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12-13 May 2026 | Maison de la Poste, Brussels

REPURPOSING OF MEDICINES 4ALL

beacon for rare diseases

Enhancing Drug Repurposing Accuracy through AI-Orchestrated Integration of Knowledge Graph Reasoning, Structure-base Model Prediction, and Gene Expression Analysis

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Introduction

Drug repurposing enables faster and lower-risk drug development. However, current AI approaches remain **fragmented**, lacking a unified framework for **multi-source evidence integration**.

To address this, we present an **AI-orchestrated platform** that integrates **three evidence layers**: **structure-based modeling** of drug–target interactions, **knowledge graph reasoning** for disease relevance, and **gene-expression analysis** for functional validation. Each component generates independent evidence and is integrated at the **decision level** for **cross-validation across heterogeneous data**.

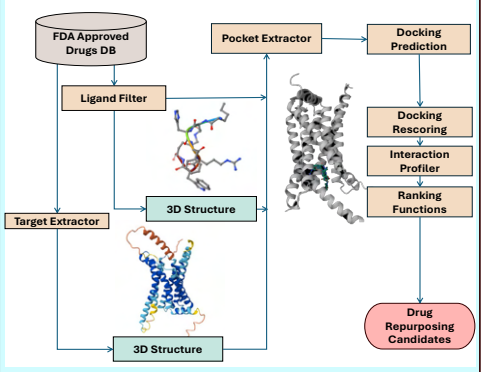
The platform supports **two workflows**: a **Drug–Target Driven** workflow that starts from existing drugs to identify new targets and indications, and a **Disease–Gene Driven** workflow that begins from disease biology to discover candidate therapeutics.

By combining **multi-modal evidence integration with bidirectional exploration**, the framework improves **robustness, interpretability, and translational relevance**, transforming fragmented AI predictions into **coherent and actionable therapeutic insights**.

Multi-Modal Evidence Platform

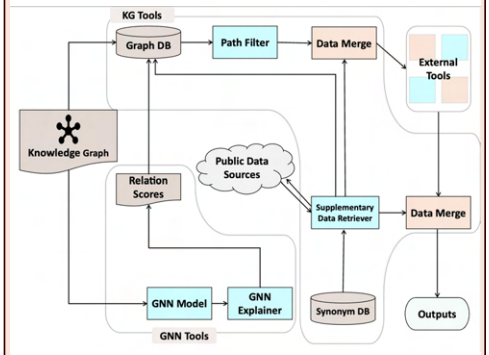
Structure-based Modeling

Structure-based prediction of drug–target interactions



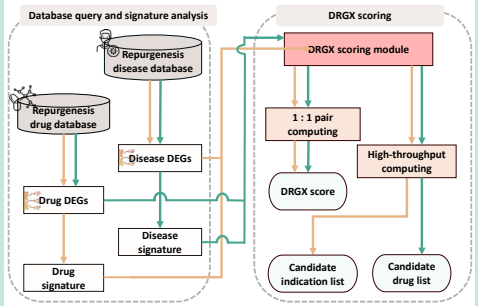
Knowledge Graph Reasoning

Knowledge graph reasoning for disease relevance



Gene Expression Analysis

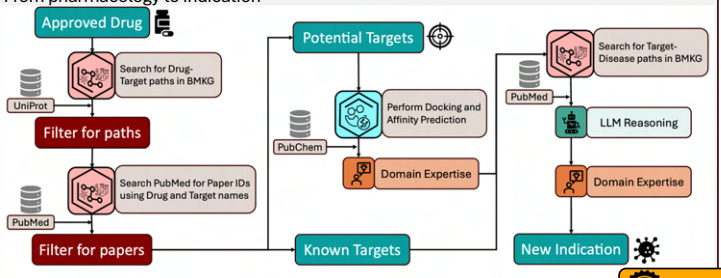
Gene-expression signature matching



Orchestrated Intelligence Workflow

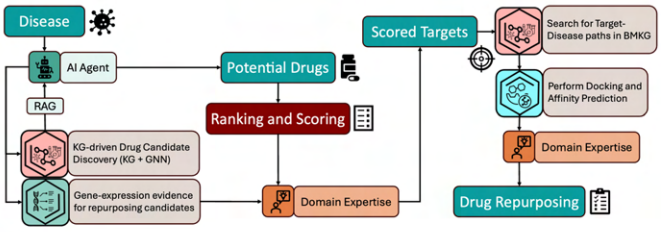
Drug–Target Driven Discovery

From pharmacology to indication



Disease–Gene Driven Discovery

From disease biology to therapeutics



Cross Validation

Translational Pipeline: From Discovery to IP

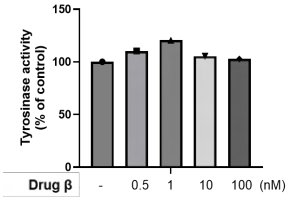
AI-identified candidates advancing across translational stages

Disease Area	Indication	Drug ID	PoC Validation	IP Protection	Pre-Clinical
Autoimmune	Vitiligo				
Fibrosis	Idiopathic Pulmonary Fibrosis				
Neurodegenerative	Spinocerebellar Ataxia (Rare)				

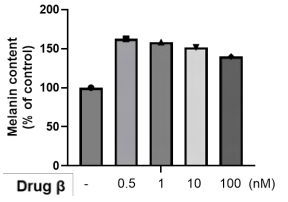
Leveraging existing safety profiles, our pipeline accelerates repurposing from AI discovery to clinical and IP outcomes.

Case Study: Drug β Repurposing

Drug β Repurposing for Vitiligo Treatment (Patented)



Drug β increased tyrosinase activity in B16F10 cells



Drug β increased cellular melanin content in B16F10 cells

We are thankful to R&D and DevOps team for their continued support in this work

From Pain to Patient Preferences: Patient Engagement in Early HTA for Repurposing Losartan in Osteogenesis Imperfecta

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BACKGROUND

- Patient engagement is essential for aligning drug development with unmet needs, and meaningful outcomes, supporting access decisions and early identification of non-viable projects. REMEDi4ALL is an EU-funded initiative for drug repurposing, embedding early health technology assessment (eHTA) and patient engagement to guide losartan's development in osteogenesis imperfecta (OI). While fracture reduction is a key clinical outcome in trials and is targeted with treatments, patients indicated that their unmet needs were not fully represented by this outcome. This work aimed to define a disease-specific, patient-informed unmet need list for OI.
- An unmet patient need is a problem or challenge that people with OI still face because current treatments or support don't fully address what matters in daily life, which can have a serious impact on overall quality of life.

METHODS

- An in-person multi-stakeholder workshop organised by REMEDi4ALL - involving five patients (including one paediatric), one caregiver, one patient representative, one clinician, and one developer - in 2024 explored patient preferences of treatments in OI using an initial, non-disease specific element list.
- The first session laid the foundation for a workshop with 31 OI patients of varying ages and severity held at the Osteogenesis Imperfecta Federation Europe (OIFE) annual meeting in June 2025.
- After presenting collected elements of unmet need (shown in Table) from patient-informed, current OI literature (1.-4.), participating patients expressed their unmet needs in facilitated group discussions.
- Subsequently, written outputs were synthesised into domains and elements by the Health Economics and Outcomes Research (HEOR) team.

Factor	Mild OI	Moderate OI	Severe OI	Children	Adolescents	Adults	Elderly
Pain	Chronic, but less severe	Persistent and debilitating	Persistent and debilitating	Emerging issue	Increasing	Major issue	High impact
Fatigue	Common	Severe	Severe	Moderate	High impact	High impact	High impact
Fracture Burden	Lower	High, frequent surgeries	Very high, frequent surgeries	Very high	High	Moderate	High
Mobility Needs	Walking aids, occasional support	Wheelchair use, rehabilitation needs	Wheelchair dependence, intensive rehab	Moderate	Increasing need	High impact	Severe
Mental Health Needs	Anxiety, hearing loss	Depression, social isolation	Depression, social isolation	Emerging	High concern	High concern	Aging-related anxiety
Employment Challenges	Some impact	High unemployment, workplace barriers	High unemployment, workplace barriers	–	–	Major concern	Retirement concerns
Dental & Hearing Needs	Hearing aids, mild dental issues	Severe malocclusion, dentinogenesis imperfecta	Severe malocclusion, dentinogenesis imperfecta	Early dental intervention	Orthodontic needs	Ongoing dental care	Hearing loss worsening
Financial Concerns	Moderate	High due to disability	High due to disability	–	–	High financial burden	Cost of caregiving
Specialized Care Needs	Moderate	High (surgery, ortho, rehab)	High (surgery, ortho, rehab)	Pediatric OI care	Transition to adult care	Multidisciplinary care	Assisted living

Table: Literature-informed list and grouping of elements of unmet need in osteogenesis imperfecta

RESULTS

- Twenty distinct unmet need elements were identified and grouped into three domains: Therapeutic Gaps (4), Clinical Care Gaps (8), Supportive and Mental Health Care Gaps (8) (shown in Figure.)
- Chronic pain, fatigue have again emerged as the most reported unresolved burden in all severity and age groups.
- Mental health challenges were consistently mentioned across workshops, reflecting its impact on daily functioning and overall quality of life.
- These elements cover a broad range of patient-centric value drivers that go beyond conventional clinical endpoints.

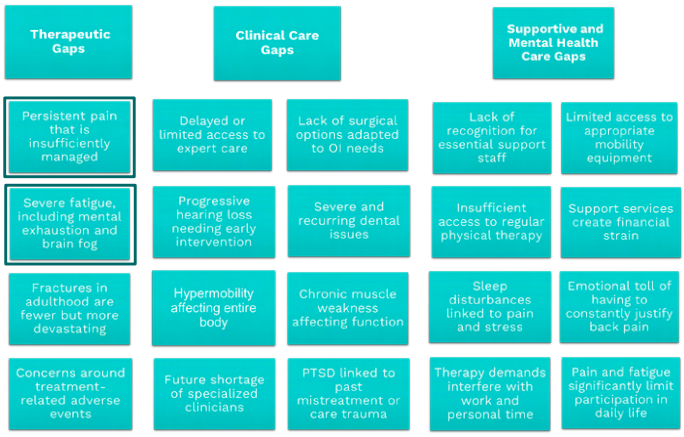

Figure: Unmet patient need elements in osteogenesis imperfecta

IMPLEMENTATION

The identified unmet needs directly informed how treatment benefits were communicated in patient-support materials, including revisions to the patient information leaflet to reflect priorities such as pain and fatigue.

This input contributed to a shift in the target product profile: while fracture reduction remained the central target for drug action, greater emphasis was placed on pain and fatigue reduction being leading therapeutic benefits. Incorporating patient perspectives early provided insights into enrolment barriers and communication needs, improved evidence generation strategies, and will support better alignment between value propositions and patient priorities.

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Association between use of furosemide and risk of Parkinson's disease

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School of Pharmacy, University of Eastern Finland[1], Finnish National Institute for Health and Welfare (THL), Finland[2]

Background and Objectives

- Furosemide was associated with lower risk of Parkinson's disease (PD) in French population.
- We studied the association between furosemide use and risk of PD in a Finnish nationwide nested case-control study, and an indication-restricted case-control study among persons with heart failure or renal failure.

Material

- 19 568 PD cases and 130 156 sex, age, and region-matched controls from the register-based Finnish study on PD (FINPARK, diagnosed 1999-2015) were included.
- A case-control study restricted to those with heart failure or renal failure was conducted, including 1 222 PD cases and 4 766 controls, matched on age, sex, region and duration of heart failure or renal failure.
- Furosemide use was identified from Prescription register (1995-2015), and exposure to furosemide was determined as ever, at least 3, 5 or 8 years before the outcome.

Methods

- Associations were studied with conditional logistic regression model adjusted for occupational social class, exposure to agricultural work, asthma/COPD, hypertension, coronary artery disease, diabetes, head injury, cancer, and number of days stayed in a hospital.
- Covariates were measured before the exposure assessment.

Results

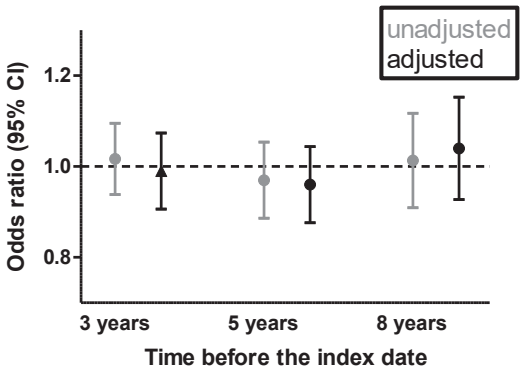
- Regardless of the study population or exposure assessment period, furosemide use was not associated with risk of PD.
- **OR 0.98; 95% CI 0.83-1.16** for furosemide use at least 5 years before the diagnosis in indication-restricted study and **OR 0.99; 95% CI 0.93-1.06**, entire FINPARK study.

Conclusion

- We found no evidence on the association between the use of furosemide and risk of PD.

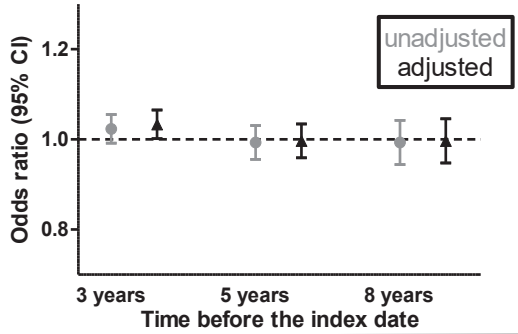
Association between the use of furosemide and PD, compared with non-users in indication-restricted design.

Furosemide use before index date	PD cases 1 222, n (%)	Controls 4 766, n (%)
3 years	662 (54.2)	2 582 (54.2)
5 years	416 (49.1)	1 567 (48.7)
8 years	203 (41.4)	735 (40.2)



Association between the use of furosemide and PD, compared with non-users in all FINPARK cases and controls.

Furosemide use before index date	PD cases 19 568, n (%)	Controls 130 156, n (%)
3 years	1 839 (9.4)	11 549 (8.9)
5 years	1 233 (7.0)	7 899 (6.7)
8 years	673 (4.7)	4 285 (4.5)



UNIVERSITY OF EASTERN FINLAND

FUNDING

This study was funded by the Michael J. Fox Foundation (grant number MJFF-023918)

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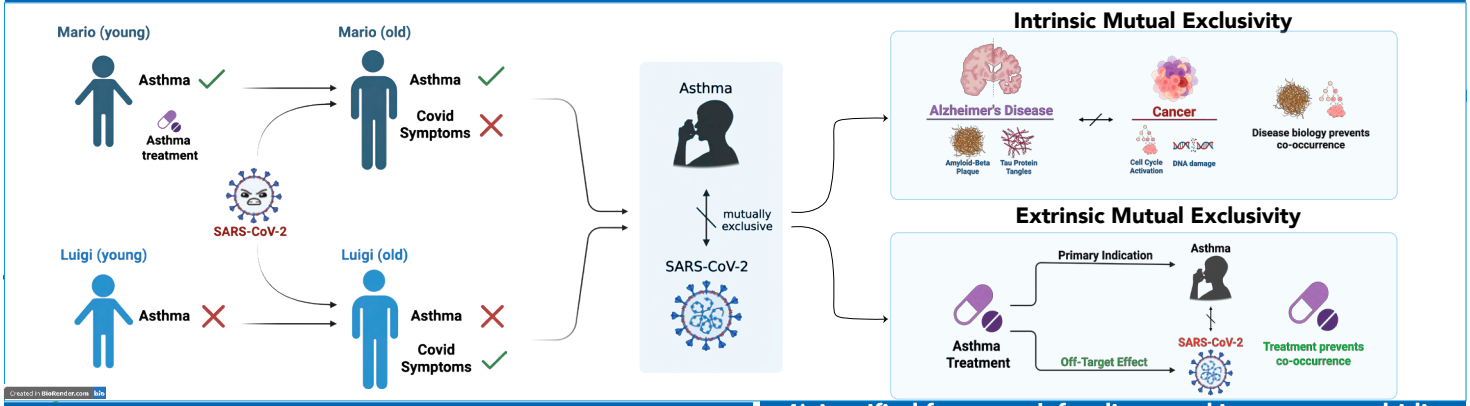
SCAN ME !

Large-scale identification of inverse comorbidity trends for in-silico drug repurposing

Flavio Passante[1,2,3,4], Ottavio Croci[1], Francesco Iorio[1,3,4]

1) Hypothesis: Some inverse comorbidity relationships might arise from drug-induced protective effects, where treatment for one disease reduces the incidence of another

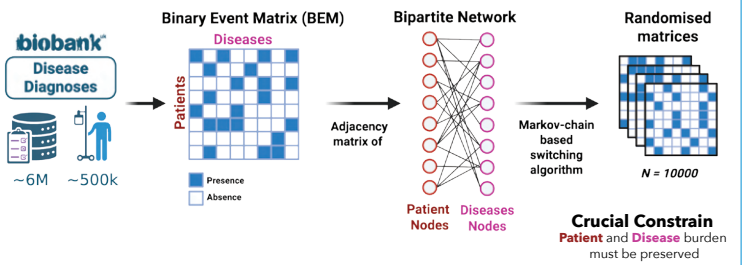


2) Drug activity as causal mechanism for mutually exclusive diseases

We aim to identify **comorbid** and **inversely comorbid disease pairs** using a unified statistical framework and investigate potential **causal mechanisms** underlying inverse comorbidity. We argue that some inverse associations arise from **drug pleiotropy,** whereby treatment for one disease reduces the risk of another.

3) Constrained null-diagnoses model generation

Phenotypes are considered **comorbid** or **inversely comorbid** only if their association is statistically stronger than expected under a null random model.

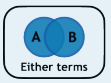


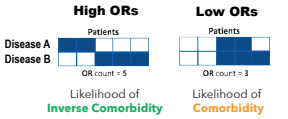
Crucial Constrain
Patient and **Disease** burden must be preserved

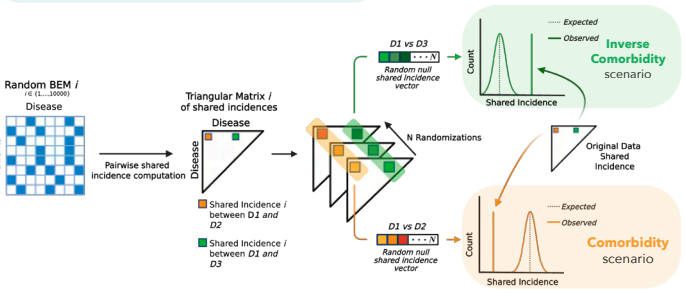
4) A unified framework for direct and inverse comorbidity analysis

Shared Incidence

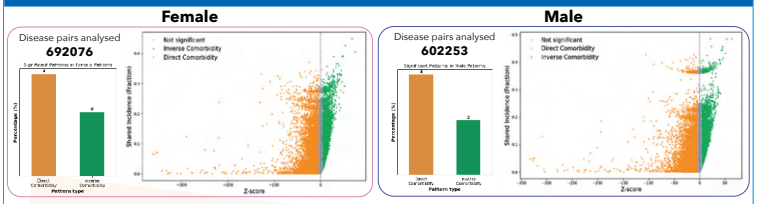
We compute, for every pair of diseases, the observed **joint count O_{ij}** of individuals presenting **either** or **both** disease *i* and-disease *j*. This allows to detect both **Co-morbidities** and **Inverse Comorbidities** with a single metric, the **Shared Incidence**

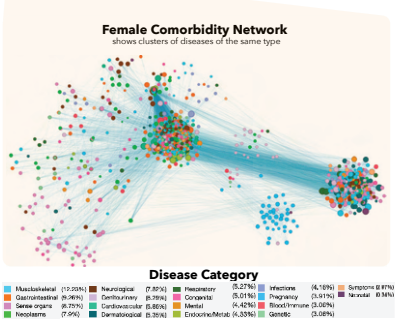




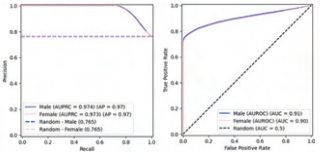


5) Comorbidity validation lends credibility to identified inverse comorbidites

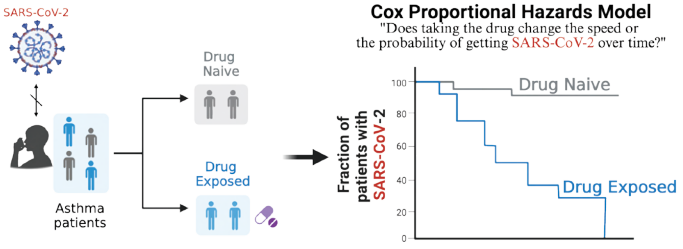




- As there are no gold-standard inverse comorbidities, we validated mutual exclusivities by validating **direct comorbidities** with **Relative Risk (RR)** and **Phi (φ) correlation**



6) Next step: inferring protective mechanisms from inverse comorbidity relationships



Conclusions

- We developed a metric able to detect **comorbidities** and **inverse comorbidities** concurrently and efficiently
- We have **validated** direct comorbidities, boosting the **confidence** for inversely comorbid associations
- We plan to exploit inverse comorbdities to identify **drug repurposing** opportunities

Affiliations

1. Computational Biology Research Centre, Human Technopole, Milan, Italy
2. University of Milan (Statale), Milan, Italy
3. European School of Molecular Medicine (SEMM)
4. NeuroCOV European research project

HT Research Centre

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2. Iorio, *et al.* Efficient randomization of biologitcal networks while preserving functional characterization of individual nodes. *BMC Bioinformatics* **17**, 542 (2016)
3. Gobbi A, et al. *BiRewire: High-performing routines for the randomization of a bipartite graph (or a binary event matrix), undirected and directed signed graph preserving degree distribution (or marginal totals)*

Radboudumc Therapy Accelerator for Rare Diseases: Turning challenges into opportunities in academic repurposing

Karin Ruijtenbeek, Maaike Oosterveer, Kevin Bos, Marga Bosch-Bouma, Najoua El Boujnouni, Anna de Goede, Rick Greupink, Rob ter Heine, Dorien Hermkens, Joanna in 't Hout, Manoe Janssen, Paul de Jonge, Sven van Laanen, Margot Neefjes, Gerty Schreibelt, Dirk Lefeber, Saskia de Wildt

Radboudumc Therapy Accelerator for Rare Diseases

From Challenges to Opportunities

Key Challenges

- Funding gaps
- Protection & patent positioning
- Regulatory complexity
- Drug supply & shortages
- Small patient populations
- Lack of Stakeholder alignment

Opportunities

- Innovative development strategies
- Creative protection
- Efficient use of existing data
- Exploring early access
- Dedicated manufacturing
- Adaptive Clinical Trial design
- Early stakeholder involvement

Collaboration is key!

