MİCE

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications. However, chronic use of NSAIDs can cause inflammation and ulcers in the small intestine (enteropathy), affecting up to 70% of patients. Enteropathy can not be treated by using antisecretory drugs, so new therapeutic targets must be identified. Lysophosphatidic acid receptor 5 (LPAR5) has high expression level in the small intestine and on immune cells, suggesting that this receptor could serve as a potential target for the treatment of enteropathy. Activation of LPAR5 has been shown to regulate the immune cell recruitment, cytokine release and the expression of tight junction proteins, but its role in regulating NSAID-induced enteropathy is unclear.

Our aim was to assess the role of LPAR5 in NSAID-induced enteropathy by using *Lpar5* knockout (KO) mice.

Enteropathy was induced with indomethacin (INDO, 40 mg/kg) in WT and *Lpar5* KO mice and animals were terminated 24 hour after INDO treatment. The severity of small intestinal inflammation was detected macroscopically, and by measuring the tissue level of different inflammatory markers (*Il6*, *Il10*, *Il1β*, COX-2, MPO, PTX3) and tight junction proteins (Claudin-1 and Occludin) by using Western blot or qPCR. The expression and localisation of *Lpar5* mRNA was determined using RNAscope technique.

INDO induced severe enteropathy, characterised by intestinal shortening, increased level of the measured inflammatory markers and decreased expression of tight junction proteins. Enteropathy was also associated with decreased *Lpar5* expression. Deletion of *Lpar5* itself did not cause intestinal inflammation, but resulted a decrease in the small intestinal level of tight junction proteins, and exacerbated the intestinal inflammation caused by INDO.

Our study shows that LPAR5 plays an important role in regulating the expression of tight junction proteins, and protects against NSAID-induced enteropathy. Therefore, activating LPAR5 could be a potential treatment for NSAID-induced enteropathy.

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69. CHRONIC RESTRAINT STRESS REDUCES INFLAMMATION, BUT NOT PAIN OR ANXIETY IN THE K/BXN SERUM-TRANSFER ARTHRITIS MURINE MODEL

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Growing evidence suggests that chronic stress plays a key role in the development and maintenance of persistent pain, but the exact mechanisms remain unclear. Rheumatoid arthritis is one of the most prevalent chronic autoimmune conditions in which the immune system attacks the joint lining, causing progressive joint damage, pain, swelling, and stiffness, most commonly in the small joints. Therefore, this study aims to investigate the effect of chronic stress on K/BxN serum-induced autoimmune arthritis mouse model combining functional measurements with in vivo imaging technique and histopathological evaluation.

Arthritis was induced by i.p. K/BxN serum (each 150 µl on Day 0 and 3), while non-arthritogenic BxN serum was injected into the control group. Chronic restrain stress (CRS; 6 hours/day for 14 days) was performed on these serum-treated male C57/Bl6 mice (12-15 weeks, 19-25g). Touch sensitivity of the paws was measured by dynamic plantar aesthesiometry, paw oedema with plethysmometer, and arthritis severity was scored using a semiquantitative visual scale. Neutrophil myeloperoxidase (MPO) activity and the vascular leakage were measured with in vivo imaging techniques. Open field test (OFT), light-dark box (LDB) and tail suspension test (TST) were performed after the stress, thymus and adrenal glands weights were collected at the end of the experiment.

Neutrophil myeloperoxidase activity and vascular leakage were reduced [g= -1,6 and g= -1,5] on 8 days of CRS in K/BxN-arthritis group, and there was evident lower arthritis severity between day 7 and 9. In contrast to that, the stress increased mechanical hyperalgesia with a medium effect from day 8 until day 13 [g= -0.6, g= -0.7]. CRS decreased time spent on the open arms, time spent in the light, and immobility time in the K/BxN-arthritis group [g ≈ 0.5 for all three measures], whereas it produced no difference in the (BxN) control group.

In conclusion, CRS could have a positive effect on inflammation in arthritis, but it increases mechanical allodynia and anxiety-like behavior, which is consistent with the current clinical studies. Furthermore, stress reduction serves as a valuable therapeutic adjunct for rheumatoid arthritis; its potential initial proinflammatory effects warrant further investigation.

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70. UNCOUPLING PROTEIN2 PROTECTS AGAINST TRPV1DRIVEN ACUTE NEUROGENIC INFLAMMATORY PAIN IN RATS INDEPENDENTLY OF CAPSAICIN-EVOKED CALCIUM INFLUX

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Background and aims: Uncoupling protein2 (UCP2) is a mitochondrial inner membrane carrier that lowers mitochondrial membrane potential and reduces reactive oxygen species production. Although UCP2 is expressed in primary sensory neurons, its role in Transient Receptor Potential (TRPV1)- mediated acute neurogenic inflammatory pain and associated mitochondrial dysfunction remains unclear. This study investigated UCP2's contribution to resiniferatoxin (RTX)-evoked thermal and mechanical hypersensitivity, mitochondrial membrane potential, and TRPV1 agonist-evoked Ca^{2+} responses in dorsal root ganglion (DRG) neurons using gene-deleted rats.

Methods: Experiments were performed on male and female UCP2 knockout ($UCP2^{-/-}$) and wildtype ($UCP2^{+/+}$) rats (12–15 weeks, 250–300 g). Acute neurogenic inflammation was induced by intraplantar RTX (0.3 µg/mL, 100 µL) into the hind paw; thermonociceptive and mechanonociceptive thresholds were assessed by hot plate and dynamic plantar aesthesiometry. Primary DRG cultures were prepared from neonatal $UCP2^{-/-}$ and $UCP2^{+/+}$ rats and were used for MitoTracker Red CMXRos confocal imaging (mitochondrial membrane potential) and fura-2 AM ratiometric Ca^{2+} imaging following capsaicin application.

Results: Baseline thermonociceptive thresholds were lower in male $UCP2^{+/+}$ rats, and mechanonociceptive thresholds were lower in females in both genotypes. RTX-induced thermal hyperalgesia and mechanical allodynia were significantly enhanced and prolonged in female $UCP2^{-/-}$ rats compared to $UCP2^{+/+}$, with no genotype difference in males. $UCP2^{-/-}$ female DRG neurons exhibited significantly elevated MitoTracker fluorescence, indicating mitochondrial hyperpolarization. Capsaicin-evoked Ca^{2+} transient amplitude and proportion of responsive neurons did not differ between genotypes.

Conclusions: UCP2 deletion selectively enhances RTX-induced acute neurogenic thermal and mechanical hypersensitivity in female, but not male rats and is associated with elevated mitochondrial membrane potential in female DRG neurons. TRPV1-evoked Ca^{2+} responses to capsaicin remained unchanged between genotypes, suggesting that UCP2 modulates acute neurogenic pain through mitochondrial control of sensory neuron excitability rather than direct Ca^{2+} alteration.

