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Development of small molecule probes as tools to study membrane receptors

Dr. Bert Beerkens

Biography

Bert graduated his master studies chemistry at Leiden University in 2018, having completed internships in Leiden and Munich. Immediately afterwards he continued his PhD studies is the group of prof. Adriaan IJzerman and dr. Daan van der Es at the Leiden Academic Centre for Drug Research (LACDR). His PhD studies comprised the synthesis, as well as biological and pharmacological evaluation, of small molecular probes for G protein-coupled receptors, in this case the adenosine receptors. This work was published in various journals, namely RSC medicinal chemistry, ACS medicinal chemistry and ACS chemical biology, and presented at various (inter)national congresses. Upon defending his dissertation in 2023, Bert continued as a postdoctoral researcher in the group of prof. Laura Heitman, investigating more thoroughly which biological pathways play a role in signaling of G protein-coupled receptors, in this case the chemokine receptors.

Development of small molecule probes as tools to study membrane receptors

Bert L.H. Beerkens, Daan van der Es & Laura H. Heitman

Membrane-bound receptor proteins have a pivotal role in the transduction of cell-cell communication, as well as cellular responses towards extracellular stimuli. Within the tumor microenvironment, signaling pathways between cells can be dysregulated due to an over- or under-expression of certain membrane receptors, increasing chances of survival for the cancerous cells. To obtain a better picture of the role of membrane receptors in cancerous conditions, our lab develops small molecule probes that allow us to label and detect membrane receptors, in particular G Protein-Coupled Receptors, in a wide variety of biochemical assays. Here we show the developmental route towards such probes, exemplified by projects on the C-C Chemokine Receptor subtype 2 (CCR2) and the Adenosine A3 Receptor (A3AR). Both GPCRs are involved in modulation of the immune response, therefore interesting targets from an immuno-oncology perspective, but also expressed on cancer cells themselves. With aid of the herein developed probes, we were able to detect CCR2 and A3AR in multiple assays, i.e. SDS-PAGE, microscopy, flow cytometry and pull-down proteomics. Altogether, this work provides new methods to study expression, localization and proteoforms of target receptors in diverse cellular backgrounds, paving the way for future investigations towards the role of membrane receptors in cancer.

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Biomarker Discovery and Disease Prediction in Autoimmune Disorders: Insights form a REFS-Based Approach

Sita Newer, Michalina Kierszka, Dominika Tomezyk, MSc Sarah Kidwai, David Rojas-Velazquez, Prof. dr. Johan Garssen, Dr. Alejandro Lopez-Rincon

Biography

Sita Newer is a dedicated and aspiring pharmacy student at University of Utrecht currently pursuing her bachelor's degree. With a keen interest in the evolving landscape of pharmacology, particularly how emerging technologies can transform drug development, delivery, and patient care. She is enthusiastic about the potential of personalized medicine, biotechnology, and machine learning to improve traditional practices in healthcare.

Biomarker Discovery and Disease Prediction in Autoimmune Disorders: Insights form a REFS-Based Approach

Sita Newer1, Michalina Kierszka1, Dominika Tomczyk1, Sarah Kidwai 1, David Rojas-Velazquez 1,2, Johan Garssen1,3, Alejandro Lopez-Rincon1,

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Introduction

Autoimmune diseases (AIDs) are complex chronic conditions characterized by an abnormal immune response against the body's own tissues, leading to inflammation and tissue damage [1]. Among the most prevalent AIDs are Systemic Lupus Erythematosus (SLE), Type 1 Diabetes (T1D), Rheumatoid Arthritis (RA), and Inflammatory Bowel Disease (IBD), all affecting various organs and presenting unique symptoms. Despite their differences, AIDs can share common disease mechanisms [2] leading to overlapping symptoms such as fatigue, joint pain and gastrointestinal symptoms [3] [4], or may co-occur in a condition called polyautoimmunity (PA) [5].

Objectives

Our goal is to identify potential biomarkers among shared genes in AIDs using machine learning. We will compare cases and controls in mRNA data to analyse how these genes contribute to the diagnosis and mechanisms of autoimmune disorders through pathway analysis.

Methods

For each AID a dataset was selected from NCBI Gene Expression Omnibus (GEO) repository [6]. This included the datasets: GSE112087 (SLE) with 60 samples, consisting of 31 cases and 29 controls, GSE123658 (T1D) with 82 samples with 30 cases and 43 controls, GSE216678 (RA) with 311 samples including 97 cases and 114 controls, and GSE186507 (IBD) with 1030 samples compromising 402 cases and 628 controls. In all datasets the controls were labelled as 0 and the cases were labelled as 1. The four datasets were merged into a single dataset (AID dataset) containing 1383 samples with a total of 16333 features. We applied the Recursive Ensemble Feature Selection (REFS) algorithm used for biomarker discovery on the AID dataset to find the most important features [7].

Results

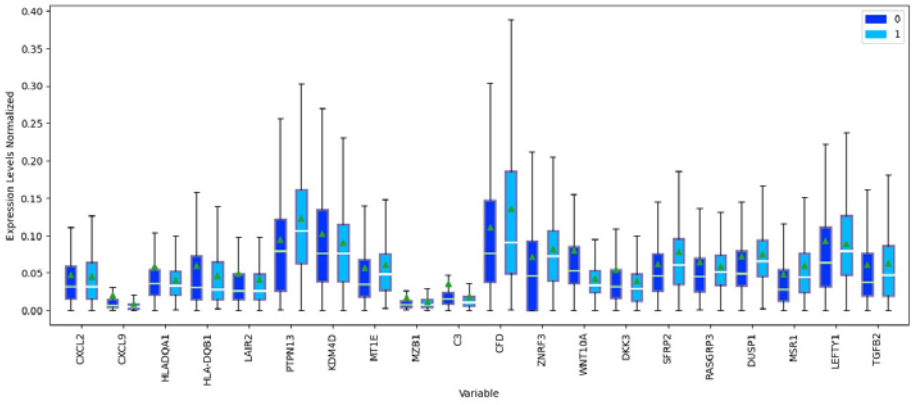
REFS selected 512 genes as the most effective feature set for distinguishing the autoimmune group from the control group in the AID dataset. We selected a subset of 327 genes, which is contained in the set of 512, to simplify the dataset. To validate this selection, we executed the validation process using the 327

genes as input, the classifier with the best performance was Multilayer perceptron with an AUC of 0.95, considered as excellent [8]. After a literature review, 20 genes were selected for biological interpretation analysis, revealing differential expression patterns between healthy and autoimmune samples. In Fig. 1 are highlighted specific genes with significant expression differences between the groups, with PTPN13, ZNRF3, SFRP2, and DUSP1 elevated in the AID group, while WNT10A and DKK3 were higher in the control group. Finally, a pathway analysis was performed on the selected 20 genes by using the gene enrichment analysis tools GOnet [9] and GSEA [10].

Conclusion

This study highlights the utility of REFS in discovering potential overlapping biomarkers for autoimmune diseases from RNA-Seq data. Pathway analysis revealed the involvement of these genes in key immune-related pathways, including MAPK [11], WNT [12], immune signalling [13], and complement signalling pathways, emphasizing their relevance in the pathogenesis of AIDs, Fig. 2. Several studies provide evidence of the connections between these pathways and autoimmune diseases, suggesting that disruptions in these signalling networks may contribute to the onset and progression of these conditions [11, 14]. Additionally, understanding these pathways could aid in the identification of novel therapeutic targets and strategies for intervention.

Figure 1: Gene expression difference between the key genes in controls (0) and cases(1) for AIDs.



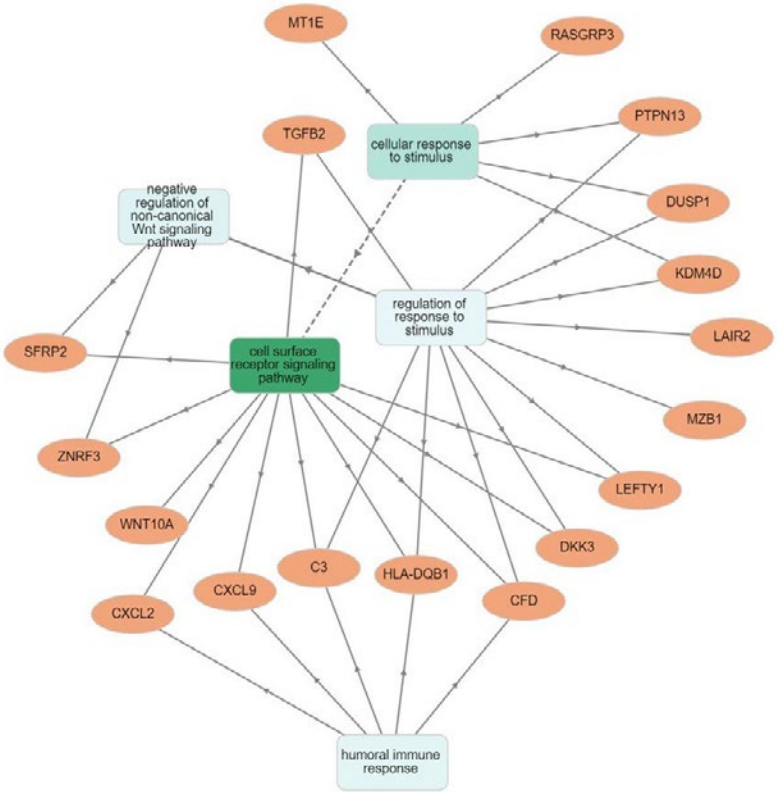


Figure 2: Immunologic pathways of key genes.

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Predicting Long COVID: A Machine Learning Approach to Identifying Genetic Biomarkers in COVID-19 Patients

Michalina Kierszka

Biography

I successfully graduated with a degree in Pharmaceutical Science from Utrecht University. For my research project, I completed an internship with the Machine Learning group at Utrecht University, where I used machine learning approaches to identify biomarkers for long COVID. This experience allowed me to develop strong skills in data analysis and biomedical research. I am passionate about integrating technology with pharmaceutical science to advance healthcare solutions. I am now continuing my career by pursuing a Master's in Drug Science at Basel University, where I aim to further my expertise in drug development and innovation.

Predicting Long COVID: A Machine Learning Approach to Identifying Genetic Biomarkers in COVID-19 Patients

Michalina Kierszka1, Dominika Tomczyk1, Sita Newer1, Sarah Kidwai1, David Rojas-Velazquez1, 2, Johan Garssen1, 3, and Alejandro Lopez-Rincon1

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2Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands
3Global Centre of Excellence Immunology Danone Nutricia Research, Utrecht, The Netherlands

Introduction

The COVID-19 pandemic has had profound and lasting impacts on global health, resulting in a significant number of individuals experiencing long COVID—a condition characterized by persistent symptoms such as shortness of breath, dyspnea, and brain fatigue or exhaustion following the acute phase of infection. Despite its widespread occurrence, the underlying biological mechanisms of long COVID remain poorly understood [1].

Objectives

This study aims to identify specific genetic biomarkers using machine learning approach to predict the onset and severity of long COVID[2]. By focusing on genes associated with inflammatory response, neurological function, and cancer (Figure 2). This will enhance the understanding of long COVID's pathophysiology, improve the diagnostic, and therapeutic strategies.

Methods

Using the Recursive Ensemble Feature Selection (REFS) algorithm [3], a comprehensive analysis was conducted of gene expression data obtained from patients with a history of COVID-19. The gene expression profiles dataset used in this study was obtained from the Gene Expression Omnibus (GEO) ascension GSE224615 [4]. This dataset includes the PBMCs blood samples collected from a cohort of 43 individuals, comprising 27 long COVID patients and 16 non-long COVID participants [5].

Results

After applying REFS to the dataset, 16 out of 18708 genes were selected as the minimum number of genes to achieve the highest accuracy (Figure 1a). In the validation process, the classifier with the best performance was ExtraTrees with an AUC of 0.97 (Figure 1b). The literature analysis focused on ten genes (Table 1) such as IGHG4, NAIPP4, TRAV8-5 (adaptive immunity [6]), MDGA1 (negative regulation of synapse [7]), KNDC1 (neuronal differentiation [8]), RP11-456K23.1, ELL2P1, SNTG2, KRT17P2, and NDRG2 that are integral to immune regulation, neuronal function, and cancer (Figure 2) [1, 9].

Conclusion

This study identifies ten potential genetic biomarkers for predicting long COVID, offering a new perspective on its pathophysiology. The observed immune dysregulation and autoimmune responses create a connection between the pathophysiology of cancer and long COVID. The similarities in these underlying mechanisms suggests that biomarkers used in the detection and management of cancer might also be relevant in patients with long COVID, reflecting comparable pathological processes. Genes related to neurological function, such as MDGA1 and SNTG2, showed alterations suggesting disruptions in synaptic development and neuronal signaling, potentially linked to neurological symptoms like brain fog in long COVID. While further research is needed, these findings suggest a multifaceted genetic basis for long COVID, involving immune dysregulation, neurological impact, and cancer (Figure 2).

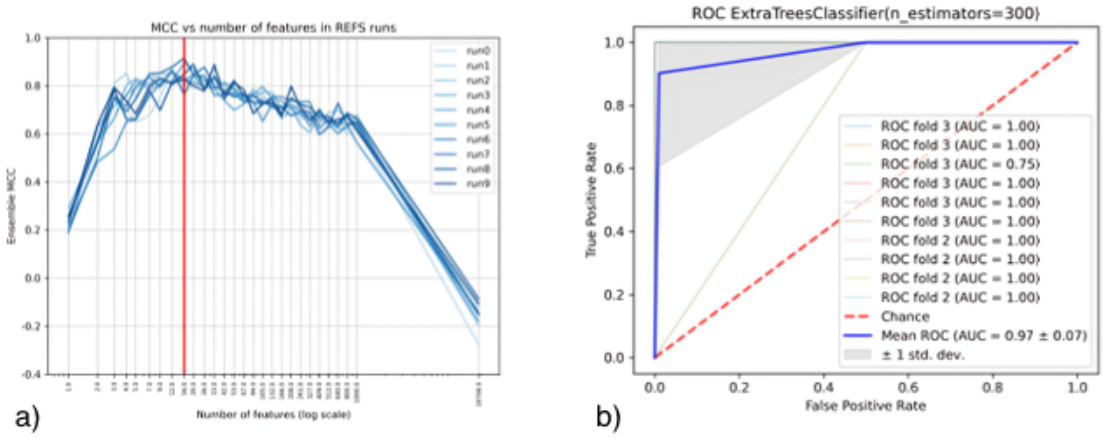

Figure 1: a) The minimum number of features that achieve the highest accuracy, b) ExtraTrees was the classifier with the best performance in the validation module.

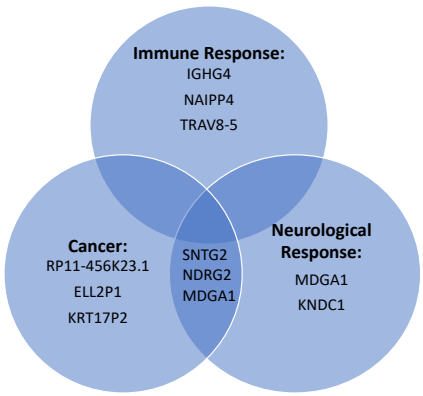

Figure 2: Overview of Three Groups of Genes: Immune Response, Neurological Response, and Cancer.

Table 1: Genes Functions and Implications in Long COVID

Gene Name	Functional Context	Key Findings
IGHG4	Immunoglobulin subclass	Persistent immune activation
NAIPP4	Belongs to NLR family	Linked to inflammasome activity
TRAV8-5	Involved in adaptive immunity	Dysregulation of T-cell response
MDGA1	Negative regulation of synapse	Neurological symptoms
KNDC1	Regulation of neuronal diferentiation	Involved in neurological symptoms
RP11-456K23.1	LncRNA found in glioblastoma	Neurological symptoms
ELL2P1	Pseudogene acting as ceRNAs	Transcriptional misregulation in cancer
SNTG2	Interacts with dystrophin	Downregulated in COVID-19 pateints
KRT17P2	Pseudogene found in leukoplakia	Immune dysregulation
NDRG2	Expressed in astrocytes	Inflammatory response

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Machine Learning Reveals New Gene Functions in Viral Respiratory Infections

Dominika Tomczyk, Michalina Kierszka, Sita Newer, Sarah Kidwai, David Rojas-Velazquez, Prof. dr. Johan Garssen, Dr. Alejandro Lopez Rincon

Biography

A fresh Bachelor degree graduate in College of Pharmaceutical Sciences at Utrecht University. Finished a 5-month Bachelor internship under the supervision of Dr. Alejandro Lopez Rincon, with the main focus on the use of machine learning for identification of predictive biomarkers for disease diagnosis and treatment. Highly interested in immunogenetics, especially the mechanisms driving human genetic susceptibility to infectious and autoimmune diseases, and exploring these subjects using innovative ways such as artificial intelligence.

Machine Learning Reveals New Gene Functions in Viral Respiratory Infections

Dominika Tomczyk1, Michalina Kierszka1, Sita Newer1, Sarah Kidwai1, David Rojas Velazquez1, 2, Johan Garssen1, 3, Alejandro Lopez-Rincon1

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Introduction

Influenza virus, human metapneumovirus, rhinovirus, and respiratory enterovirus are the leading causes of acute respiratory infections worldwide [1, 2, 3, 4]. Seasonal epidemics caused by these viruses suggest that some people are more sensitive to viral infections than others. This indicates the existence of yet-unidentified, otherwise healthy individuals, who are genetically predisposed to develop respiratory infections. As there are no vaccines against rhinovirus and human metapneumovirus, and the vaccination rates against influenza remain low, such individuals may be constantly exposed to the contagious agents [1, 4]. Therefore, there is a high need for biomarkers capable of identifying such genetically predisposed populations. Objectives. Use machine learning to identify the overlapping differently expressed genes in patients infected with either influenza, enterovirus/rhinovirus, or human metapneumovirus, and investigate whether these genes are related to the host antiviral response or, if not, whether they can contribute to human genetic susceptibility to viral respiratory infections. Methods. Data Processing. 133 RNAseq samples from BioProject PRJNA660611 (GEO accession: GSE157240 [5]) were processed (Figure 1). Subjects included 65 adults infected with influenza virus, 31 with enterovirus/rhinovirus, 17 with human metapneumovirus, and 20 asymptomatic controls. All infected patients were merged into one group (n = 113) and controls were kept separately (n = 20).