RARE-NL HUB FOR RARE DISEASES AND DRUG REPURPOSING

CHDR Centre for Human Drug Research

ombion Centre for Animal-free Biomedical Translation

PHARMA DELTA

HAN BIOCENTRE

ccmo

DMT HUMAN ORGAN AND DISEASE MODEL TECHNOLOGIES

DCRT Dutch Center for RNA Therapeutics

D A R E NL

pivotpark

cpbt

oost nl

FAST

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Email: therapyacceleratorrarediseases@radboudumc.nl

E+ ALLIANCE:

A united voice for Rare and Complex Epilepsies

José Ángel Aibar Moreno, Sandra Silva Arrieta, Irena Bibic, Isabella Brambilla, Ana Cantó Martínez, Emma del Rey, Marita Gunn Sandness, Malgorzata Kosla, Carol Anne Partridge.

WHO IS E+A?

A united alliance of patient groups, families, clinicians, researchers and advocates with more than **50 associations**.

OVER 50 ASSOCIATIONS UNITED:

The network currently comprises over 50 global associations with a strategic goal to grow to **100 members.**

GLOBAL REACH:

The Alliance maintains 5 global collaborations with active presence in **Europe, USA, Latin America,** and **Australia.**

VALUES:

Hope — Strength — Union

Full Members

Direct voice, voting rights, and leadership initiatives

Associated Members

Rights to propose ideas, discuss strategy, and collaborate on projects.

The E+A SURVEY:

IDENTIFYING THE TRUE BURDEN

E+ complex and rare Epilepsies alliance SURVEY

The survey is designed to collect robust data to move beyond seizure frequency and understand the holistic impact of rare epilepsies.

FOUR CRITICAL DOMAINS

DAILY CHALLEN-GES | **CAREGIVER NEEDS** | **QUALITY OF LIFE** | **ACCESS TO CARE**

MARCH 10th

Data Collection

DRIVING POLICY CHANGE

Survey findings will be used to launch the Patient's Rights Charter at the 16th European Epilepsy Congress in Athens, on September 2026.

PART 1

PART 2

YOUR VOICE IS IMPORTANT!

Help us capture the full picture of Rare and Complex Epilepsies - beyond seizures.

Join and share the E+A Survey today!

Drug Repurposing to Modulate eEF1A2-expression in a Rare Neurodevelopmental Disorder

Tamsin Baxter, Richard Elliott, Neil Carragher and Cathy Abbott

University of Edinburgh

The Clinical Problem

De novo heterozygous missense mutations in eEF1A2 cause a rare neurodevelopmental disorder.

Symptoms include epilepsy, developmental delays and autism.

Only symptomatic treatments available and drug resistance is common.

High mutational heterogeneity limits genotype-phenotype conclusions.

Therapeutic Rationale

Evidence suggests a partial loss-of-function mechanism.

Increasing wildtype eEF1A2 could counteract this.

Gain-of-function could be targeted by reducing mutant protein.

High-throughput drug repurposing facilitates both approaches.

Assay Design

Stable HEK293T cells expressing GFP-tagged eEF1A2 and DsRED global translation control were generated.

This dual-reporter system allows high-throughput screening of compounds that selectively alter eEF1A2 expression.

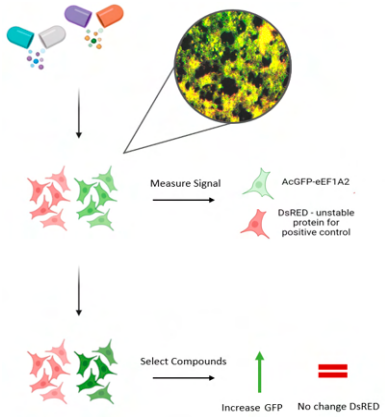


Fig 1. Dual-reporter HEK293T cell line enables measurement of eEF1A2 expression (GFP) alongside a global translation control (DsRED).

References

Péladeau et al., Nat Commun (2020) on eEF1A2 upregulation.

Giorgio et al., Hum Mutat (2021) on drug screening strategies.

Acknowledgements

Thanks to my supervisors Cathy Abbott and Neil Carragher, the members of the Abbott group and Richard Elliott from the phenotypic screening team for their help and guidance so far in this project. This work is funded by Medical Research Scotland and Healx.

High-throughput Screening Identifies Compounds that Increase eEF1A2

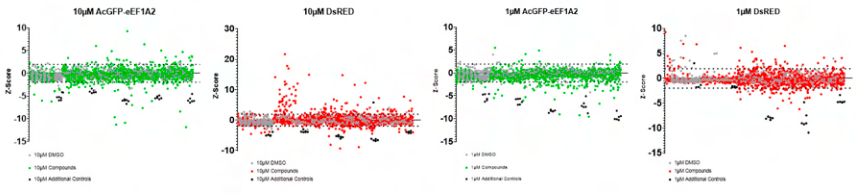


Fig 2. Z-scores for GFP (eEF1A2) and DsRED (global translation) across 1,400 FDA-approved compounds. Dotted lines show hit thresholds (z ≥ 1.95 for GFP, unchanged DsRED). 33 compounds selectively increase eEF1A2 without affecting global translation.

Dose-dependent Increase in eEF1A2

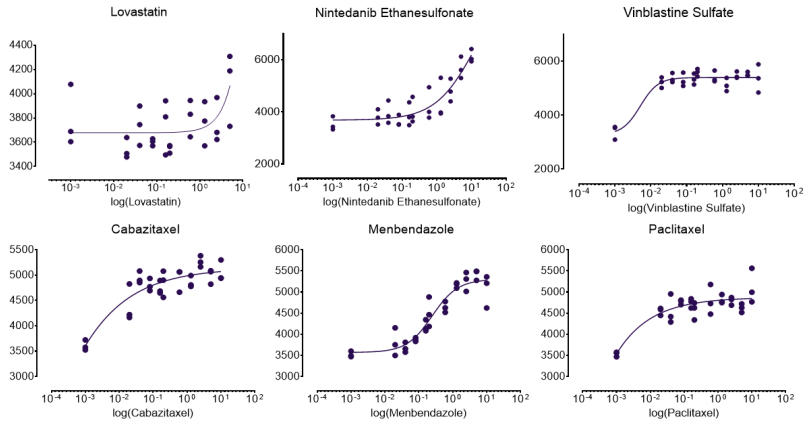


Fig 3. Dose-response activity for six representative compounds identified from the high-throughput screen. Response measured as GFP-tagged eEF1A2 fluorescence. Curves show reproducible, dose-dependent increases in eEF1A2.

Top Hits Increase Endogenous eEF1A2

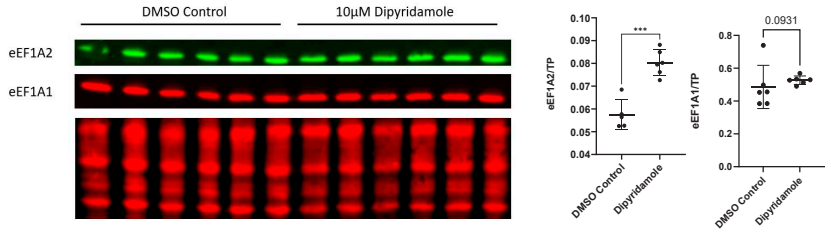


Fig 4. Treatment with selected hits increases endogenous eEF1A2 in stable cell lines and SH-SY5Y cells. Quantification plotted as mean ± SD.

Conclusions

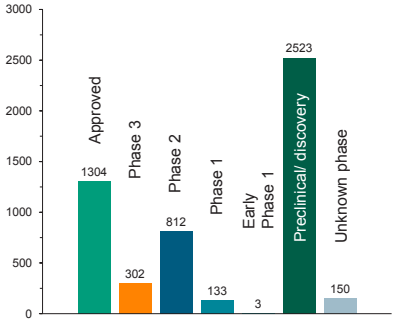
This platform enables rapid identification and validation of FDA-approved compounds that selectively increase eEF1A2, providing a potential route to the first targeted therapy for EEF1A2-related neurodevelopmental disorders.

Establishing the Remedi4All harmonized repurposing set as a community-shared, high-quality drug repurposing resource

Maria Kuzikov, Jeanette Reinshagen, Johanna Huchting, Axel Pahl, Katja Herzog, Leonie von Berlin, Yojana Gadiya, Andrea Zaliani, Flavio Ballante, Brinton Seashore-Ludlow, Michaela Vallin, Adelinn Kalman, Kun Qian, Hanna Axelsson, Elin Asp, Jonne Rietdijk, Marianna Tampere, Päivi Östling, Zaiurrehman Tanoli, Tero Aittokallio, Ola Spjuth, Annika Jenmalm-Jensen, Philip Gribbon

Fraunhofer ITMP
Fraunhofer Institute for Translational Medicine and Pharmacology ITMP
Karolinska Institutet
FIMM Institute for Molecular Medicine Finland
SciLifeLab
Uppsala Universitet
REPURPOSING OF MEDICINES 4ALL

Drug collection

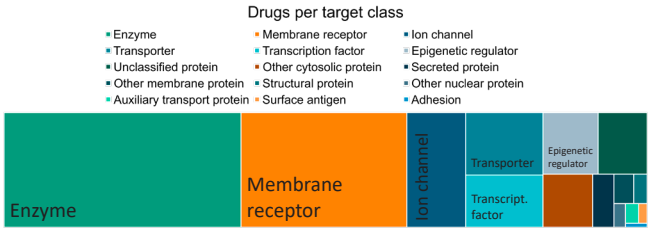


Number of repurposing set compounds by clinical phase.

REMEDi4ALL screening partners share a compound set that comprises approved drugs, clinical candidates, and well-characterized chemical probes.[1,2]

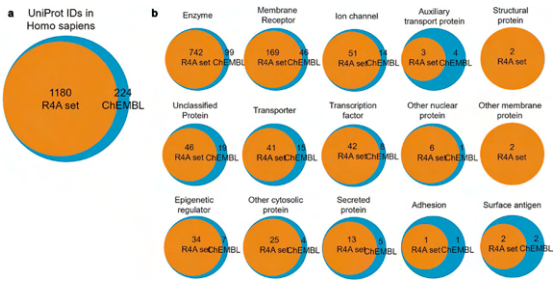
Public data

To leverage all associated biomedical knowledge, we have built efficient workflows to access and integrate molecular, bioactivity, and clinical data from public repositories.[2]

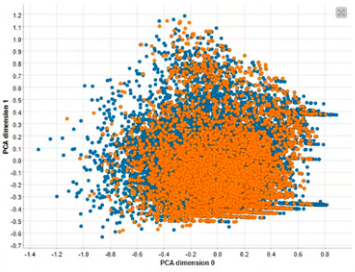


Biological target class coverage of the repurposing set .

REMEDi4ALL harmonized repurposing set



Overlap of druggable proteome in Homo sapiens between the REMEDi4ALL repurposing set[2] and ChEMBL chemical compounds for targets.

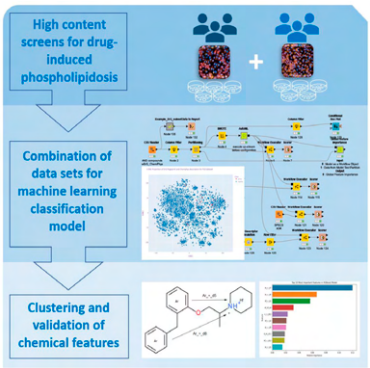


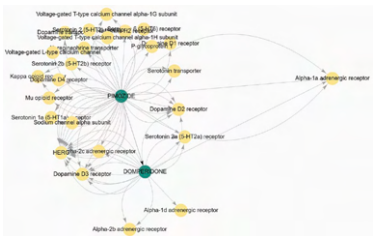
Chemical space coverage of the repurposing set (orange) compared to ChEMBL clinical compounds (blue).

- ✓ **High-quality material & virtual knowledge platform[4]**
- ✓ **Broad coverage of drug targets**
- ✓ **Harmonized and extensive chemical and biomedical annotation**

Liability screening

To identify potential liabilities, we are systematically profiling the repurposing set in cross-site screening programs.[3]

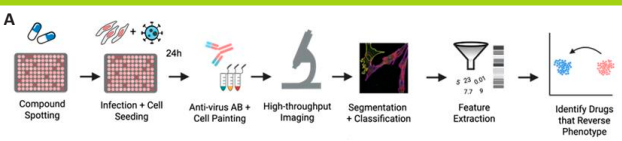


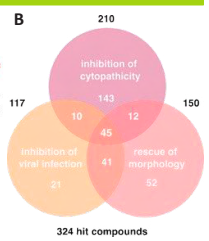


Data exploration example: Target space overlap analysis using the example of Pimozide and Domperidone

Disease-specific screening

- ✓ Novel antiviral repurposing candidates identified
- ✓ False-positives efficiently deprioritized through liability annotation





A Schematic overview of our adapted Cell Painting (CP) workflow to screen the REMEDi4ALL repurposing set[2] for anti-SARS-CoV-2 effect.[5]
B Venn diagram depicting the overlap of compounds identified as hits across the CP and two other anti-SARS-CoV-2 assay readouts.[5]

From clinical anecdotes to data-driven drug repurposing

The REMEDi4ALL repurposing set is a compound collection available for testing at partner laboratories. All related knowledge is brought together in a single virtual platform which allows researchers to easily explore, analyze, and reuse existing data, helping them identify promising new uses for known drugs more quickly and efficiently.

1 Corsello SM, Bittker JA, Liu Z, et al. *Nat Med.* **2017**. doi:10.1038/nm.4306
2 Reinshagen J, Seashore-Ludlow B, Gadiya Y, et al. *Database (Oxford).* **2025**. doi:10.1093/database/baaf081
3 Kuzikov M, Kalman A, Karki R, et al. Patterns **2026**. doi:10.1016/j.patter.2025.101453
4 https://chembioatlas.serve.scilifelab.se/
5 Asp E, Rietdijk J, Tampere M, et al. *biorxiv (preprint)* **2025**. 10.1101/2025.08.28.672794

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Rational Drug Repurposing Targeting GLP-2R in Short Bowel Syndrome

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[2] Institute of Chemical Sciences and Technologies "Giulio Natta" (SCITEC) – CNR, Italia
[3] Department of Translational Medicine and Surgery, Catholic University of the Sacred Heart, Roma, Italia
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[5] Department of Medicine and Ageing Sciences, Università "G. d'Annunzio" Chieti-Pescara, Chieti, Italia
[6] Department of Innovative Technologies in Medicine and Dentistry, Università "G. d'Annunzio" Chieti-Pescara, Chieti, Italia
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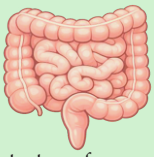
BACKGROUND

Short Bowel Syndrome (SBS) is a **rare disease** and a major cause of intestinal failure, often linked to Crohn's disease, and requires parenteral nutrition due to reduced absorption. It has high morbidity, mortality, and costs, with no available biomarkers and limited therapies[1].

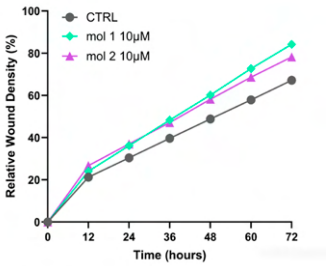
Current treatment relies mainly on teduglutide, a glucagon-like peptide-2analogue, though alternatives remain under development and have limitations[2].



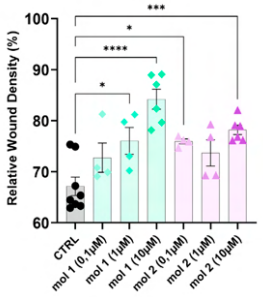
In this context, **drug repurposing** is a cost-effective strategy for rare diseases like SBS, allowing new therapeutic uses for approved drugs with known safety profiles[3]. This study explores in silico-driven drug repurposing combined with experimental evaluation as a cost-effective strategy, using structural analysis of the GLP-2 receptor and virtual screening of approved drugs from the ZINC database. Artificial Intelligence supports this approach by improving predictions of drug-target interactions and accelerating therapeutic discovery.

IN VITRO EVALUATION

Experimental validation of the identified candidate compounds was performed using a scratch assay in Caco-2 cells to assess their effects on epithelial wound healing.



Cells were treated with two selected molecules (mol 1 and mol 2) at different concentrations (0.1 µM, 1 µM, and 10 µM), and wound closure was monitored over time. Both compounds increased wound density over time, with a more pronounced effect observed at later time points.



Quantification of wound closure at 72 hours in Caco-2 cells treated with mol 1 and mol 2 at different concentrations. Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparisons test.
*p < 0.05, ***p < 0.001, ****p < 0.0001 vs control.

CONCLUSION

The AI-supported drug repurposing strategy enabled the identification of potential modulators of the GLP-2/GLP-2R complex with high affinity and favorable interaction profiles, through an innovative *molecular glue* approach aimed at stabilizing the receptor-peptide interface. In vitro results confirmed the biological activity of the selected compounds, showing improved wound healing in Caco2 cells. Overall, this study highlights the potential of drug repurposing as a rapid and effective strategy for developing new therapies for rare disease, such as short bowel syndrome.

REFERENCES

(1) Pironi L.. Nutrition in Clinical Practice 38 (2023): S9-S16.
(2) Amiot A. et al. Clinical nutrition 32.3 (2013): 368-374.
(3) Pirolli et al. Sci Rep 13.1 (2023): 1494
(4)McNutt AT. et al. J Cheminform 17.1 (2025): 28

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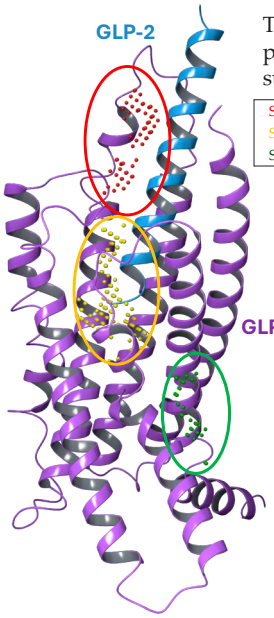
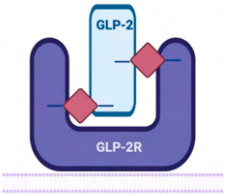
IN-SILICO DRUG REPURPOSING



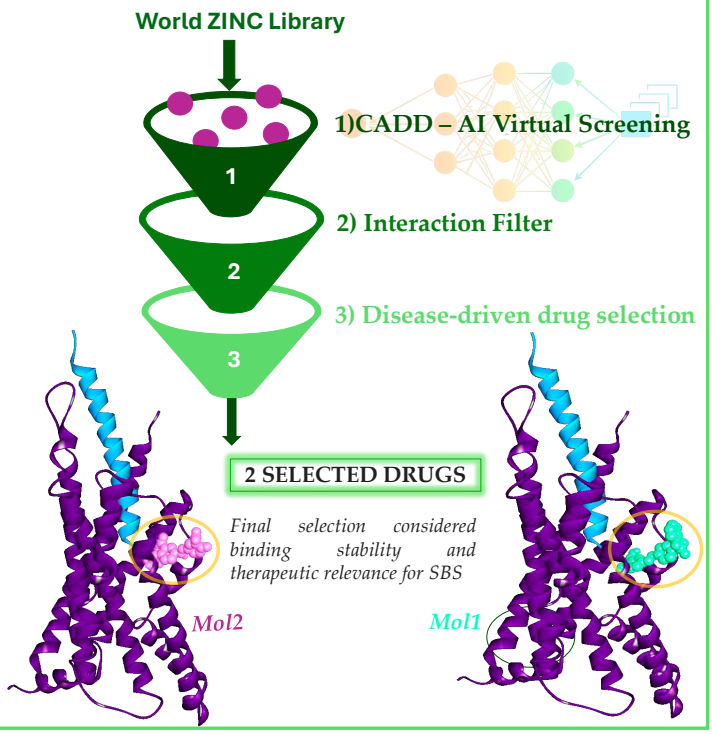
Three potential binding site have been predicted and characterized on the surface GLP2-GLP2R complex

Site 1	Orthosteric	Dscore 0.779	SiteScore 1.139
Site 2	Orthosteric	DrugScore 0.772	SiteScore 0.815
Site 3	Allosteric	DrugScore 0.791	SiteScore 0.756



The dual orthosteric sites support a *molecular glue strategy*, enabling the identification of compounds that simultaneously bind the receptor-peptide interface, stabilizing the complex and promoting receptor activation.