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71. THE NOVEL MULTI-TARGET DRUG CANDIDATE (SZV-1287) IMPROVES ENDOTOXIN-INDUCED ACUTE AIRWAY INFLAMMATION IN MICE

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SzV-1287, a novel drug candidate patented by our research group for the treatment of neuropathic pain, has successfully completed Phase Ia clinical trials. It irreversibly inhibits the enzyme copper-containing amine oxidase 3 (AOC3), also expressed in the lung and produces tissue irritants activating Transient Receptor Potential Ankyrin1 (TRPA1), a receptor involved in airway inflammation. It also exerts direct TRPA1 and Vanilloid 1 (TRPV1) antagonist activity and additionally, its metabolite, oxaprosin is a cyclooxygenase inhibitor. We investigated the effect of SzV-1287, comparing with the reference compound selective AOC3 inhibitor LJP-1207 or dexamethasone (DEXA) in a mouse model of acute airway inflammation.

Pneumonitis was induced by intratracheal administration of 0.25 mg/kg endotoxin (lipopolysaccharide: LPS; E. coli O111:B4; in 60 µl phosphate buffer saline (PBS)) in female and male C57BL/6J mice. Treatment groups were randomized into i) PBS+vehicle (Kolliphor ip.), ii) LPS+vehicle, iii) LPS+SzV-1287 (20 mg/kg ip.), iv) LPS+LJP-1207 (20mg/kg ip.), or LPS+DEXA (5 mg/kg i.p.). Respiratory functions were measured by restrained plethysmography in conscious mice 24 h after induction, and lungs were then excised under anaesthesia. Lung inflammation was assessed by gadolinium-based magnetic resonance imaging and by histopathological analysis of hematoxylin-eosin and CD68-immunostained lung sections.

LPS-induced body weight loss was prevented by SzV-1287 and unaltered by LJP-1207. Inflammation resulted in a significantly decreased tidal volume, minute ventilation, functional residual capacity, peak inspiratory, expiratory and tidal-mid expiratory flow, as well as an increased gadolinium uptake which were counteracted by SzV-1287 and DEXA. LPS-evoked perivascular edema was alleviated by both SzV-1287 and LJP-1207, whereas CD68+ macrophage infiltration was reduced only by SzV-1287.

The multi-target drug SzV-1287 improves acute airway inflammatory parameters more effectively than the selective AOC-3 inhibitor reference compound, which can partially be contributed to its additional TRPA1/V1 ion channel and COX inhibition. Thus SzV-1287 represents a promising new therapeutic potential for this indication.

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72. MITOCHONDRIAL UNCOUPLING PROTEIN 2 REGULATES INFLAMMATORY CARDIO-PULMONARY DYSFUNCTIONS: IN VIVO RAT STUDIES

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Uncoupling protein 2 (UCP2) is expressed in the inner mitochondrial membrane of lymphocytes and macrophages as well as other tissues including the lung. Besides regulating ATP synthesis, it is also involved in reactive oxygen species elimination. Recently increasing attention has been focused on its protective role in cardiovascular diseases. However, little and contradictory knowledge is available on the role of UCP2 in airway inflammation. Our aim is to elucidate UCP2 involvement in i) endotoxin (lipopolysaccharide: LPS)-induced acute pneumonitis and ii) chronic cigarette smoke exposure (CSE)-induced irritative airway inflammation models using UCP2 gene-deficient ($Ucp2^{-/-}$) and wildtype ($UCP2^{+/+}$) rats.

$Ucp2^{+/+}$ and $Ucp2^{-/-}$ rats were randomized into 2 subgroups receiving LPS or PBS (controls) intratracheally. 24 hours after induction airway function was measured by plethysmography. Lungs were exised, weighed, and fixed in formalin for histopathologic evaluation. In the second series of experiment $Ucp2^{+/+}$ and $Ucp2^{-/-}$ rats were exposed to 6 months of CSE, their counterparts served as intact control animals. Pulmonary and cardiac functions as well as echocardiography were measured at the beginning of the experiment and after 2, 4 and 6 months of CSE. Morphological analysis was performed by in vivo microCT. At the end of the protocol animals were terminated and lungs were harvested for histopathologic evaluation.

LPS induced a significant body weight loss and lung edema 24 h after induction in both $Ucp2^{+/+}$ and $Ucp2^{-/-}$ rats. LPS increased breathing frequency, peak inspiratory and expiratory flow, as well as mid-expiratory flow and decreased tidal volume, inspiratory and expiratory time. Interestingly, LPS-induced airway functional alterations were less severe in male, but more aggravated in female $Ucp2^{-/-}$ rats. CSE induced transient changes in respiratory parameters; frequency and peak expiratory flow increased, while tidal volume and inspiratory and expiratory time decreased in $Ucp2^{-/-}$, but not in wildtype rats after 4 months of CSE. Ejection fraction and tricuspid annular plane systolic excursion decrease -characteristic to left and right ventricular dysfunction- developed in intact Ucp2-/- rats already after 2 months, which deteriorated upon 6 months of CSE in both wildtype and Ucp2-/- animals. Interestingly, low attenuation area/total lung volume characteristic to emphysema was more pronounced in CSE-exposed Ucp2+/+.

The LPS-induced airway alterations in $Ucp2^{-/-}$ rats are sex-dependent having UCP2 deletion an aggravating effect in females. Lack of UCP2 is associated with deteriorated right ventriucular function, which is aggravated by CSE.

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73. TOLL-LIKE RECEPTOR 5 (TLR5) PROTECTS AGAINST NON-STEROIDAL ANTI-INFLAMMATORY DRUG-INDUCED ENTEROPATHY IN MICE

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Non-steroidal anti-inflammatory drugs (NSAIDs) are among the world's most widely used medications to alleviate pain, inflammation and fever. However, chronic use of these drugs has been associated with enteropathy, which refers to damage occuring predominantly in the distal small intestine, affecting up to 70% of patients. Currently, no proven effective treatment or prevention is known for this condition. In contrast to NSAID-induced gastropathy, enteropathy can not be treated by using antisecretory drugs, hence there is urgent need of identifying new therapeutic targets. While previous studies have emphasised the role of Toll-like receptor 4 (TLR4) and Toll-like receptor 2 (TLR2) in enteropathy, the functional significance of TLR5, a receptor for bacterial flagellin maintaining intestinal homeostasis, remains elusive.

Accordingly, the objective of our study was the investigation of the role of TLR5 in NSAID-induced enteropathy.