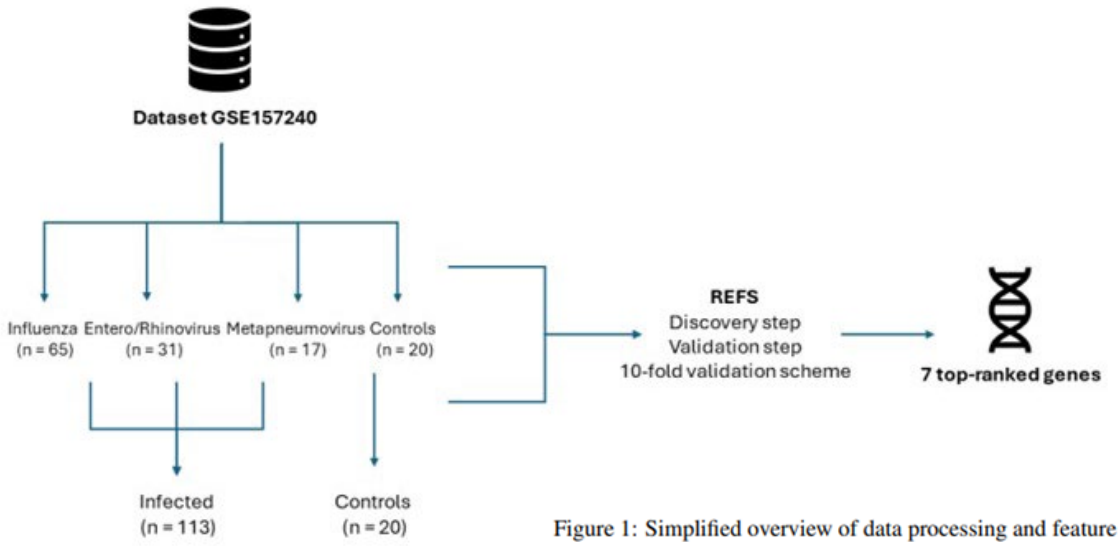

Figure 1: Simplified overview of data processing and feature selection of the study.

Feature Selection

The Recursive Ensemble Feature Selection (REFS) consists of two steps: discovery and validation [6]. First, REFS uses 8 discovery classifiers, each ranking the features independently. As an output, the discovery step gives the minimum number of features with the highest accuracy of classification (top-ranked genes). This output is validated using 5 different classifiers, that were not used 1 in the ranking step, in a 10-fold cross-validation validation scheme. The final output is a diagnostic measure, given by the Area Under the Curve (AUC) [7] of the 5 validation classifiers after the 10-fold scheme is completed.

Results

Out of 19,203 genes, REFS identified 7 top-ranked differently expressed genes common in all three infections, with an AUC of 0.76, and std of 0.11. Five genes were grouped either as those related to antiviral response (two genes - IFI27 and CARD17P) or those possibly related to susceptibility to viral respiratory infections (three genes - NOG, MT2A, and ID3). Two genes remained of unknown function and could not be grouped.

Conclusion

In this study, we uncovered possible novel functions of NOG, MT2A, and ID3 genes that, if proven true, could be potential biomarkers of human susceptibility to viral respiratory infections. This is especially important as respiratory viruses still cause seasonal epidemics and affect millions of people worldwide [1]. Identification of genetically predisposed populations is crucial for disease prevention and the management of future outbreaks. Populations with a genetic predisposition to develop (severe) respiratory viral infections, even if otherwise healthy, should be taken special care of in subsequent pandemics, especially as there are still no vaccinations against many respiratory viruses.

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Learning from success; analysis of matched molecular pairs networks of approved drugs per target class

Helle van den Maagdenberg, Professor J. G. Coen van Hasselt, Professor Piet Hein van der Graaf, Professor Gerard van Westen

Biography

After my bachelor Bio-Pharmaceutical Sciences at Leiden University (2016-2019), I chose to start the master computer science (2019-2021) with a specialization in Bioinformatics to broaden my horizon and learn more about computational drug discovery. During the master program I worked on a research project to develop a deep-learning model for cell tracking at LIACS. I finished my master with a thesis project at LAP&P. The topic was to explore and create a framework for determining parameter identifiability for a series of Target Mediated Drug Disposition models with different biomarkers and parameter sets through simulation. I am currently doing my PhD research under the supervision of Prof.Dr. Gerard van Westen, Prof.Dr. Coen van Hasselt and Prof.Dr. Piet van der Graaf on the topic of virtual drug discovery. The aim of the project is to couple computational chemistry and pharmacology modelling methods in a single approach to virtual drug discovery.

Learning from success; analysis of matched molecular pairs networks of approved drugs per target class

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2 Certara, Canterbury, UK.

Introduction

Selecting the most promising hit or lead compounds to pursue remains a challenge despite numerous efforts to characterize the optimal drug profile. Many such efforts focus on retrospective analysis of approved drugs, including the well-known 'rule of thumb' Lipinski's rule of five (Ro5) [1]. The rise of computational approaches, such as virtual screening and generative drug design further increases the need for automated selection of promising structures. The current literature predominantly focuses on optimal drug properties in a broad sense. However, there are only a few efforts to classify favourable properties for specific drug classes or targets, such as antibacterial drugs [2] or kinase inhibitors [3].

Objectives

Here we aim to identify the optimal drug physicochemical and ADME profile per target class of approved drugs in the ChEMBL [4] database.

Methods

We retrieved and filtered approved drugs from the ChEMBL database. All available bioactivity for the selected drug targets was subsequently obtained from Papyrus [5], a curated bioactivity dataset. For each target and the associated bioactivity data, matched molecular pairs were identified. These pairs were then used to create networks where each node represents a compound, and edges connect matched molecular pairs. In each network, the approved drugs were identified, and the distances (in steps) from the approved drug then served as a marker for the development state. A global analysis of physicochemical properties was performed, examining how these properties change with increasing distance from the approved drugs, thus mimicking a temporal analysis of early, middle and late-stage compounds.

Results

Initial results show a decrease in drug-likeness (QED [6]) with distance from the approved drugs in the matched-molecular pairs networks. This shows that the networks capture some of the trends typically

observed during drug development. However, trends within target networks deviate from the overall trend observed across all targets and drugs. This suggests that the most favourable drug property profile may vary across different drug target classes.

Conclusion

In conclusion, preliminary results show that matched-molecular pair networks can facilitate the temporal analysis of approved drugs and their development trajectories. The next step will be the analysis of the physiochemical and ADME properties per target class to identify an optimal drug profile per target class.

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The effects of sevuparin on LPS-induced inflammation in healthy volunteers

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Biography

Qualifications
2021 Medical doctor (MD), MSc, Vrije Universiteit, Amsterdam, the Netherlands
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PhD student, project leader and research physician
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Introduction

In search of novel treatments for acute systemic inflammation disorders such as sepsis and endotoxemia, heparin and its derivatives have been suggested as potential candidates. Heparin, aside from its anticoagulant properties, is also known to possess properties that modify inflammation. Sevuparin is a low-anticoagulant heparinoid under development as a treatment for distinct disorders with acute systemic inflammation such as sepsis, severe malaria, and endotoxemia. This proof of mechanism study in healthy volunteers was designed to evaluate the potential of sevuparin on experimental endotoxemia.

Objectives

To evaluate the safety and tolerability of sevuparin and the effects of sevuparin on local and systemic lipopolysaccharide (LPS)-induced inflammation.

Methods

This was a randomized, double-blind, placebo-controlled phase 1 study in healthy participants. Participants received one of three intravenous sevuparin doses or placebo as a continuous infusion of 6 hours and were challenged with intradermal LPS (4 injections of 5 ng) in part 1 and with intravenous LPS (1 ng/kg as a bolus injection) in part 2. The local LPS response was evaluated using imaging techniques for skin perfusion and erythema and by performing flow cytometry and cytokine analysis on blister fluid retrieved from skin suction blisters. The systemic LPS response was evaluated by measuring vital signs, circulating cytokine levels, leukocyte subsets, C-reactive protein, and by performing flow cytometry.

Results

A total of 71 participants were randomized to study treatment in part 1 and part 2. Sevuparin was well-tolerated. A dose-dependent increase in activated partial thromboplastin time (APTT) was observed in the active treatment groups, without clinical relevance in the study population. Sevuparin did not significantly modulate the local LPS response. In the systemic LPS challenge, sevuparin significantly increased the circulating basophil, neutrophil, and lymphocyte counts at the highest dose level. The sevuparin effects on lymphocyte counts were confirmed by immunophenotyping of circulating immune cells. Furthermore, the LPS-induced relative tachypnea was suppressed to a near-significant elevation in respiratory rate at the highest dose level. For other systemic measures, sevuparin did not significantly differ from placebo.

Conclusion

In this study, sevuparin primarily modulated LPS responses of select leukocyte populations and the LPS-

driven relative tachypnea in healthy volunteers. Based on the observed pharmacodynamic effects along with the advantageous safety profile, further exploration of sevuparin as a treatment for acute systemic inflammatory disorders such as sepsis and endotoxemia is envisaged.

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Association of positive end-expiratory pressure ventilation with midazolam metabolism: a prospective observational study in COVID-19 and non-COVID-19 intensive care patients

MSc Johanna Kramer-Abma, Dr. Jos le Noble, Dr. Annemariek Driesen, MSc. Kimberly Shudofsky, Dr. Paddy Janssen, Dr. Emmeke Wammes-van der Heijden

Biography

Johanna Kramer-Abma was born in Bolsward, one of the Frisian eleven cities. From 2008 till 2015 she studied Pharmacy in Groningen. After working as a pharmacist in two hospitals (Nij Smellinghe, UMCG), she started training as a hospital pharmacist at VieCuri Medical Center (Venlo) and Maastricht University Medical Center in January 2020. During this training, she chose Clinical Pharmacology as her differentiation. Her registration research focused on the influence of positive end-expiratory pressure (PEEP) ventilation on the metabolism of midazolam. Secondly the effect of COVID-19 on midazolam metabolism was studied, due to clinical experience that very high doses of midazolam were required in these patients. This research took place in the intensive care unit at the VieCuri Medical Center. Since January 2024 Johanna has become a hopital pharmacist and is currently working at the Martini Hospital in Groningen. This year she plans to complete her training as a Clinical Pharmacologist.

Association of positive end-expiratory pressure ventilation with midazolam metabolism: a prospective observational study in COVID-19 and non-COVID-19 intensive care patients

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Introduction

Midazolam is a widely used sedative in intensive care patients due to its favourable kinetics. However, the half-life and thus the duration of sedation may be prolonged in several cases.

Objectives

Post End-Expiratory Pressure (PEEP) can lower cardiac output, thereby reducing liver blood flow. We hypothesized that high PEEP could therefore decrease midazolam metabolism. Secondly, we investigated whether there was a difference in midazolam metabolism between COVID-19 and non-COVID-19 intensive care patients with mechanical ventilation.

Methods

Patients with and without COVID-19, admitted to the ICU, treated with continuous infusion of midazolam and ventilated with PEEP were included. Midazolam and 1-OH midazolam plasma concentrations were measured in residual material during steady state of midazolam. The metabolic rate 1-OH-midazolam/midazolam (1OH-MDZ/MDZ), corrected for molecular weight, was calculated for all included samples. Samples were assigned to low PEEP (PEEP ≤10 cmH2O) or high PEEP (PEEP >10 cmH2O). In both a cross-sectional analysis and a generalized estimation equations (GEE) analysis, the effect of PEEP and COVID-19

on the metabolism of midazolam was studied. The metabolic rate 1OH-MDZ/MDZ was corrected for different covariates.

Results

50 patients were included, resulting in 166 samples for the GEE-analysis. The cross sectional median 1OH-MDZ/MDZ metabolic ratio was 0.058 (0.031-0.115) and 0.061 (0.022-0.135) in the low and high PEEP groups, respectively. No association was found between PEEP category and the metabolic rate of midazolam. COVID-19 was however associated with a 35% faster midazolam metabolism, despite a negative association of CRP with midazolam metabolism.

Conclusion

Our study suggests no need to adjust the midazolam dose to the normal PEEP settings in the ICU. Future studies should include more variation in PEEP settings alongside direct measurements of liver blood flow to determine whether PEEP does indeed not influence midazolam metabolism.
However, in ICU patients with mechanical ventilation and COVID-19, higher doses of midazolam or other sedation strategies should be taken in consideration due to an increased metabolism of midazolam.

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Serious hypokalemia induced by abiraterone following discontinuation of prednisolone: a case report

Angelique Egberts, Tijn van Assen, Anja Roerade, Quirine van Rossum - Schornagel

Biography

Angelique Egberts is a pharmacist at the Franciscus Gasthuis & Vlietland hospital and clinical pharmacologist in training at the Leiden University Medical Center. In 2018, she obtained her PhD degree with her thesis entitled 'Delirium in Old Age: Pathophysiological and Pharmacological Aspects'. She continued her scientific research career and is involved in several research projects.

Serious hypokalemia induced by abiraterone following discontinuation of prednisolone: a case report

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Introduction

Abiraterone, an androgen biosynthesis inhibitor approved for the treatment of metastatic hormone-sensitive prostate cancer (mHSPC), can induce a state of mineralocorticoid excess, which is associated with hypokalemia, hypertension and edema. To minimize these side effects, abiraterone is used in combination with predniso(lo)ne.

Objective

As the number of abiraterone prescriptions increases, it is important for physicians and pharmacists to be aware of the side effect profile of abiraterone and the need to combine abiraterone with predniso(lo)ne.

Methods

We present a case of abiraterone-induced grade 4 hypokalemia (<2.5mmol/l) following discontinuation of prednisolone and explain the underlying mechanism.

Results

Case report: A 74-year-old man with mHSPC, treated with androgen deprivation therapy, abiraterone and prednisolone since 2020, presented with malaise and hypertension (179/98 mmHg) in 2023. Laboratory findings showed hypokalemia (2.0 mmol/L) for which no cause could be identified: the patient was not receiving diuretics, had no diarrhea or hypomagnesemia, and no relevant drug changes had occurred in the preceding days. Hypokalemia was treated with oral potassium replacement therapy and hypertension with enalapril. One month later, laboratory findings again showed hypokalemia (2.0 mmol/L) and potassium replacement was restarted. The side effect profiles of all drugs in use were reviewed and revealed that abiraterone can cause hypokalemia in more than 10% of the patients. However, it remained unclear why the serious hypokalemia presented three years after the start of abiraterone, while potassium levels were always within the normal range. A new medication review led to the conclusion that prednisolone was unintentionally discontinued during a hospital admission 1.5 months before the first measured hypokalemia.

Underlying mechanism: Abiraterone inhibits 17-alpha-hydroxylase/C17,20-lyase (known as CYP17) which leads to a decrease in testosterone and cortisol production. Decreased cortisol levels result

in an increased release of adrenocorticotropic hormone (ACTH) which in turn leads to increased mineralocorticoid production by the adrenal gland. This causes enhanced sodium reabsorption and potassium excretion, which can lead to hypokalemia, hypertension and edema. Predniso(lo)ne suppresses ACTH release and thus the side effects caused by mineralocorticoids.

Conclusion

Physicians and pharmacists should be aware that hypokalemia is a common side effect in patients treated with abiraterone. Despite that predniso(lo)ne will minimize the development and severity of hypokalemia, still more than 10% of the patients treated with the combination will develop it.
We therefore recommend to add abiraterone to a clinical decision support system, like a clinical rule for hypokalemia that links low potassium levels directly to medication, to alert health care providers in case of hypokalemia. In addition, we encourage physicians to write the indication on the predniso(lo)ne prescription, so that this drug is not stopped unintentionally.

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Oral folinic acid prophylaxis prevents pemetrexed-induced neutropenia: results from a randomized clinical trial

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Biography

I recently finished my hospital pharmacy residency with a specialization in oncology. I have a particular interest in lung cancer and am currently pursuing a PhD in this field.
I like the dual role of providing personalized care on one side while conducting practical research that can swiftly reach the patient to improve patient care in general. I believe there still is room for improvement in the oncological treatment with classical chemotherapy agents which is the main topic of my PhD.

Oral folinic acid prophylaxis prevents pemetrexed-induced neutropenia: results from a randomized clinical trial

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Introduction

Pemetrexed is a cornerstone in the treatment of non-small cell lung cancer. Although this drug is generally well-tolerated, a substantial part of the patients receiving pemetrexed experiences dose- or treatment-limiting toxicities, the foremost being neutropenia. Grade III/IV neutropenia has reported incidences up to 26% and can lead to hospitalization, treatment interruption or even death. Based on in vitro and preclinical data pemetrexed induced neutropenia can be prevented by treatment with prophylactic treatment with folinic acid. The main objective of this study is to evaluate the effect of oral folinic acid on preventing pemetrexed-associated neutropenia.

Methods

A multicenter, open label, double arm, randomized trial was performed. Fifty patients, requiring pemetrexed-containing therapy were randomized in a 1:1 ratio to either receive oral folinic acid 24 hours after pemetrexed administration for 3 days or receive standard of care without folinic acid. All patients in both arms received suppletion with folic acid and vitamin B12. The primary endpoint was the difference in neutrophil count between both groups after the first cycle of chemotherapy at nadir. Secondary endpoints were the neutrophil count after the second cycle of chemotherapy, grade neutropenia (according to the Common Terminology Criteria for Adverse Events v5), efficacy of oncological treatment, renal function and the incidence of dose delays and reductions of pemetrexed.

Results

In total, 24 patients were included in the folinic acid group and 26 patients in the control group. No differences in baseline characteristics were observed between both groups. For the primary endpoint a higher absolute neutrophil count ($p<0.01$) after the first cycle of chemotherapy was observed in the folinic acid group (median: 3.79; IQR: 2.22 – 4.93) compared to the control group (median: 1.85; IQR: 1.43 – 3.78).
Secondary a higher neutrophil count ($p=0.01$) was also observed after the second cycle of chemotherapy in the folinic acid group (median: 2.60; IQR: 2.03 – 4.41) compared to the control group (median: 1.76; IQR: 0.87 – 2.73). The incidence of grade I neutropenia after the first cycle of chemotherapy was 4% in the folinic acid group vs. 27% in the control group ($p=0.04$). The incidence of grade II neutropenia was 0% in the folinic acid group vs. 15% in the control group. ($p=0.05$). After the second cycle of chemotherapy the incidence of grade III neutropenia was 5% in the folinic acid group vs. 29% in the control group ($p=0.08$). No differences were observed in efficacy of oncological treatment, dose reductions, delays

discontinuation of treatment. No difference in renal function between both groups was observed. Also no serious adverse events related to the treatment with folinic acid were observed.

Conclusion

Prophylaxis with oral folinic acid is effective in preventing pemetrexed-associated neutropenia and should be incorporated in the standard of care. Prospective evaluation after implementation may serve to validate our findings with respect to real-world reduction in toxicity and efficacy of lung cancer treatment.