Virtual screening of the library was performed using GNINA[4], that integrates traditional docking approaches with convolutional neural network (CNN)-based scoring to improve the accuracy of binding pose and affinity predictions. The identification of GLP-2/GLP-2R modulators was carried out through a structure-based virtual screening strategy on both orthosteric and allosteric sites, using the "World" subset of the ZINC database. Compounds were subsequently prioritized based on CNN_VS scores and favorable interaction profiles.



The HIV protease inhibitor nelfinavir synergizes with cisplatin to kill platinum-resistant ovarian cancer cells through DNA damage and double-stranded DNA–triggered inflammatory signaling

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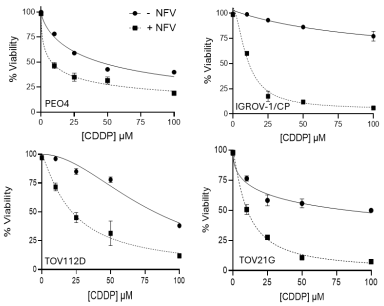
Background

Epithelial ovarian cancer (EOC) is a highly lethal malignancy characterized by a profoundly immunosuppressive tumour immune microenvironment (TIME), rendering it largely refractory to immunotherapy, including immune checkpoint inhibitors (ICIs). Standard platinum (Pt)-based therapies, such as cisplatin (CDDP), predominantly induce non-inflammatory cell death, limiting anti-tumour immunity. Here, we show that combining CDDP with the repurposed HIV protease inhibitor nelfinavir (NFV) induces synergistic cytotoxicity in EOC by activating an integrated immunogenic cell death (ICD) program. This includes DNA damage with impaired repair, suppression of survival Akt signaling, induction of ER stress, mitochondrial dysfunction, activation of caspases-4, -8, and -3 and gasdermin E, accumulation of cytoplasmic double-stranded (ds) DNA, inflammatory signaling, and release of lactate dehydrogenase (LDH) and damage-associated molecular patterns (DAMPs) — hallmarks of regulated lytic ICD, confirmed *in vivo* by prophylactic vaccination studies. **The ICD induced by CDDP/NFV can reprogram ovarian tumours from immune silent to checkpoint responsive.**

Key Findings

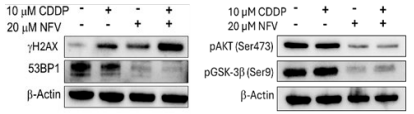
Fig.1: NFV potentiates Pt-induced cytotoxicity in EOC cells resistant to clinically achievable CDDP concentrations



Pt-resistant EOC cells (PEO4, TOV112D, IGROV-1/CP, TOV21G) treated with CDDP (3 h) followed by NFV (20 µM, 72 h). Viability was assessed by microcytometry; IC50 and Bliss synergy scores were calculated, yielding scores ranging from >10 (moderate) to >20 (strong).

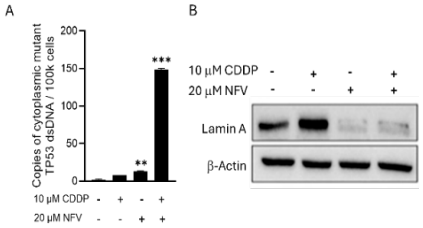
Key Findings (cont'd)

Fig.2: CDDP/NFV combination treatment increases DNA damage, impairs DNA repair, and suppresses Akt signaling



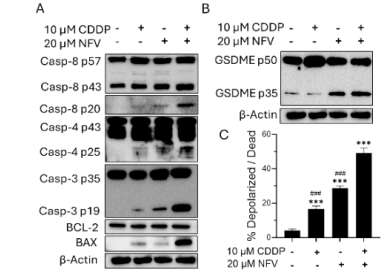
Western blots (WB) from IGROV-1/CP cells treated with CDDP (3 h) followed by NFV (72 h). DNA damage (γ-H2AX), DNA repair (53BP1), and Akt signaling markers (p-Akt, p-GSK3β) were assessed; similar results were observed in PEO4 cells.

Fig.3: CDDP/NFV toxicity in Pt-resistant EOC cells is associated with inhibition of Lamin A maturation — a key component of the nuclear envelope integrity — and promotion of cytoplasmic DNA leakage



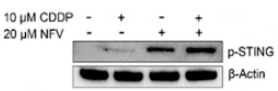
(A) Pt-resistant PEO4 EOC cells were harvested, and the cytosolic fraction was isolated. DsDNA was extracted using the Qiagen EZ1/2 DNA extraction kit. Droplet digital (dd) PCR was performed to quantify PEO4-specific *TP53* mutant copies; **p<0.01/***p<0.001 vs. untreated cells (one-way ANOVA/Tukey's post-hoc test). (B) Lamin A expression was assessed by WB.

Fig.4: CDDP/NFV drives cross-talk between apoptotic and pyroptotic mediators, culminating in a hybrid cell death program



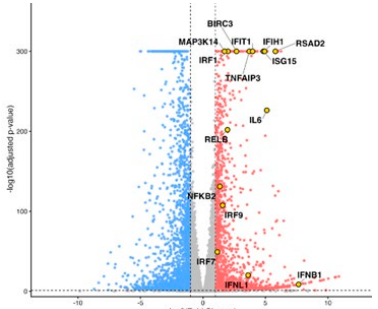
IGROV-1/CP cells were treated with CDDP for 3 h, followed by NFV for 72 h. Protein expression at the end of the experiment was analyzed by WB. Similar results were obtained in PEO4 cells. Casp-8, caspase-8; Casp-4, caspase-4; Casp-3, caspase-3; GSDME, gasdermin E.

Fig.5: Cytoplasmic dsDNA activates the cGAS–STING pathway, which is known to drive inflammatory cytokine and type I interferon (IFN) production



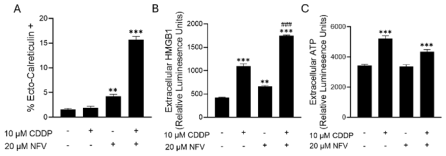
Pt-resistant IGROV-1/CP EOC cells were treated with CDDP for 3 h and/or NFV for 48 h. Cells were then harvested and processed for WB analysis using antibodies against phospho-STING (Ser366) with β-actin as a loading control.

Fig.6: CDDP/NFV drives transcriptional immune–inflammatory activation, marked by upregulation of genes linked to IFN and tumour necrosis factor (TNF) signaling



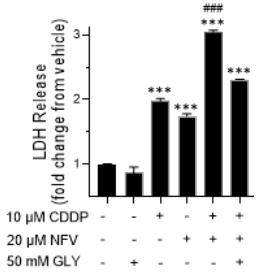
Differential gene expression in CDDP/NFV-treated IGROV-1/CP cells. The x-axis shows $\log_2$ fold change, and the y-axis $-\log_{10}$ adjusted p-value. Genes with FDR < 0.05 and $\log_2$ FC ≥ 1 are highlighted (upregulated in red, downregulated in blue). Selected immune-related genes are shown as yellow dots with black labels.

Fig.7: CDDP/NFV-treated EOC cells display abundant hallmark markers of regulated lytic ICD (a.k.a. DAMPs)



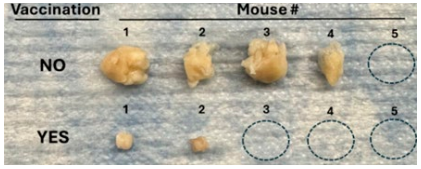
PEO4 cells were treated with vehicle, CDDP, NFV, or the combination. (A) Ecto-CRT was assessed by flow cytometry using an anti-CRT antibody. (B) HMGB1 levels were measured by ELISA. (C) Extracellular ATP was quantified by ELISA. **/ ***, p < 0.01/0.001 vs. vehicle; ###, p < 0.001 vs. CDDP (one-way ANOVA/Tukey).

Fig.8: Lytic plasma membrane (PM) disruption is evidenced by lactate dehydrogenase (LDH) release — a surrogate of PM rupture — in CDDP- or NFV-treated cells. Combination treatment elicits greater LDH release, which is partially attenuated by glycine, a well-established cytoprotective molecule



IGROV-1/CP cells were treated with vehicle, CDDP, NFV, the combination, or the combination plus glycine (GLY). CDDP (3 h) was followed by NFV (72 h) plus GLY. LDH release in supernatants was quantified by ELISA. ***p < 0.001 vs. control; ###p < 0.001 vs. CDDP/NFV/GLY.

Fig.9: Vaccination with CDDP/NFV-treated mouse ID8 EOC cells (days 1 and 7; ~85% dying cells) prevented or delayed tumour growth upon contralateral live-cell challenge (day 14), with 60% of mice remaining tumour-free at day 80, indicating durable tumour-specific protective immunity



Tumor growth following prophylactic vaccination with CDDP/NFV-treated mouse EOC ID8 cells. Data are shown from 5 vaccinated and 5 unvaccinated animals. Empty circles indicate animals that did not develop tumours within 80 days after live-cell challenge.

Conclusions

Together, the anti-HIV agent NFV and the Pt derivative CDDP induce:

Conclusions (cont'd)

- Synergistic cytotoxicity in Pt-resistant ovarian cancer (PROC) cells
- DNA damage associated with suppression of DNA repair and Akt survival signaling pathways
- ER stress signaling, including GRP78, IRE1α, p-eIF2α, CHOP, and PUMA (data not shown)
- Loss of Lamin A, compromising nuclear envelope integrity and promoting cytoplasmic dsDNA accumulation
- Caspase-8/4/3, driving apoptosis (caspase-8), ER stress–associated inflammatory signaling (caspase-4), and caspase-3–mediated execution with GSDME cleavage and secondary pyroptosis
- Caspase-8/3–dependent cell death as it is inhibited by caspase inhibitors, with concomitant loss of GSDME cleavage (data not shown)
- Regulated lytic cell death associated with DAMP release, LDH secretion, and immune–inflammatory gene activation
- Prophylactic vaccination with CDDP/NFV-treated dying EOC cells that delays or prevents tumour growth upon rechallenge, indicating ICD with *adjuvanticity* and *antigenicity* consistent with durable anti-tumour immune memory

Impact

EOC survival has improved only marginally over the past three decades, and poor responsiveness to immunotherapy remains a major unmet clinical need. The synergistic induction of regulated lytic ICD by CDDP/NFV enhances tumour cell death while increasing adjuvanticity and antigenicity, leading to remodeling of the TIME and potentially promoting durable anti-tumour responses. These alterations in the TIME may restore sensitivity of PROCs to ICIs and enable more sustained disease control. Finally, **our findings support the feasibility of repurposing the HIV therapeutic agent NFV as part of the treatment strategy for EOC**.

A NEW USE FOR AN OLD COMPOUND: MICRODOSING LSD TO REDUCE CENTRALIZED PAIN SYMPTOMS

Evidence From A Fibromyalgia Mouse Model

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[2] Faculty of Medical & Health Sciences, Tel Aviv University.
[3] Carvin Medicines, Israel. E-mail: sarciniegas@rossvet.edu.kn

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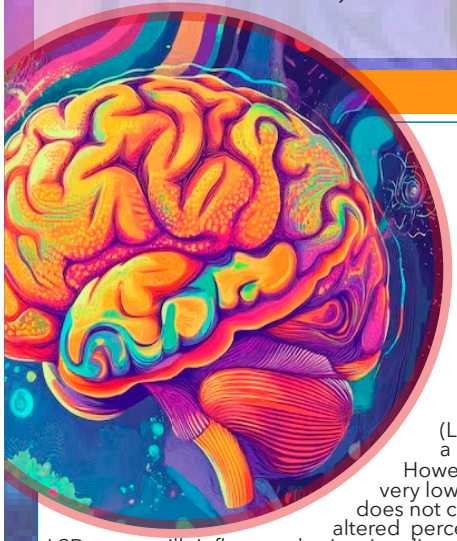


INTRODUCTION

Central pain conditions involve altered processing of pain signals in the brain and spinal cord, leading to persistent pain along with fatigue, mood changes, sleep disturbances, and reduced well-being. Fibromyalgia is a common example, and current treatments often offer limited relief.

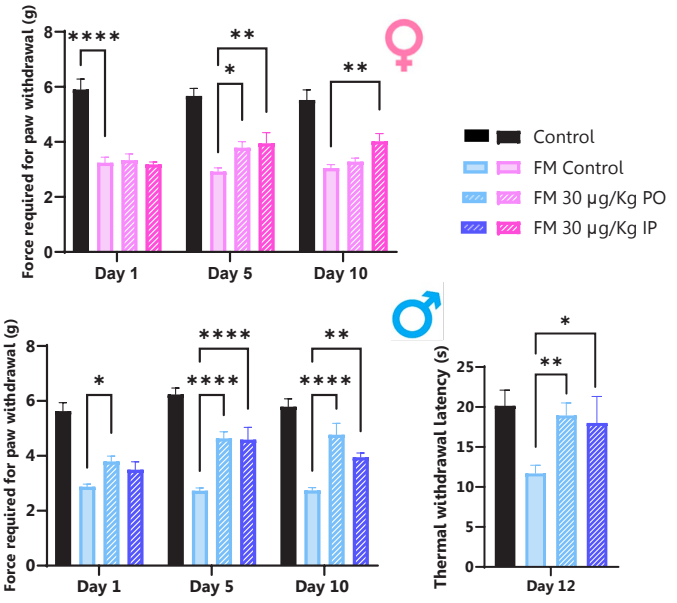
Lysergic acid diethylamide (LSD) is commonly known as a psychedelic substance. However, when administered at very low doses (microdoses), LSD does not cause hallucinations or altered perception. At these low doses, LSD may still influence brain signaling pathways involved in pain perception, mood regulation, and emotional processing. This suggests that microdosed LSD could have potential benefits for central pain conditions that involve both sensory and emotional components.

AIMS

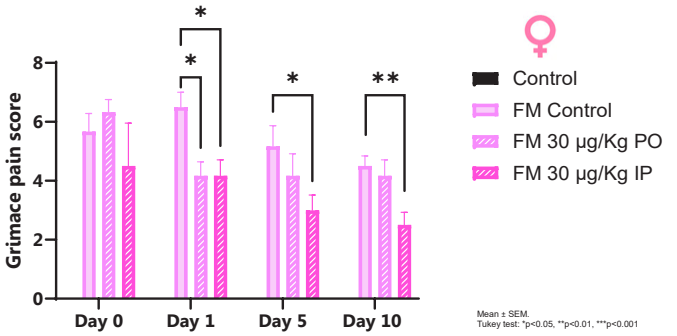
The aim of this study was to determine whether repeated microdoses of LSD can reduce pain and improve mood-related behaviors in an animal model of central pain, while evaluating sex differences, route of administration, and overall tolerability. Overall, this work seeks to support the potential repurposing of microdosed LSD as a novel, patient-relevant approach for managing central pain conditions.

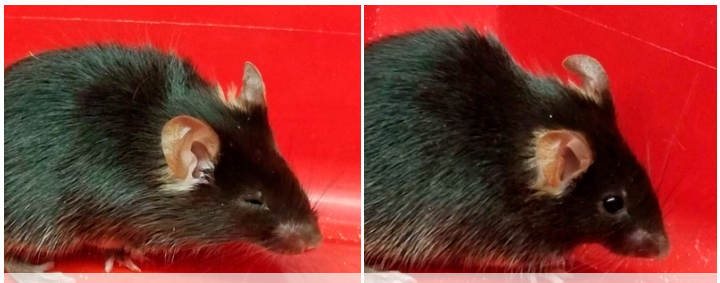
PAIN SENSITIVITY

Microdosed LSD reduces mechanical allodynia and thermal pain sensitivity in a fibromyalgia mouse model in a sex- and route-dependent manner



REPEATED MICRODOSES OF LSD REDUCE FACIAL PAIN EXPRESSION IN FEMALE FIBROMYALGIA-MODEL MICE

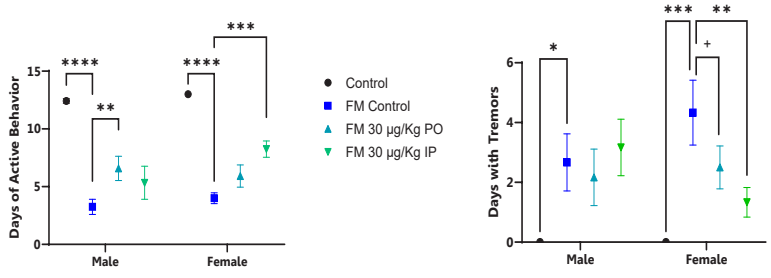




Mouse Grimace Scale (facial pain expression)
Representative images illustrating spontaneous pain assessment.

MICRODOSED LSD IMPROVES MOOD-RELATED ACTIVE BEHAVIOR IN FIBROMYALGIA-MODEL MICE

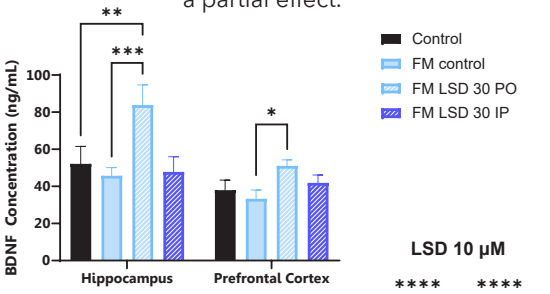
Fibromyalgia-model mice showed reduced activity and tremors compared to controls, and microdosed LSD improved active behavior in a sex- and route-dependent manner.



NEUROPLASTICITY

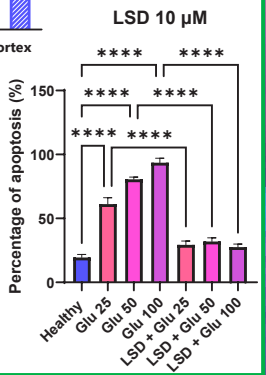
MICRODOSED LSD INCREASES BDNF LEVELS IN PAIN-RELATED BRAIN REGIONS OF MALE MICE

FM animals showed reduced BDNF compared to controls. Oral LSD significantly increased BDNF levels, while intraperitoneal administration showed a partial effect.



LSD DEMOSTRATES FUNCTIONAL NEUROPROTECTION

Reduced glutamate-induced neuronal apoptosis supports the increase in BDNF, suggesting that LSD promotes neuroplastic and neuroprotective brain resilience.



Conclusion

Microdosed LSD improved pain sensitivity and mood-related behaviors in a central pain model without major safety concerns. Behavioral benefits were accompanied by increased BDNF and protection against glutamate-induced neuronal apoptosis providing evidence that LSD engages neuroplastic and neuroprotective pathways relevant to centralized pain. Overall, this work supports further investigation of serotonergic drug repurposing strategies as patient-relevant therapies for centralized pain disorders.

Empowering Change: Findings from a National Workshop on Drug Repurposing for Cancers and Rare Diseases in Malaysia

Fikri Taib[1], Yolanda Augustin[1,2], Nafeesa Mat Ali[2], Chee Han Lim[3], Karina Yong[3], Yoke Ling Chee[3], Sanjeev Krishna[1,2], Ivy Chung[1]

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[3]*Third World Network, Kuala Lumpur, Malaysia*

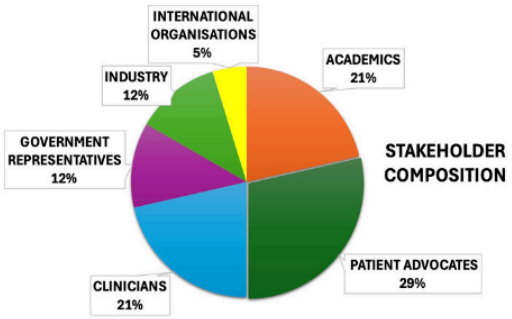
BACKGROUND

- Drug repurposing uses existing medicines for new indications and can provide a **cost-effective route** to expand access to treatment particularly for cancers and rare diseases.
- In Malaysia, a national ecosystem for drug repurposing is being developed through **multistakeholder engagement** across academia, government, industry, funders and patient advocacy groups.
- In October 2025, **a national roundtable was convened** to identify barriers, enablers, and practical strategies for advancing drug repurposing for cancers and rare diseases in Malaysia.

METHODS

Multi-stakeholder national roundtable with structured breakout discussions and thematic analysis

Convened Roundtable → Plenary Session → Breakout Discussions → Data Collection → Thematic Analysis



RESULTS

Stakeholder Perspectives

*"The question is not whether Malaysia can become a **regional** drug repurposing hub, but how."*

Policy/industry participant quote

"Return on investment is often easier to measure, but patient benefit is not. We need to rethink how we value the benefits of repurposing for families, caregivers, and communities."

Patient advocate quote

Thematic Analysis

Theme 1: Governance and Coordination

Fragmented responsibilities across organisations create barriers but stakeholders are ready to collaborate through a national coordination mechanism.