Enteropathy was induced with a single high dose of indomethacin (IND, 30 mg/kg) in C57BL/6 mice. TLR5 signaling was blocked using TH1020, a potent and selective TLR5 antagonist (10 µg/mouse). 24 hour after IND treatment, animals were terminated and the severity of enteropathy was assessed. Intestinal TLR5 and flagellin levels were determined using qPCR and Western blot, respectively. TLR5 levels were also determined in naproxen-treated rats.

On the basis of our results, NSAID enteropathy was associated with decreased TLR5 expression, and elevated ileal levels of flagellin. Inhibition of TLR5 by TH1020 itself did not resulted in intestinal inflammation, however it exacerbated the intestinal inflammation caused by IND treatment.

In conclusion, our results suggest that TLR5 receptor has a pivotal regulatory role in NSAID-induced enteropathy as a protective factor, hence the activation of TLR5 might be a potential therapeutic target in the treatment of NSAID-induced enteropathy.

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74. ACUTE SYSTEMIC INFLAMMATION INDUCES ASTROGLIOSIS IN THE BRAIN: IN VIVO OPTICAL IMAGING USING GFAP-LUC TRANSGENIC MICE

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Glial fibrillary acidic protein (GFAP) is predominantly expressed in astrocytes in the brain, and it is upregulated in different inflammation-related neuropathologies such as chronic pain. Studying in vivo astrocyte activation in animal models of these diseases in a time-dependent manner is important to determine their role in the pathophysiology. In this study, brain astrocyte activation was investigated in vivo in tumor necrosis factor alpha (TNF-a)- and lipopolysaccharide (LPS)-induced systemic and carrageenan- and resiniferatoxin (RTX)-induced local inflammation models, as well as in complete Freund's adjuvant (CFA)-induced chronic joint inflammation model using GFAP-luciferase (luc) transgenic mice.

TNF-a (63 and 250 ug/kg) and LPS (0.5, 1 and 2 mg/kg) were injected intraperitoneally (i.p.), carrageenan (20 ul, 30 mg/ml), RTX (20 ul, 0.1 ug/ml) and CFA (20 ul, 1 mg/ml) subcutaneously into the right hind paw in 8-12-week-old male and female GFAP-luc transgenic mice. CFA was also injected into the tail root in the same volume on days 0 and 1 to boost the systemic inflammatory response. Mechanonociception was determined by aesthesiometry thermonociception by increasing temperature hot plate, paw edema by plethysmometry, GFAP expression in the brain by luciferase substrate CycLuc1 (cyclic alkylamino-luciferin, 7.5 mg/kg, i.p.)-derived in vivo bioluminescent imaging.

250 ug/kg TNF-a, as well as all LPS doses induced significant bioluminescent signal increase in the brain at 24 h compared to their self-control baseline values and the vehicle-injected controls indicating strong GFAP upregulation without dose-dependency. Carrageenan and CFA induced significant mechanical hypersensitivity and edema of the hind paw at 6 and 24 h and between days 2 and 21, respectively. RTX induced significant thermal hypersensitivity at 10 min and mechanical hypersensitivity of the hind paw at 90 min compared with vehicle-injected controls. However, pain-related behavior was not associated with in vivo GFAP upregulation in the brain at any of the time points examined.

TNF-a- and LPS-induced systemic inflammation mouse models are appropriate for self-control follow-up study of astroglia activation in the brain as an important neuroinflammatory component of systemic inflammation. However, localized peripheral inflammation (acute/chronic neurogenic/non-neurogenic) does not evoke in vivo detectable astrocyte activation with this technique. This platform might be a useful tool to examine the effects of potential astrocyte inhibitors in the future.

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75. T AND B LYMPHOCYTES CONTRIBUTE TO PAIN MECHANISMS TRIGGERED BY CHRONIC IMMOBILIZATION IN MALE MICE

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Chronic stress induces and aggravates many chronic pain conditions, such as widespread musculoskeletal pain in fibromyalgia, but the pathophysiological mechanisms are not fully understood. Autoimmunity and neuroimmune interactions have been considered as primary causes, where T and B lymphocytes play key roles. Therefore, the involvement of these cells in chronic stress-induced pain, anxiety and depression-like behaviour was investigated using lymphocyte-deficient mice.

Chronic restrain stress (CRS; 6 hours/day for 14 days) was performed on recombination activator gene deficient ($Rag1^{-/-}$) mice on both sexes compared to their C57/Bl6 wild-type (WT) controls (10-12 weeks old, 18-28 g). Touch sensitivity of the paws was measured by dynamic plantar esthesiometry, cold sensitivity by detecting withdrawal latency from icy water. Open field test (OFT), light-dark box (LDB) and tail suspension test (TST) were performed after the stress, thymus and adrenal glands weights were detected at the end of the experiment.

Without stress, no difference was found in $Rag1^{-/-}$ mice compared to their WTs in all examined parameters. CRS caused 60-70% and 40-50% decrease in mechanical and cold sensitivity in WT males and females, but his mechanical hyperalgesia was significantly lower in male $Rag1^{-/-}$ animals. Stress decreased the time spent in the middle zone of the OFT and the light of the LDB and time spent immobility in TST with no effect of gene deletion. The thymus weights of non-stressed Rag1-/- mice were significantly lower than those of WTs, and stress further reduced thymus weight in all groups.

These results suggests, that T and B lymphocytes might have a crucial role in chronic immobilization-induced mechanical pain responses in male mice.

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76. ANALYSIS OF PACAP AND OTHER BIOACTIVE FACTORS IN THE BLOOD PLASMA OF WOMEN FOLLOWING MISCARRIAGE

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The pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic neuropeptide with anti-inflammatory, anti-apoptotic and antioxidant properties. The neuropeptide plays an important role in regulating the female reproductive system. Our previous studies examined the changes of PACAP level during pregnancy and lactation.

The aim of this study was to compare plasma PACAP-38 levels in women undergoing spontaneous miscarriage (n=28) compared to control women having elective termination of pregnancy (n=44), and to analyse various bioactive factors (IL-1α,IL-1β,IL-1RA,IL-2,IL-6,IL-10,IL-17α,MCP-1,FGF2,G-CSF,IP-10,PDGF,VEGF). These factors play key roles in implantation, placentation, and angiogenesis; therefore, we examined their changes in relation to PACAP-38 levels.

Plasma samples from the HUN-REN RCNS Perinatal Biobank were analysed using sandwich ELISA for PACAP-38 quantification, while the multiplex Luminex assay was applied to measure cytokines, chemokines, and growth factors. Statistical analyses focused on group comparisons and correlation patterns between PACAP-38 and the measured bioactive factors.