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A novel kinase degrader-based payload for the development of potent Degrader Antibody Conjugates (DACs)

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Biography

Martine started her career as a Research Scientist at Genmab BV, followed by investigating cell cycle regulators involved in extra cell divisions of the body wall muscle of the nematode C. elegans, at the group of Prof. van den Heuvel at Utrecht University. Her work at Netherlands Translational Research Centre (NTRC) focused on profiling cancer drugs on a broad panel of cancer cell lines and finding synergy. Martine has a strong affinity for cell biology and a hands-on approach. At Crossfire Oncology she works on generating and characterizing Degrader Antibody Conjugates (DACs) using multiple cellular and biochemical techniques.

Introduction

Antibody drug conjugates (ADCs), despite their suboptimal therapeutic window, are a great therapeutic success. To broaden their applicability, a search for payloads with a more favorable safety profile is ongoing. Recently, heterobifunctional degraders have gained great interest as payloads, and degrader antibody conjugates (DACs) are now seen as a novel therapeutic modality.
Heterobifunctional degraders consist of a small molecule ligand that binds a target (protein of interest or POI), a spacer and an E3 ligase ligand, which together induce proximity of the target protein and the E3 ligase, leading to catalysis of target degradation. Owing to their catalytic activity, degraders can have better potency than the equivalent inhibitors, making them suitable as ADC payload [1]. The main advantage of degrader payloads is their ability to specifically target validated tumor drivers, unleashing novel mechanisms of action (MoA) for the selective killing of cancer cells. In combination with their rapid clearance from circulation this endows DACs with a superior therapeutic profile compared to ADCs with classical payloads that are confined to a limited number of MoA and are known to induce off-tumor toxicity because of the long payload half-life in circulation.

Objectives

Since many FDA-approved targeted therapies are based on the inhibition of protein kinases, we reasoned that degraders of these kinases could be interesting as DAC payloads. Kinase DACs could bring enhanced targeting and therefore better therapeutic window to a field where classic ADC payloads have often shown substantial toxicities. As many heterobifunctional degraders show poor cell membrane penetration, their cytotoxic potential could be increased by coupling them to an antibody. Following endocytic uptake of a DAC, the degrader payload is released intracellularly, resulting in degradation of the target kinase and subsequentially cell death.

Methods

To identify kinase degrader payloads, we present a workflow based on a platform called Energetically Privileged Ligands (EPriLs). EPriLs are macrocycle scaffolds that bind non-covalently in the kinase ATP pocket. Their unique binding mode avoids contact with acid positions where resistance to kinase inhibitors frequently occurs. EPriL macrocycles can be decorated appropriately to rationally design specific inhibitors for many therapeutically relevant kinases and provide synthetic handles to couple them to VHL or CRBN ligands to generate effective kinase degraders.

Results

Here we describe how EPriL kinase degraders can be developed into effective DACs, using consecutive libraries of EPriL ligands, spacers, E3 ligase ligands and linkers. First, suitable degraders are identified,

based on rapid and deep target degradation and potent antiproliferative activity on target cell lines. Degraders are then transformed into maleimide-linked degraders using convenient attachment of enzymatically cleavable linkers. In a medium throughput fashion, these maleimides are coupled to antibodies to generate DACs, which are tested for stability and biological potency.

Conclusion

Applying this workflow to various well-validated kinase targets in oncology resulted in a promising DAC targeting a cell cycle kinase. The kinase degrader payload on the DAC has favorable ADME properties, clear potentiation compared to the parent ligand, and can be easily conjugated to an antibody.

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Viral GPCR US28 as a driver of oncogenic extracellular vesicle secretion in brain cancer

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Biography

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Viral GPCR US28 as a driver of oncogenic extracellular vesicle secretion in brain cancer

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Introduction

US28 is a vial G protein-coupled receptor (vGPCR) encoded by the human cytomegalovirus. In glioblastoma, a fast-growing and aggressive form of brain cancer, it has been demonstrated that US28 expression enhances oncogenic signaling pathways. US28 has also been linked to the emerging field of extracellular vesicles (EVs). These are nanosized membrane enclosed vesicles that contain heterogenous bioactive cargo which were shown to display multi-faceted cancer-promoting functions.

Objectives

We propose that US28 modulates EV secretion and/or composition via pathways that require elucidation to further understand the GPCR-mediated onco-modulation.

Methods

To determine whether US28 itself and its presence in glioma cells changes EV secretion or composition, Western blotting looking at various EV markers was employed. For localization studies of US28 and with EV markers of interest, immunofluorescence imaging was used. EVs were isolated from conditioned media using ultrafiltration and size exclusion chromatography. Further, luminescence-based assays were performed to determine molecular determinants of US28 that are responsible for EV secretion and/or cargo selection.

Results

Our results showed the presence of US28 in EVs derived from glioma cells, which seems partially dependent on constitutive activity and intracellular cycling. Further, a comprehensive EV marker panel indicated an US28-dependent change in either EV particle amount or content upon its expression in glioma cells.

Conclusion

Our findings identify US28 as a regulator of EV secretion and/or cargo selection, changes that may explain its reported oncomodulatory role.

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A novel and promising ruthenium-based PACT treatment for uveal melanoma

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Biography

Dr. Daria Kotova is a Postdoc in the group of Professor Sylvestre Bonnet in Leiden Institute of Chemistry (LIC). She is a biochemist who defended her PhD thesis in Molecular Biology with a strong physiological basis. For her PhD, she studied redox processes in brain cells in vivo and has an extensive experience in developing optogenetic tools and implementing them in various pathological models. During that time she published 15 papers in peer-viewed journals. In the Metals in Catalysis, Biomimetics & Inorganic Materials (MCBIM) group at LIC, she is working on optimizing photoactivated chemotherapy for in vivo use in cancer treatment.

A novel and promising ruthenium-based PACT treatment for uveal melanoma

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Background and Objective

Uveal melanoma (UM) is a rare eye tumor and the most prevalent intraocular cancer in adults, affecting approximately 4.3 individuals per million worldwide1, with the highest incidence in Northern Europe, reaching up to 10 cases per million2. Primary UM treatments, though effective, often lead to a diminished quality of life due to vision loss, and 50% of patients develop liver metastases3. The PACT4EYE project aims to create an innovative treatment method known as photoactivated chemotherapy (PACT) for UM. This approach uses a patented ruthenium-based prodrug (Ru-MTI) that becomes toxic when activated by green or red light, releasing the cancer-targeting agent MTI (MicroTubule polymerization Inhibitor) and a non-toxic, ruthenium-containing, photocleavable protective group. The technique employs laser light at the tumor site to activate the prodrug locally, effectively destroying the tumor with minimal side effects. This method is unique due to its dioxygen-independent bond cleavage photoreaction, differentiating it from current UM treatments that rely on oxygen activation. Ru-MTI can be activated even in hypoxic tumor tissues, which are generally more resistant to other treatments. Current study explores the potential of this new PACT compound in cancer therapy, particularly for treating uveal melanoma.

Materials and Methods

Primary in vitro experiments were carried out to evaluate the efficacy of Ru-MTI in both normoxic and hypoxic conditions by conducting photocytotoxicity tests on various cancer cell lines, analyzing compound uptake, and determining the mode of cell death. In vivo studies were conducted to explore biodistribution and anticancer activity using a subcutaneous tumor model in mice, aiming to determine the optimal treatment regimen.

Results and Discussion

Our findings demonstrate the strong therapeutic potential of PACT treatment with Ru-MTI, which can be activated by both green and red light. We confirmed that the photocytotoxicity results from microtubule polymerization inhibition, leading to cancer cell death through apoptosis. Additionally, we showed that Ru-MTI demonstrates high efficacy under both normoxic and hypoxic conditions. The compound's enhanced water solubility also supports more versatile drug administration, aligning with the observed rapid blood clearance and confirmed good biosafety in in vivo studies using a mouse model.

Conclusions

Ru-MTI is a promising drug for the treatment of various cancer types, particularly uveal melanoma. These results are encouraging for further pre-clinical in vivo studies of specific drug formulations. The PACT4EYE

project focuses on developing and refining this treatment approach, facilitating the translation of this technology to human applications.

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A modular nanoparticle vaccine platform for simultaneous antigen and adjuvant delivery to antigen-presenting cells

Anne de Dreu

Biography

Anne de Dreu obtained her Bachelor's and Master's degree in Biomedical Engineering from the Eindhoven University of Technology (TU/e). She then pursued a PhD in the Precision Medicine research group, also at the TU/e . She is currently a fourth year PhD candidate under the supervision of Prof. Willem Mulder and Dr. Roy van der Meel. Her research focusses on the development of fusion proteins that are used in immune-modulating nanoparticles.

A modular nanoparticle vaccine platform for simultaneous antigen and adjuvant delivery to antigen-presenting cells

Anne de Dreu

Abstract

As became evident during the COVID-19 pandemic, a vital need exists for flexible and safe platforms that enable swift vaccine manufacturing and deployment. Here, we present a modular vaccine platform based on apolipoprotein A1 (apoA1) nanoparticles (aNPs) with avidity for antigen-presenting cells. The vaccine aNPs' composition can be finetuned by incorporating apoA1-antigen fusion proteins and various adjuvants. In vitro, apoA1-ovalbumin fusion proteins and aNPs were efficiently taken up by dendritic cells. Subsequent activation of ovalbumin-specific CD8+ T cells was confirmed with flow cytometry. We investigated the in vivo behaviour of our aNPs upon intravenous or intramuscular administration and found that injected aNPs mainly accumulate in lymph nodes, bone marrow and spleen. We then vaccinated mice with apoA1-ovalbumin aNPs containing a single adjuvant, or combinations of multiple adjuvants. We observed increased numbers of ovalbumin-specific CD8+ T-cells in the spleens of mice vaccinated with aNPs containing multiple adjuvants. Additionally, significantly more antibodies against ovalbumin were raised for aNPs that contained multiple adjuvants compared to aNPs that contained only one adjuvant, and to aNPs mixed with adjuvants currently in clinical use. With this, we have taken important steps in establishing a modular vaccine platform that elicits a strong cellular and humoral immune response.

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Enhancing clinical decision-making: the role of ex vivo drug sensitivity profiling in pediatric precision medicine.

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Biography

Marlinde Schoonbeek is a PhD candidate at the Princess Máxima Center of paediatric oncology. She focuses on advancing current pre-clinical models for determining drug sensitivity in neuroblastoma and other pediatric solid tumors. She established ex vivo short-term cultures as well as organoid models to be compared with PDX models of the ITCCP4 consortium. Next to this, she improves drug sensitivity screens by an advanced microscopy-based viability readout.
Next to research, Marlinde is a junior ambassador of the Dutch TOPX network for ambitious woman in life sciences, in which she enjoys organizing and moderating events and panel discussions. Within the institute, Marlinde is a part of Ace, a community promoting authenticity for excellence in science by organizing role model lunches, coaching programs and workshops.

Enhancing clinical decision-making: the role of ex vivo drug sensitivity profiling in pediatric precision medicine

Introduction

Despite advancements in identifying targetable driver genes in pediatric cancer, precision medicine's efficacy is hindered by inadequate models to promptly predict response to therapy. In vivo studies are costly and subjected to ethical considerations. Additionally, the establishment of patient-derived xenografts (PDXs) and organoids often takes too long to be beneficial for the patient. Preclinical models for precision medicine must be rapid to guide clinical decisions, feasible to establish and reliable in predicting drug response.

Objectives

This study proposes ex vivo short-term drug screening of tumor material as a solution to timely determine tumor drug sensitivities, focusing on solid tumors.

Methods

The ITCCP4 consortium established 350 pediatric cancer PDX models, from which 50 pan-solid models were selected for ex vivo short-term screening. In addition, PDX models were selected to build a pan-cancer organoid repository. The patient, PDX and organoid models were profiled using whole exome sequencing, low-coverage whole genome sequencing, RNA-sequencing, and methylation arrays. Compound sensitivity was determined for ex vivo short-term and organoid models (140-224 compounds).

Results

In ex vivo short-term screening is a rapid method, as drug sensitivity profiles were determined within 14 days after receiving the primary sample. Ex vivo pan-cancer short-term screens were successfully performed in 37 out of 50 cases (74% success rate), demonstrating the feasibility of the model. The cohort of samples covers eight tumor types, such as neuroblastoma (36%), Ewing sarcoma (19%), osteosarcoma (8%) and hepatoblastoma (5%). The protocol demonstrated high reproducibility in drug sensitivity profiling, with tumors from a single model harvested from two distinct PDXs showing a correlation of $r = 0.95$. As expected, drug sensitivity-based hierarchical clustering of ex vivo short-term screens show aggregation based on tumor type. Consistent with literature, we find significantly lower sensitivity for idasanutlin in TP53 mutated patients compared to TP53 wildtype patients. In addition, 5 PDX-derived neuroblastoma organoids were established (50% establishment success). Drug sensitivities of NB ex vivo

short-term screens show a strong correlation with organoid screens derived from the same patient (r = 0.72 to 0.96, n = 4).

Conclusion

This study demonstrates that ex vivo short-term screens are a suitable model to guide clinical decisions in pediatric solid tumors. This model is fast enough to benefit the patient, feasible to establish from solid tumors and drug sensitivities correlate with those of organoids and known genetic events. Currently, ex vivo short-term screens are performed on patient samples in the clinic, and ex vivo drug sensitivities are correlated with clinical outcome. Ex vivo short-term screens hold significant promise for broad adoption in clinical practice, timely facilitating personalized interventions for pediatric cancer patients.

Fig. 1: 37 ex vivo pan-cancer short-term screens were successfully performed, across 8 tumor types (74% success rate)

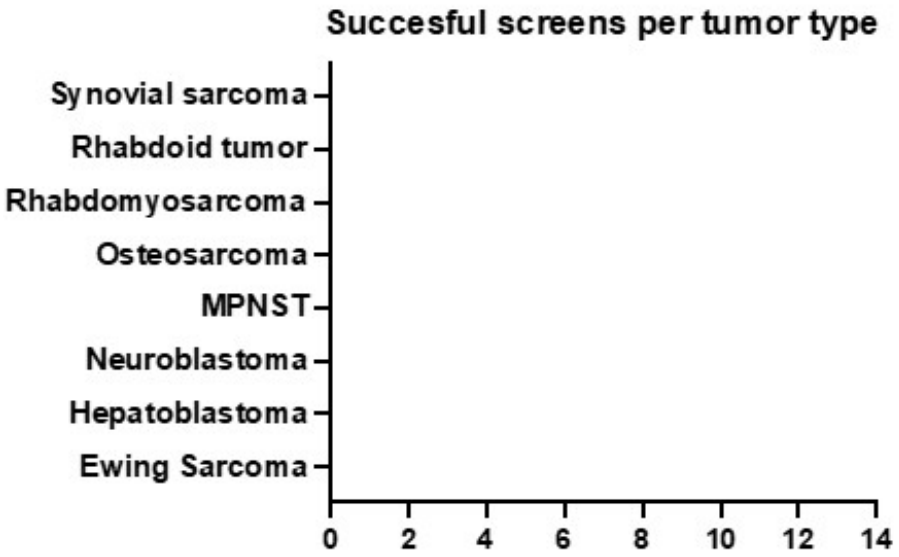


Fig. 2: Drug sensitivity-based hierarchical clustering of ex vivo short-term screens partly show clustering based on tumor type.

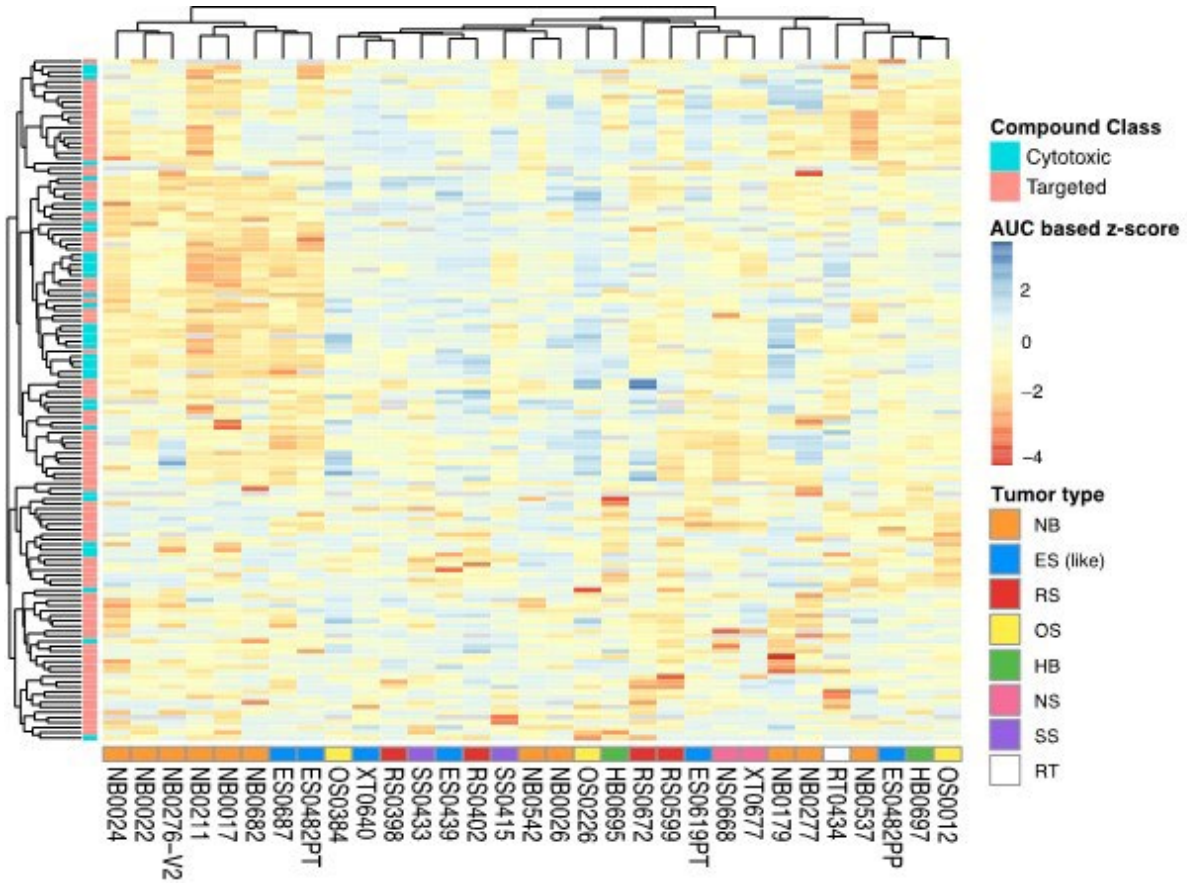
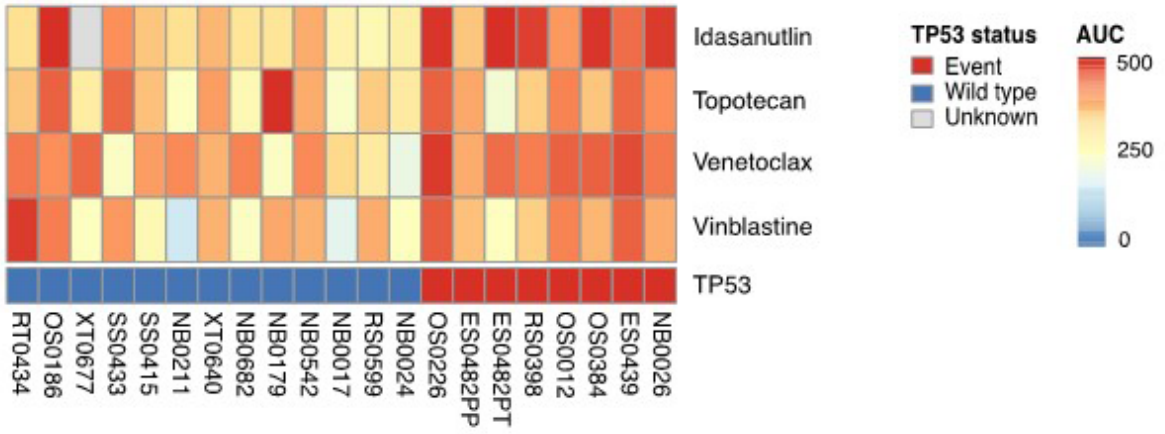


Fig. 3: We find significantly lower sensitivity (i.e. higher AUC) for idasanutlin in TP53 mutated patients compared to TP53 wildtype patients.