Theme 2: Drug Candidate Selection & Industry Engagement

Uncertainty in prioritising candidates and engaging industry slows development, while clearer processes and incentives could support collaboration.

Theme 3: Commercial Incentives & Manufacturing

Limited incentives for off-patent drugs and manufacturing uncertainty hinder development; domestic manufacturing capacity could support scale-up and access.

Theme 4: Clinical and Research Capacity

Malaysia has strong academic and research foundations, as well as a mature clinical trial ecosystem.

Theme 5: Funding Constraints

Existing support is short-term and fragmented; sustainable, strategically aligned funding models are needed for long-term programme development.

Theme 6: Regional & ASEAN Competitiveness

Strong opportunities exist for regional collaboration, regulatory harmonisation, and shared translational platforms.

Strategic Priorities for Impact

- Integrate patient and community perspectives into research and policy
- Strengthen clinical translational pathways from bench to bedside
- Align regulatory frameworks for repurposed therapies
- Establish sustainable funding models for scale-up
- Leverage international collaborations and proven global models

CONCLUSION

- Malaysia has strong scientific foundations and motivated stakeholders for drug repurposing.
- Progress depends on a coordinated national strategy, sustainable funding, early patient engagement, and regional collaboration.
- These steps could accelerate equitable access to repurposed therapies and position Malaysia as a regional hub for impactful drug repurposing.

Reference:
1. Krishnamurthy N et al. BMC Health Serv Res. 2022
2. Garcia Diaz M et al. Front Pharmacol. 2025
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Unlocking drug repurposing potential: Dutch multi-stakeholder perspectives on policy mechanisms

Menke Winckers[1,2,3], Kristóf Gyöngyösi[1,4] , Zsuzsa Réka Pozsár[1,4], Wim Goettsch[2,5], Zoltán Kaló[1,4], András Inotai[1,4], Dalma Hosszú[1,4]

BACKGROUND

Sustainable policy frameworks to support drug repurposing implementation remain underdeveloped. This study examines how Dutch stakeholders across the drug repurposing field, perceive and consider or utilise push and pull mechanisms to strengthen the viability of drug repurposing within national and European pharmaceutical systems.

METHODS

Semi-structured interviews (n=9) were conducted with six different stakeholder groups (funders, regulators, HTA experts, manufacturers, patient representatives and researchers) active and located in the Netherlands. Interviews were based on the **REMEDi4ALL Catalogue of Actions**[1], which outlines potential policy measures to support drug repurposing.

Interview data were analysed thematically to identify stakeholder perspectives on endorsed and/or underutilised policy mechanisms.

FINDINGS

Theme 1: Regulatory Pathway & Evidence Requirements

The regulatory system is not the main barrier but lacks predictability and alignment.

No need for a dedicated repurposing pathway → **current framework is sufficient if used flexibly**

Strong demand for:
- **clear guidance and phase-specific support**
- **transparent roadmap of evidence requirements**
- Clear overviews of existing evidence

Real-world data (RWD):
- seen as a promising **supplement** to traditional evidence generation
- **limited acceptance due to uncertainty and bias concerns**

Developers require:
- **predictable evidence expectations**
- early insight into **pricing and reimbursement implications**

Barriers arise from **uncertainty** (Industry) **and lack of coordination** (Academia), not from regulation itself

Theme 2: Health Technology Assessment (HTA), Pricing & Reimbursement

Access is constrained by fragmented decision-making across national HTA bodies, pricing, and insufficient reimbursement models.

HTA

Same standards should apply → **no separate framework for repurposed drugs**
- Low budget impact may waive full economic evaluation

National evidence requirements can create barriers, as repurposing projects often rely on existing evidence

Pricing

Broad consensus on a **"middle-ground" pricing model**:
- above generics, below originators

Support for **indication-based pricing**
- feasibility remains questionable

Ongoing tension:
- affordability vs. sufficient incentives

Reimbursement

Lack of **reimbursement-linked exclusivity** undermines business cases

Need for:
- **indication-specific reimbursement mechanisms**
- **long-term certainty for manufacturers**

Regulatory approval alone is insufficient; **misalignment in downstream decision-making regarding financials limits access**

Theme 3: Economic Incentives & Market Facilitators

Viable financial incentives are essential but currently inadequate.

Public funding is crucial but:
- **fragmented across development stages**
- need for **continuity ("funding chain")**

Strong consensus:
- **exclusivity is required to attract investment**, especially for off-patent drugs

Concerns:
- feasibility and enforcement of proposed EU exclusivity mechanisms

Alternative incentive models:
- Managed entry agreements (MEAs)
- Subscription or volume-based models
- One-off payments or royalties

Without credible return on investment, **repurposing remains commercially unattractive**

Theme 4: Education and dissemination, Demand & Collaboration

System-level coordination is a key enabler of successful repurposing.

Gaps in regulatory and HTA knowledge among **academia/SMEs**, who are not expected to master these areas

Need for:
- **early-stage guidance and support structures**
- centralised initiatives (e.g. REMEDi4ALL, REPO4EU)

Demand & implementation

Enablers:
- **inclusion in clinical guidelines and formularies**
- indication-based prescribing

Off-label use remains necessary → should be **better monitored, not restricted**

Collaboration

Public–private partnerships are essential

Increasing need for:
- **alignment between regulators, HTA bodies, and payers**

EU-level coordination valuable, but:
- pricing and reimbursement remain **nationally determined**

Effective repurposing depends on **coordinated action across stakeholders**, not isolated interventions

CONCLUSION

From the perspective of the Dutch ecosystem, drug repurposing is not primarily hindered by a lack of scientific opportunities, but by systemic **misalignment across regulation, HTA, pricing, reimbursement, and incentive structures**. Stakeholders emphasised the need for **coordinated pull mechanisms**, rather than isolated reforms, to enhance feasibility and sustainability.

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1) Inotai A, Hosszú D, Gyöngyösi K, Pozsar ZR, Winckers M, Lo D, *et al.* Catalogue of actions to address policy barriers of drug repurposing. *Value Health.* 2025;28(Suppl 1):S37.

AFFILIATIONS & ACKNOWLEDGEMENTS

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2 Utrecht University, Netherlands
3 Erasmus University Rotterdam, Netherlands
4 Semmelweis University, Hungary
5 Zorginstituut Nederland

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PROTEOMIC IDENTIFICATION OF HSP90 CO-CHAPERONES AS THERAPEUTIC TARGETS FOR DRUG REPURPOSING IN PLATINUM RESISTANT CANCERS

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BACKGROUND

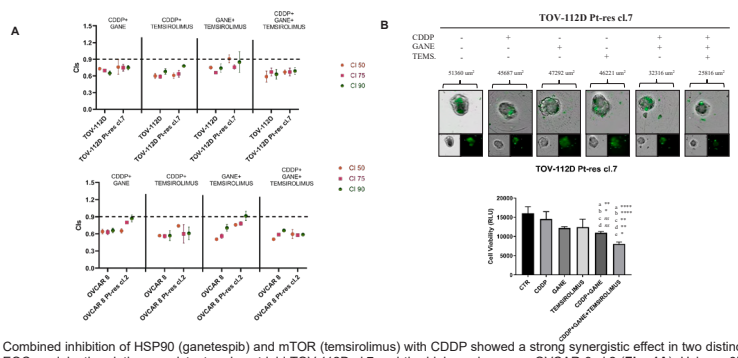
Platinum-based chemotherapies, such as cisplatin (CDDP), are widely used for the treatment of epithelial ovarian cancer (EOC), non-small cell lung cancer (NSCLC), and head and neck squamous cell carcinoma (HNSCC) [1]. However, intrinsic or acquired resistance often limits their therapeutic efficacy [2]. Using proteomic and phosphoproteomic approaches, we identified heat shock protein 90 (HSP90) and the mammalian target of rapamycin (mTOR) pathway as potentially activated pathways through which cells acquire platinum chemoresistance, thereby contributing to the identification of new therapeutic targets [3,4].

AIM

This study evaluates the efficacy of the triple-drug combination ganetespib (HSP90 inhibitor), temsirolimus (mTOR inhibitor), and CDDP in different platinum-resistant models including EOC, NSCLC and HNSCC [4]. Concurrently, it investigates putatively resistance-associated targets through proteomic profiling of the HSP90 interactome. Given the absence of FDA-approved HSP90 inhibitors, the study emphasizes co-chaperones as alternative therapeutic targets to circumvent drug resistance and enhance treatment efficacy.

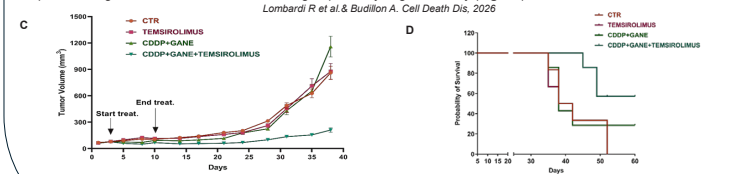
RESULTS

1. *In vitro* and *in vivo* synergistic antitumor effect of CDDP, GANETESPIB and TEMSIROLIMUS combination in EOC models



Combined inhibition of HSP90 (ganetespib) and mTOR (temsirolimus) with CDDP showed a strong synergistic effect in two distinct EOC models: the platinum-resistant endometrioid TOV-112D cl.7 and the high-grade serous OVCAR 8 cl.2 (**Fig. 1A**). Using a 3D microtissue assay, we demonstrated that the CDDP/ganetespib/temsirolimus combination strongly inhibited microtissue formation compared to single or double treatments, confirming its potent synergistic activity (**Fig. 1B**).

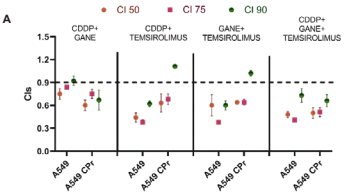
Lombardi R et al.& Budillon A. Cell Death Dis, 2026



The CDDP/ganetespib/temsirolimus combination was tested in NSG mice bearing tumors from platinum-resistant TOV-112D cl.7 cells. The triple treatment almost completely suppressed tumor growth compared with all other groups (**Fig. 1C**) and was associated with a significant improvement in overall survival (**Fig. 1D**).

Lombardi R et al.& Budillon A. Cell Death Dis, 2026

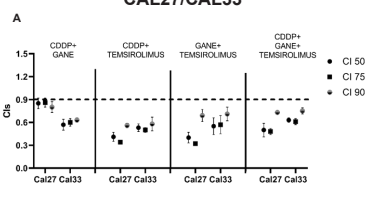
2. Validation of results in A549 CPr



Similar synergism of ganetespib and temsirolimus combined with CDDP was observed in the platinum-resistant NSCLC isogenic model A549 CPr, especially with the triple combination (**Fig. 2A**).

Lombardi R et al.& Budillon A. Cell Death Dis, 2026

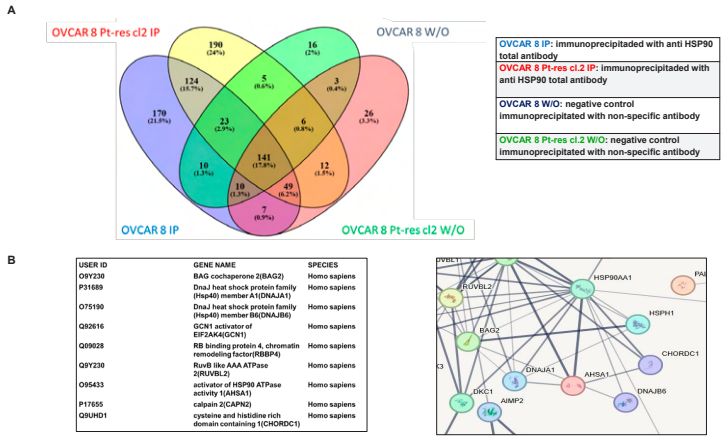
3. Confirmation of results in CAL27/CAL33



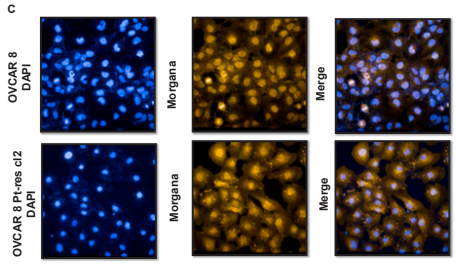
The triple combination also showed a synergistic anti-proliferative effect in intrinsically platinum-resistant HNSCC cell line Cal27 and Cal33 (**Fig. 3A**).

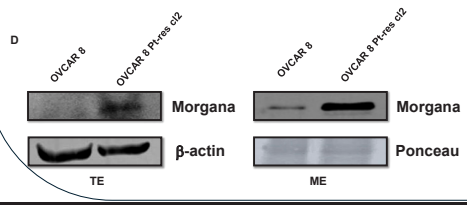
Lombardi R et al.& Budillon A. Cell Death Dis, 2026

4. Identification of Resistance-Linked Proteins via HSP90 Co-Immunoprecipitation



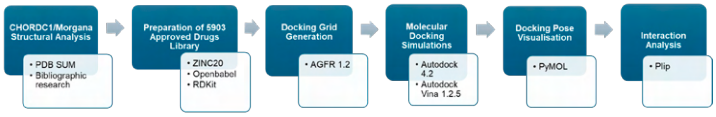
Given the absence of clinically approved HSP90 inhibitors, we performed proteomic profiling of HSP90 complexes in EOC OVCAR 8 Pt-res cl.2 to directly map HSP90's functional network, identifying its client proteins specific to the resistance context. We generated Venn diagram which allowed visualization of shared and unique protein subsets, thereby facilitating the identification of proteins specifically enriched in resistant versus sensitive cells (**Fig. 4A**) . Enrichment analysis, performed exclusively on proteins identified by mass spectrometry in resistant cells (190 proteins), highlighted key co-chaperones, including Morgana (CHORDC1), BAG2 and AHSA1. STRING network analysis further confirmed their functional connection to HSP90, suggesting their role in resistance-associated processes (**Fig. 4B**).





Immunofluorescence (**Fig. 4C**) and Western blot (total extract, TE) (**Fig. 4D**) analysis corroborate the overexpression of Morgana in the OVCAR 8 Pt-res cl2 model compared to parental cells. Moreover, we also demonstrated an increased secretion of Morgana in the medium (ME) of OVCAR 8 Pt-res cl2 cells compared to sensitive ones, highlighting its extracellular activity (**Fig. 4D**).

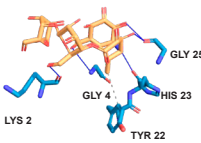
5. Virtual Screening to identify compounds able to block the binding between MORGANA and HSP90



A

B. ENERGY 1	B. ENERGY 2	AUTODOCK COMPOUND (A)	Approved	Approved indications
-12.45	-12.88	A_1 COMPOUND	YES	Type 2 diabetes mellitus
-10.97	-11.45	A_2 COMPOUND	YES	Serious bacterial infections
-10.41	-10.3	A_3 COMPOUND	YES	Interstitial cystitis
-10.33	-9.99	A_4 COMPOUND	YES	Bacterial infections
-9.94	-9.96	A_5 COMPOUND	YES	Hypertension, heart failure
B. ENERGY 1	**B. ENERGY 2**	**AUTODOCK VINA COMPOUND (B)**	**Approved**	**Approved indications**
-8.438	-8.463	B_1 COMPOUND	YES	Chronic hepatitis C (antiviral combination)
-8.03	-7.972	B_2 COMPOUND	YES	Chronic myeloid leukemia Ph+
-7.84	-7.625	B_3 COMPOUND	YES	HER2+ breast cancer
-7.613	-7.579	B_4 COMPOUND	YES	Immune thrombocytopenia, etc.
-7.547	-7.545	B_5 COMPOUND	YES	Type 2 diabetes mellitus
-7.547	-7.514	B_6 COMPOUND	YES	Photodynamic therapy in tumors
-7.532	-7.552	B_7 COMPOUND	YES	Vitamin deficiencies, nutraceutical use
-7.526	-7.541	B_8 COMPOUND	YES	asthma, allergic rhinitis
-7.509	-7.545	B_9 COMPOUND	YES	Migraine

B



Interaction Analysis. The image illustrates a molecular docking example showing how compound A_1 engages the target interface (CHORD2).

The CHORD2 domain of MORGANA, which mediates interaction with HSP90, was modeled with Modeller 10.2 using the CHORDC1 structure as a template, and the HSP90–CHORD2 complex was predicted by molecular docking. This analysis identified a potential binding pocket suitable for virtual screening of a 5,903-compound library using AutoDock 4.2 and AutoDock Vina 1.2.5, each run in duplicate. Among the top-ranked hits, 9 FDA-approved molecules were consistently identified by AutoDock Vina and 5 by AutoDock (**Fig. 5A**). Interaction profiling with the PLIP tool revealed key binding residues and interaction patterns, including hydrogen bonds and Van der Waals contacts, within the predicted pocket (**Fig. 5B**).

METHODS

Cell proliferation assay. Cell proliferation was quantified using the Sulforhodamine B (SRB) assay, which measures total protein content as an indicator of cell growth. **3D microtissue assay.** Cell viability in 3D models was evaluated using microtissues co-cultured with NIH fibroblasts for 96 hours. Viability was measured using the CellTiter-Glo® 3D Cell Viability Assay (Promega), with luminescence read on the Opera Phoenix system (PerkinElmer). ***In vivo* xenograft studies.** Mice were randomized into four treatment groups (n=6) once tumors became palpable. Treatments were administered intraperitoneally once per week and included cisplatin (2.5 mg/kg in PBS), Ganetespib (30 mg/kg in 10% DMSO, 40% PEG, 5% Tween 80, 50% ddH_2O), and Temsirolimus (20 mg/kg in 10% DMSO, 40% PEG, 5% Tween 80, 45% ddH_2O). **Co-immunoprecipitation followed by LC-MS/MS**. The immunoprecipitation was performed using an anti-HSP90 antibody (Stressmarq). Peptides were analyzed by high-resolution LC-MS/MS Mass Spectrometry (Orbitrap Q-Exactive Plus) and data were processed using Proteome Discoverer and matched against the UniProt/Homo Sapiens database for protein identification. Bioinformatic analyses comprised Venn diagram generation with Venny to compare datasets across conditions, functional enrichment assessment via DAVID software and protein–protein interaction network analysis using STRING. **Protein extraction and western blotting.** The cell pellet was lysed and clarified by centrifugation while medium (ME) was collected and concentrated at 4,000 × *g* by centrifugation with Amicon Ultra Filter 10 Kda. Equal amount of protein, monitored by Bradford assay, was separed on 10% SDS polyacrilamide gel electrofoporesis (PAGE). **Immunofluorescence assay.** Cells were cultured for 48 h, then fixed and permeabilized. After blocking, samples were incubated with anti-CHORDC1 primary antibody (Genetex), followed by Alexa Fluor 568–conjugated secondary antibody. Images were acquired using the Opera Phenix system and analyzed with Harmony software. **In *silico* virtual screening.** In *silico* virtual screening targeting the CHORDC1–HSP90 interface was performed following structural analysis of CHORDC1/MORGANA using PDB SUM and literature review. A library of 5,903 approved drugs was obtained from ZINC20, OpenBabel and RDKit tools. Docking grids were generated using AGFR 1.2, and molecular docking simulations were carried out with AutoDock 4.2 and AutoDock Vina 1.2.5 [5] [6]. Docking poses were visualized using PyMOL, and compound–target interactions were analyzed with PLIP.

CONCLUSIONS

- Our findings highlight the therapeutic potential of a triple-drug combination targeting HSP90 and mTOR pathways to overcome platinum resistance across multiple tumor types, including EOC, NSCLC, and HNSCC [4].
- Given the lack of FDA-approved HSP90 inhibitors, the identification of Morgana, BAG2 and AHSA1 as putative resistance-associated HSP90 co-chaperones represents a promising strategy to overcome current therapeutic limitations in platinum-resistant cancers.
- Our data demonstrate that Morgana protein is overexpressed and abundantly secreted in OVCAR-8 Pt-resistant cells compared with their sensitive counterparts and Virtual screening of the Morgana–HSP90 complex led to the identification of 14 FDA-approved compounds, many with non-chemotherapeutic indications.
- Validation of the additional co-chaperones, BAG2 and AHSA1, is currently ongoing. In parallel, *in silico* analyses of their complexes with HSP90 will expand this strategy and enable a broader, comparative evaluation.
- Compounds emerging from these analyses will be prioritized through high-throughput screening to identify candidates with optimal efficacy and safety profiles. Altogether, this approach underscores the potential of drug repurposing as a rapid and cost-effective strategy to accelerate the development of novel therapeutic options aimed to overcome platinum resistance.

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#iDR26

Hypothesis-driven drug repurposing identifies new potential low-toxicity treatment strategies for acute myeloid leukemia patients

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Can **non-oncological** drugs with adverse effects on blood cells be **repurposed** as novel therapies in myeloid **leukemia**?