Compared with gestational age-matched control pregnancies, plasma samples from women experiencing spontaneous miscarriage showed significantly elevated MCP-1 levels, and significantly decreased IL-8, FGF-2, and PACAP-38 levels. Differences in PACAP-38 were particularly pronounced in samples collected between gestational weeks 9-13. In the spontaneous miscarriage group, plasma PACAP-38 levels showed a strong positive correlation with VEGF and a strong negative correlation with IL-1β.

Our findings highlight altered PACAP-38 and cytokine profiles in spontaneous miscarriage. Future work will focus on elucidating the role of PACAP-38 in various reproductive disorders and pregnancy pathologies, such as preeclampsia and intrauterine growth restriction.

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77. THE PROTECTİVE EFFECTS OF METHYLENE BLUE ON PMCA İN ALCOHOL-RELATED PANCREATİC AND HEPATİC İNJURY

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Introduction: Alcohol-related diseases can damage multiple organs, particularly the pancreas and the liver. Alcohol-induced acute pancreatitis (AP) and alcoholic hepatitis (AH) are major complications of excessive alcohol consumption. Ethanol and fatty acids impair the expression and function of the cystic fibrosis transmembrane conductance regulator (CFTR), an ion channel essential for epithelial homeostasis in both organs. This dysfunction also destabilizes the CFTR–plasma membrane Ca^2 -ATPase (PMCA)-calmodulin complex, resulting in impaired intracellular Ca^2 regulation and toxic Ca^2 overload that contributes to disease progression. Pharmacological strategies that restore epithelial Ca^2 handling may therefore offer therapeutic benefit. Methylene blue (MB), an antioxidant influencing intracellular Ca^2 regulation, may therefore support PMCA-mediated protection in alcohol-related pancreatic and liver injury.

Aim: We aim to investigate the potential beneficial effects of MB on PMCA and to examine its protective role in models of AP and AH.

Methods: MB effects on PMCA and CFTR were studied in human and mouse pancreatic ductal and mouse liver organoids (hPO, mPO, mLO). In vitro injury was induced by pre-treating organoids with 100 mM ethanol (EtOH) and 200 uM palmitic acid (PA) for 24 hours. Organoids were analyzed using microfluorimetry, immunofluorescence (IF), qPCR, and mitochondrial potential assays (TMRM, JC-10), while cytotoxicity was assessed with a 3D viability assay. In vivo AP was induced by EtOH and palmitoleic acid, while AH was induced by a Lieber–DeCarli EtOH diet with binge alcohol. Disease severity was evaluated by histology, Oil Red O staining, serum analysis, and ketone body measurement.

Results: MB significantly enhanced PMCA-mediated Ca^2 extrusion in hPOs, mPOs and mLOs after EtOH-PA exposure, accompanied by stronger apical PMCA4 localization. Mitochondrial membrane potential measurements confirmed MB's protective role in maintaining mitochondrial function. Gene expression analysis showed no significant changes in CFTR, PMCA4, or ORAI1 expression between EtOH-PA and MB treated groups. In vivo, MB reduced pancreatic injury in acute pancreatitis, lowering serum amylase and histological scores. In an alcoholic hepatitis model, MB decreased serum ALT/AST levels and hepatic lipid accumulation. Together, these results indicate that MB protects against ethanol- and fatty acid-induced pancreatic and liver injury through regulation of PMCA activity and stabilization of mitochondrial function.

Conclusion: Our findings demonstrate that MB protects pancreatic and liver tissues from ethanol- and fatty acid-induced injury by enhancing PMCA activity, reinforcing apical PMCA expression, and preserving mitochondrial function. These results highlight MB as a promising translational candidate for mitigating alcohol-induced acute pancreatitis and alcoholic hepatitis, although further studies are required to fully establish its therapeutic potential.

78. İNVESTİGATİON OF THE ROLE OF ENDOCANNABİNOİDS İN INDOMETHACIN-İNDUCED ENTEROPATHY İN MİCE

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Chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) frequently leads to damage of the distal small intestine, for which no effective therapy is currently available. The endocannabinoid system (ECS) plays a crucial role in maintaining gastrointestinal homeostasis. In our previous studies, increasing the levels of the two main endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), attenuated NSAID-induced gastric injury. However, inhibition of AEA metabolism with URB597 did not reduce the severity of NSAID-induced enteropathy.

The aim of this study was to determine whether inhibition of monoacylglycerol lipase (MAGL)—a key enzyme involved in 2-AG metabolism—or direct administration of AEA could alleviate indomethacin (IND)-induced enteropathy in mice.

C57BL/6 mice were treated with a single high dose of IND (30 mg/kg p.o.) or its vehicle (1% hydroxyethylcellulose). In the first experiment, animals received JZL184 (4 or 16 mg/kg p.o.) or its vehicle, while in the second experiment mice were treated with AEA (1 or 10 mg/kg p.o.), administered three times. Twenty-four hours after IND treatment, animals were sacrificed, and small intestinal length was measured. Tissue levels of inflammatory mediators and tight junction (TJ) proteins were determined, and AEA and 2-AG concentrations were quantified by LC–MS.

JZL184 treatment significantly increased 2-AG levels in the small intestine, as expected, but did not affect the severity of enteropathy. In contrast, AEA treatment significantly reduced the tissue levels of inflammatory mediators (COX-2, MPO, IL-1β) in enteropathic animals.

Our findings suggest that inhibition of endocannabinoid metabolism alone is not sufficient to alleviate NSAID-induced enteropathy. However, direct administration of AEA significantly reduces intestinal inflammation and may represent a promising therapeutic approach for the treatment of enteropathy. Further studies are needed to clarify the mechanisms underlying the lack of efficacy of metabolism inhibitors.

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79. MULTIMODAL INSIGHT INTO THE PATHOMECHANISM OF FIBROMYALGIA: NEURODEVELOPMENTAL, NEUROINFLAMMATORY, AND PAIN MATRIX CONNECTIVITY ALTERATIONS

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Fibromyalgia (FM), the most prevalent chronic primary pain condition affects 2-3% of individuals worldwide. Its pathophysiology remains elusive due to patient heterogeneity and lacking objective biomarkers. Recent studies highlight central sensitization and psychological distress, while multi-omics profiling implicates inflammation; however, their interplay in sustaining chronic symptoms is poorly defined. This multimodal study integrated baseline and acute pain-evoked functional Magnetic Resonance Imaging (fMRI), psychological profiling, and peripheral blood mononuclear cell (PBMC) transcriptomics to elucidate central, psychological and peripheral mechanisms in FM.