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Pilot Study: Is Autism Spectrum Disorder Related with Formula?

David Rojas-Velazquez, Ting Chia Liu, Sarah Kidwai, Johan Garssen, Alejandro Lopez-Rincon

Biography

Currently a PhD candidate in the pharmacology laboratory at Utrecht University, where I apply machine learning methods to discover biomarkers for the diagnosis of autoimmune diseases.

Pilot Study: Is Autism Spectrum Disorder Related with Formula?

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Introduction

Autism Spectrum Disorder (ASD), characterized by social communication deficits and repetitive behaviors, may have a genetic link [1, 2]. Key factors affecting neurological development include breastfeeding and the baby's social environment [3, 4]. Additionally, the gut microbiome, often differentiated in those with ASD who also experience intestinal issues, is a potential contributing factor to ASD development [5].

Objectives

We will employ machine learning-based feature selection algorithms to detect taxonomic differences between breast milk and formula. Subsequently, we will investigate whether these identified taxa exhibit any relationship with Autism Spectrum Disorder (ASD) by examining two relevant datasets.

Methods

Table1 details three datasets used to study taxa differences between breast milk and formula, and two ASD-related datasets to explore potential links between these taxa and ASD. All datasets were downloaded from NCBI repository.

Table 1: Characteristics of the selected datasets

Accession Number	Experiment	Samples	Labels	Taxa (features)
PRJNA633365 [6]	breast milk - formula	65	22 - breastfeed 43 - formula-feed	1,202
DRA007599 [7]	breast milk - formula	60	24 - breastfeed 36 - formula-feed	2,521
PRJNA515307 [8]	breast milk - formula	40	20 - breastfeed 20 - formula-feed	1,779
PRJNA578223 [9]	ASD	96	48 - healthy-control 48 - ASD	18,749
PRJNA624252 [10]	ASD	50	20 - healthy-control 30 - ASD	3,249

Feature Selection

We applied the methodology described in [11] which combines a DADA2-based script and the Recursive Feature Selection (REFS) algorithm designed for biomarker discovery and consists of four phases:

- dataset selection criteria
- raw data processing
- feature selection
- testing

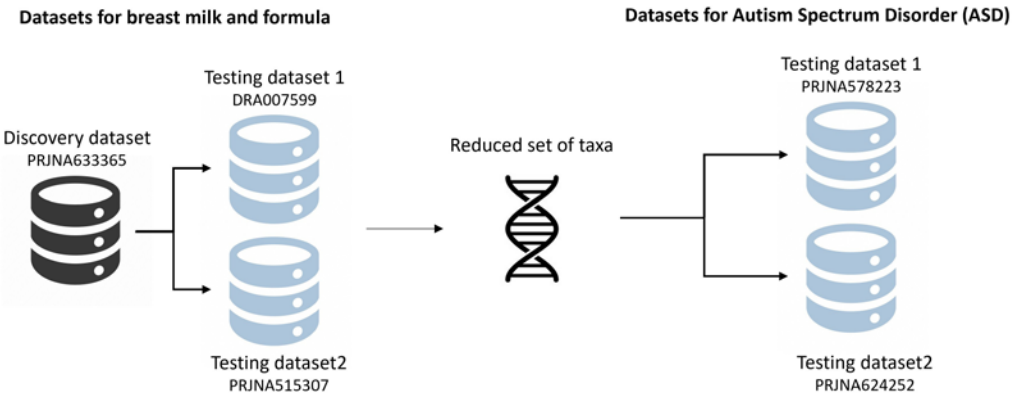
The methodology was applied to identify taxa differences between breast milk and formula using three independent datasets: one as a discovery dataset (PRJNA633365) and the remaining two for testing (DRA007599 and PRJNA515307). For the ASD experiments, we used the same methodology applied to two ASD-related datasets (PRJNA578223 and PRJNA624252), but in this case, we replaced the feature selection phase with the taxa resulting from the previous experiment, Fig.1.

Results

After applying the methodology to the three datasets related to breast milk and formula, nine bacteria were identified in the discovery dataset PRJNA633365, at genus level: Cutibacterium, Lactobacillus, Bacteroides, Bifidobacterium, Bifidobacterium, Lactobacillus, Bilophila, and Romboutsia; at order level: Enterobacterales. Five out of nine and three out of nine were found in the testing datasets DRA007599 and PRJNA515307 respectively.

In all cases, the algorithm with the best performance after the testing phase was KNeighbors with AUCs of 0.77 for PRJNA633365, 0.63 for DRA007599 and 0.75 for PRJNA515307. In the ASD experiments, we found two out of nine for PRJNA578223 and tree out of nine for PRJNA624252. For both cases, the algorithm with the best performance was the Multi-layer Perceptron with AUCs of 0.67 for PRJNA578223 and 0.62 for PRJNA624252. The bacteria found in the ASD datasets are, at genus level, Bacteroides and Bilophila in both datasets and Lactobacillus only in PRJNA624252. The literature shows Bacteroides and Lactobacillus are increased [12, 13] in people diagnosed with ASD, in contrast with Bilophila which is decreased [13].

Figure 1: General overview of the experiments in this study using the methodology described in [11].



Conclusion

We applied a reproductible methodology that makes use of machine learning methods to identify taxa differences between breast milk and formula. These results were used to study a possible relationship between these taxa and ASD. The results obtained in this pilot study are promising. While further research with more experimentation is necessary, this study provides the first steps to explain the roles that breast milk and/or formula play in the development of ASD and other conditions.

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Machine Learning Analysis of Gut Microbiome Profiles in Infants from Different Feeding Practices

TingChia Liu, Sarah Kidwai, David Rojas-Velazquez, Johan Garssen, Alejandro Lopez-Rincon

Biography

I am currently pursuing a Master's degree in Bioinformatics and Biocomplexity, with a keen interest in understanding the intricate relationships between microbial communities and human health. My academic journey has equipped me with a robust foundation in data analysis, and complex systems, which I am now applying to cutting-edge research in microbiome studies.
My research project focuses on the infant microbiome and its impact on health. This topic is particularly fascinating as it explores how early-life microbial communities influence developmental outcomes and long-term health. Through this project, I aim to uncover critical insights that could inform better infant healthcare strategies.

Machine Learning Analysis of Gut Microbiome Profiles in Infants from Different Feeding Practices

Ting Chia Liu1, Sarah Kidwai1, David Rojas-Velazquez1,2, Johan Garssen1,3, Alejandro Lopez-Rincon1

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Introduction

The importance of breastmilk in infant health is well-established, recent research suggests that early nutrition affects the infant's gut microbiome, development of their immune system, and their health throughout life. Feeding patterns significantly influence the gut microbiota in infants, and several studies have compared the effects of human milk versus formula milk on the infant microbiome.

Objectives

We will use Machine learning-based feature selection algorithms to identify taxonomic differences between breast milk and formula, and analyze its relationship with infant health.

Methods

Data

Table 1 details the three datasets, downloaded from NCBI repository, used in this study.

Accession Number	Samples	Labels	Taxa (features)
PRJNA633365 [1]	65	22 - breastfeed 43 - formula-feed	1,202
DRA007599 [2]	60	24 - breastfeed 36 - formula-feed	2,521
PRJNA515307 [3]	40	20 - breastfeed 20 - formula-feed	1,779

Feature Selection

We applied a methodology that combines a DADA2-based script and the Recursive Feature Selection (REFS) algorithm designed for biomarker discovery [4], to identify taxa differences between breast milk and formula using three independent datasets: one as a discovery dataset (PR- JNA633365) and the remaining two for testing (DRA007599 and PRJNA515307).

Results

After applying the methodology to the three datasets, nine bacteria were identified in the dis- covery dataset PRJNA633365. At the genus level, these bacteria are: Cutibacterium, two Lactobacillus, Bacteroides, two Bifidobacterium, Bilophila, and Romboutsia. At the order level, Enterobacterales were identified. After searching the resulting bacteria in the testing datasets DRA007599 and PRJNA515307 we found five out of nine and tree out of nine respectively. For the tree datasets, the algorithm with the best performance after the testing phase was KNeighbors with AUCs of 0.77 for PRJNA633365, 0.63 for DRA007599 and 0.75 for PRJNA515307. In Fig. 1 is illustrated the effectiveness of the nine bacterias in making the difference between the breastfeed and formula-feed groups.

Certain microbes provide protection to gut health, while others are more prevalent in infants with allergic diseases. For example:

- Cutibacterium and Bacteroides play a protective role in atopic dermatitis (AD) [5, 6].
- Bilophila increases in infants with AD [7].
- Bifidobacterium serves as a biomarker for investigating allergic infants [8], including those with asthma [9] and cow's milk allergy [10].
- Lactobacillus is used as a probiotic in the treatment of allergic rhinitis [11]. Both Lactobacillus and Bifidobacterium seem to be protective against the development of food allergies (FA) [6].

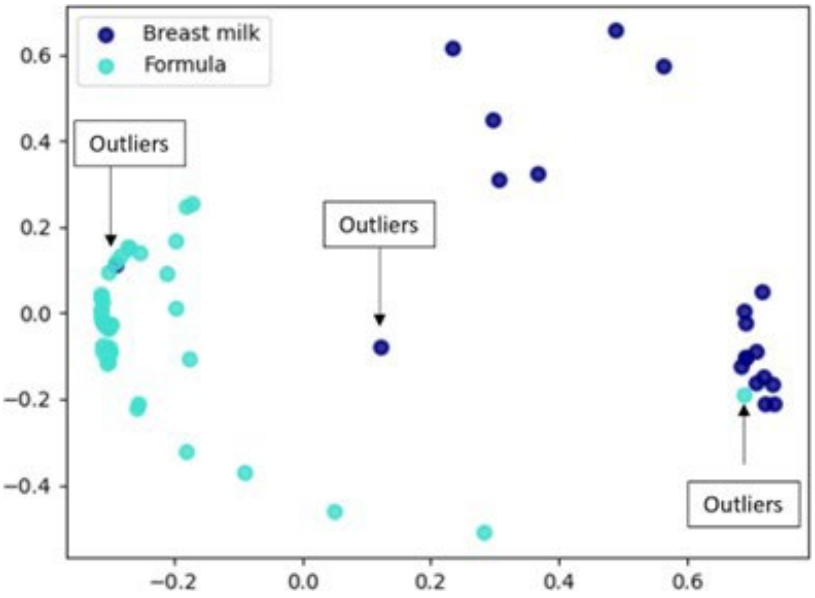

Figure 1: PCA plot using rbf kernel.

- Romboutsia is found more frequently in patients with neurodevelopmental disorders (NDD) [12]. Specifically, Romboutsia timonensis has been associated with autism diagnosis [13].
- Phylum Proteobacteria class and Gammaproteobacteria (Enterobacterales order) may be associ- ated with the development of allergic respiratory diseases [7].

Conclusion.

We identified taxonomic differences between breast milk and formula by applying a methodology to analyze microbiome datasets. The literature analysis of the resulting taxa show that breast-fed infants have a higher abundance of beneficial microbes, such as Bifidobacterium and Lacto- bacillus, which protect against allergic diseases and infections. The next steps of this work will be to expand the analysis to more datasets and study the relationship between infant health and the microbiome present in breast milk and formula.

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Development of a 2,3-Difluorosialic Acid-Based Covalent Neuraminidase Probe

Lemeng Chao

Biography

Lemeng Chao obtained her bachelor's degree in 2015 from Nankai University, China, with a thesis titled A Mesoporous Silica Nanoparticles (MSNs) Based Multi-responsive Drug Delivery System; She started her master's study in Utrecht University in the same year, during which she carried out two researches: The Release Study of Macromolecules Loaded Biodegradable Nanogels, and Development of Fluorophore Probes for the Determination of Specificity and Activity of Influenza Virus Neuraminidase. Her review of the master writing project resulted in a publication in Angewandte Chemie. In 2018, she started her PhD project at Utrecht University titled Molecular Tools to Determine, Image, and Perturb the Activity of Influenza Viral Neuraminidase, in which she focused on the synthesis of difluorosialic acid-based probes and novel neuraminidase inhibitors. She was also involved in collaborative research to study paramyxovirus, resulting in two publications in Plos Pathogen.

Development of a 2,3-Difluorosialic Acid Based Covalent Neuraminidase Probe

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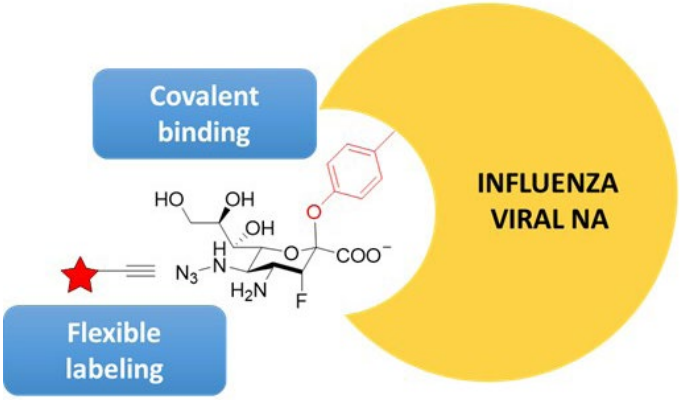
Neuraminidase (NA), one of the major surface glycoproteins of influenza A virus, is an important diagnostic biomarker and antiviral therapeutic target. Probing NA provides important information of influenza virus biology, that can monitor the emergence of drug-resistant strains and guide the development of novel drugs and vaccines. However, there is still a lack of covalent NA probes with enhanced specificity and higher stability and this limits the in depth exploring of NA.
Based on the covalent NA inactivators, 2,3-difluoro sialic acids (DFSA) synthesized by Wennekes and colleagues1, we modified DFSA with a azide mini-tag to converted it into a covalent probe. In this study, we chemically synthesis of 5-azidoacetamide-2,3- difluoro sialic acid (5N3-DFSA) as a covalent probe. Reactivation assay had shown the probe binding to NA without cleavage in 6 hours. The NA protein inhibited by 5N3-DFSA could be labeled with a reporter via the CuAAC reaction, which also proofed the covalent binding of the probe to the NA protein. Besides, the probe also kept an inhibition activity on NA with a IC50 value from 50 to 60 μM. With these properties, we expect the DFSA probes to be promising tools in labeling, visualizing and mobilizing NA proteins and Influenza virus particles.



Acknowledgement

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Rerouting aNPs to specific immune cells using a modular apoA1 fusion protein platform

Ir. Koen De Bruin, Ir. Ayla Hokke

Biography

Koen de Bruin is a third year PhD student in the Precision Medicine group lead by Willem Mulder and Roy van der Meel at the Eindhoven University of Technology. His work focusses on apolipoprotein A1 fusion proteins for immunomodulatory nanomedicine.

Rerouting aNPs to specific immune cells using a modular apoA1 fusion protein platform

Lipid nanoparticles (LNPs) are widely used for delivering therapeutic payloads such as RNA and small molecules, yet reaching any preferred cell types beyond the liver remains challenging. Our group developed the apolipoprotein-based lipid nanoparticle (aNP) platform, which mimics high-density lipoprotein (HDL) and utilizes apolipoprotein A1 (apoA1)'s affinity for myeloid cells to deliver therapeutic payloads like RNA and small molecules to the innate immune system. Here, we expand our scope beyond the myeloid cell compartment by functionalizing the aNP platform using fusion proteins of apoA1 with a nanobody (VHH) against CD8. Formulated VHHCD8 aNPs targeted to CD8+ T cells were stable and spherical, and in vitro experiments on mouse splenocytes showed binding of VHHCD8 aNPs to CD8+ T cells. Following intravenous injections in mice, radiolabeled VHHCD8 aNPs showed a shorter half-life compared to control formulations, and higher accumulation in lymphoid organs like the spleen and lymph nodes. Cell-specific analysis of aNP biodistribution shows clear rerouting of VHHCD8 aNPs to CD8+ T cells in all analyzed organs. This research shows the potential of rerouting aNPs to selected immune cell types using this modular scaffold with easily exchangeable nanobodies, and can ultimately be applied in precision immunotherapy.

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Exploring poly(2-oxazoline)-based lipids for nucleic acid delivery

Sulan Luo, Zlata Nagorna, Stef van den Berg, Heyang Zhang, Joachim Van Guyse

Biography

Sulan Luo obtained her Master of Science in Pharmaceutics from Sun Yat-sen University in 2020. Since 2023, she has been doing a PhD program at Leiden University, where her research focuses on the use of poly(2-oxazoline) in nucleic acid delivery.

Exploring poly(2-oxazoline)-based lipids for nucleic acid delivery

S. Luo, Z. Nagorna, S. van den Berg, H. Zhang, J. F. R. Van Guyse
Division of BioTherapeutics, LACDR, Leiden University, the Netherlands

Introduction

Lipid nanoparticles (LNPs) are the most clinically mature technology for RNA delivery, with a proven track record in the COVID-19 vaccination campaigns, and currently under investigation for cancer therapies. A crucial component herein are the PEG-lipids (polyethylene glycol), which affect stability, immunogenicity, biodistribution and gene expression. Despite their benefits, current LNP technologies face several challenges. Issues such as liver tropism, immunogenicity, and limited stability in complex biological media remain, prompting research into novel LNP components.1 Recently, we demonstrated the utility of poly(2-oxazoline)-lipids in LNP-mediated RNA delivery, exhibiting comparable performance to PEG-LNPs, yet displaying attenuated immune responses.2 To elucidate the origins of attenuated immunogenicity, we investigated the in vitro uptake and transfection performance of LNPs with different PEG, POx, and lipid combinations upon serum incubation. Interestingly, while the systems feature a similar performance upon direct incubation, serum preincubation revealed notable differences, suggesting the impact of linker between polymer and lipid on LNP stability and performance.