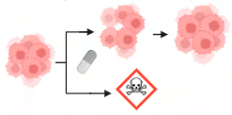
BACKGROUND

Acute myeloid leukemia (AML)

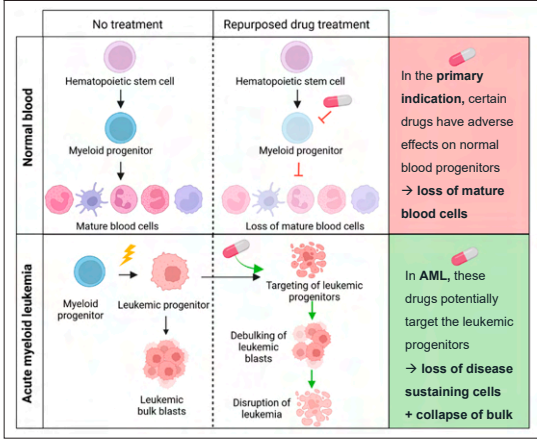
- Malignancy of blood and bone marrow
- Higher age = worse prognosis
- 5-yrs survival (>60 yr) is <10%
- 1% of new cancer incidences

Leukemia inhibits normal blood cell production

Treatments for older patients have high toxicity and often result in relapse



HYPOTHESIS



SUMMARY

Hypothesis-based selection ensure refined compound evaluation compared to unbiased drug libraries
- 10 of 21 single drugs showed patient specific effect

Identified repurposed combinations with antileukemic effect
- **Chloramphenicol** (antibiotic)**+valproic acid** (antiepileptic)
- **Chloramphenicol + phenylbutazone** (NSAID)

Combinations **target leukemic progenitors** potentially lowering treatment toxicity and supporting therapeutic benefit

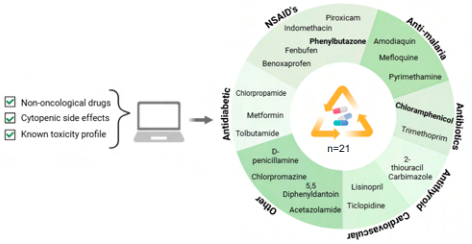
Combinations have **promising effect in relapse/refractory** disease, holding great potential for treatment of more patients

Ongoing work

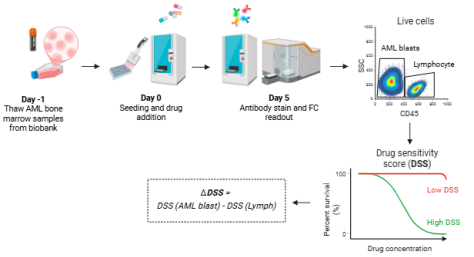
- In vivo testing
- Biomarker evaluation

RESULTS

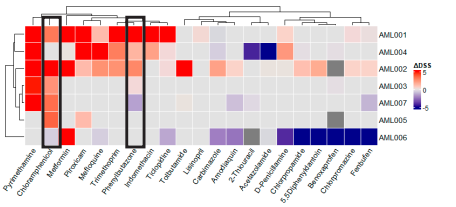
1. Hypothesis-based identification of potential drugs for repurposing



2. Testing antileukemic activity of hypothesis-selected compounds by high-throughput drug screening

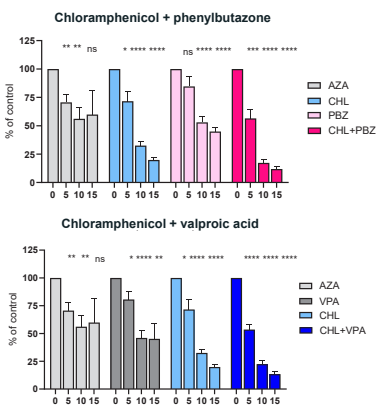


Drug screening workflow performed with bone marrow samples from AML patients. The drug sensitivity score (DSS) of phenotypic populations was used to determine the ΔDSS.



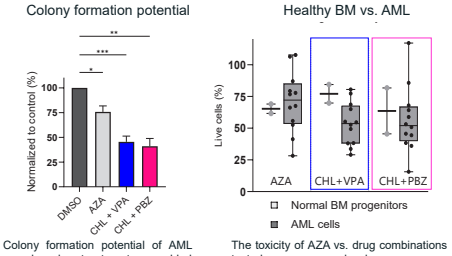
Heatmap of ΔDSS for drugs tested on patient samples (n=7). Multiple single drugs present antileukemic effects in a patient-specific manner. We selected chloramphenicol (**CHL**) and phenylbutazone (**PBZ**), black boxes, for further testing.

3. Combinations of low-toxicity drugs enhance antileukemic effects in long-term cultures



Drug effects on the expansion of AML patient samples (n=12) during 15 days of culture. Azacitidine (**AZA**) used as a treatment control. CHL tested in combination with PBZ and with valproic acid (**VPA**). VPA is an anti-epileptic drug commonly investigated in novel repurposing combination strategies.

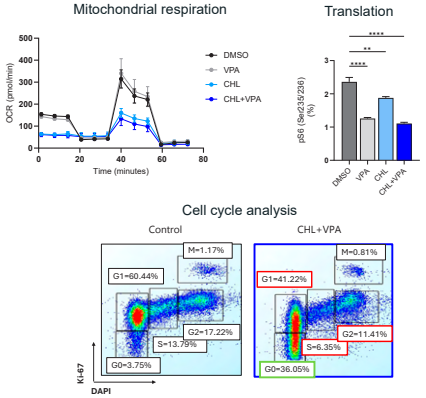
4. Combination treatments target leukemic progenitors and show therapeutic window



Colony formation potential of AML samples when treatments are added directly to the Methocult. Readout day 14 by colony counting.

The toxicity of AZA vs. drug combinations tested on normal bone marrow progenitors compared to AML blasts.

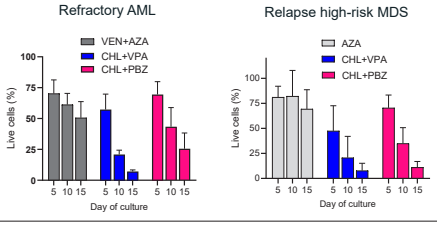
5. CHL +VPA combination decreases OXPHOS-dependency, protein translation, and cell cycling



The AML cell line MOLM-13 was exposed to drugs for 72 hrs. Seahorse determined changes in mitochondrial respiration as a measure of OXPHOS, staining for phosphorylated ribosome S6 was performed to measure specific translation activity, and co-staining with Ki-67 and DAPI was used to investigate the treatment effect on the cell cycle.

6. Additional therapeutic potential of repurposed combination treatments

Ex vivo treatment of AML samples (n=5) refractory towards standard treatment with venetoclax (**VEN**) + AZA shows strong sensitivity to our combination treatments (**Left**). Additionally, we observed a great treatment effect in patient samples from another myeloid malignancy, MDS (n=4), after relapse from AZA treatment (**Right**).



ACKNOWLEDGEMENT & FUNDING

This project has received funding from The Danish Cancer Society grant R343-A19704, the Danish Comprehensive Cancer Center national research center for precision medicine in blood cancers, grant R223-A13071, Advokat Bent Thorbergs Fond, and Læge Sofus Carl Emil Friis og Hustru Olga Doris Friis' legat.

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BRIC Biotech Research & Innovation Centre

Danish Cancer Society

KNÆKCANCER

Advokat Bent Thorbergs Fond

Læge Sofus Carl Emil Friis og Hustru Olga Doris Friis' Legat

IDR26: NAVIGATING THE FUTURE
12-13 May 2026
Brussels

Formulation of 3D-Printed Losartan Potassium Tablets and Orodispersible Films

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Scope

There is growing evidence that the well known anti-hypertensive drug, Losartan may mitigate systemic disease progression in children with recessive dystrophic epidermolysis bullosa[1]. 3D printing (3DP) addresses challenges like individualized dosing, drug application difficulties, patient individualized drug therapy, drug repurposing and drug shortages.

Aim:

- **Develop 3D printed Losartan Potassium preparations**
- **Determine 3DP parameters**
- **Observe the influence of parameter changes on quality**

Materials and Methods

Materials

Losartan Potassium (LP) (Zhejiang Huahai Pharmaceutical Co., Ltd) was used with Polysorbate 80 (Caesar & Loretz GmbH) in Curablend® gel tablet excipient base and Curablend® Orodispersible Film (ODF) excipient base (CSS; CurifyLabs Oy, Helsinki, Finland). Three different LP dosage forms (printlets) were developed using 3D printing as shown in **Table 1**.

Table 1: Composition of 3D printed Losartan Potassium preparations

Preparation	Losartan Potassium Proportion (% m/m)	Polysorbate Proportion (% m/m)	Excipient Base Proportion (% m/m)	Printable doses
2% LP gel-tablets	2	1	97	4-20mg
10% LP gel-tablets	10	2.5	87.5	20-100 mg
0,5% LP ODF	0,5	1	98.5	1- 4.5 mg

Method of Preparation

Gel-tablet: Losartan Potassium and Polysorbate 80 were weighed into a mixing jar with CuraBlend® gel tablet excipient base mixed at 2800 rpm in a Gako planetary mixer for 10 minutes. During this time the base melts through internal friction and thereby forms a viscous liquid.
ODF preparation: Losartan Potassium was suspended with Polysorbate 80; then ODF excipient base was gradually added to the pestle and mortar.

Each preparations were transfered into a dedicated syringe and placed into a printer for printing them into the Blister. An order was created for each specific preparation and dose.

Results

- **Preparation specific Blueprint created with the 3DP parameters.**
- **At total of 226 printlets were printed.**
- **Printlet weights were analysed with a built-in scale.**
- **All printlets met the Ph. Eur. (2.9.5.) Uniformity of Mass of Single-Dose Preparations requirement.**

The doses of the LP were adjusted by the size of the printlets. The printed tablets and ODF can be seen in **Figure 1.** The used printing parameters are detailed in **Table 2.**

Figure 1: Losartan 3D printed preparations

Table 2: Printing parameters and printed amounts of Losartan Potassium printlets

Preperation	Nominal density	Retraction amount	Extrusion temperature	Printed doses :Number of printlets
2% LP gel-tablets	1.14 g/ml	0.27 mm	41°C	4 mg: 43
				9 mg: 5
				10 mg: 8
				12 mg: 5
				20 mg: 33
10% LP gel-tablets	1.156 g/ml	0.3 mm	41°C	20 mg: 36
				50 mg:16
				100 mg: 16
0,5% LP ODF	1.1 g/ml	0.15 mm	25°C	1 mg: 20
				2 mg: 20
				2.5 mg: 5
				4.5 mg: 19

The content of 4 mg and 20 mg 2% LP gel tablets was measured using RP-HPLC. **(Table 3).**

Fulfilled specifications:

- **Ph. Eur. 2.9.6.:** Uniformity of Content of Single Dose Preparations
- **Ph. Eur. 2.9.40.:** Uniformity of Dosage Units: Content Uniformity

Table 3: Results of HPLC analysis of 3DP 2% LP

2% LP gel-tablet dosage form Nominal API content	Mean API content (quantified by HPLC)	Content Uniformity (Ph. Eur. 2.9.40) Acceptance Value (Specification: <15)
4 mg	97.67%	7.38 (fulfilled)
20 mg	97.65%	6.21 (fulfilled)

Discussion

Three different 3D printed Losartan Potassium preparations are produced in a feasible and reliable manner. Further analytical methods must be developed and validated, and tests have to be carried out to ensure the quality, safety and efficacy the dosage forms.

References
[1] Yildiz, G., Schauer, F., Gewait, S., Reinecke, K., Reimer-Taschenbecker, A., Schwegler-Brief, A., Ott, H., Schreiner, C., Grishina, O., Murrell, D., Stäter, B., Zahn, T., Nyström, A., & Bruckner-Tuderman, L. (2024). Safety and tolerability of losartan in treat recessive dystrophic epidermolysis bullosa in children (REPLACT): an open-label, single-arm, phase 1/2 trial. eClinicalMedicine, 77. https://doi.org/10.1016/j.eclinm.2024.102900

SHORTENING **BURULI ULCER** TREATMENT IN WESTERN AFRICA: AN UPDATE ON THE **BLMs4BU** TRIAL

BLMs4BU
Beta-lactam containing regimen to shorten Buruli Ulcer therapy

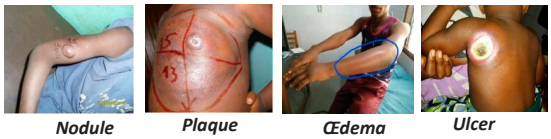
#ICDR26

Roch Christian Johnson [rochchristianjohnson@gmail,com], Emma Sáez-López, Esaï Sèdjro Anagonou, Perrin Catraye, Akimath Habib, Lotus Hotegni, Marlène Alaye, Godwin Gérard Kpoton, Nadège Elegbede, Line-Marlène Ganlonon, Elena Dacal, Zaida Herrador, Anna Gine-March, Isra Cruz and Santiago Ramón-García and **BLMs4BU Consortium blms4bu.org**

BACKGROUND

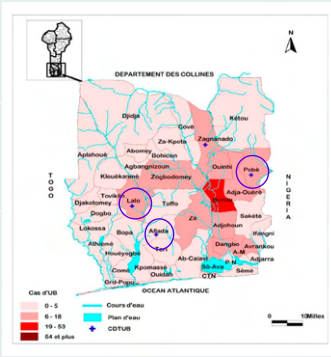
- WHO Buruli Ulcer (BU) standard treatment: oral rifampicin and clarithromycin for 8 weeks
- Need for hospitalization in certain cases with an impact on household income, patient adherence, etc.

Positive impact on BU control expected from a shorter, effective, all-oral regimen based on a combination of Amoxicillin/Clavulanate + standard treatment



Nodule *Plaque* *Œdema* *Ulcer*

STUDY SITES



STUDY GOAL

To assess whether BU treatment could be ***shortened from 8 to 4 weeks*** by co-administration of amoxicillin/clavulanate with current rifampicin-clarithromycin

Primary efficacy outcome: CURE; lesion healing without recurrence & **without excision surgery** 12 months after start of treatment

STUDY DESIGN

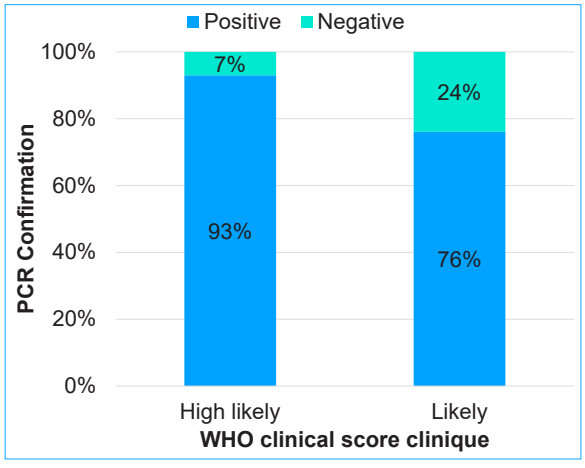
- BLMs4BU clinical trial: multi-country, multi-site, randomized, controlled, open label, non-inferiority (RCA4 vs RC8)
- Screening and eligibility : WHO Clinical score (Very Likely - Likely)
- Stratified by disease severity category I, II, III
- Blind assessment of lesions and need for major excision surgery by an external expert panel (week 14 post-treatment)

12 months follow-up with wound care and disability prevention
10 scheduled visits
Clinical and Safety assessments
PK/PD
IS*2404* qPCR for *M. ulcerans*
Bacterial clearance

RESULTS

Main features

- 152 participants recruited
- 51 % children <15 yo
- 57 % female
- 44 % Cat. III
- 89 % PCR positive
- 66 % Highly Likely score
- 74 % CDTLUB Pobè
- No single MES needed as per TEP assessment



- High PCR confirmation rate
- BU WHO clinical score: valid tool in places where PCR diagnostic capacities are not readily available

RCA4 safety

Only mild, transient adverse events have been observed, with no serious adverse events related to the study medication

GOAL: Short, all-oral treatment—faster healing, less hospitalization, lower costs, aligned with WHO Road Map for NTDs

No rescued patients

Funding and support:
tres cantos OPEN LAB FOUNDATION
anesvad FOUNDATION
CuresWithinReach
GSK
UCL

Partners:
CIRED
PNLLUB MINISTERE DE LA SANTE
Raoul Follereau
Institut Pasteur de Côte d'Ivoire
DAHW
KCCR
Instituto de Salud Carlos III
Universidad Zaragoza
anesvad FOUNDATION
araid

Repositioning yesterday's drug for tomorrow's vision:
Drug repurposing to promote corneal endothelial regeneration

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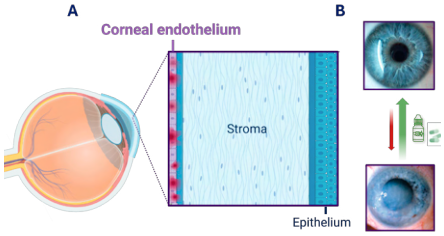


Fig. 1: The human cornea.
(A) A schematic representation of the human cornea, which consists of three cellular layers. The corneal endothelium is the innermost layer.
(B) Comparison of a healthy cornea, which preserves transparency, and a diseased cornea from a patient with Fuchs endothelial corneal dystrophy (FECD), showing loss of corneal clarity. The development of an eye drop formulation may serve as a promising alternative treatment to a corneal endothelial transplantation.

Corneal endothelial dysfunction leads to corneal opacification: one of the most common causes of corneal blindness worldwide

PROBLEM
Global shortage of donor corneas: only 1 available for every 70 patients on the waiting list

SOLUTION
Discovery of a small molecule compound with the ability to enhance the regenerative capacity of corneal endothelial cells

METHODS
A high throughput screening (HTS) pipeline to identify small molecules that promote corneal endothelial cell growth

RESULTS
13 of the 33 primary repurposing hits (1.6% primary hit rate) are currently confirmed and tested in secondary confirmation assays

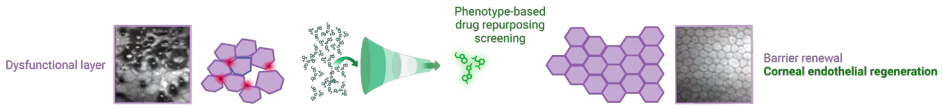


Fig. 2: Drug repurposing as a potential strategy to identify small molecules for corneal endothelial barrier renewal.
The corneal endothelium is a monolayer of hexagonal cells. The general consensus states that these human corneal endothelial cells (HCEnCs) are arrested at the G1/S transition of the cell cycle. Therefore, cell loss is not compensated by cell division. To date, there are no FDA- or EMA-approved drugs that stimulate the regeneration of these cells in patients with corneal diseases, such as Fuchs endothelial corneal dystrophy (FECD). In that regard, we explore the repurposing of drugs to promote corneal endothelial regeneration, i.e. proliferation and migration.

1. *Background* - Corneal endothelial research

The ***human corneal endothelium*** is a single layer of hexagonal cells located on the innermost surface of the cornea and plays a ***crucial role in safeguarding corneal transparency***.

Dysfunction of this critical layer leads to corneal edema, i.e. bullous keratopathy and eventually progresses to ***corneal blindness***. An endothelial transplantation is currently the only available treatment option for patients suffering from corneal endothelial dysfunction. However, the ***global shortage of donor tissue*** forces R&D into exploring other potential solutions, such as a ***pharmacological alternative***, which aims to restore vision without the need for graft tissue.

To address this alternative treatment option, we developed -- ***a high throughput screening (HTS) pipeline*** -- to identify small molecules that ***promote corneal endothelial cell growth***.

Specifally, we aim to explore the repositioning, i.e. ***repurposing of existing drugs***. This strategy holds the potential to facilitate the drug discovery process in preclinical reseach focused on corneal endothelial regeneration.

2. *Methods* - HTS screening

Primary screening
Enzo® and Prestwick® drug repurposing chemical libraries

Image-based phenotypic proliferation assay of B4G12 corneal endothelial cells: Endpoint nuclear count analysis (Hoechst)

3 concentrations: 50 µM, 5 µM and 50 nM

↓

Hit identification
Hit threshold: top 2% of the hit ranking

↓

Confirmation screening
Drug repurposing primary hit selection

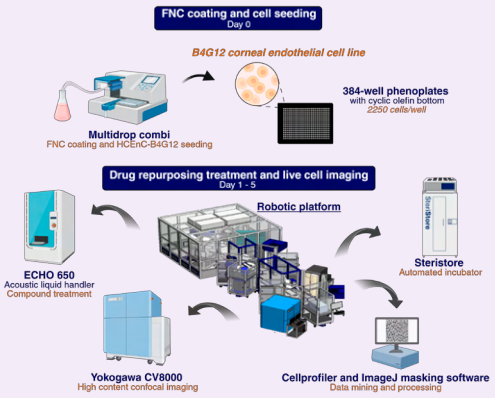
Image-based phenotypic proliferation assay of B4G12 corneal endothelial cells: Time-lapse analysis for 5 days

Concentration range: 50 µM - 10 nM

↓

Secondary confirmation assays
Drug repurposing secondary hit selection

In vitro scratch wound migration assay: Time-lapse analysis for 5 days



3. *Results* - Small molecule drug discovery for corneal endothelial regeneration

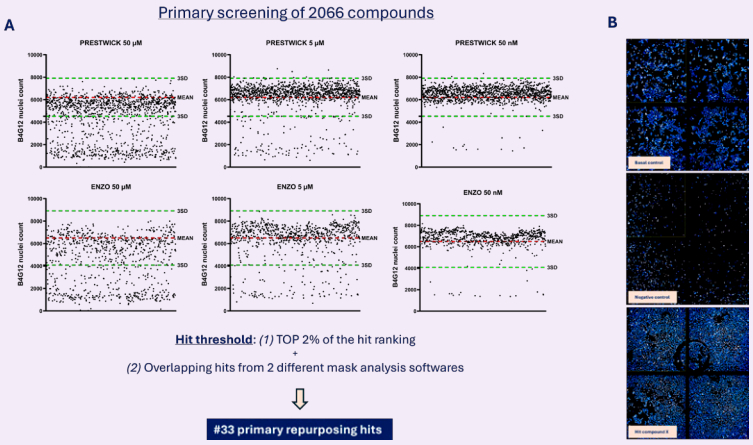


Fig. 3: Primary screening of the Enzo® and Prestwick® repurposing chemical libraries.
(A) Graphs represent the results of the primary screening using the ENZO® and PRESTWICK® chemical libraries, each tested at three different concentrations. Data from three biological replicates are presented as dot plots, with each dot representing the mean value of a specific compound. The dotted line indicates the mean of the control DMSO treated group. ***(B)*** Example of the endpoint nuclear count analysis. Four fields per well are covering more than 80% of the total well surface in a 384-well plate. Representative images compare the DMSO control, the negative control (staurosporine), and compound X, the latter significantly enhances the growth of the HCEnC-B4G12 cells.