Adults meeting the 2016 American College of Rheumatology FM classification criteria (n=33) and matched healthy controls (HC, n=29) were recruited from 3 centres in Hungary (Budapest, Hévíz, Pécs). Participants completed peripheral blood mononuclear cell (PBMC) sampling; a subset underwent fMRI (resting-state, heat-pain stimulation, post-stim. resting-state), and psychological assessment via clinical interviews, Mérei-based Rorschach assessment and validated self-report questionnaires (e.g., FIQR, STAI, BDI). PBMC RNA underwent next-generation sequencing for transcriptomic analysis. Differential expression and pathway analyses were performed using Ingenuity Pathway Analysis (IPA) and pathway databases (KEGG, GO, Reactome). Statistical analyses used R and GraphPad Prism. This study was approved by the Hungarian National Ethics Committee (ETT-TUKEB, BMEÜ/3788-1/2022/EKU)

fMRI showed altered baseline integration of sensory areas with default-mode network and pain regions (thalamus, amygdala) in FM. Acute pain enhanced DMN-frontoparietal network connectivity, suggesting neurodevelopmental changes. FM patients had consistently higher symptom burden, functional impairment and reduced well-being. Somatic focus and inflexibility were observed, with constricted processing and impaired cognitive-affective integration, aligning with the fMRI results. Several inflammation- and neurodevelopment-related signaling pathways (IL-12 signaling in macrophages, neutrophil degranulation, netrin signaling, synaptic long term potentiation) were altered in FM patients' PBMCs.

These findings demonstrate altered central pain processing, interoceptive dominance with reduced epistemic openness in FM, and omics analysis suggests dysregulated immune-neural crosstalk, hyperactive innate immune mechanisms, and impaired synaptic plasticity. FM emerges as a biologically sustained, psychologically modulated condition, warranting development of novel therapeutic guidelines and identification of diagnostic/prognostic biomarkers to improve future treatment regimens.

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80. COMPARISON OF PRECLINICAL MODELS OF TRIGEMINAL SENSITIZATION

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The mechanisms behind primary headache disorders are still not fully understood; however, activation of the trigeminovascular system and neurogenic inflammation are key factors in pain development. Given the lack of optimal translational models, it is necessary to characterize and compare different in vivo paradigms to identify key mediators and novel therapeutic targets. Inflammatory, mediator-based, and neuropathic orofacial or periorbital pain models are widely used as surrogate models to investigate pain mechanisms related to primary headaches. Our goal was to compare four in vivo models of trigeminal sensitization based on different mechanisms.

Experiments were performed on adult male Sprague-Dawley rats using two inflammatory models (subcutaneous injection of Complete Freund's Adjuvant [CFA] into the whisker pad; repeated epidural infusions of an "inflammatory soup" containing 2 mM histamine, bradykinin, serotonin, and 0.2 mM prostaglandin E2 [IS]), one mediator-based model (repeated intraperitoneal administration of glyceryl trinitrate [GTN]), and one neuropathic model (partial infraorbital nerve ligation [pIONL]). Mechanical pain thresholds were measured using von Frey filaments of logarithmically increasing force.

The CFA model showed a high success rate (~76%) and produced pronounced and stable allodynia (18.30 g → ~6 g). The IS model had a lower success rate (~45%) and caused a more moderate decrease in mechanical pain threshold (18.30 → ~11 g). In the GTN model, 67% of animals exhibited reduced mechanical thresholds, with average values dropping from 18.30 g to ~12 g. The pIONL model demonstrated a 53% success rate and stable sensitization (18.30 → ~10 g).

All four models are appropriate for testing trigeminovascular activation-induced allodynia, making each suitable for studying different pathophysiological mechanisms. However, the extent and stability of allodynia vary significantly across models; therefore, when testing potential drug candidates, the limitations and underlying mechanisms of each model should be considered while interpreting results.

81. INVESTIGATION OF FUNCTIONAL EXPRESSION OF TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 AND VANILLOID 1 ION CHANNELS IN ENDOMETRIOSIS-DERIVED EPITHELIAL AND ENDOMETRIAL STROMAL CELL LINES

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Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) receptors are non-selective cation channels activated by inflammatory mediators, and have been implicated in several pathologies. Our research group has previously demonstrated the mRNA and protein expression of these ion channels in deep infiltrating endometriosis; as well as their correlation with pain severity, however, their exact role remains unclear. Due to the lack of reliable animal models, two- and three-dimensional primary human endometriosis-derived cell cultures serve as translational models to investigate the pathophysiology of endometriosis.

In this study, we aimed to investigate the mRNA expression and functional activity of TRPA1 and TRPV1 in human immortalized 12Z endometriosis epithelial and HESC endometrial stromal cell lines.

mRNA expressions were analyzed using RNAscope *in situ* hybridization and quantitative PCR (qPCR). Receptor functionality was assessed by measuring Fura-2 AM-based Ca^{2+} influx in response to the TRPV1 agonist capsaicin (CAPS: 300 and 1000 nM) and TRPA1 activator allyl-isothiocyanate (AITC: 50 and 200 µM) in monocultures and in 1:1 co-culture of 12Z and HESC cells. TRPA1-and TRPV1-specific fluorescent signals were detected in both 12Z and HESC using RNAscope, which was confirmed by qPCR. The TRPA1 agonist AITC (200 µM) induced a significant Ca^{2+} influx in both monocultures and co-cultures. This response was effectively inhibited by the selective TRPA1 antagonist A-967079 (1 µM) in monoculture experiments. The TRPV1 agonist CAPS evoked a significant Ca^{2+} influx in 12Z cells at of 1000 nM concentration, whereas in HESC, 300 nM CAPS induced influx response. In contrast, no functional TRPV1-mediated Ca^{2+} signal was detected in the 12Z: HESC co-culture.

These findings demonstrate, for the first time, the functional expression of TRPA1 and TRPV1 channels in both 12Z and HESC cell lines in mono-and co-cultures.

Further studies are planned to elucidate the potential estrogen-mediated sensitization of the TRPV1 in these cell models, as well as to investigate the effects of receptor agonists on cell viability and proliferation.

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82. INHİBİTİON OF THE FRACTALKINE CX3CR1 RECEPTOR PREVENTS HYPERALGESİA AND NEUROİNFLAMMATİON İN PASSİVE TRANSFER-TRAUMA MOUSE MODEL OF COMPLEX REGİONAL PAİN SYNDROME (CRPS)

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Complex Regional Pain Syndrome (CRPS) is a persistent, severe, chronic pain condition that develops following a minor limb injury (sprain, fracture or dislocation). The most characteristic symptoms are hyperalgesia, edema and autonomic dysfunction. Pathophysiology is unknown, but based on our research, autoimmunity, complex sensory-immune-vascular interactions, and neuroinflammation play a role. Since the current therapy for CRPS is unsatisfactory, there is a need to identify new drug targets. In our previously developed CRPS passive transfer-trauma mouse model, we investigated the role of the fractalkine inflammatory chemokine receptor 1 (CX3CR1) expressed on microglia and macrophages, which may be a new therapeutic pathway in the treatment of CRPS.