Objectives

Within this study, we assessed the in vitro performance of LNPs formulated with different PEG and POx-lipid combinations upon serum incubation. The goal of this study is to correlate lipid-polymer structure to LNP performance and attributes, such as immunogenicity, stability, expression efficiency and biodistribution. Our preliminary results indicate a correlation between stability and the polymer-lipid component in LNPs.

Methods

mRNA-LNPs (ONPATTRO® formulation) were synthesized by ethanol injection, mixing the lipid components with mRNA in citrate buffer (pH 3, 50 mM) at an N/P ratio of 5, followed by overnight dialysis. LNPs were characterized for encapsulation efficiency (Ribogreen assay), size, and polydispersity (dynamic light scattering). Cellular uptake (Cy5) and protein expression (EGFP) in HepG2 and Hela cells were analyzed via flow cytometry after 24 hours. Additionally, LNPs were pre-incubated in 10% FBS or PBS for 2 hours to assess the impact of serum on their in vitro performance.

Results

All LNPs were homogenous (PDI <0.24), with sizes around 130 nm and encapsulation efficiencies > 85%. In vitro experiments revealed a similar transfection efficiency across formulations when applied directly to Hela and HepG2 cells. Serum preincubation, however, boosted transfection efficiency in 3 out of 4 formulations. One formulation displayed a comparable performance regardless of preincubation, highlighting its relative stability to other formulations. Based on these results, we hypothesize that LNP stability in serum can be affected by the choice of linker between lipid and polymer.

Conclusion

Our results indicate that the linker between polymer and lipid can impact the overall stability of LNPs upon serum exposure, which we hypothesize to be an important factor in the immunogenicity and liver tropism displayed by current technologies. Further research employing a library approach is ongoing to identify structure activity relationships.

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Unraveling the influence of structural attributes on the biological performance of synthetic nanoparticles

Xinye Gao, Bonan Zhao, Jeroen Bussmann, Matthias Barz, Heyang Zhang

Biography

Xinye Gao is a researcher with a background in immunopharmacology, holding a Master's degree from Shandong University and a Bachelor's degree in Pharmacy from Yantai University. She has gained experience in the construction and functional study of CAR-NK cells, focusing on enhancing NK cell efficacy through gene modifications and lentivirus packaging. Her research has included exploring the role of RB1 in hepatocellular carcinoma and comparing NK cell functions under different cytokine conditions. She is proficient in experimental techniques such as flow cytometry, cell proliferation assays, and RNA sequencing data analysis. Currently, her research focuses on drug delivery, specifically optimizing lipid nanoparticle (LNP) formulations and studying LNP properties in zebrafish models.

Unraveling the influence of structural attributes on the biological performance of synthetic nanoparticles

Xinye Gao, Bonan Zhao, Jeroen Bussmann, Matthias Barz, Heyang Zhang

Introduction

The physicochemical properties of nanoparticles, including size, morphology, and surface characteristics, significantly influence their circulation, biodistribution, and therapeutic efficacy following systemic administration. Understanding the relationship between nanoparticle attributes and their biological performance is crucial but challenging, primarily due to the instability of certain nanoparticles (e.g., lipid nanoparticles, micelles) in the dynamic and complex physiological environment. To comprehensively explore the impact of structural attributes on biological performance, we synthesized polypept(o)ide-based brush-like nanoparticles as a versatile platform, allowing for precise control over the properties, and thus facilitating a thorough investigation of their behavior both in vitro and in vivo.

Method

Polypept(o)ide-based nanoparticles composed of poly-L-lysine backbone and polysarcosine side chain, with defined size, morphology and surface charge were synthesized, and characterized by dynamic light scattering and fluorescence correlation spectroscopy, with respects to size and stability in buffer and human plasma. The morphology of nanoparticles was observed by atom force microscopy. Using Alexa fluor 647 labeled nanoparticles, in vitro cellular uptake was assessed on different cell lines (Hela & Raw264.7 cell) by flow cytometry. Further, the in vivo behavior was visualized and quantified in zebrafish embryos, including circulation and biodistribution.

Objectives

This study aims to evaluate the influence of structural attributes of synthetic nanoparticles on their biological performance.

Results

Three synthetic brush-like nanoparticles (hydrodynamic radius of 11.0, 17.6, and 33.0 nm) with neutral surface charges remained stable after 24 hours' exposure to human plasma. The densely packed polysarcosine shell prevented significant cellular internalization across all cell lines tested. After exposure to human plasma, there was no detectable aggregation in all particles, regardless of the surface charge (-17.7, -2.5, and 30.5 mV), while a slight increase in hydrodynamic radius (approximately 3 nm compared to PBS) suggests minimal protein adsorption. In vitro, cationic nanoparticles exhibited significantly higher cellular uptake compared to their zwitterionic and anionic counterparts, with relative mean fluorescence intensity (rMFI) increasing by 600-fold in RAW264.7 cells and 2000-fold in HeLa cells compared to

negatively and neutrally charged nanoparticles. However, these in vitro findings were not observed in zebrafish embryos following microinjection into the duct of curvier, and no significant differences in circulation half-life or biodistribution were found.

Conclusion

Our findings suggest that the size and surface charge of brush-like polymer nanoparticles do not significantly influence their circulation in vivo. However, in vitro studies revealed differences in cellular uptake among charge-controlled nanoparticles, though the underlying causes for these observations require further investigation.

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Multicompartment Polyion Complex Micelles Facilitate RNA Therapeutics

Prof. Dr. Matthias Barz, Marco Hehmann, Dr. Heyang Zhang

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Abstract

While lipid formulation represents the leading option in the clinical applications of RNA therapeutics, several challenges, such as limited stability and limited loading capacity (L. Xu Adv. NanoBiomed Res. 2022), pose the need of polymeric delivery systems. Our previous work has demonstrated polypept(o)ide-based polyion complex micelles (PICMs) as a safe, efficient and versatile platform for nucleic acid (e.g., siRNA, mRNA) delivery. While we highlighted the potential of PICM in siRNA delivery, the insufficient endosomal escape efficiency limited the further application (Capelôa, Macromol. Rapid. Commun. 2022). This can be remedied by a multicompartment PICMs which contain a polycationic core to complex nucleic acid and a hydrophobic compartment for coloading of endosomal escape enhancing drugs while an outer shielding layer provides stability against degradation. By use of pSar (poly(N-methyl glycine)) as biocompatible and biodegradable PEG-alternative promoting longer circulation times, polybenzylglutamic acid as hydrophobic block encapsulating desloratadine (DES) and polylysine for nucleic acid binding the Barz Lab developed an siRNA/DES loaded PICM. In the collaboration with Schneider and coworkers the control groups, including siRNA-LNPs, failed to show comparable knockdown efficiencies of the targeted microfibrillar associated protein5 (MFAP-5) in the cancer associated fibroblasts (CAFs) of the tumor microenvironment (TME) of a hepatocellular carcinoma (HCC) mouse model (Schneider et al. Adv. Mater. 2024). These findings highlight the potential of a coloaded PICM and the boosting of endosomal escape to improve gene therapy. Schwiertz et al. (Biomacromolecules 2024) demonstrated the morphological versatility of PICMs by varying the linear structure towards miktoarm stars comprising 3 or 6 arms of the outer shielding layer. While the authors presented an increased stability and enhanced shielding effect compared to the linear equivalents, conversely, the transfection efficacy suffered as a result. As described above this shortcoming can be remedied by coloading of cationic amphphilic drugs (CADs) like DES. The versatile polypept(o)ide based coloaded PICMs will be further exploited to study the influence of each blocklength, segment composition, architecture and drug linkage. Taking into account the findings from the previous investigations on the structure activity relationship, the optimized PICMs hold the potential to expand the current state of nucleic acid delivery.

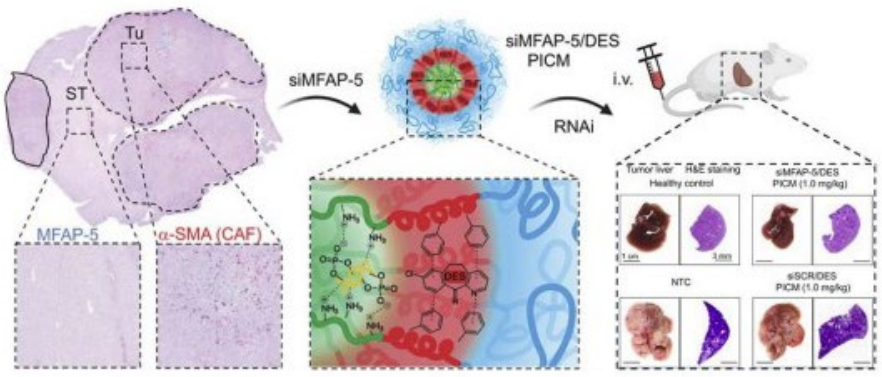

Figure 1. Schematic overview of studying siRNA/DES PICM in syngeneic implantation model of HCC (Schneider et al., Adv. Mater. 2024).

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Physiologically-based pharmacokinetic modelling for novel radiopharmaceuticals using a multilevel object-oriented modelling methodology

Dr. Ramona Bouwman, Dr. Govert de With

Biography

Dr. de With is associated with the application of ionising radiation since he started working at NRG in 2008. Since 2017 he is responsible for the R&I activities on medical applications. This includes research in the field of physiological and dosimetric modelling. His work is dealing with the development of models for prediction/estimation of the uptake of radiopharmaceuticals in human tissue using pharmacokinetic models. Furthermore, Govert is responsible for the Dutch National Dose register in the Netherlands. In this role he extensively contributed to the development of harmonised procedures containing specifications and guidance for the collection and registration of radiological data.

Physiologically-based pharmacokinetic modelling for novel radiopharmaceuticals using a multilevel object-oriented modelling methodology

Introduction

NRG PALLAS is a producer of medical isotopes which are used for more than 30 000 patients daily. Currently NRG PALLAS is building the new PALLAS reactor in Petten to support the production of medical isotopes. NRG PALLAS contribute to the innovation of novel radiopharmaceuticals (RPs) via the FIELD-LAB project.

Todays novel therapeutic RPs are focussing on alpha emitting nuclides. To assess dosimetric impact of these novel radiopharmaceuticals we developed a multilevel object-oriented physiologically based pharmacokinetic (PBPK) model for the treatment of neuroendocrine tumours, based on a PBPK model previously developed by Kletting et al. In this model, which is implemented in PhysPK, the full chain of events following intravenous administration are included: eg. extravasation, binding, internalization and release. To study the usefulness of the model for alpha therapy we have performed a simulation to study the results for an alpha emitting nuclide including her progeny.

Material and Methods

In this study we have used a pre-therapeutic scan (from literature) which provide patient specific uptake of the RP in healthy and tumorous tissue and simulated a treatment with Lu-177-DOTATATE and Pb-212-DOTAMTATE. The model including patient specific details is used to simulate therapy with a total administration of 145 nmol DOTA(M)TATE of which 10 nmol is labelled with either Lu-177 or Pb-212. The dissociation constant and dissociation rate of 0.52 nmol l-1 and 0.013 min-1 respectively are used for both radiopharmaceuticals. Subsequently we compared the amount of radioactive compound accumulated in the liver, spleen, kidney and the tumour at the different sub-tissue levels. For each organ the time integrated activity (TIA) is determined and the fraction located in the vascular, interstitial and cellular space is estimated.

Results

The TIA of Pb-212 was found to be between the 20 and 26% of the Lu-177 TIA for the evaluated tissues. The distribution of the radiopharmaceutical between the different sub-tissue levels was found to be comparable for each radiopharmaceutical including the progeny. This is expected because both the physiological and pharmacokinetic parameters are equal. However, although the fraction of receptors bound with DOTA(M)TATE remains equal, the fraction bound with radioactive DOTATATE declines more quickly for Pb-212 and progeny due to differences in decay time. This means that the time in which the total dose is delivered to the tissue is shorter for Pb-212.

Conclusion

Although further validation is needed, the multilevel PBPK model demonstrated to be useful for applications in targeted alpha therapy. One of the subsequent steps to include in the model are the inclusion of different dissociation parameters for different ligands and the impact of recoil. This allows us to conduct dosimetric evaluations of such novel radiopharmaceutical before it's actual clinical use.

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The multi-colored role of microRNA-33a-3p in lipid metabolism and atherosclerosis development

Melanie Modder, Vimal Ramachandran, Patrick Rensen, Peter Tontonoz, Hani Najafi-Shoushtari, Sander Kooijman

Biography

My research aims at identifying novel pharmacological targets for cardiometabolic diseases. The focus is on the regulation of lipid metabolism of which we often make use of the APOE*3-Leiden.CETP mice, as a well-established model for human lipoprotein metabolism and atherosclerosis development.

The multi-colored role of microRNA-33a-3p in lipid metabolism and atherosclerosis development

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Background

Genetic knockdown and therapautic inhibition of miRNA-33a (miR-33a) have been shown to reduce atherosclerosis development in mice, but also induce metabolic problems such as liver steatosis and increased susceptability to obesity. Importantly, miR-33a is processed into two strands after transcription: miR-33a-3p and miR-33a-5p, of which the latter is more abundantly expressed. Therefore, previous experiments predominantly described the effects of lowering miR-33a-5p levels on lipid metabolism and atherosclerosis development, while the role of miR-33a-3p is still quite unknown. Here, we investigated the effects of increased miR-33a-3p levels on lipid metabolism and atherosclerosis in different settings, including hematopoietic and whole-body overexpression, and treatment with a miR-33a-3p mimic in APOE*3-Leiden.CETP mice.

Material and Methods

miR-33a-3p-transgenic (Tg) mice were generated by CRISPR/Cas9 to knock-in miR-33a-3p at the ROSA26 locus and were crossed with Ldlr-/- mice. After western-type diet feeding, plasma lipid levels and atherosclerosis development were assessed in miR-33a-3p Tg Ldlr-/- mice, in Ldlr-/- mice transplanted with miR-33a-3p Tg bone marrow, and in APOE*3-Leiden.CETP mice treated with a liver-targeted miR-33a-3p mimic.

Results

miR-33a-3p Tg Ldlr-/- mice had lower plasma triglyceride (-38%) and LDL-cholesterol (-37%) levels than Ldlr-/- mice. Accordingly, miR-33a-3p overexpression reduced atherosclerotic lesion size (-50%) and number of severe lesions (-47%). Interestingly, Ldlr-/- mice transplanted with miR-33a-3p Tg bone marrow tended to have bigger atherosclerotic lesions and had more severe lesions (+98%) compared to Ldlr-/- mice transplanted with wild-type bone marrow, while plasma lipid levels were unchanged. In APOE*3-Leiden.CETP mice, a well-established model for humanized lipoprotein metabolism, treatment with a liver-targeting miR-33a-3p mimic attenuated postprandial plasma triglyceride (-51%) and non-HDL-cholesterol (-27%) levels. Long-term miR-33a-3p mimic treatment in these mice resulted in more stable atherosclerotic lesions, containing less macrophages and more collagen, while lesion size and severity were similar compared to saline-treated controls.

Conclusion

Our findings reveal heterogenous effects of increasing mir-33a-3p levels in different tissues. Whole-body overexpression of miR-33a-3p has a protective effect on atherogenesis, which is partly due to effects on the liver, while hematopoietic miR-33a-3p overexpression seems to oppose this response.

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Pharmacological profiling for CCR5 antagonists

Yao Yao

Biography

I am a PhD candidate at the Division of Medicinal Chemistry, Leiden University, studying the antagonists for CC chemokine receptors.

PHARMACOLOGICAL PROFILING FOR CCR5 ANTAGONISTS

Yao, Y1, Ortiz Zacarias, N.V1, Heitman, L.H1,2

1 Division of Medicinal Chemistry, LACDR, Leiden University, the Netherlands
2 Oncode Institute, Leiden University, The Netherlands

Aims

CC chemokine receptor 5 (CCR5), belongs to the class A family of G protein-coupled receptors (GPCRs). Like most chemokine receptors, CCR5 has multiple endogenous ligands and is involved in many inflammatory and autoimmune diseases, making it a compelling therapeutic target. Notably, CCR5 serves as a primary co-receptor for human immunodeficiency virus (HIV) infection. So far, only one CCR5 orthosteric antagonist, Maraviroc, is currently available in the market for the latter indication.
Intracellular allosteric antagonists are an interesting novel avenue to target receptors, as they bind to distinct binding sites from endogenous/orthosteric ligands. Thereby, they do not compete with but modulate the affinity/efficacy of endogenous ligands, as well as potentially lead to probe dependence and bias signaling.
Hence, a deeper understanding of promising CCR5 orthosteric and allosteric antagonists at the pharmacological level before the clinical application is needed.

Methods

For this study, three known orthosteric antagonists (Maraviroc, TAK779, and BMS-813160) and six intracellular allosteric antagonists from diverse chemical scaffolds were selected (CCR2-RA-[R], JNJ-27141491, LUF7722, LUF7654, LUF7684, and LUF7686). These were then profiled in different functional assays, i.e. [35S]GTPγS binding, β-arrestin recruitment, and whole-cell impedance assay, yielding potency values of the antagonist set. Moreover, two CCR5 endogenous ligands, CCL3 and CCL5, were used in all assays to assess the so-called probe-dependence of these antagonists.

Results

All nine antagonists inhibit CCR5 in different functional assays when stimulated by chemokines CCL3 and CCL5, with pIC50 values ranging from 5.8 to 10. Of note, the orthosteric antagonists were more potent than the allosteric antagonists, with low nanomolar potency.
In β-arrestin recruitment, most allosteric modulators (except CCR2-RA-[R]) showed probe-dependence, with a preference for CCL3 induction. Among the allosteric compounds, LUF7686 was the most potent in inhibiting [35S]GTPγS binding (pIC50 7.0 with CCL3, and pIC50 7.2 with CCL5), while LUF7721 was the most potent in inhibiting the β-arrestin recruitment (pIC50 7.6 with CCL3). LUF7721 preferred β-arrestin recruitment to [35S]GTPγS binding, when induced by both chemokines. Interestingly, while CCR2-RA-[R] and JNJ-27141491 exhibited lower potencies, these compounds displayed a preference for inhibition of CCR5 upon induction by CCL5 than by CCL3 in the [35S]GTPγS binding assay.

Conclusion

In conclusion, this study provides a comprehensive pharmacological characterization of CCR5 orthosteric and allosteric antagonists. Furthermore, it offers valuable insights for the understanding of CCR5 antagonism, and ultimately helps the development and clinical utilization of CCR5 antagonists.