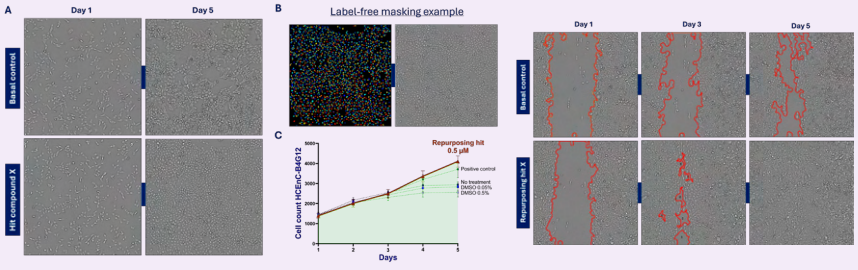


Fig. 4: Confirmation screening of the primary hit selection.
(A) Digital phase contrast phenotypic proliferation assay over 5 days. Images show an example of the HCEnC-B4G12 cell confluency after seeding versus endpoint for the basal control and after treatment with hit compound X. ***(B)*** Example of the Cellprofiler customized masking pipeline for cell counting. ***(C)*** Graph illustrates the growth of HCEnC-B4G12 cells over 5 days for a selected repurposing hit X compared to the control conditions. The compound treated cells exhibit enhanced growth rates.

Fig. 5: In vitro scratch wound migration assay as a secondary confirmation assay.
Optimization of an imageJ customized scratch wound masking pipeline. Representative images of the scratched area were captured at 1, 3 and 5 days post-treatment. Cells treated with compound X exhibited significantly enhanced wound closure compared to the control group.

4. *Conclusion* - Future repurposing directions

Our findings show **promising repurposing results for the stimulation of corneal endothelial regeneration**, showing trends similar to Y-27632 (25 µM) and chroman-1 (10 nM), 2 ROCK inhibitors used as evidence-based, positive controls for boosting the regenerative capacity of corneal endothelial cells.

Moreover, our results highlight the relevance for future compound characterization and downstream pathway identification as well as the need to test the confirmed hits on primary corneal endothelial cell culture.

Perceived impact and actionability of barriers to drug repurposing: Insights from a multi-stakeholder policy survey

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INTRODUCTION

- Drug repurposing (DR) - identifying new therapeutic indications for existing approved or investigational drug substances - has emerged as an important strategy to reduce development costs in comparison with de novo drug discovery.[1]
- Policy barriers to successful DR remain pervasive across the drug development ecosystem.[2]
- A systematic literature review undertaken by the REMEDi4ALL Horizon Europe project identified 33 policy-related barriers.[3]

METHODS

- We developed an online policy survey.
- Participants were asked to weigh the impact and actionability of all barriers from their perspectives, using 5-point categorical scales.
- The barriers were grouped by categories, and participants were allowed to skip whole themes (therefore not every participant weighted every barrier).
- The responses of the participants were converted into numerical values.
- The barriers were prioritized by a weighted combination of the scores from the two domains.

RESULTS: SURVEY COMPLETION

- Participants' characteristics are shown in *Table 1.*, completion rates for each theme can be seen on *Figure 1.*

Table 1. Characteristics of the participants

Total participants	60	100%
Participants by stakeholder group		
HTA, healthcare payer, regulator	11	18.3%
Patient representatives	6	10.0%
Funders (Philanthropic or public funder of DR)	12	20.0%
Pharmaceutical industry (pharmaceutical companies, biotech and SME, industry association, consultant, venture capitalist)	17	28.3%
Researchers (researchers, academia and clinicians)	14	23.3%
DR Expertise/perspective of participants by geographical distribution		
EU countries before 2004 (EU15)	31	51.7%
EU countries after 2004 (EU13)	12	20.0%
Other (USA, Switzerland, Ukraine, UK)	17	28.3%

HTA - health technology assessment; SME - small-medium sized enterprises; EU - European Union; USA - United States of America; UK - United Kingdom

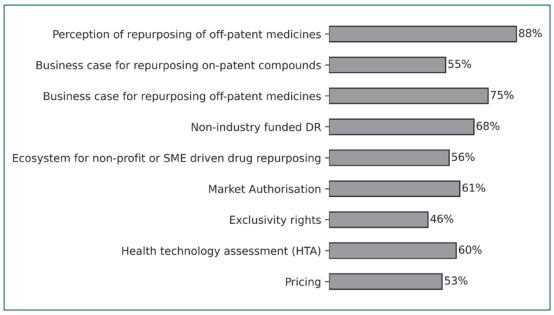

Figure 1. Response rates for each theme, in order of their appearance in the survey (% of all respondents)

OBJECTIVES

- To prioritize the identified barriers with the involvement of multiple stakeholder groups.
- To develop a shortlist of barriers that are considered most important based on the result.
- To examine the differences between stakeholder groups' perceptions on the most important barriers.

RESULTS: BARRIER SHORTLIST

- The final shortlist contained 22 barriers.
- The highest ranked barriers varied across different stakeholder groups, with the patient representatives' preference showing the greatest divergence from other groups' preferences.

Table 2. Shortlist of Barriers and their Heatmap based on Stakeholder Groups' Perceptions of the Barriers

Barrier	HTA, healthcare payer, regulator	Patient representatives	Funders	Pharmaceutical industry	Researchers
Generic pricing mechanisms are often applied to off-patent repurposed medicines.	red	grey	grey	red	red
Limited market protection and data exclusivity options for repurposed medicines.	grey	grey	grey	red	yellow
Limited incentives to turn off-label use to on-label to ensure access to a wider patient population.	red	grey	orange	yellow	white
Insufficient return on investment is anticipated for repurposing off-patent medicines.	grey	white	white	orange	orange
Competitors can benefit from the DR investment in case of off-patent medicines by cross-label prescribing and dispensing.	grey	white	orange	white	orange
Limited, incomplete and fragmented funding is available for non-profit DR at different stages.	grey	orange	red	grey	white
Indication-based differential pricing for repurposed medicines is problematic.	white	grey	grey	orange	orange
Evidence requirement for HTA is not designed for off-patent DR and is of high burden.	white	white	white	red	white
For label-extension, there is a need for marketing authorisation holder's involvement for non-MAH developers.	orange	grey	red	white	red
There is a lack of clarity/limited awareness on evidence requirements for some off-patent drug repurposing cases.	orange	grey	red	white	white
Enforcement of market protection for repurposed medicines is difficult because of cross-label prescribing and dispensing.	grey	grey	grey	yellow	white
Lack of findable, accessible, interoperable, and reusable (FAIR) data (especially proprietary data) for DR.	grey	yellow	white	white	yellow
No tailored or predictable technology appraisal process exists for off-patent DR.	white	yellow	yellow	orange	white
Incentives for market authorization of repurposed medicines in paediatric indications are not proportionate to the required efforts for evidence generation.	yellow	grey	white	yellow	white
Evidence generation is burdensome for the market authorisation of off-patent medicines.	orange	grey	white	white	red
Enforcement of patent protection for repurposed medicines is difficult and costly.	grey	grey	grey	white	yellow
DR research of off-patent medicines is perceived as less-innovative, less robust or less attractive compared to de novo drug development.	yellow	red	white	white	white
Cost of DR development is perceived to be disproportionally high compared to the risks and potential revenues.	red	white	white	white	white
Limited options for patent protection of repurposed medicines.	grey	grey	grey	white	white
Public-private partnerships in funding DR are complex and not always possible.	grey	orange	white	grey	white
The know-how needed for DR may not be available for non-profit entities or SMEs.	grey	red	white	white	white
Originator companies often lack incentives to repurpose on-patent compounds due to low expected return on investment and strategic business decisions regarding their disease portfolio.	grey	red	grey	white	white

Colour code: grey - answered by less than 50% of participants from the stakeholder group; white - ranked 10th or lower within the stakeholder group; yellow - ranked between 7th and 9th within the stakeholder group; orange - ranked between 4th and 6th within the stakeholder group; red - ranked among the top 3 within the stakeholder group

DR - drug repurposing; HTA - health technology assessment; MAH - market authorisation holder, SME - small medium-sized enterprise

POLICY IMPLICATIONS

- Prioritization of barriers can facilitate the development of solutions by focusing on the most critical challenges first.
- Recognition of stakeholder groups' different perceptions on the impact and actionability of barriers is essential in creating a shared multi-stakeholder understanding when developing policy recommendations to address the most pressing obstacles to DR.

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INTEGRATING *IN SILICO* PRIORITIZATION AND DRUG REPURPOSING TO TARGET NOTCH1 IN CHRONIC LYMPHOCYTIC LEUKEMIA

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is an incurable B-cell malignancy characterized by the accumulation of mature but functionally impaired lymphocytes. Although **targeted therapies** such as venetoclax and ibrutinib have significantly improved patient outcomes, **treatment resistance** remains a major clinical challenge. The **NOTCH signaling pathway** is **constitutively activated** in CLL cells and contributes to cell survival, proliferation and therapy resistance, making it a **promising target** for therapeutic intervention. Moreover, the NOTCH1 receptor was identified as strongly associated with CLL by the *in-silico* disease-association platform OpenTargets, underscoring the rationale for further investigation into its role in CLL pathogenesis.

MATERIALS AND METHODS

***In vitro/ex vivo* efficacy assessment**

Compounds inhibiting the distinct steps of the NOTCH signaling pathway were assayed for cytotoxic activity *in vitro* using the **MEC-1** and the venetoclax resistant **MEC-1 VER** chronic lymphocytic leukemia (CLL) cell lines. Additionally, their activity was assessed *ex vivo* in **primary CLL cells** (N=14) obtained from patients after informed consent. Cytotoxic effects were quantified via **resazurin-based metabolic activity assays**.

NF-κB signaling assay

Given the constitutive activation of the NF-κB signaling pathway in CLL, compound-mediated effects on NF-κB nuclear translocation were further evaluated by AMNIS ImageStream **imaging flow cytometry**. **Nuclear translocation** of NF-κB p65 was measured in MEC-1 cells, which were treated with each compound and incubated for 1 h, then **activated with PMA/ionomycin** (10 mM/1 μM) and incubated for another hour. The cells were subsequently fixed and their nuclei and p65 stained with DAPI & AlexaFluor 647, respectively. Translocation is determined by the shape similarity and colocation of DAPI and AF647.

RESULTS

NOTCH Signaling Inhibitors Are Cytotoxic to CLL Cell Lines

Because NOTCH1 is not directly druggable due to its mode of action, inhibitors targeting distinct steps of the signaling pathway were selected.

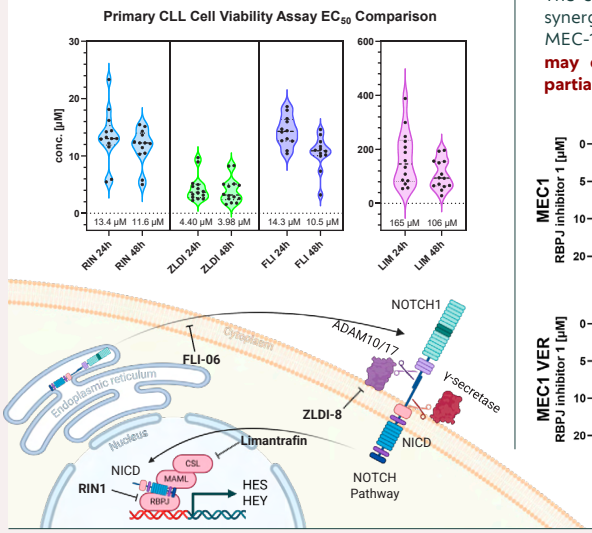
In 24 h resazurin-based viability assays, ADAM17 inhibitor **ZLDI-8 demonstrated the highest potency**, with EC_{50} values of 2.93 μM in MEC-1 cells and 4.8 μM in **venetoclax-resistant MEC-1 VER** cells (EC_{50}[venetoclax] ≈ 71 μM).

RBPJ-inhibitor-1 (RIN1) exhibited **minimal sensitivity to venetoclax resistance**, with EC_{50} values (24h/48h) of 20.3 μM/12.5 μM and 18.2 μM/14.9 μM in MEC1 and MEC1 VER cells, respectively.

Drug / EC_{50}[μM]	MEC1 (24h)	MEC1VER (24h)	MEC1 (48h)	MEC1VER (48h)
RIN1	20.3	18.2	12.5	14.9
ZLDI-8	2.93	4.8	2.78	5.1
FLI-06	10.1	23.9	4.5	4.1
Limantrafin	57.4	142	34.7	73
Venetoclax	**9.6**	**70.9**	**6.8**	**18.9**

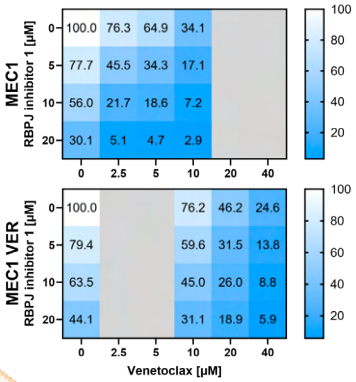
Primary CLL Cells Are Susceptible to NOTCH Signaling Inhibition

RIN1, ZLDI-8, and FLI-6 exhibit **dose and time dependent cytotoxicity** in patients' primary CLL cell lines *ex vivo*. The mean effect of most compounds proved to be **comparable to MEC1** cells.



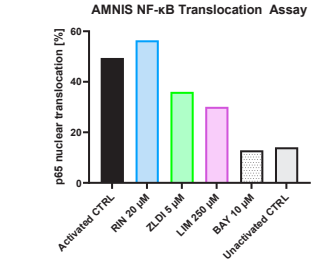
RIN1 Exhibits Synergistic Cytotoxic Activity in MEC1 and MEC1 VER Cell Lines

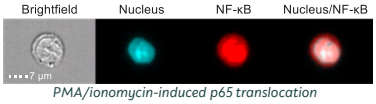
Synergistic interactions between RIN1 and venetoclax were evaluated after 48 h using a resazurin-based viability assay. The combination treatment produced a synergistic effect in both MEC-1 and MEC-1 VER cells, indicating that **RIN1 may enhance venetoclax efficacy** and **partially overcome resistance.**



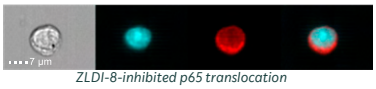
Identified Compounds Suppress PMA/ionomycin Stimulated NF-κB Translocation in MEC1 Cells

Assessment of NF-κB signaling by imaging flow cytometry, based on **p65 nuclear translocation**, revealed compound-specific effects on pathway activity. **ZLDI-8 exhibited the highest potency**, whereas limantrafin was only effective at substantially higher concentrations.





PMA/ionomycin-induced p65 translocation



ZLDI-8-inhibited p65 translocation

CONCLUSION

Collectively, these findings demonstrate that pharmacological **inhibition of NOTCH signaling exerts cytotoxic effects in both CLL cell lines and primary patient samples**, supporting the pathway as a **relevant therapeutic target**. The differential effects on NF-κB signaling indicate that these inhibitors act through distinct and potentially complementary mechanisms that are not solely dependent on NF-κB modulation. Overall, these results highlight the potential of targeting NOTCH signaling **to improve treatment efficacy** and address venetoclax resistance in CLL. Further studies in physiologically relevant ***in vivo* models**, alongside detailed mechanistic investigations, are warranted to validate these findings and support the **development of more effective targeted therapeutic approaches for CLL**.

ACKNOWLEDGEMENTS

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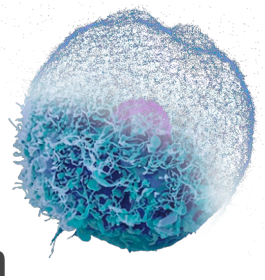

TwinCell: Large Causal Cell Model for Reliable and Interpretable Therapeutic Target Prioritisation

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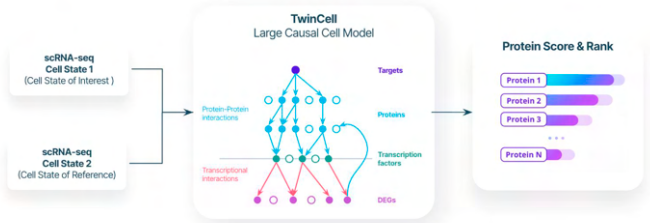
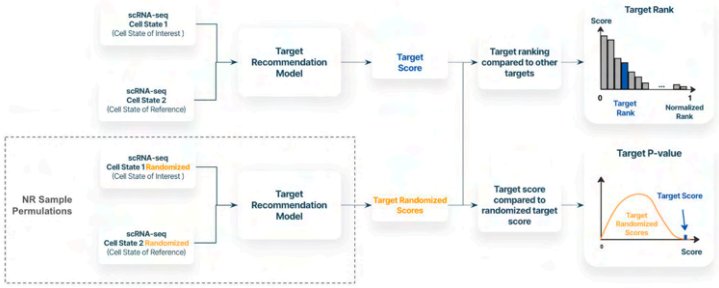
1. Context & Challenges

- Drug development is a complex process requiring many decisions, starting with selecting the **right target** for a specific indication
- Target identification is a **critical area in drug discovery**, where challenges like **cellular heterogeneity** and disease context persist
- Recent advances in single-cell and spatial molecular biology have generated **massive datasets** capturing **compound effects**
- Models trained on these data frequently **fail to generalize** or struggle to outperform simpler linear methods
- Current methods specifically fail to addresses the common "**popularity bias**" challenge found in recommendation algorithms

2. TwinCell & TwinBench methods

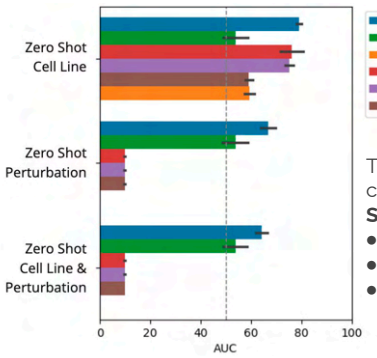


- TwinCell is a Large Causal Cell Model that predicts which driver genes will maximise the transition between two cell states
- From from single-cell foundation models and *in vitro* perturbation data, TwinCell learns a causal cell type specific interactome.
- Twincell predicts which **driver genes** are connected with disease- and cell-type-specific differentially regulated genes



- TwinBench is a benchmarking framework to assess virtual cell models performances
- TwinBench is the first benchmark to correct for popularity bias, heavily present in high dimensional data such as omics data.

3. *In vitro* performance

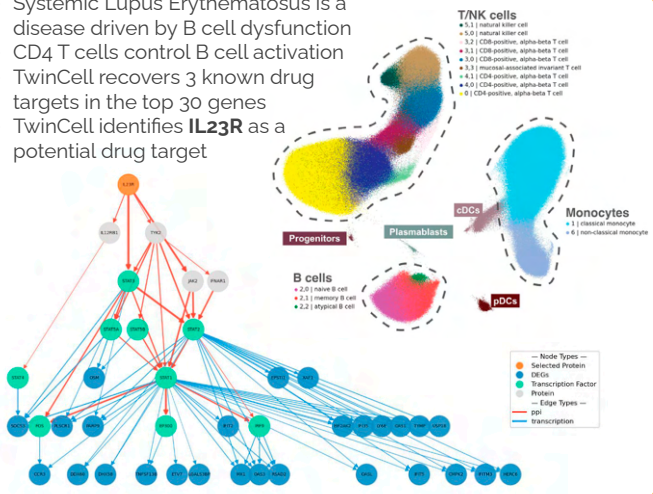


TwinCell with TwinBench correction **outperforms SOTA methods** in 3 scenarios

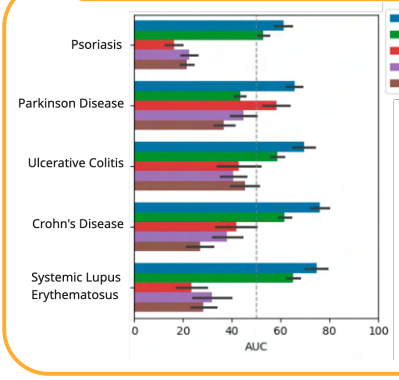
- unseen cell lines
- unseen perturbations
- combination of both

4. *In vivo* performance

- Systemic Lupus Erythematosus is a disease driven by B cell dysfunction
- CD4 T cells control B cell activation
- TwinCell recovers 3 known drug targets in the top 30 genes
- TwinCell identifies **IL23R** as a potential drug target



5. Generalization



- TwinCell retrieves **approved drug targets** for multiple indications
- TwinCell performance is **independent of the tissue**
- TwinCell **outperforms SOTA methods**

6. Conclusions

- Target identification is the **critical starting point** of every drug discovery project
- TwinCell recovers **known targets** as high ranking genes, and generates a **new target hypothesis** for the treatment of SLE
- TwinCell **outperforms SOTA methods** *in vitro* and **generalizes** to new cell types and tissues *in vivo*

Phenotypic screening for mitochondrial therapeutics in patient-derived cells for neurological disorders

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Background

Mitochondrial dysfunction is a key pathomechanism in multiple neurological conditions and restoring mitochondrial function is an attractive therapeutic target. Modulators of mitochondrial function have been successfully identified using phenotypic screening in Parkinson's Disease patient-derived fibroblasts[1]. This study therefore sought to determine whether mitochondrial phenotypic screening could identify beneficial compounds in fibroblasts derived from three different neurological disorders; the primary mitochondrial disorder Leigh Syndrome, the repeat expansion disorder Friedreich's Ataxia in which the expansion affects a primarily mitochondrial protein, and the neurodegenerative condition Huntington's Disease caused by a repeat expansion which does not directly affect a mitochondrial protein.