Since CRPS is more common in women, we used female C57BL/6J mice in our experiments and treated them daily intraperitoneally (*i.p.)* with purified IgG fractions from CRPS patients or healthy volunteers. On day 0, we performed a plantar skin-muscle incision to induce a minor injury. The role of the fractalkine receptor was examined in CX3CR1 gene-deficient and wild-type mice treated with the receptor antagonist AZD8797 (80 µg/kg *i.p.*/day).

The paw mechanonociceptive threshold was measured by dynamic plantar aesthesiometry and volume by plethysmometry. The density of microglia and astrocytes in pain-related regions of the central nervous system (somatosensory cortex, periaqueductal gray matter, and spinal cord dorsal horn) was analyzed by ionized calcium-binding adapter molecule 1 (Iba1) and glial fibrillary acidic protein (GFAP) immunohistochemistry.

The plantar incision resulted in 45–50% mechanical hyperalgesia, which persisted throughout the 7-day experiment in the CRPS IgG-treated group. CX3CR1 gene deficiency and AZD8797 antagonist treatment significantly reduced the CRPS IgG-induced mechanical hyperalgesia. Microglia immunoreactivity was significantly reduced in the somatosensory cortex and the dorsal horn of the spinal cord by CX3CR1 gene knockout and antagonism (AZD8797), and in the periaqueductal gray matter by CX3CR1 gene knockout. Astrocyte activation was significantly reduced in the somatosensory cortex by the antagonist treatment.

Specific regulation of CX3CR1 may play a role in the development of chronic pain and neuroinflammation associated with CRPS. Inhibition of CX3CR1 may provide a basis for understanding the molecular mechanisms of CRPS and may represent new therapeutic options in the treatment of CRPS.

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83. HIGH-RESOLUTION SPATIAL TRANSCRIPTOMICS REVEALS SEGMENT-SPECIFIC SPINAL CORD ALTERATIONS IN A MOUSE MODEL OF COMPLEX REGIONAL PAIN SYNDROME

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Complex Regional Pain Syndrome (CRPS) is a chronic pain condition characterized by persistent unilateral limb pain, swelling, and autonomic dysfunction. While immune mechanisms, particularly autoantibody-mediated processes are increasingly implicated, molecular changes within the spinal cord remain poorly defined. This study aimed to characterize spatially resolved transcriptional alterations in the spinal cord associated with CRPS.

We investigated spinal cord transcriptional alterations using a passive-transfer trauma (PTT) mouse model combined with high-resolution spatial transcriptomics. Female C57BL/6 mice received daily intraperitoneal injections of IgG from CRPS patients together with a plantar skin–muscle incision, while controls received IgG from healthy donors. Mechanical sensitivity was assessed over five days. Lumbar spinal cord segments (L3–L5) were analyzed using the Xenium HD (10x Genomics) platform. Data were processed in R (v4.4.1) with Seurat (v5.3.0). Differential expression was assessed using a two-sided Wilcoxon test with Benjamini–Hochberg correction (FDR < 0.10, $|\log_2 FC| \geq 0.8$) followed by functional annotation and Ingenuity Pathway Analysis (IPA).

CRPS IgG-treated mice developed significantly greater mechanical hyperalgesia within 24 hours post-injury. Spatial transcriptomics revealed cell type–specific changes, including downregulation of Unc13c (astrocytes, oligodendrocytes), Hoxd11 (interneurons), and Mecom (endothelial cells). Injured versus contralateral regions showed upregulation of Rmst, Lamp5, Hpcal1, Cacna2d2, Kcnh5, and Dner, and downregulation of Opalin, Sox10, and Gjc3. Pathway analysis highlighted neuroinflammatory signaling and altered Sox10-associated myelination, indicating region-specific spinal cord remodeling in CRPS.

These findings provide novel spatially resolved insights into spinal cord–specific mechanisms underlying CRPS and highlight potential molecular targets for therapeutic intervention.

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84. EXTRACELLULAR MATRIX, VASCULAR AND CELLULAR INFLAMMATION AND PAIN-RELATED GENE EXPRESSION CHANGES IN DEEP INFILTRATING RECTOSIGMOID ENDOMETRIOSIS

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Rectosigmoid deep infiltrating endometriosis (DIE) is a severe, chronic, and painful inflammatory disorder, responsible for up to 90% of intestinal endometriosis cases. Although complex neurovascular and immune interactions are likely to contribute to disease development, its exact pathophysiological mechanisms remain enigmatic. Since current treatment options provide limited efficacy, DIE represents an unmet medical need. Thus, identifying key molecular mediators and signaling pathways is crucial for the development of novel therapeutic strategies.

In this study , an unbiased transcriptomic analysis of DIE lesions (n=9) was performed using next-generation mRNA sequencing, and the results were compared to normal recto-sigmoid bowel wall (n=13) and healthy endometrium (n=9). Bioinformatic analysis identified differentially expressed (DE) genes using the KEGG, GO and Reactome database, and the ingenuity pathway analysis (IPA) software identified disease-related pathways and networks. Our results were compared to the gene expression microarray data of the publicly accessible Turku Endomet database.

A total of 31 DE genes were identified in our DIE sample cohort relative to both healthy endometrium and normal bowel wall samples. Functional annotation classified these genes into three main cathegories: extracellular matrix (ECM)-related genes (n= 8), growth factor and inflammation-associated genes (n=8), and intracellular signaling pathway components (n=14). Among these, the cartilage oligomeric matrix protein (COMP), immunoglobulin-like and fibronectin III. domain 1 (IGFN1) and cartilage intermediate layer protein 2 (CILP2) showed the highest fold-changes. Notably, IGFN1 and CILP2 have not been previously implicated in endometriosis. IPA analysis revealed that these DE genes are primarily associated with cell migration, cell proliferation, collagen trimerization, ECM organization and scar tissue formation. Comparisons with the Turku Endomet dataset revealed similar expression patterns, further supporting our findings.

Our study provides the first integrated transcriptomic view of differentially expressed canonical pathways, biological functions and gene networks in DIE. These findings highlight the role of local inflammation, vascularization and ECM organization, as well as cell growth and migration in ectopic endometrial lesions. Our data contribute to a better understanding of the molecular mechanisms related to DIE progression and may facilitate the identification of novel therapeutic targets.