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Exploring Patient Experiences and Preferences in the Context of Drug Recalls: A Qualitative Study

Msc Pieter Annema

Biography

Pieter Annema is a hospital pharmacy resident at Jeroen Bosch Hospital, where he is also pursuing a PhD focusing on the impact of drug recalls on patients.

Exploring Patient Experiences and Preferences in the Context of Drug Recalls: A Qualitative Study

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4. Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, the Netherlands

Introduction

In a previous study we demonstrated that drug recalls occur regularly in the Netherlands, with some resulting in patients having to change medication. However, research on the ensuing impact of a drug recall on patients is currently absent.

Objectives

This qualitative study sought to elucidate the experiences and preferences of patients concerning drug recalls in the Netherlands.

Methods

Two focus groups were conducted involving patients that experienced a drug recall, employing purposive sampling through an outpatient and a community pharmacy. The sessions were audio-recorded and transcribed verbatim. A thematic analysis approach was used for the analysis of the transcripts, which is currently still ongoing.

Results

The focus group sessions were attended by 13 patients. Patients exhibited varying responses to drug recalls. Some patients displayed minimal concern and proceeded through the recall unaffected. Conversely, others reacted with heightened concern, prompting them to actively engage in the process. Furthermore, patients collectively experienced challenges with the clarity of recall communication. Patients expressed a desire for elucidation regarding the definition and ramifications of a contaminated pharmaceutical product, encompassing both the immediate effects and the potential long-term repercussions stemming from their historical and present utilization of such products. Most patients emphasized the importance of timely, transparent communication through qualified personnel.

Conclusion

Preliminary conclusions are that healthcare providers must realize that patients can respond differently to recalls and that good communication is essential.

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Association of Urinary Epidermal Growth Factor with Kidney Outcomes and Effects of SGLT2 Inhibition: Results from CANVAS and CREDENCE

Erik Moedt, Akihiko Koshino, Dr. Niels Jongs, Dr. Wen Ju, Dr. Michael Hansen, Prof. dr. Stephan Bakker, Prof. dr. Hiddo Heerspink

Biography

Erik Moedt is a PhD-candidate with a background in pharmacy, focusing on biomarkers for personalized medicine in patients with diabetes and CKD.

Association of Urinary Epidermal Growth Factor with Kidney Outcomes and Effects of SGLT2 Inhibition: Results from CANVAS and CREDENCE

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5 Department of Nephrology and Rheumatology, Graduate School of Medical Sciences, Kanazawa University

Introduction

Epidermal growth factor (EGF) is involved in the regenerative processes of kidney tubular cells. Higher urinary EGF (uEGF) levels are associated with a reduced risk of kidney failure in observational studies. The SGLT2 inhibitor canagliflozin reduces the risk of kidney failure in patients with type 2 diabetes (T2D).

Objectives

We investigated the association of baseline uEGF and change in uEGF from baseline to week 52 with kidney outcomes and assessed the effect of canagliflozin on uEGF.

Methods

We performed a post-hoc analysis of the combined CANVAS and CREDENCE trials, which assessed efficacy and safety of canagliflozin versus placebo in those with T2D +/- albuminuric chronic kidney disease. The primary outcome was defined as a 40% decline in eGFR, kidney failure, or death due to kidney failure. Hazard ratios were estimated using multivariate Cox regression. We examined the effect of canagliflozin 100 mg/d on change from baseline in uEGF at years 1 and 3 in 2038 CREDENCE patients (year 3 CANVAS samples were not available) using a repeated measures model. The association of change in uEGF from baseline to week 52 with kidney outcomes was assessed in 4633 patients from the combined cohort.

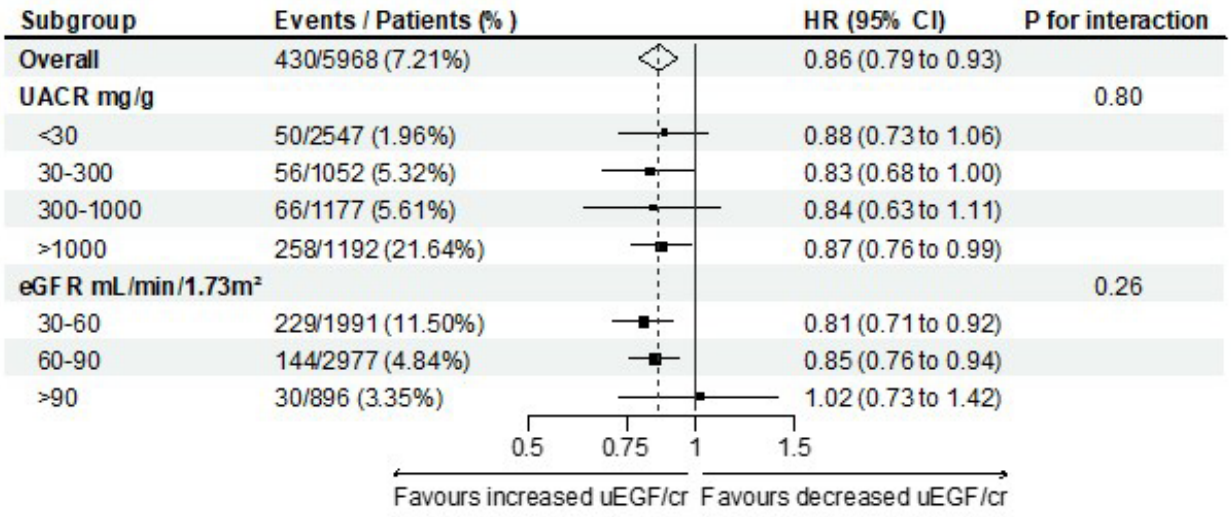
Results

We analyzed data for 3521 (81.3% of the total cohort) CANVAS patients (mean age 62.8 years, eGFR 77.0 mL/min/1.73m2, median UACR 11.6 mg/g) and 2457 (55.8% of the total cohort) CREDENCE patients (mean age 63.2 years, eGFR 57.0 mL/min/1.73m2, median UACR 918 mg/g), with available urine samples. Every doubling in baseline uEGF/Cr was associated with a reduced risk of the kidney outcome (HR 0.86 [95% CI 0.79, 0.93]; p<0.001). This association was consistent across baseline eGFR and UACR subgroups, as depicted in figure 1. The uEGF/Cr change from baseline to year 1 was -12.5% ([95% CI -15.6, -9.1]; p<0.001) in the placebo group and -5.4% ([95% CI -8.7, -1.9]; p=0.002) in the canagliflozin group, corresponding to a between-group difference of 8.1% ([95% CI 1.1, 15.6]; p=0.016). The effect of canagliflozin on uEGF was more pronounced at week 156 with a between-group difference of 16.5% ([95% CI 2.1, 32.8]; p=0.016). Every doubling in change in uEGF from baseline to week 52 was associated with a reduced risk of the kidney outcome (HR 0.75 [95% CI 0.69, 0.81]; p<0.001).

Conclusion

Both higher baseline uEGF/Cr and greater increase in uEGF/Cr from baseline to week 52 were significantly associated with lower risk of kidney disease progression in patients with T2D with and without CKD. Canagliflozin consistently attenuated the uEGF/Cr decrease compared to placebo.

Figure 1:



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*Mitochondrial uncoupling with BAM15 prevents weight gain, lowers plasma cholesterol and attenuates atherosclerosis development in APOE*3-Leiden.CETP mice*

Jamie Van Der Vaart, MSc Christopher L. Axelrod, Prof. Dr. Patrick C.N. Rensen, PhD Robin van Eenige, PhD Sander Kooijman

Biography

Jamie I. van der Vaart is a PhD candidate at the Leiden University Medical Center (LUMC) in the group of Patrick Rensen. She obtained both her bachelor Biomedical Sciences (magna cum laude) and her master in Biomedical Sciences (cum laude) at the University of Amsterdam, specializing in Experimental Internal Medicine. She previously completed internships at UMC Utrecht and TNO, where she contributed to innovations in research into Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) by investigating the acetylation process of the Farnesoid X Receptor (FXR). Her current research focuses on the development of new therapeutics to combat atherosclerotic cardiovascular disease. More specifically she investigates the effects of the mitochondrial uncoupler BAM15 and the effects of glucagon on lipid metabolism.

Mitochondrial uncoupling with BAM15 prevents weight gain, lowers plasma cholesterol and attenuates atherosclerosis development in APOE*3-Leiden.CETP mice

Jamie I. van der Vaart1,2, Christopher L. Axelrod3, Patrick C.N. Rensen1,2, Robin van Eenige1,2, Sander Kooijman1,2

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2. Einthoven Laboratory for Experimental Vascular Medicine;
3. Integrated Physiology and Molecular Medicine Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA, USA.

Background and Aim

The global obesity pandemic has led to a high prevalence of obesity-associated diseases including atherosclerotic cardiovascular disease. A century ago, 2,4-dinitrophenol was developed as an effective weight loss strategy, as it increases energy expenditure through mitochondrial uncoupling. However, 2,4-dinitrophenol was rapidly banned due to adverse effects, hyperthermia and even mortality. Recently, BAM15 was identified as a mitochondria-targeted small molecule protonophore, with wider tolerability in vitro, and potency to reverse diet-induced obesity in mice (Axelrod, EMBO Mol Med 2020). Here, we investigated the effects of BAM15 on body weight gain, dyslipidemia and atherosclerotic cardiovascular disease in APOE*3-Leiden.CETP mice, a well-established model for human-like cardiometabolic diseases.

Methods

Female APOE*3-Leiden.CETP mice were fed a Western-type diet (containing 16% fat, 0.15% cholesterol) with or without 0.1% w/w BAM15. Body weight, body composition, and 4h-fasted plasma total cholesterol and triglyceride levels were monitored. Very-low-density lipoprotein (VLDL) production and VLDL catabolism were assessed, and atherosclerotic lesion size was quantified within the aortic valve area.

Results

In all experiments, BAM15 attenuated body weight gain (-1.9 g) by preventing fat accumulation (-40%). BAM15 consistently lowered plasma total cholesterol levels (approx. -50%), as explained by a lowered hepatic VLDL production (-52%), without affecting plasma triglyceride levels. BAM15 increased VLDL-triglyceride-derived fatty acid uptake by subcutaneous and gonadal white adipose tissue (+36% and +85%, respectively), in line with increased uncoupled respiration and heat production. Likely as a compensatory effect, BAM15 decreased fatty acid uptake by subcapsular and interscapular brown

adipose tissue (-46% and -36%, respectively). Collectively, BAM15 decreased atherosclerotic lesion size (-34%) and improved lesion stability.

Conclusions

BAM15 attenuates atherosclerosis development in APOE*3-Leiden.CETP mice by lowering circulating cholesterol, without the typical adverse side effects of 2,4-dinitrophenol. We therefore anticipate that BAM15 is a promising therapeutic tool to combat obesity and atherosclerotic cardiovascular disease in humans in the future.

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(S)-Opto-prop-2 analogs to dynamically control β2-adrenergic receptor signaling with light

Shuang Shi, Simone.A.H Does, Yangzhi Cao, Dr. Yang Zheng, Dr. Maikel Wijtmans, Dr. Henry F. Vischer, Dr. Rob Leurs

Biography

Shuang Shi, a 4th-year PhD candidate at Vrije Universiteit Amsterdam, gets her scientific training in the lab of Professor Leurs at the Amsterdam Institute for Molecules, Medicines and Systems (AIMMS). Specializing in pharmacology, Shuang's research extends across the regulation of G-protein coupled receptors, notably the photo pharmacology and fundamental regulation of beta-adrenergic receptor signalling. She also contributes to mutagenic studies using pharmacology methodology to decipher the interactions between compounds and targets.

(S)-Opto-prop-2 analogs to dynamically control β2-adrenergic receptor signaling with light

Shuang Shi, Simone A.H. Does, Yangzhi Cao, Yang Zheng, Maikel Wijtmans, Henry F. Vischer, Rob Leurs

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Introduction

Beta-blockers are used for numerous acute and chronic conditions such as various heart problems, hypertension, and glaucoma. These beta-blockers antagonize agonist-induced β2-adrenergic receptor activity. Previously, we have reported the azologization of the FDA-approved beta-blocker propranolol to yield the photoswitchable antagonist (R/S)-opto-prop-2 that displays 600-fold increased binding affinity upon illumination with 360 nm light.

Objective

This research aims to design and characterize photoswitchable (S)-Opto-prop-2 analogs that switch to high-affinity cis isomer upon illumination of the trans isomer with red-shifted wavelengths and appropriate half-lives. Such photoswitchable antagonists would ultimately allow local control of their bioactivity in the body.

Methods

For this research, various photoswitchable (S)-Opto-prop-2 analogs were designed, synthesised, photochemically characterized, and pharmacologically tested in a FRET-based EPAC biosensor assay to measure β2-adrenergic receptor-induced cAMP production. All photoswitchable ligands were tested in both agonist and antagonist modes in one.

Results

The illumination wavelengths required to obtain the cis isomer are mostly 520 nm. Conversely, cis isomers are illuminated at 434 or 520 nm to revert to trans isomers. As a result, most of the ligands can be illuminated with longer wavelengths than UV light. The PSScis and PSStrans values (PSScis % = the percentage of cis isomers after illumination of trans isomer) are found to be above 50%. The half-lives of the ligands are long enough to perform pharmacological assays. All photoswitchable ligands antagonized isoprenaline-induced β2-adrenergic receptor activity. Further pharmacological research is currently ongoing.

Conclusion

This study provides possible candidates for photoswitchable beta-blockers with appropriate half-lives and lower-energy switching wavelengths compared to (S)-Opto-prop-2.

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Do distinct pharmacological properties of seven histamine H3 receptor isoforms affect their regulatory functions in the brain?

Meichun Gao, Mabel Dekker, Jasper Ooms, Prof.Dr. Rob Leurs, Dr. Henry Vischer

Biography

My name is Meichun Gao. I am currently a PhD student from Vrije Universiteit Amsterdam. In our group, we are interested in investigating the biological role of the histamine H3 receptor and its isoforms, aiming to develop small molecules to modulate these isoforms.
I completed a bachelor's degree at China Pharmaceutical University and subsequently pursued a master's degree at Xiamen University in China. This educational background has provided a strong foundation in pharmaceutical sciences and enriched my current research endeavours.

Do distinct pharmacological properties of seven histamine H3 receptor isoforms affect their regulatory functions in the brain?

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Introduction

The histamine H3 receptor (H3R) is a presynaptic G protein-coupled receptor that regulates the synthesis and release of various neurotransmitters in the brain, and is consequently considered to be a potential target for a range of neurological conditions. The H3R displays constitutive activity, and the inverse agonist pitolisant (Wakix®) has been approved as anti-narcolepsy drug in 2016. In human, seven H3R splicing variants have been identified that conserve the seven-transmembrane helical structure of GPCRs but vary in the length of their intracellular loop 3 and/or C-terminal tail. Nonetheless, H3R drug discovery and lead optimization has been exclusively focused on the H3R-445 reference isoform in the last two decades.

Objectives

This study aims to pharmacologically characterize all seven H3R isoforms, and assess whether these isoforms have distinct properties that may potentially influence their regulatory functions.

Methods

The binding affinity of H3R agonists and inverse agonists for the seven H3R isoforms (H3R-445, H3R-453, H3R-415, H3R-413, H3R-373, H3R-365, and H3R-329) are assessed using radioligand binding assays. To investigate H3R-mediated signaling, we employ a FRET-based EPAC biosensor to measure intracellular cAMP levels and utilize Nanoluciferase-based complementation technology to monitor β-arrestin1/2 recruitment by the H3R isoforms.

Results

In this study, we pharmacologically characterize the seven H3R isoforms. The H3R-453, H3R-415, H3R-413 display similar binding affinities for agonists and inverse agonists as the H3R-445. In contrast, H3R-329, H3R-365, and H3R-373 have increased agonist affinities but decreased inverse agonist affinities as compared to H3R-445, which is linked to the higher constitutive activity of the three shorter isoforms. In addition, histamine-stimulated H3R-329 and H3R-365 were found to interact with β-arrestin1/2 in a more transient manner as compared H3R-445, H3R-415, and H3R-413, whereas H3R-373 and H3R-453 were unable to recruit β-arrestin1/2. These different interactions of the isoforms with β-arrestin1/2 are hypothesized to affect their desensitization upon histamine stimulation.

Conclusion

The observed differences in pharmacology and regulation by β-arrestins between longer and shorter H3R isoforms should be considered in future drug discovery programs and may shed more light on their presynaptic receptor functions in the brain.

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Replacing A Radioactive With A Non-Radioactive Method To Reliably Measure Kidney Function

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Introduction
Accurate assessment of kidney function by measuring glomerular filtration rate (mGFR) is crucial for diagnosing chronic kidney disease, determining kidney donation eligibility, adjusting drug doses, and evaluating the renal hemodynamic profile of drugs. The mGFR and its correction for effective renal plasma flow (ERPF) can be determined after administering 125I-iothalamate and 131I-hippuran via a continuous infusion ("warm method") [1, 2]. The use of radioactive material is unfavorable due to the radioactive burden for patients and personnel and the environment. To explore the possibility of replacing these radiolabeled compounds with their non-radioactive isotopologues in the clinic ("cold method"), we must confirm that measurement of non-radioactive iothalamate and hippuran results in the same mGFR values compared to the "warm method".

Objectives
To determine whether mGFR and ERPF can be accurately assessed by measuring non-radioactive iothalamate and hippuran.