Hypothesis

This project aimed to test the hypothesis that compounds to rescue mitochondrial function can be identified using high content imaging of mitochondrial phenotypes in fibroblasts derived from patients with Leigh Syndrome, Friedreich's Ataxia and Huntington's Disease

Methods

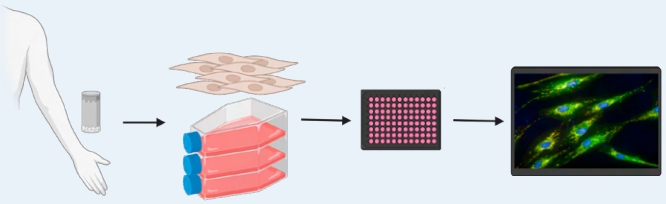


Figure 1. Overview of methods. Fibroblasts derived from healthy control and patient donors were expanded, plated into multi-well plates and assessed using high content imaging, plate reader and respirometry assays to characterise mitochondrial phenotype

High content imaging (HCI) assays: Cells grown in glucose or galactose containing media and stained with mitotracker green to visualise all mitochondria, Tetramethyl rhodamine methyl ester (TMRM) to visualise mitochondria with a membrane potential, and lysotracker to visualise lysosomes. Hoechst was used to stain nuclei. Images captured on an Opera Phenix confocal microscope were analysed using Harmony software to segment individual mitochondria and lysosomes.

Compound testing: Cells were plated into 96 well plates and grown in galactose (LS and HD) or glucose (FA) containing media for 24 hours before the addition of compounds for a further 24 hours before HCI assays.

Table 1: Demographics of healthy control and patient donors whose fibroblasts were used in this study

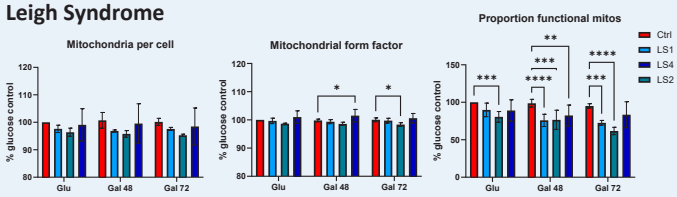
Controls	Gender	Age	Patients	Gender	Age	Mutation
Control 1	M	3	LS1	M	2	SURF1 C.370G>A AND C.751+1G>A
Control 2	F	2	LS2	F	6	SURF1 C.351T>G (P.TYR117*), C.312_321 DEL10INSAT
Control 3	M	7	LS3	F	8	SURF1 (C.845_846DELCT)

Controls	Gender	Age	Patients	Gender	Age	GAA repeat
Control 1	F	29	FA 1	F	36	330/380
Control 2	M	34	FA 2	M	30	541/420

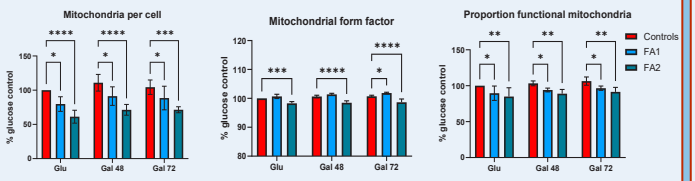
Controls	Gender	Age	CAG repeat	Patients	Gender	Age	CAG repeat
Control 1	M	49	15	HD 1	M	50	17:44
Control 2	M	50	16:19	HD 2	M	49	15:46
Control 3	M	55	12:17	HD 2	F	56	16:43

Mitochondrial phenotypes measured in patient derived fibroblasts

Leigh Syndrome



Friedreich's Ataxia



Huntington's Disease

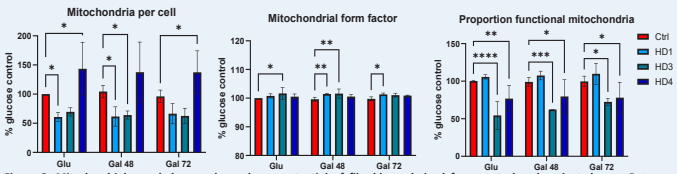
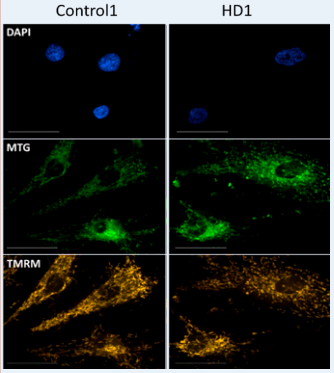


Figure 2: Mitochondrial morphology and membrane potential of fibroblasts derived from control and patient donors. Data are normalised to that of control cells in glucose conditions. Error bars indicate SD and data are from at least 75 cells per condition in each of 3 biological replicates. Significance was analysed by ANOVA with Šidák's multiple corrections test. *<0.05, **<0.01, ***<0.001, ****p<0.0001.



Fibroblasts derived from LS patients display no change in the overall number of mitochondria but a lower proportion of mitochondria with a functional membrane potential.

Fibroblasts derived from FA patients display significantly reduced numbers of mitochondria as well as a lower proportion of mitochondria with a functional membrane potential.

Fibroblasts derived from HD patients display alterations in number of mitochondria which are specific to each patient and a lower proportion of mitochondria with a functional membrane potential in two of three patient cell lines.

Mitochondrial network complexity is varied among individuals with each condition.

Figure 3: Fibroblasts from HD patients and control donors. Cells in glucose conditions stained with Hoechst to visualise nuclei (DAPI), mitotracker green to stain all mitochondria (MTG), and TMRM to stain only mitochondria with a membrane potential. Scale bar represents 50 µm

A769662 enhances the proportion of functional mitochondria in cells from patients with Leigh Syndrome, Friedreich's Ataxia and Huntington's Disease

Tool compounds were selected for reported effects on the mitochondria; Ursodeoxycholic Acid, A769662, Urolithin A and Mitochonic Acid. Compounds were tested in patient derived fibroblasts using the imaging paradigm described. The AMPK activator A769662 enhanced the proportion of functional mitochondria in control and LS patient cells. This activity is likely mediated by A769662 effect on mitophagy.

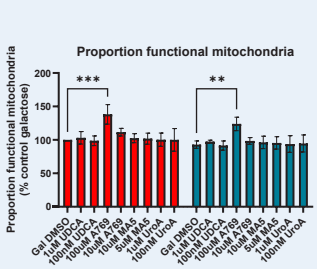


Figure 4: Small molecule effects on LS patient derived fibroblasts. Tool compound screen of Ursodeoxycholic acid (UDCA), A769662 (A769), Urolithin A and Mitochonic acid in 3 LS patient-derived fibroblast cells (3 control lines combined in red, 3 patient lines combined in blue). Data are mean of 3 biological replicates and error bars show SD. Data are normalised to DMSO vehicle control for each experiment. Two-way ANOVA with with Holm-Šídák's multiple comparison test *=p<0.05,**=p<0.01, ****=p<0.0001

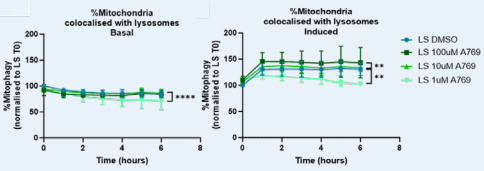


Figure 5: **A769662 enhances induced mitophagy in LS patient derived fibroblasts**. Mitophagy over 6 hours in LS cells pretreated for 24 hours with DMSO or 100, 10, 1 µM A769662 in basal and induced (1 µM Oligomycin, 4 µM Antimycin A) conditions. Mitophagy calculated as percentage of mitochondria colocalised with lysosomes and normalised to T0 of the DMSO condition for each LS patient. Lines represent the mean of three technical and three biological replicates for each cell line. Error bars represent standard deviation. Data analysed by Two-way ANOVA, with Holm-Šídák's multiple comparisons test for treatment effects * = p>0.05, ** = p>0.01, *** = p>0.001, **** = p>0.0001.

A769662 enhances the proportion of functional mitochondria in FA and HD patient derived fibroblasts, indicating potential in multiple neurological disorders

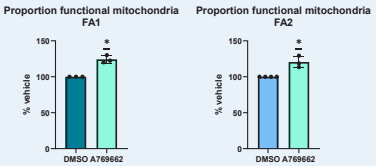


Figure 6: A769662 increases the proportion of functional mitochondria in FA patient derived fibroblasts. Proportion of TMRM positive mitochondria in FA patient cells after 24 hour treatment with either DMSO or 100 µM A769662. Data are mean of 3 biological replicates and error bars show SD. Data normalised to DMSO vehicle control for each experiment. One sample t test, *=p<0.05.

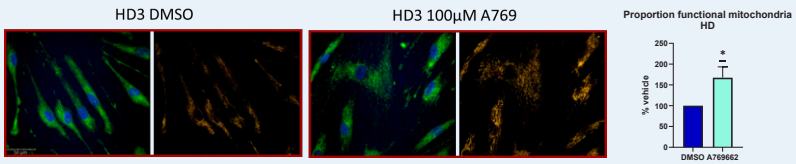


Figure 7: A769662 increases the proportion of functional mitochondria in HD patient derived fibroblasts. HD patient derived fibroblasts grown in galactose conditions for 24 hours followed by 24 hour DMSO (left panel) or 100 µM A769662 (right panel) treatment also in galactose conditions. Cells stained with Hoechst to visualise nuclei (blue), mitotracker green to stain all mitochondria (green) (left box of each panel) and TMRM to stain only mitochondria with a membrane potential (right image in each panel). Scale bar represents 50 µm. Quantified as proportion of TMRM positive mitochondria. Data are mean of 3 biological replicates and error bars show SD. Data normalised to DMSO vehicle control for each experiment. One sample t test, *=p<0.05.

Conclusions: High content imaging used to identify altered mitochondrial phenotypes in patient derived fibroblasts is applicable to multiple neurological disorders. This platform is sensitive to modulation of mitochondrial phenotypes by small mlecules, as indicated by the effects of the AMPK activator A769662.

Our next aims will be to:

- **Confirm whether the effect of A769662 confers functional benefit using respirometry and investigate action via mitophagy in FA and HD**
- **Characterise larger cohorts of patient derived fibroblasts for mitochondrial phenotype and effects of AMPK activation**
- **Generate induced neuronal precursor cells from these cell lines and develop induced neurons and astrocytes in which to confirm compound effects in a disease relevant cell type**

Acknowledgments

I would like to acknowledge Professor Mortiboys and her lab, collaborators at SITraN and the donors who make this work possible.

Thanks to the funders of this work.

References

1. Mortiboys et al. Ursocholanic acid rescues mitochondrial function in common forms of familial Parkinson's disease. Brain 2013 136:10

MPxPISA: Screening of 1000s of FDA drugs against 1000s of targets

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Maximilian Gerwien[1,3]

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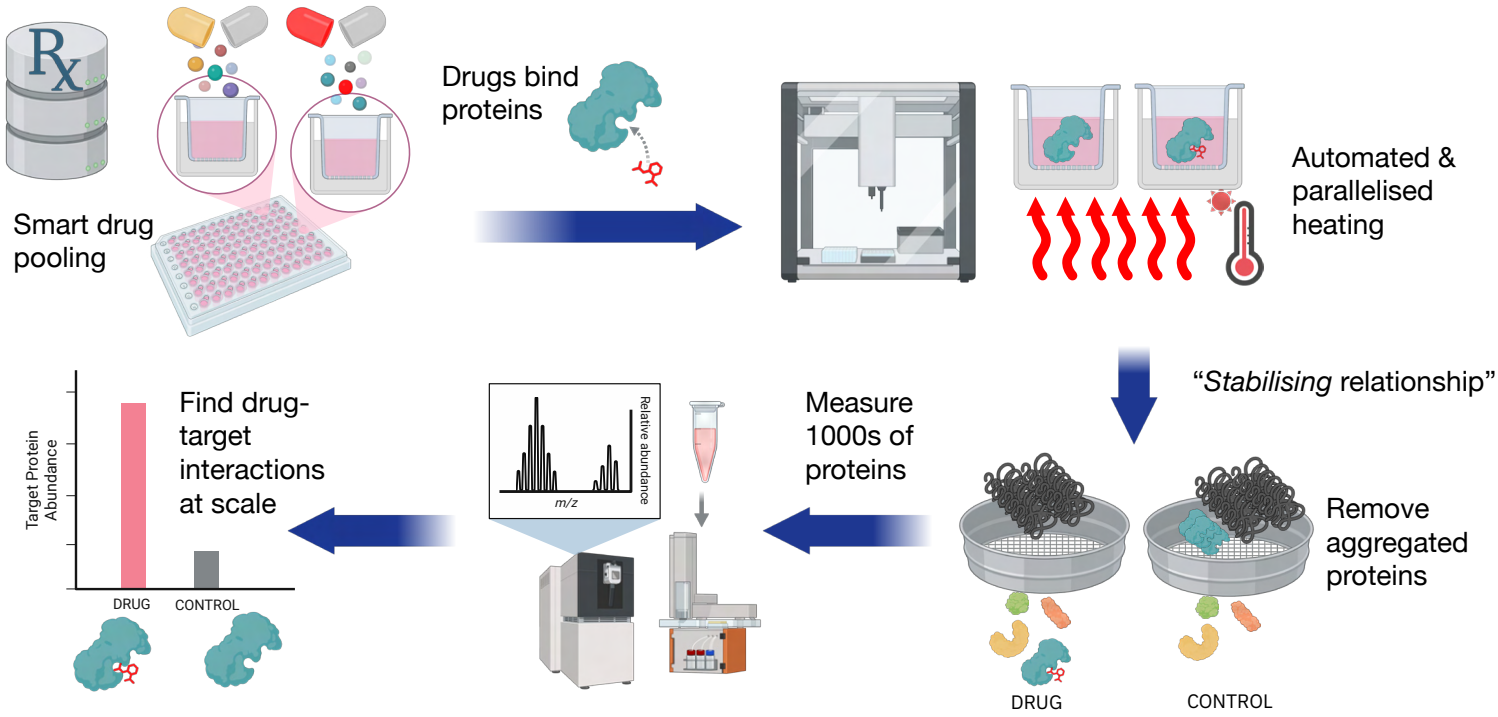
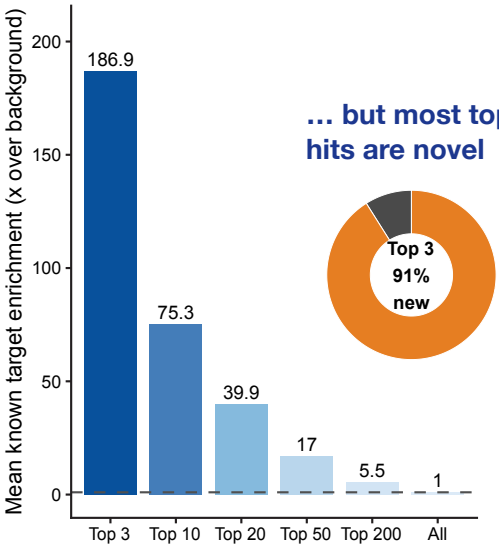
- Many **drugs** work by interacting with **proteins**.
- Identifying those protein targets helps explain how drugs work, why they fail, and how they might be **repurposed**.
- Current target-identification methods are too slow for **large drug collections**.

- **MPxPISA** makes target discovery **fast and scalable** using drug pooling, automation and parallelisation.
- **3,200 drugs** in under **10 days** - many FDA-approved.
- Data can be explored in simple **web app**!

MPxPISA: Target discovery of pooled drugs in a hot proteome bath



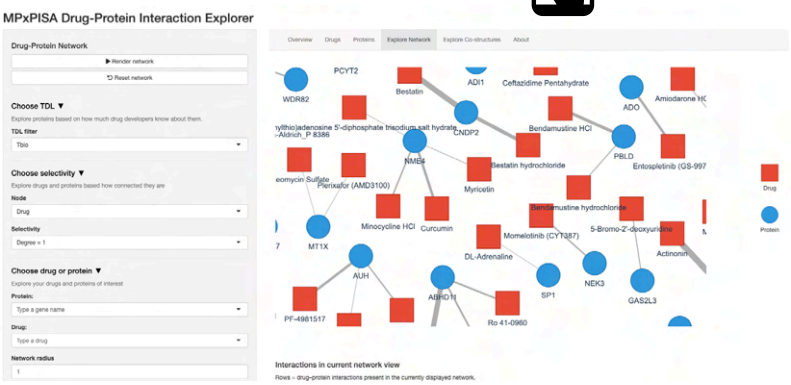
Known drug targets are enriched among top-ranked protein hits…



… but most top hits are novel

MPxPISA Web App

- Use the **Drugs** tab to explore how selected drugs affect proteins
- Use the **Proteins** tab to find drugs that interact with a protein of interest
- Use the **Explore Network** tab to view connections across the wider drug–protein landscape
- Use the **Explore Co-structures** tab to inspect predicted 3D co-structures for selected new drug–protein interactions
- Find me for a live demo of the MPxPISA App!



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Repositioning Saracatinib for Fibrodysplasia Ossificans Progressiva (FOP): From Molecular Discovery to the STOPFOP Clinical Trial

Presented by: Dr Andrew J. Rankin (FOP Friends Trustee)

Eleanor Williams[1], Jana Bagarova[2], Georgina Kerr[1], Vincent A. Verheij[3], Clemens Stockklausner[4], Paul B. Yu[2], E. Marelise W. Eekhoff[3], Alex N. Bullock[1]

1 University of Oxford, 2 Harvard University, 3 Amsterdam University Medical Center, 4 Klinikum Garmisch-Partenkirchen

Abstract

Fibrodysplasia Ossificans Progressiva (FOP) is an ultra-rare genetic disorder characterised by progressive heterotopic ossification (HO), leading to severe disability and reduced life expectancy. There is currently no approved treatment. FOP is caused by mutations in the receptor kinase ALK2, resulting in overactive bone morphogenetic protein (BMP) signalling and abnormal bone formation. FOP Friends supported research to identify an ALK2 inhibitor, and drug repurposing screens of existing clinical kinase inhibitors identified saracatinib (AZD0530) as a promising candidate. Saracatinib is now being evaluated in the European STOPFOP Phase 2 clinical trial.

Fibrodysplasia Ossificans Progressiva (FOP)

FOP is one of the **rarest genetic diseases**, affecting approximately 1 in 1–2 million individuals worldwide.

Symptoms begin in early childhood, with episodic, painful inflammatory soft tissue swellings, known as **"flare-ups".**

Many develop **heterotopic ossification** where soft tissues (muscles, tendons, and ligaments) are transformed into bone.

Flare-ups can be triggered by **minor trauma or inflammation**, such as bruises or viral infections.

Currently, there are **no effective therapies.**

Surgery is not possible as it can trigger flare-ups.

Progression and severity of FOP are variable and impossible to predict, but disability is cumulative and life expectancy is shortened.

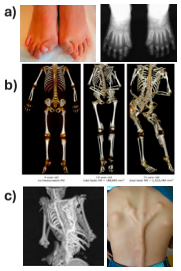

Figure 1 Clinical Presentations of FOP. **a)** Characteristic malformed great toe and radiograph. **b)** Whole body computed tomography images from representative subject. **c)** Extensive heterotopic ossification on the back of FOP patients. Adapted from Pignolo *et al.*, 2011, Pignolo *et al.*, 2019 and Kaplan *et al.*, 2008.

How does a gene mutation lead to faulty bone formation?

BMP pathway signalling drives the **differentiation** of precursor cells into **skeletal tissue**.