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85. INVESTIGATING THE ROLE OF ANGIOTENSIN II RECEPTOR TYPE 1 IN OPIOID ANTINOCICEPTIVE TOLERANCE

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Introduction: Opioid analgesics are the cornerstones in the management of moderate to severe pain, however their effectiveness is hampered by side effects such as analgesic tolerance. Angiotensin II receptor type 1 (AT1) blockers (ARBs), particularly telmisartan, have been shown to alleviate chronic pain and delay the development of morphine tolerance. However, the effect of ARBs on opioid antinociceptive tolerance development, the localisation of AT1 in the spinal cord, and possible co-localisation of AT1 and μ-opioid receptor (MOR) remains to be fully elucidated.

Aim: To determine the extent to which ARB treatment influences morphine antinociceptive tolerance; explore the localisation of AT1 and its possible co-localisation with MOR in the spinal cord.

Methods: Male Wistar rats (180-250 g) were treated with different combination of subcutaneous morphine (31.08 μmol/kg), oral telmisartan (20 μmol/kg) or their respective vehicles for 9 days. On day 10, all animals were treated with different doses of morphine to construct dose-response curves (DRC) of morphine. The rat tail-flick assay was used to test the antinociceptive effect. After plotting the DRCs, animals were sacrificed, and their spinal cords were removed for further analysis. In the spinal dorsal horn the fluorescent intensity of MOR was measured. To investigate the colocalization of AT1 and MOR, naïve rats were used. Histological sections were analysed using confocal microscopy.

Results: Chronic morphine administration for 9 days resulted in a rightward shift and a slight flattening in its DRC, indicating the development of morphine antinociceptive tolerance. After the chronic co-administration of morphine and telmisartan, morphine retained its maximal effect in the investigated dose-range and a leftward shift was observed in the DRC. The fluorescent intensity of MOR in the spinal dorsal horn did not differ between treatment groups. The AT1 was localised in the outer laminae of the spinal dorsal horn and double -positive MOR+/AT1+ structures were also detected.

Conclusion: ARB treatment preserved the maximal effect of morphine and reduced the rightward shift of morphine's DRC in an opioid tolerance model *in vivo*. Spinal MOR fluorescent intensity was unaffected by chronic treatment with morphine or ARB. AT1 localises mainly in the dorsal horn of the spinal cord in the proximity of MOR.

86. CAUSES, PATHOPHYSIOLOGY AND TREATMENT OF CHRONIC FATIGUE SYNDROME

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Chronic fatigue syndrome (CFS) is a debilitating illness that occurs in up to 1% of infections by certain viruses, last for 3-6 months after which it gives place to chronic pain. In a mouse model of the condition in which polyinosinic:polycytidylic acid (P(I:C)) was used as a trigger, we studied animals challenged with this immunostimulant or vehicle controls, for 3 weeks. We evaluated the function of the immune system (IS), central nervous system (CNS) and energy homeostasis (EH) as the literature suggests that these are the key players in the condition.

We judged functioning of the IS from repeated blood corticosterone measurements and from the terminal weights of immune organs; the functioning of the CNS from the concentrations of monoamine neurotransmitters, β-endorphin and Brain Derived Neurotropic Factor (BDNF) measured in the dorsal and ventral hippocampus and in trunk plasma terminally; and the functioning of the EH based on daily body weight, physical activity, energy consumption measurements and terminal concentrations of ATP and its metabolites in plasma and tissue.

We calculated pairwise correlations between the above parameters and performed principal component analysis (PCA) on the physical activity values of our mice and human and canine Iditarod participants.

The first important observation that we made is the presence of large amplitude periodic changes in energy intake and usage in healthy animals, with two maxima per week. What gives the importance of these is that the measured effects can be explained if and only if these are considered. Namely, P(I:C)-injected animals *move* significantly *less* and do not consume significantly different amount of sugar water compared to controls, and yet, their *body weights* significantly *decrease* following immune stimulation. The frequency of oscillation of *sucrose intake* in immune-challenged animals increases significantly, and this is the only significant difference when comparing the frequency values between immune challenged and control groups or before and after injection.

Additionally, according to PCA results, the murine body's daily sampled movement as a function of time can be well modelled as a linear time-invariant discrete-time control system.

Based on the strong and significant correlations between the measured parameters (for example, between physical activity and plasma BDNF) and on changes over time, we conclude that the source of metabolic oscillations is neurogenesis in the dentate gyrus, which is part of two overlapping control loops, one for energy intake and one for energy usage, that viruses that typically cause CFS interfere with neurogenesis through decreasing Protein Kinase R activity, leading to decoupling of the two central loops, that the transition from CFS to chronic pain appears when the cycles of β-endorphin production also get affected and that there is a natural restoring mechanism for CFS originating from the microbiota, through the microbiota-gut-brain axis.

87. USAGE OF INTERLEUKINE-1 SIGNALING TO ALLEVIATE STRESS-INDUCED PAIN SENSITIZATION IN MICE

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Chronic stress is a key contributor to the development and persistence of chronic pain conditions such as fibromyalgia (FM), for which effective pharmacological treatments remain limited. Neuroinflammatory mechanisms, such as proinflammatory cytokines like interleukin-1 (IL-1), have been implicated in stress-induced pain sensitization. In the present study, we investigated the direct IL-1 receptor blockade using the recombinant IL-1 receptor antagonist anakinra and the MCC90, a pharmacological inhibitor of the NLRP3 inflammasome responsible for IL-1 release. We tested the potential analgesic effect of anakinra and MCC950 in two different stress-induced FM mouse models.

Chronic restraint stress (CRS) and intermittent cold stress (ICS) were applied. Mechanical pain sensitivity and cold tolerance of the hind paw were measured continuously. In the CRS model, mechanical thresholds were measured weekly, whereas in the ICS model, measurements were performed following the stress protocol. Anakinra (10 mg/kg), MCC950 (10 mg/kg), or vehicle administered daily intraperitoneally from the beginning of each stress protocol. To exclude nonspecific behavioral effects, the potential sedative properties of MCC950 were evaluated using the open field test (OFT).

CRS induced a progressive mechanical hyperalgesia, reaching a maximum threshold reduction of approximately 15–20% by the second week of stress. Meanwhile, ICS led to mechanical hyperalgesia up to that extent on the second day after the stress protocol. Anakinra could attenuate CRS-induced, but not ICS-induced hyperalgesia. MCC950-treated mice did not develop mechanical hyperalgesia in either stress model. In contrast, stress exposure induced a significant cold hyperalgesia of approximately 80%, which emerged during the first week of CRS and immediately after ICS protocol, which was not affected by anakinra or MCC950 administration. Neither Anakinra nor MCC950 exhibited a sedative effect in the OFT.