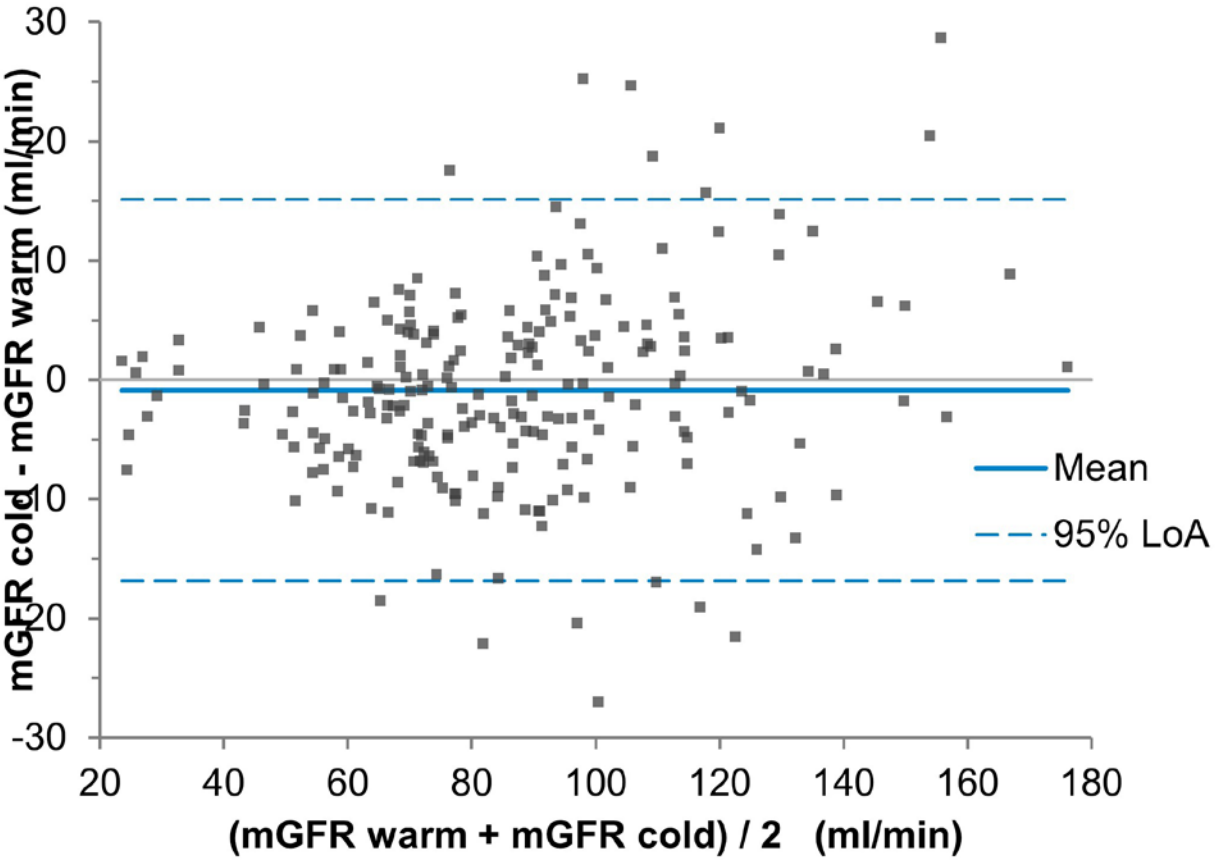
Methods
The 222 patients in this study were kidney donors, kidney transplant recipients, and patients with autosomal dominant polycystic kidney disease, scheduled for a kidney function test between July 2021 and March 2022. The 125I- and 131I- radioactivity levels were measured in serum, urine, and infusion solution, of 222 patients, using a gamma counter. These samples were re-analyzed for iothalamate and hippuran concentration using a validated LC-MS/MS method. The mGFR and ERPF, determined using both methods, were compared for each patient. Bland-Altman plots and Passing-Bablok regression were used to determine bias (unbiased when 95% CI included 0 and 1 for intercept and slope respectively). We also determined the accuracy of the corrected mGFR, and this was considered accurate when ≥80% of measurements were within 30% (P30) and ≥50% were within 10% (P10).

Results
Bland-Altman analysis showed a mean difference of -0.9 ml/min (95% CI -2.0, 0.2) in corrected mGFR when comparing the methods (figure 1). Passing-Bablok regression revealed Y=1.1x-5.0 (slope 95% CI: 1.0, 1.1; Y-intercept 95% CI: −8.0, -2.0) as shown in figure 2. The P10 was 73% and the P30 was 100%. For the ERPF the Bland-Altman analysis showed an absolute mean difference of +65 ml/min (95% CI 60, 71). Passing-Bablok regression revealed Y=1.4x−38 (slope 95% CI: 1.3, 1.4; Y-intercept 95% CI: -53, -23).

Conclusion
No major differences were seen between mGFRcold and mGFRwarm. These results indicate that mGFR can be assessed from trace amounts of non-radioactive iothalamate and hippuran. The ERPFcold showed a systematic and proportional overestimation in comparison to the ERPFwarm. This is probably due to a limitation of the warm method, which is reliant on the radiochemical purity of 131I-hippuran

when measuring radiation of 131I (free 131I and 131I-hippuran), as opposed to the cold method which specifically measures hippuran [3].

Figure 1 Bland-Altman plot of the difference between mGFRwarm and mGFRcold



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In silico identification and chemical remodelling of tick protein epitopes for vaccine antigen development.

Stepan Denisov, Amine Jmel, Wouter Peeters, Michalis Kotsyfakis, Hans Ippel, Tilman Hackeng, Ingrid Dijkgraaf

Introduction

Ingrid Dijkgraaf obtained her MSc degree (Organic Chemistry) at Wageningen University. After obtaining her PhD degree and various postdoc positions, she started in 2011 at the Department of Biochemistry of Maastricht University where she is currently working as a full professor in biomimetic chemistry. In 2014 she was a Fulbright Scholar at The Scripps Research Institute, La Jolla, USA. Her research focusses on the design and synthesis of peptides and proteins that can be used for molecular imaging, drug development, and studying molecular mechanisms in the field of cardiovascular diseases and oncology. Therefore, structure-activity relationship studies of polypeptides are performed. For her research, she peeks at Nature, especially at hematophagous parasites, as they express a vast variety of anticoagulant, anti-inflammatory, immuno-modulatory, and vasodilating proteins that evade or counteract host defence mechanisms. These organisms represent major sources of lead compounds for development of pharmacological tools and potentially useful therapeutic agents.

In silico identification and chemical remodelling of tick protein epitopes for vaccine antigen development

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Ticks and tick-borne diseases are severe burdens for healthcare systems and for animal husbandry amounting to billions of dollars in economic losses worldwide. Due to climate change, ticks' habitats are expanding, which makes the need for novel ways of tick control as pressing as never before. One of the desirable strategies is the development of anti-tick vaccines to elicit acquired tick resistance. The challenge in the development of such vaccines often lies in the inherently low immunogenicity of tick proteins, which they acquired through millions of years of evolution. Here we present a pipeline for the development of anti-tick vaccine antigens (ATVA) which utilizes AlphaFold2 structure modelling of tick proteins, in silico identification of antigenic epitopes by protrusion-based algorithms, their chemical remodeling and multimerization. The pipeline was applied to the tick salivary lectin pathway inhibitor (TSLPI) from I.scapularis as it plays a crucial role in B.burgdorferi transmission. Bioinformatic analysis showed the presence of a 10-residue β-hairpin designated as an antigenic epitope. This peptide was synthesized using solid-phase peptide synthesis and cyclized to preserve its secondary structure as in the parent protein, which was confirmed by NMR and CD spectroscopy. Further, the cyclized epitope was tetramerized using the chemically synthesized lysine wedge. Both monomeric and tetrameric epitopes were coupled to the carrier protein KLH and used in mice immunization experiments. ELISA analysis showed that the tetrameric epitope caused a 100-fold higher titer of TSLPI-specific antibodies than the monomeric construct. Then, rabbits were immunized by the TSLPI or tetrameric TSLPI epitope and challenged by ticks. Animals immunized by epitopes showed a higher level of TSLPI-specific antibodies, lower tick weights and egg hatching rate compared with the control and TSLPI. Finally, the applicability of a proposed pipeline on the tick transcriptome level was explored revealing dozens of potential new candidates for ATVA.

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Cohort study on drug survival and tolerability of adalimumab biosimilar transitioning: pharmaceutical properties do matter.

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Introduction

Wieland Müskens studied medicine between 2010 and 2017. During this period he also obtained his bachelor's degree in pharmacy. In 2021 he received his PhD with the thesis entitled: "Implementing innovations in rheumatic care; results from introducing a biosimilar and an eHealth application in daily clinical practice". In 2020 he started training as a rheumatologist. He also started training as a clinical pharmacologist in 2023.

Cohort study on drug survival and tolerability of adalimumab biosimilar transitioning: pharmaceutical properties do matter.

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Background

Since the compound patent on the bio-originator of adalimumab expired, several adalimumab biosimilars (BS) have been introduced. Extensive research shows equivalence in effectiveness and safety, both for new patients and after transitioning from originator to biosimilar. However, different pharmaceutical properties might drive differences in tolerability. Patents on the modernised adalimumab bio-originator expired on different moments in time as effect of evergreening. This included reduced volume (0.4 ml instead of 0.8ml) and absence of citrate for the modernised bio-originator. This means that earlier adalimumab biosimilars differ in pharmaceutical properties from newer ones. Our aim is to describe the difference in outcomes between patients transitioning from the modernised bio-originator (0.4ml/no citrate) to BS1 (0.8ml/citrate) and from BS1 to BS2 (0.4ml/ no citrate) for all patients receiving treatment with adalimumab in the Sint Maartenskliniek in the Netherlands.

Methods

In this retrospective cohort study of patients with a clinical diagnosis of RA, PsA or axial SpA receiving adalimumab, two cohorts were identified. Patients who transitioned from the modernised bio-originator to BS1 (cohort 1) in 2021 and patients who transitioned from BS1 to BS2 (cohort 2) in 2023. Cohort entry was defined as the first time patients receive BS1 (cohort 1) or B2 (cohort 2) after the respective transition. The primary outcome was the 12 month drug survival of BS1 (after switching from bio-originator) and BS2 (after switching from BS1). Discontinuation of the biosimilar was counted as an event, except when the reason was pregnancy or remission. A multivariate cox regression analysis with a variance estimator was used, to adjust for confounding and account for data dependency because patients could be included in both cohorts. Secondarily, reasons for discontinuation were investigated separately for inefficacy and adverse events using hazard ratios (HR).

Results

In cohorts 1 and 2, 983 and 1082 patients transitioned from bio-originator to BS1 or from BS1 to BS2 respectively, with 659 patients in both cohorts (table 1). Drug survival rates at 12 months were 73% (95%CI: 70% to 76%) for cohort 1 and 90% (95%CI: 88% to 92%) for cohort 2 ($p<0.001$) (figure 1A). The adjusted HR was 0.32 (95%CI:0.26-0.40) in favour of the newer BS2. The HR for discontinuation due to inefficacy between cohorts was 0.50 (95%CI 0.37-0.67) with better drug survival for cohort 2. For discontinuation due to adverse events the HR was 0.20 (95%CI 0.14-0.28) with better drug survival for cohort 2 (Figure 1B and 1C). The main tolerability issue was that BS1 injections were being experienced by patients as more painful than the bio-originator.

Conclusion

Adalimumab BS1 (0.8ml/citrate) has a significantly lower drug survival rate compared to BS2 (0.4ml/no citrate), and the difference is mainly driven by lower tolerability. These findings suggest that, when transitioning biosimilars, pharmaceutical differences can have an important impact on drug survival, and this should be taken into account.

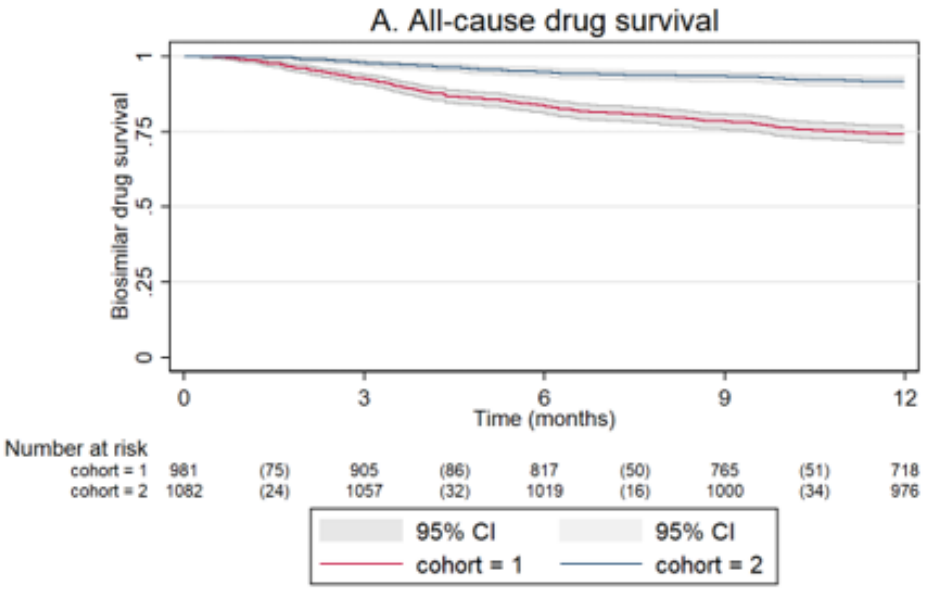
Table 1. Baseline characteristics of both cohorts

Characteristics	Cohort 1: switch to BS1 (n=983)	Cohort 2: switch to BS2 (n=1082)*
Diagnosis, n (%)		
RA	441 (44.9%)	524 (48.4%)
PsA	313 (31.8%)	326 (30.1%)
axSpA	229 (23.3%)	232 (21.4%)
Female, n (%)	526 (53.5%)	563 (52.0%)
Age in years, mean (sd)	56.6 (14.7)	56.3 (14.5)
Disease duration in years, median (25^{th}-75^{th} percentile)	8.1 (4.3-16.5)	7.0 (3.5-16)
Disease activity, mean (sd)		
DAS28-CRP, RA	2.2 (1.6-2.9) (121 missing)	2.1 (1.6 - 3.0) (118 missing)
DAS28-CRP, PsA	1.9 (1.6-2.7) (128 missing)	2.0 (1.5-2.6) (111 missing)
ASDAS, axSpA	1.8 (1.3- 2.4) (114 missing)	2.2 (1.6-2.8) (115 missing)
Previous bDMARDs n (%)		
0	782 (79.6%)	901 (83.3%)
1	171 (17.4%)	146 (13.5%)
2	22 (2.2%)	24 (2.2%)
3 or more	8 (0.8%)	11 (1.0%)
Duration of adalimumab use in years, median (25^{th}-75^{th} percentile)	3.4 (1.7-6.0)	2.6 (1.1-5.5)
Adalimumab standard dose, n (%)**	543 (54.9%)	628 (58.0%)
Adalimumab DDD% , mean (sd)	80% (0.24)	81% (0.24)
Concomitant csDMARD use, n (%)	442 (44.7%)	530 (49.0%)

*659 (61%) of the patients in cohort 1 also belong to cohort 2.

**The standard dose of adalimumab is 40mg once every 2 weeks (100%).

Abbreviations: RA Rheumatoid Arthritis, PsA: Psoriatic Arthritis, axSpA: axial Spondyloarthritis, DAS28-CRP: Disease Activity Score in 28 joints calculated with C-reactive protein, ASDAS: Ankylosing Spondylitis Disease Activity Score, bDMARD: biological disease modifying anti-rheumatic drug, csDMARD: conventional synthetic disease modifying anti-rheumatic drug, DDD%: percentage of the Daily Defined Dose, sd: standard deviation.



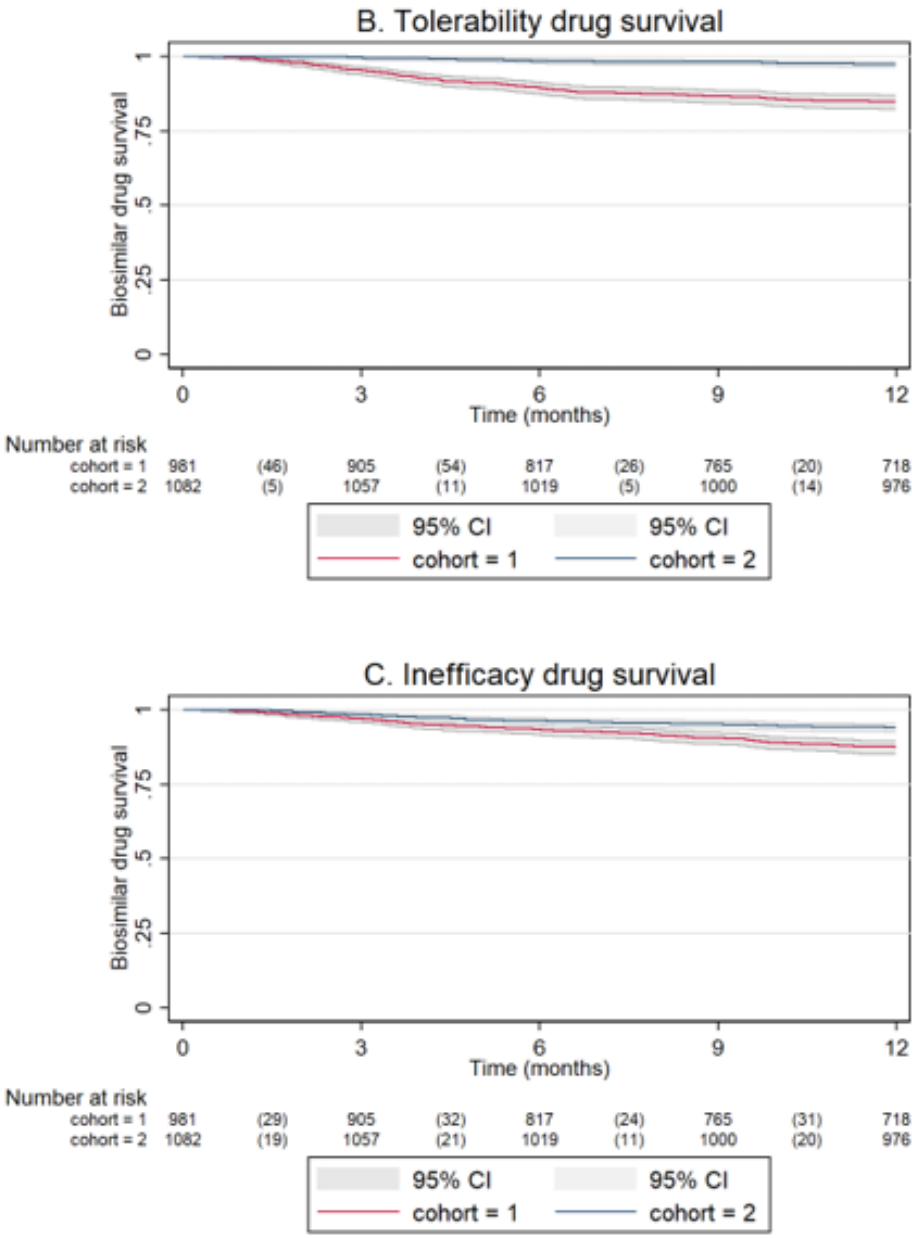


Figure 1. Kaplan-Meier curves showing the drug survival of the two biosimilars. Switching from originator to biosimilar 1 (0.8ml/citrate) (cohort 1) and from biosimilar 1 to biosimilar 2 (0.4ml/no citrate) (cohort 2) (A). Divided into discontinuation due to adverse events (B) and inefficacy (C).

Abbreviations: CI Confidence Interval.

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Comparative effectiveness of anti-hyperlipidemic drugs monotherapy in primary prevention of cardiovascular disease

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Biography

Xuechun Li is in her 3rd year of the Ph.D. program in PharmacoTherapy, -Epidemiology and -Economics, Groningen Research Institute of Pharmacy, University of Groningen. Her research interests are in Pharmacoepidemiology. Her current research mainly focuses on adherence and drug patterns of anti-hypertensive and anti-hyperlipidemic drug monotherapy in the real world.