FOP is caused by **mutations in the *ACVR1* gene,** which encodes the BMP type I receptor kinase **ALK2.**

About 97% of patients carry the **gain-of-function mutation R206H.**

Activating mutations in ALK2 **increase BMP signalling via:**

- hypersensitivity to BMPs
- abnormal response to Activin A

This leads to abnormal bone formation **(heterotopic ossification).**

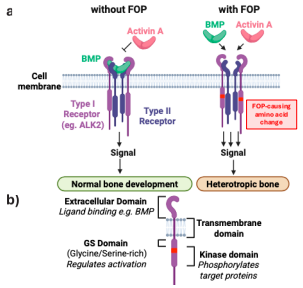

Figure 2. BMP signalling in FOP. **a)** Canonical and aberrant BMP signalling. Under physiological conditions, BMP ligands activate type II and type I receptors (e.g., ALK2), leading to regulated signalling and normal bone development. In FOP, mutant ALK2 results in hyperactive signalling, including aberrant activation by Activin A, driving heterotopic ossification. **b)** Domain organisation of the ALK2 receptor(Shore et al., 2006). Created with BioRender.com.

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Pignolo RJ *et al.* (2011) Orphanet J Rare Dis 6:80; Pignolo RJ *et al.* (2019) Orphanet J Rare Dis 14:98; **Kaplan FS *et al.*** (2008) Best Pract Res Clin Rheumatol 22:191–205; **Shore EM *et al.*** (2006) Nat Genet 38:525–527; **Williams E *et al.*** (2021) JCI Insight 6; **Smilde BJ *et al.*** (2022) BMC Musculoskelet Disord 23:519.

FOP Friends- supporting FOP research

FOP Friends was founded to raise awareness of FOP and related conditions.

The organisation provides **support** to individuals living with FOP and their families in the UK, bringing the **community** together.

It also **funds** the **FOP research team** at the University of Oxford.

Repositioning a kinase inhibitor, saracatinib, for FOP

Protein kinases are key therapeutic targets and among the most successful targets in drug discovery

Drug repositioning identifies **new therapeutic uses** for existing or previously tested drug candidates that have undergone clinical trials and recorded safety data

It enables **faster, more cost-effective** drug development —especially for **rare diseases**

Hypothesis: Existing approved or clinically tested kinase inhibitors may target **ALK2**

Laboratory screening for ALK2 inhibitors

ALK2 kinase domain was **screened** against a library of ~150 clinically tested kinase inhibitors.

Saracatinib is an **orally bioavailable kinase** inhibitor, originally developed for ovarian cancer.

Preferential inhibition of ALK2 was observed (Fig. 3a).

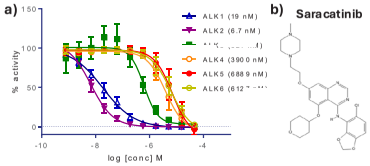

Figure 3. Screening identifies saracatinib as a potent inhibitor of ALK2. **a)** IC50 inhibition curves for saracatinib against purified recombinant ALK1-6 proteins, containing the GS and kinase domains (Fig. 2b), were determined using a LANCE Ultra TR-FRET kinase assay (PerkinElmer). Reactions contained 10 nM kinase, 50 µM peptide substrate, and 10 µM ATP. Data shown are plotted as mean ± SD (n = 3 independent replicates). **b)** Chemical structure of saracatinib.

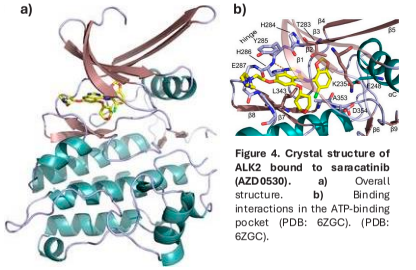

Figure 4. Crystal structure of ALK2 bound to saracatinib (AZD0530). **a)** Overall structure. **b)** Binding interactions in the ATP-binding pocket (PDB: 6ZGC). (PDB: 6ZGC).

Structural analysis

Crystal structure of saracatinib bound to the ALK2 kinase domain revealed the molecular basis of inhibition (Fig. 4).

Saracatinib binds and locks ALK2 in an inactive state, preventing abnormal signaling.

This explains its selectivity and supports the development of targeted FOP therapies.

In vivo studies (mouse model)

Saracatinib **blocked heterotopic ossification** and preserved range of motion (Fig. 5).

Moderate doses appear effective and well-tolerated, with no impact on neonatal growth, suggesting **potential for long-term use** in FOP to reduce both **flare-ups and ongoing disease progression.**

Figure 5. In vivo efficacy of saracatinib in the FOP-knockin mouse model. Neonatal Acvr1[R206H]FlEx/+] mice were injected with a low dose of Ad.Cre (1 × 108 PFU i.m. P7) and treated with 25 mg/kg/d saracatinib or vehicle orally for 28 days (grey shaded region) and observed for a total of 90 days. Representative mice are shown with Micro-CT, and Alizarin red hindlimb prep.

(Williams *et al.*, 2021)

STOPFOP phase 2a clinical trial

Preclinical studies suggest saracatinib may be a potential therapy for FOP.

The **Saracatinib Trial to Prevent Fibrodysplasia Ossificans Progressiva (STOPFOP)** is an investigator-sponsored study in Europe evaluating its safety and efficacy in patients with FOP.

Key challenges of FOP research:

- extremely small patient population
- sensitivity to invasive procedures that may trigger flare-ups
- slow, variable disease progression

To manage these issues:

- study uses a **randomized controlled design with an open-label extension** and **comparison to natural history data**
- relies on **non-invasive imaging** (low-dose CT and 18F-NaF PET-CT)
- restricts participants to patients with the **classic ALK2 R206H mutation** to **reduce variability**

Study design

Phase IIa clinical trial

Placebo-controlled, double-blind

3 Phases:

- 1st phase: 6-month RCT phase
- 2nd phase: 12-month open label phase
- 3rd phase: optional extension

3 Centres:

- Amsterdam, the Netherlands
- Garmisch-Partenkirchen, Germany
- London, United Kingdom

Outcome measures

- Changes in heterotopic bone, detected by low dose CT
- FOP disease activity detected by 18F-NaF PET-CT
- Joint mobility through Range of Motion assessment
- FOP disease activity and overall daily functioning through questionnaires
- Safety and tolerability

Data are currently being analysed, and results are expected soon

(Smilde *et al.*, 2022)

www.stopfop.com

Discussion

Active research and ongoing clinical trials are bringing much-needed hope to people living with FOP and their families.

If confirmed as safe and efficacious, saracatinib **could provide a rapidly translatable therapy,** supported by extensive safety data from >28 clinical trials involving over 600 patients.

In the future, effective peri-operative treatment may also make **surgical intervention** to remove heterotopic bone a possibility.

Acknowledgements

We gratefully acknowledge funding from **FOP Friends and the UK and France FOP community.** We sincerely thank the patients and families who participated in the STOPFOP study.

We also thank **Helen Bedford-Gay** and **Christopher Bedford-Gay** of **FOP Friends.**

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 821600. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

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Registered charity in England and Wales 1147704 and Scotland SC04950

#StrongerTogether

No-one should have to live with the fear, pain, isolation, and loss of independence caused by FOP. Help us to help those living with FOP.

To learn more about FOP or find out ways you can help, visit: **www.fopfriends.com**

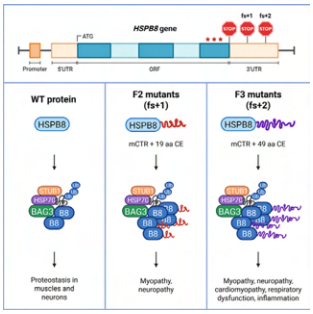
Cure MFM13 and Drug Repurposing Strategies for Myofibrillar Myopathy Type 13

Sylwia Szwec, Ania Kordala, Matthew McLeod, Julia Mielcarz, Todd King

Myofibrillar Myopathy Type 13 (MFM13) with Rimmed Vacuoles

MFM13 (OMIM #621078) is an **ultra-rare**, currently incurable autosomal dominant disorder caused by **frameshift mutations in the *HSPB8* gene**, first described by Ghaoui et al. in 2015.

The disease is **slowly progressive** and characterized by **muscle wasting** and increasing weakness, with symptom onset typically occurring in early adulthood (20s-40s). Approximately **60 cases have been reported worldwide.**



Adapted from Zhou W. et al. 2026 (MFM13 review, in collaboration with Cure MFM13).

Rare frameshift mutations in the third exon of *HSPB8* cause the protein to become longer than normal, adding an extra tail of 19 or 49 amino acids at its C-terminus.

Under basal conditions, HSPB8 maintains protein homeostasis in muscle and nerve cells by forming the CASA complex with BAG3, HSPA, and STUB1, facilitating the removal of damaged proteins.

However, the elongated mutant form of HSPB8 tends to aggregate, sequesters other components of the CASA complex, and reduces its overall activity. As a result, this impairment leads to muscle damage (myopathy) and may also be associated with neuropathy, cardiomyopathy, and respiratory involvement.

Who We Are

We are **Cure MFM13, the only advocacy group** dedicated entirely to addressing the challenges of MFM13.

Our mission is to **improve the lives of all people affected by MFM13 and their families**.

We do this by accelerating the **drug development process**, **building a strong and empowered community,** and **advocating for the community.**

Research Projects Include:

Biomarker development to monitor disease progression over time and **treatment response.**

Identification of currently approved **therapies** that can alleviate symptoms of MFM13, for example via a **drug repurposing screen.**

We Are Developing the Following Research Tools:

Fibroblasts - GM26098, GM26579, GM26096, GM28283 (available from Coriell Institute)

iPSCs - iPSCs derived from fibroblasts, carrying HSPB8 c.515dupC | p.P173Sfs*43 mutation (to be available from Coriell Institute soon)

Antibody against mutant HSPB8 - antibody targeting the HSPB8 c.515dupC | p.P173Sfs*43 frameshift variant, currently undergoing final quality control testing (*We are seeking a partner interested in including our antibody against frameshift mutant HSPB8 (fs_mut HSPB8) in their catalog*)

Mouse Model - C57BL strain carrying HSPB8 c.515dupC | p.P173Sfs*43 mutation with humanized C-terminus (in collaboration with the International Institute of Molecular and Cell Biology, Warsaw, Poland, and the Czech Centre for Phenogenomics, Prague, Czechia)

Key Challenges in Primary Drug Repurposing Screen

Which model is most suitable for testing small molecules?

Keep in mind:

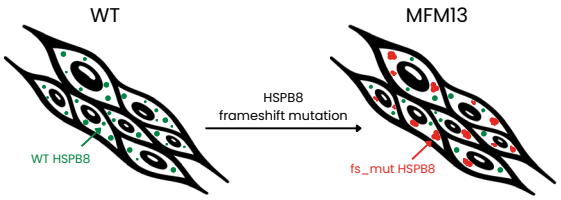
- MFM13 predominantly affects skeletal muscle tissue
- MFM13 is an adult-onset, slowly progressing disease, with damage accumulating over decades

Potential cell models:

Patient-derived fibroblasts	fs_mut *HSPB8* C2C12 (muscle cell model)	Patient-derived primary myoblasts	iPSCs-derived myoblasts / skeletal muscle
easy to culture	easy to culture	physiological relevance	physiological relevance
rapid growth	rapid growth	clinical relevance	clinical relevance
low cost	low cost	relatively low cost	difficult to culture
limited disease phenotype	limited translatability	difficult to culture	time-consuming
not a relevant tissue	murine origin	limited availability	expensive

How to identify hits?

MFM13 molecular mechanism



WT HSPB8 level ↓
fs_mut HSPB8 level ↑
→ HSPB8 aggregation ↑ → Autophagy ↓

Potential readouts:

- Decrease in protein aggregates and fs_mut HSPB8 level
- Rescue of impaired autophagy
- Improvement in muscle cell differentiation and morphology

Library:
- Al-Tahan, S. et al. (2019) *'New family with HSPB8-associated autosomal dominant rimmed vacuolar myopathy'*, Neurology Genetics.
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Newsletter

Website

Sildenafil as a repurposed therapy for Leigh syndrome: design of the upcoming SIMPATHIC trial

Drs. T.A. Braam[1], Dr. A. Müller[1,2], Drs. E.G. Lara[4], Dr. Ir. J. IntHout[4], Prof. dr. B. P. C. van de Warrenburg[5], Prof. Dr. C.D. van Karnebeek[1,2,6,7], Dr. M.C.H. Janssen[3]

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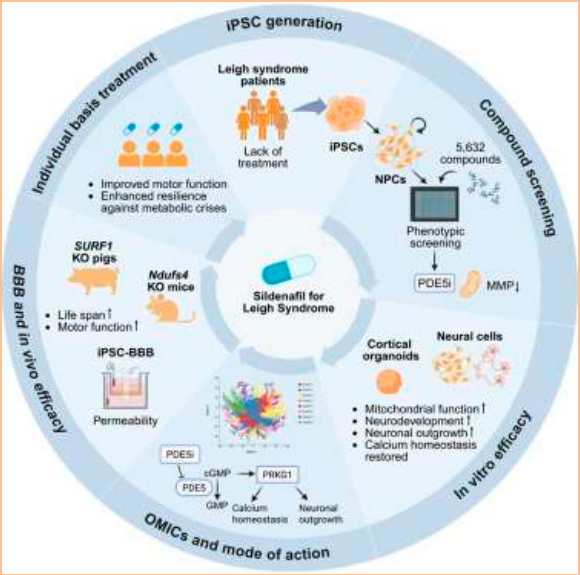
Introduction

- Drug repurposing is a key strategy in development of therapies for patients with rare diseases and high unmet medical needs
- The SIMPATHIC consortium aims to identify shared molecular and clinical mechanisms across disorders
- Leigh Syndrome (LS) is a rare, pediatric, neurometabolic disease caused by pathogenic variants in mitochondrial respiratory chain complexes, including complex I (*SURF-1)* and complex V *(MT-ATP6).*
- Sildenafil was identified as a candidate to be tested clinically in Leigh Syndrome[1]. (Pre)clinical data indicate that PDE-5 inhibition improves mitochondrial function and reduces disease burden[1]



Aim

To test the efficacy and safety of Sildenafil in *MT-ATP6*-related Leigh Syndrome in a multi-center, randomized, placebo-controlled, withdrawal design

Method - Trial Development

Collaboration of multiple stakeholders

Clinicians | Translational researchers | Regulatory agencies | Patients and representatives

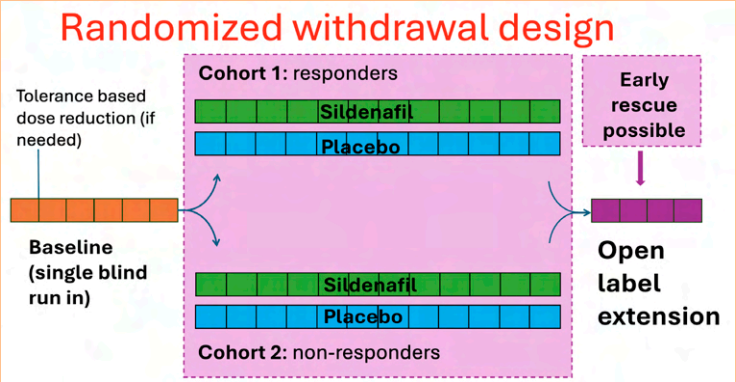
Inclusion criteria

- Genetically conformed *MT-ATP6* – related LS
- Characteristic LS abnormalities on MRI
- Age ≥6 months, weigt ≥6kg

Exclusion criteria

- Retinitis pigmentosa or other retinal abnormalities
- Cardiac abnormalities, reduced cardiac output, or arrhythmias

Trial Design



- 6-month open-label run-in period
- 12-month double-blind intervention phase (sildenafil vs placebo)
- 4-month open-label extension (OLE)

Primary analysis (cohort 1)
--> Proportion of responders in sildenafil vs placebo group

Non-responders (cohort 2) followed observationally
--> exploratory analyses: preventive / late-term effects

Blinding maintained until start of OLE due to post-hoc responder classification

Discussion

- This study can serve as a blueprint for similar trial efforts in other rare diseases
- High level of evidence generation on treatment efficacy in small patient populations

1) Zink et al, 2026, PMID 41819105

This project has received funding from the Horizon Europe research and innovation programme under grant agreement No 101080249

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DRUG REPURPOSING IS OMNIPRESENT IN DRUG DEVELOPMENT, YET WITH UNTAPPED POTENTIAL

Nikoleta Efthymiopoulou[1], Alicia Soler [1], Pan Pantziarka[2], Donald Lo[1], Martin de Kort[1]

[1]EATRIS, [2]Anticancer Fund (ACF)

INTRODUCTION

When we talk about drug repurposing (DR) we tend to focus on a handful of well-known examples creating the impression that DR is rare, a "one in a million" event rather than a common development pathway. But is this perception accurate? Is DR happening more frequently than we realise? As the literature remains largely anecdotal, we conducted a systematic analysis of the true scale of drug repurposing of already approved drugs to ask:

- How often does drug repurposing occur? What proportion of approved molecules has been repurposed?
- Are most cases of DR "obvious"? How often are expanded indications unanticipated?
- Who is driving DR? Is it primarily the original sponsor as part of product lifecycle management, or do other sponsors step in to expand uses in new indications?
- What is the market impact of drug repurposing?

METHODOLOGY

- **Data source & scope:** We conducted a systematic analysis of the GlobalData[1] pharmaceutical database, starting with ~4,500 drugs approved between 1924 through April 2025.
- **Filtering strategy:** We searched for drugs with more than one indication. We then restricted to ~1,500 drugs with verifiable approval dates in the EU, US, and Japan, and consolidated to parent molecules (i.e. active molecule independent of salt form). Manual curation confirmed ~550 cases that received drug approvals for one or more new therapeutic uses subsequent to the original approval date. The remaining cases were excluded as "false positives," including near-identical indications (e.g. osteoporosis vs post-menopausal osteoporosis), combination products, vaccines, allergen extracts, and incomplete historical records.
- **Limitations:** This estimate likely underrepresents the true extent of drug repurposing due to missing data (e.g. approvals outside the EU, US, and Japan), exclusion of combination products, and limitations in indication coding.

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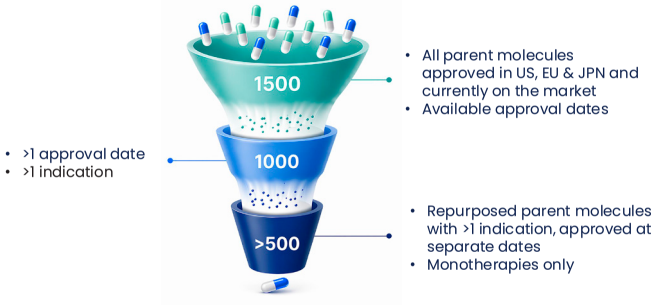
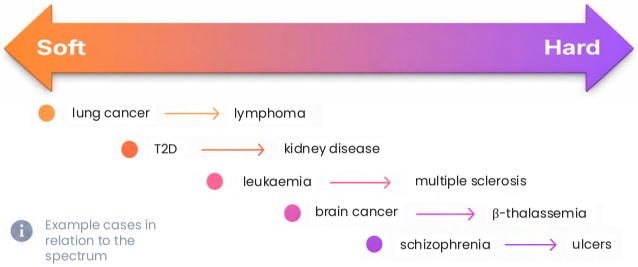
The REMEDi4ALL project has received funding from the European Union's Horizon Europe Research & Innovation programme under grant agreement No 101057442. Views and opinions expressed in this presentation are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

RESULTS & DISCUSSION

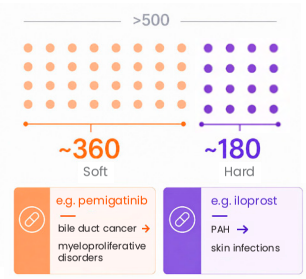
Out of 1500 approved drugs, >500 have been repurposed



The 500 repurposing cases span a spectrum from soft to hard

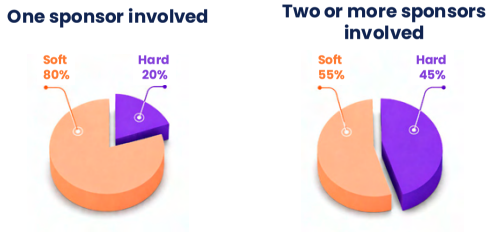


1 in 3 are "hard" repurposing cases



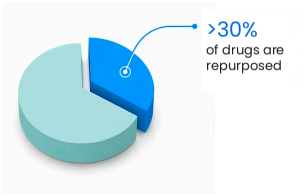
→ We defined **"hard"** cases as repurposing to a distinct therapeutic indication/area, a different target/mechanism, and/or a different mode of therapeutic benefit.

Hard repurposing happens more often when multiple sponsors are involved



→ "Sponsor" is the company that submitted for either the original drug approval or for the subsequent repurposed indication(s).

Repurposing has significant market impact



The European generics market reached **~€210 billion** in 2024.[3,4]

Assuming rough proportionality, even a 10–30% market share represents **~€20–60 billion**.

→ Because prescriptions in the EU and US are not indication-based, it is not straightforward to calculate pharmaceutical sales specific to DR indications.

CONCLUSIONS

- Fully one third of all approved medicines have been repurposed, representing a significant proportion of therapies available to patients
- These findings are comparable to those by Akodad et al. [2] for approvals after 1985 only
- 1 in 3 of these repurposing cases can be considered unanticipated at the time of original approval ("hard")
- "Hard" repurposing opportunities are more often discovered by a different sponsor
- DR represents a significant share of pharmaceutical sales
- Thus, DR is already a core part of modern drug development, but can be much further exploited especially for rare and neglected diseases

#iDR26

INTERNATIONAL DRUG REPURPOSING CONFERENCE

Navigating the future

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