These findings demonstrate that NLRP3 inflammasome-IL-1 inhibition selectively attenuates stress-induced mechanical sensitization across multiple FM rodent models, supporting NLRP3-IL-1 signaling as a potential therapeutic candidate for the treatment of FM-like pain.

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88. MIMICKING THE SPINAL ENDOGENOUS ADRENERGIC SYSTEM AND MODULATING VOLTAGE-GATED CALCIUM CHANNELS IS A PROMISING STRATEGY TO HALT NEUROPATHIC PAIN

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Introduction: The low efficacy of current medications for neuropathic pain (NP) necessitates the development of novel therapeutics, repurposing of existing drugs, and identification of effective drug combinations to improve therapeutic outcomes. The adrenergic system is among the key modulators of spinal nociception. Phenylephrine (PE), is considered a canonical α_1-adrenoceptor (α_1-AR) agonist. However, our previous data indicate that PE facilitates the release of cytosolic noradrenaline (NA), which would be relevant to the spinal pain modulation. Pregabalin (PGB), an anticonvulsant that modulates voltage-gated calcium channels (VGCCs) containing $\alpha_2\delta$ subunit, is among the first-line treatment approaches for NP. Indeed, it has a slow onset of action and low efficacy at therapeutic doses, as reflected by the number needed to treat. Furthermore, dose escalation is limited by dose-dependent adverse effects. Therefore, investigating PGB-based drug combinations to achieve effective pain relief while minimizing unwanted side effects is of clinical value. Aims: To investigate the antiallodynic potential of intrathecally and orally administered PE, alone and in combination with PGB, in a rat model of mononeuropathic pain. Uncovering the underlying mechanisms of PE in the spinal cord in the case of a positive outcome of per se intrathecal PE. Methods: Mononeuropathic pain was induced in rats via partial sciatic nerve ligation (pSNL). Tactile allodynia was assessed by the dynamic plantar aesthesiometer. PE was administered intrathecally (1, 3, 10, or 30 nmol/rat) to evaluate dose-dependent antiallodynic effects. To assess ARs mediating PE effects, the selective α_1- and α_2-AR antagonists prazosin and idazoxan, respectively, were used. In a separate cohort, oral PE (5 mg/kg) was administered alone and in combination with oral PGB (25 mg/kg). NA release was assessed in mouse spinal tissue. Results: Intrathecal PE (30 nmol/rat) significantly attenuated tactile allodynia in a prazosin- and idazoxan-reversal manner, pointing to the α_1- and α_2-ARs involvement. As a novelty, the oral administration of the combination of PE (5 mg/kg) and PGB (25 mg/kg) produced a significant antiallodynic effect with fast onset, whereas the individual compounds failed to show an effect at the test doses. Furthermore, our results indicate that the effect of PE is indirect and depends on cytosolic NA release from spinal tissue, as demonstrated by stimulation-induced release experiments in mice. Conclusions: PE produces NA-dependent antiallodynic effects in rats with NP via activation of spinal ARs, likely by activating α_2-receptors at presynaptic regions alongside activation of α_1-receptors at inhibitory interneurons. Concurrent activation of the spinal adrenergic system with VGCS modulation represents a promising strategy for the management of NP, warranting further translational investigation. Fund: TKP 2021 EGA-25

89. IMPORTANCE OF BIOSIMILAR MEDICINES IN MODERN HEALTHCARE – A REGULATORY PERSPECTIVE

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Biosimilar medicines have emerged as transformative therapeutics, offering highly similar yet cost-effective alternatives to originator biologic drugs. By maintaining comparable safety, efficacy, and quality, biosimilars address critical barriers in healthcare access, particularly in low- and middle-income countries. These therapies have been instrumental in expanding patient access to essential biologics for conditions including oncology, autoimmune disorders, and diabetes, where treatment costs have historically limited availability. Recent developments in biosimilar medicine production and regulation have significantly improved their clinical feasibility. Advances in analytical characterization, pharmacokinetic and pharmacodynamic assessments, and optimized clinical trial pathways have enhanced the efficiency of biosimilar development without compromising safety or effectiveness. Regulatory frameworks established by the European Medicines Agency (EMA), World Health Organization (WHO), and U.S. Food and Drug Administration (FDA) have standardized evaluation protocols, ensuring comparability with reference biologics while streamlining approvals. Strategic regulatory adaptations, such as tiered or stepwise clinical requirements, have demonstrated potential to reduce development time and costs, further enabling global access. Case studies illustrate the tangible benefits of biosimilars: reduced treatment costs by 20-65%, expanded patient availability, and enhanced sustainability of healthcare systems. Moreover, ongoing innovations in manufacturing processes, post-translational modification monitoring, and batch-to-batch consistency optimization highlight major improvements in quality assurance and therapeutic reliability. Despite these advances, challenges remain regarding interchangeability, clinician familiarity, and regulatory harmonization across regions. Addressing these barriers through coordinated educational efforts, policy alignment, and expanded prequalification programs is critical for maximizing the public health impact of biosimilars.

In conclusion, biosimilar medicines represent a paradigm shift in biotherapeutics, combining scientific rigor with economic and clinical benefits. Continued innovation, regulatory evolution, and global collaboration are essential to fully realize their potential in improving patient outcomes and healthcare equity worldwide.

90. THE REGULATORY LANDSCAPE OF ADVANCED THERAPY MEDİCİNAL PRODUCTS

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Advanced Therapy Medicinal Products (ATMPs), including gene therapies, somatic cell therapies, and tissue-engineered products, pose unique regulatory challenges due to their complex nature and potential for revolutionary treatments, necessitating adaptive regulatory pathways that can ensure a good framework for their development and approval. The CMC (chemistry, manufacture, control) regulatory compliance strategy for Advanced Therapy Medicinal Products has unique characteristics: gene therapy, cell therapy and tissue engineered products require a high level complexity of biological manufacturing processes and considering this, require tight CMC schedule for appropriate control on drug substance and drug product development.

The new ATMP guideline for clinical trials was published in 2025, which provides guidance on the structure and data requirements for clinical trial applications for exploratory and confirmatory trials with investigational advanced therapy medicinal products (ATMPs) and shows a good perspective towards Marketing Authorisation Applications (MAA) of the field.

Some of the key areas of focus for future ATMP developments and regulations include: personalized medicine and the use of ATMPs to treat rare diseases, the development of combination products that integrate ATMPs with other therapies, decentralized manufacturing in special cases (for example CAR-Ts).

To support ATMP developers, several platforms exist at EMA, including National Scientific Advice, ATMP classification procedure, ATMP certification and Prime Scheme which can give support for ATMP developers throughout the unique development pathway of these products.

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