Comparative effectiveness of anti-hyperlipidemic drugs monotherapy in primary prevention of cardiovascular disease

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Introduction

Anti-hyperlipidemic drug treatments are effective in reducing the risk of cardiovascular disease while different classes have different effects.

Objectives

We aim to assess the real-world comparative effectiveness of anti-hyperlipidemic monotherapies for primary prevention of cardiovascular events.

Methods

Patients aged 18 years and older, who initiated primary prevention with anti-hyperlipidemic monotherapy, were selected from the University of Groningen IADB.nl dispensing database. In the ITT analysis, all 18,375 patients were included. For the PP analysis, we considered both all 18,375 patients independent of adherence (PPIA) and the subset of 11,247 adherent patients (PPA). Exposures were anti-hyperlipidemic drug monotherapy including simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin and fibrates. Study outcome was the time to first prescription of acute cardiac drug therapy measured by valid drug proxies to identify a first major cardiovascular event. We applied inverse probability of treatment-weighted (IPTW) analysis using Cox regression and time-varying Cox regression with simvastatin as the reference category to estimate the average treatment effect hazard ratios (HR) and their corresponding 95% confidence intervals (CI).

Results

Atorvastatin users exhibited higher hazards compared to simvastatin users, with an ITT-IPTW adjusted HR of 1.27 (95% CI: 1.15 to 1.40). In the PP analyses, the HR for atorvastatin users remained elevated: PPIA-IPTW HR was 1.47 (95% CI: 1.29 to 1.68), and PPA-IPTW HR was 1.41 (95% CI: 1.18 to 1.69). Similarly, pravastatin users showed higher hazards than simvastatin users in the PP analyses, with PPIA-IPTW HR of 1.41 (95% CI: 1.14 to 1.74) and PPA-IPTW HR of 1.56 (95% CI: 1.20 to 2.04).

Conclusion

Despite a primary prevention subgroup for lipid treatment, confounding by severity most likely attributed

to both atorvastatin and pravastatin users having higher rates of cardiovascular events over a long-term follow-up period compared to users on simvastatin monotherapy in the prevention of cardiovascular events. Similar findings were observed among patients concurrently prescribed medications for diabetes, rheumatoid arthritis, and asthma/COPD. These results were derived from both ITT and/or PP analyses, assessing the comparative effectiveness of anti-hyperlipidemic drug monotherapy in primary prevention of cardiovascular events. Users of rosuvastatin, fluvastatin, and fibrates exhibited comparable rates to those using simvastatin.

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Real-life study on the clinical pharmacokinetics of enteral lormetazepam as adjunct sedative in ICU patients admitted for severe COVID-19 pneumonia

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Biography

Jos le Noble is an Internal Medicine and Intensive Care Specialist at the VieCuri Medical Center Noord-Limburg. He has also a postion at the Department of Pharmacology Toxicology of the Maastricht University.
His primary interest is Critical Care Nephrology and Pharmacology. He follows a fellowship program for clinical pharmacologist at the MUMC+.

Real-life study on the clinical pharmacokinetics of enteral lormetazepam as adjunct sedative in ICU patients admitted for severe COVID-19 pneumonia

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Background and Objective

Lormetazepam is a benzodiazepine which has been shown to have sedating properties and is primarily subjected to phase II metabolism and conjugation. This pharmacological profile makes lormetazepam an attractive add-on sedative potentially reducing the necessity for other sedatives during critical drug shortages as occurred during the pandemic coronavirus disease 2019 (COVID-19).
This study investigates lormetazepam as an adjunct sedative alternative to midazolam for mechanically ventilated patients with COVID-19 disease. We aimed to determine the clinical pharmacokinetics of enterally administered lormetazepam and provide dosing recommendations.

Methods

Critically ill patients with COVID-19 requiring mechanical ventilation and deep sedation were included in April 2020. Lormetazepam 2 mg every 12 hours was administered enterally. Blood samples were collected to quantify lormetazepam and its glucuronide. Pharmacokinetic analysis was conducted using a one-compartment model with the Edsim + + KinPop plugin.

Results

A total number of 15 patients were analyzed in this study. Briefly of which 11 (73%) were male, with a mean (± SD) age of 61.6 ±13 years, and a median (IQR) admission body mass index of 29 (IQR 27.4-31.1). The primary pharmacokinetic parameters (mean ± CV%) for absorption constant (ka), volume of distribution (V/F) and clearance (CL/F) were 6.4 1/h, 207 L/70 kg and 14.5 L/h/kg0.75, respectively. Median values for volume of distribution (Vd/F) and clearance were 2.64 L/kg and 2.53 ml/kg/min, with a half-life of 10.7 hours. The median ratio of lormetazepam metabolite to parent drug exposure was 11.5. Trough-guided dosing suggested alternatives of 0.92 mg three times daily, 1.62 mg twice daily, or 5.36 mg once daily.

Conclusion

This is the first report describing the pharmacokinetics and metabolism of lormetazepam in critically ill COVID-19 patients on mechanical ventilation using a newly developed pharmacokinetic model. The main finding of our study was that COVID-19 has no significant effect on the primary pharmacokinetic variables of lormetazepam clearance and half-life. In our population pharmacokinetic model, covariates of renal function and CRP, a measure of inflammation, did not show a relationship with the pharmacokinetics of lormetazepam. We provided practical recommendations for implementing alternative lormetazepam enteral dosing regimens. This study provides important novel data on the clinical pharmacokinetics of lormetazepam, which has implications for ICU patients and future sedative strategies.

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Safety, pharmacokinetics and pharmacodynamics of a 6-hour long target-controlled DMT infusion in healthy volunteers

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Biography

Katelijne van der Heijden finished her medical degree at Erasmus Medical Centre Rotterdam in 2018, whereafter she started training to become a psychiatrist. She interrupted this training to pursue a PhD in pharmacology at the Centre for Human Drug Research in Leiden, which focuses on phase 1 clinical trials with psychedelic compounds.

Safety, pharmacokinetics and pharmacodynamics of a 6-hour long target-controlled DMT infusion in healthy volunteers

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Background

Emerging evidence indicates that the psychedelic DMT, may exert neuroprotective effects during acute ischemic stroke1. However, several studies have demonstrated high inter-individual pharmacokinetic (PK) variability2, which possibly influences DMTs pharmacodynamic (PD) effects, thereby complicating its future use in stroke patients. This PK variability is hypothesized to be primarily caused by inter-individual variability in DMTs primary metabolism by the MAO-A enzyme3. Therefore, this study investigated the PK and PD of a 6-hour intravenous target-controlled DMT infusion and the influence of MAO-A activity on DMTs PK.

Methods

DMT fumarate was administered to healthy subjects in a randomized, double-blind, placebo-controlled (ratio DMT: placebo of 8:2), single ascending dose study. DMT was administered intravenously as a 30-second bolus + 6-hour target-controlled infusion in 3 dose levels of 1.5mg + 0.105 mg/min, 7.5mg + 0.525 mg/min and 5.0mg + 0.7875mg/min. Plasma DMT levels were assessed at baseline, during (n=10 timepoints) and after infusion (n=5 timepoints). MAO-A samples were collected 2.5 hours prior to dosing. The VAS Bowdle, adaptive tracking, body sway and resting state EEG were repeatedly performed pre- and post-dose and analysed up to 24 hours post-dose with a mixed model of covariance analysis.

Results

12 female and 17 male volunteers, aged 19-57 years, both psychedelic experienced and inexperienced, were included. Plasma exposures demonstrated dose proportionality and moderate inter-individual variability. Mean Cmax (CV%) were 4.58 (62%), 25.3 (37%) and 35.9 (35%), while mean AUClast were 14.2 (48%), 85.9 (25%) and 157.6 (35%) ng/mL per increasing dose level. No significant correlation between MAO-A activity and AUClast (r=-0.076; p=0.74), AUCinf (r=0.0040; p=0.88) or half-life (r=-0.098; p=0.71) was demonstrated. No significant PD effects were observed for dose level 1. Compared with placebo, DMT statistically significantly increased mean VAS Bowdle "feeling high" for dose levels 2 and 3 (+0.31 logmm2 (SD 0.14); p=0.0345 and +0.63 logmm2 (SD 0.14); p=0.0001), while it solely increased VAS

"external perception" (+0.40 logmm2 (SD 0.10); p=0.0005) and "internal perception" (+0.12 logmm2 (SD 0.04); p=0.0047) for dose level 3. Decreases in adaptive tracking of 4.2 (95%CI= -7.6,-0.8; p=0.0175) and 4.9% (95%CI= -8.5,-1.4; p=0.0082) and body sway of 59.9 (95%CI= 22.8,108.3%; p=0.0012) and 56.6% (95%CI= 19.1, 105.9%; p=0.0025) were observed for dose levels 2 and 3. Lastly, decreases in occipital alpha EEG power on Pz-01 of 38.4% (95% CI= -57.8%, -10.2%; p=0.0139) and Pz-02 of 35.7% (95% CI= -56.6%, -4.8%; p=0.0291) were only observed for dose level 3.

Conclusion

Plasma exposures of DMT demonstrated moderate interindividual variability resulting in moderate variability in PD measures. Most PD measures demonstrated similar effects at plasma exposure of 25 and 36 ng/mL, with only VAS Feeling high and adaptive tracking displaying dose dependent effects. No association between AUClast, AUCinf and half-life and MAO-A activity was demonstrated, however only one MAO-A sample was taken. Together, these results demonstrate that the observed moderate PK variability necessitates the identification of potential sources of PK variability and further elucidation of the function the MAO-A enzyme to support rational dose and infusion scheme selection for future proof-of-mechanism studies in patient populations.

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GlycoGenius: A Tool for Automated Glycomics Data Analysis and Visualization

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Biography

Hector is a PhD student from the University of Groningen and the Federal University of Rio de Janeiro (Brazil) and has done glycobiology related work since the beginning of his bachelor in Biophysics, in 2013. Studying the impact of hyperglycemia on colorectal cancer, he published his first article in 2017.
From 2019 and onwards, Hector started developing work on glycans analysis through mass spectrometry, publishing in 2021 a paper showing the detailed differences between the glycome of cancer cells cultured in high-glucose and regular glucose conditions, with the possible mechanism to achieve these changes described using by metabolomic analysis.
Currently, he has been studying the impact of glucose spikes on the metabolism of cancer cells; prospecting for glycans biomarkers for colorectal cancer in plasma of mice and patients; and, of course, developing GlycoGenius in hopes of making glycomics analysis easier for the glycobiology community, among other side projects.

GlycoGenius: A Tool for Automated Glycomics Data Analysis and Visualization

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Introduction

Liquid-chromatography or capillary electrophoresis coupled to a mass spectrometer (LC-MS or CE-MS) has become the ultimate tool for unveiling compositions and structures of complex molecules in biological specimens, especially for the analysis of proteins, peptides and glycans. Manual analysis of LC/CE-MS results, which can contain information on hundreds to thousands of compounds, takes unsurmountable amounts of time, hence the urgent need for automated data analysis tools. Although several software tools are available for proteomics, most tools for glycomics lack a series of desirable features. This includes accurate identification of compounds, needless of fragmentation data (MS2), visualization of identification and quantification results and minimization of false positive rates.
In this study, we present GlycoGenius, a software tool that enables, not only an efficient data visualization supporting quantification and identification assessment, but also automating the whole analysis workflow, minimizing false positive identifications, and allowing for much faster and reliable data analysis of glycomics LC-MS/MS data than other already existing software tools.

Methods

The software was completely written using Python, the package Pyteomics provided access to the raw MS data and tkinter package was used to develop the Graphical User Interface (GUI).
Other packages, such as Pandas, Dill, SciPy and Numpy were used for calculations and data exporting to excel files. The program currently contains over 14000 lines of code.

Results

GlycoGenius is able to be run in a command-line interface (CLI) as well as on a GUI, allowing for intuitive use, with plenty of options for data visualization. The program automatically identifies glycans in the spectra, checking if the identified compound is indeed the target one, based on the isotopic peak distribution, charge state and monoisotopic peaks. MS2 data can also be analyzed and annotated

automatically. The analysis includes several quality controls, such as isotopic fitting score, curve fitting score, signal-to-noise ratio and maximum PPM error that allows to filter the data dynamically. The results are saved as proprietary filetypes that can be opened on GlycoGenius graphical user interface for visualization/exporting. The results can be exported to excel files that can be easily pipelined to Metaboanalyst, an online tool for automated statistical analysis, and graph can be saved in high resolution.

In test runs GlycoGenius was able to provide a bigger number of identifications for N-Glycans from different datasets when compared to the existing solution closest to its capabilities, GlycReSoft.

Conclusions

An easy-to-use tool for automated glycomics data analysis has been developed. The source code is openly available at GitHub and further developments are ongoing, allowing for potential implementation of new features new analysis methods such as glycopeptide identification.

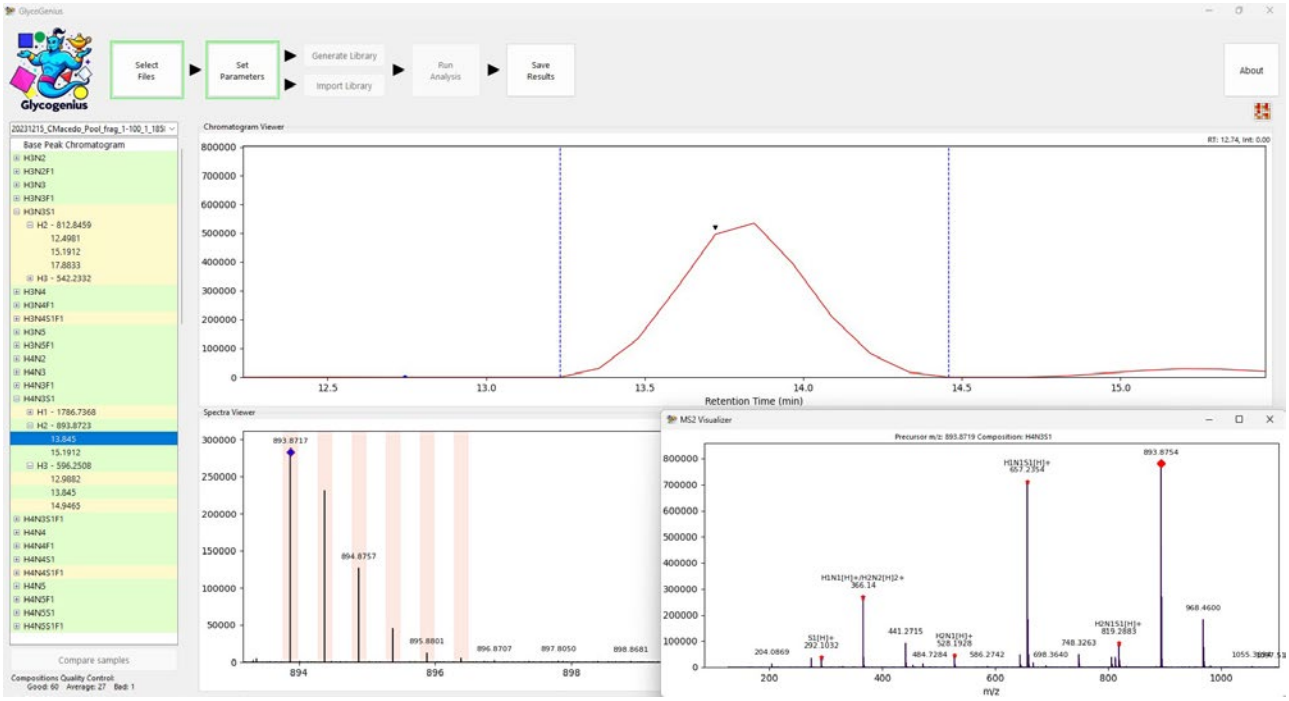

Figure 1. GUI of GlycoGenius. On the top, the necessary steps for completing a workflow are provided in an organized manner to guide the user. On the main window, the EICs of glycans traced by the program can be seen and analyzed in depth.

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Photoswitchable small-molecule ligands to optically modulate chemokine receptors

Justyna Adamska, Sophie Bérenger, Xavier Gómez-Santacana, Sabrina de Munnik, Niels Hauwert, Tamara Mocking, Sara Lopes-Van den Broek, Marta Arimont, Iwan de Esch, Henry F. Vischer, Maikel Wijtmans, Rob Leurs

Biography

PhD candidate in Molecular Pharmacology at Vrije Universiteit Amsterdam, specializing in GPCR pharmacology and photopharmacology. Experienced in studying Class A and adhesion GPCR signaling.

Photoswitchable small-molecule ligands to optically modulate chemokine receptors

Justyna Adamska, Sophie Bérenger, Xavier Gómez-Santacana, Sabrina M. de Munnik, Niels Hauwert, Tamara Mocking, Sara Lopes-Van den Broek, Marta Arimont, Iwan de Esch, Henry Vischer, Maikel Wijtmans, Rob Leurs

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Photopharmacology allows the optical modulation of protein activity with light-responsive molecules such as azobenzene derivatives.1 This technology can be used to investigate the biological function of G protein-coupled receptors (GPCRs).
In this study we developed functionally light-responsive ligands to optically modulate chemokines receptors such as CXC chemokine receptor 3 (CXCR3) and atypical chemokine receptor 3 (ACKR3). Those GPCRs play crucial role in T-cell function and are associated with inflammatory diseases and cancer. 2,3 Design and synthesis of photoswitchable compounds were inspired by the CXCR3 antagonist VUF11211 and a patent of an ACKR3 agonist. 4,5 The CXCR3 photoswitchable ligand VUF16338 has been identified as a key compound from a library of eleven analogs. VUF16338 inhibits CXCL11-induced G protein activation by CXCR3 with a 10-fold inhibitory potency shift between dark and irradiated states. For ACKR3 VUF25471 has been selected as tool compound and has been found to recruit β-arrestin2 to ACKR3 with 10-fold potency shift between dark and irradiated states. Both developed compounds are new complementary phototools to study the photopharmacology of the CXCR3 and ACKR3 receptor.

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Profiling of a neoantigen-driven recall immune response in human skin: A randomized, double-blind, placebo-controlled study with KLH

MD Micha Ronner, PhD Manon Jansen, MD Mahdi Saghari, PhD Wieke Grievink, MD, PhD Naomi Klarenbeek, PhD Sefina Arif, PhD Matthijs Moerland

Biography

Started with his biomedical bachelor at Utrecht University and finished his biomedical master education at Utrecht University in 2017, after which he went to medical school at Maastricht University, which was completed in 2021. From 2021 until present day, Micha has been working as a clinical scientist/research physician at the Center for Human Drug Research in Leiden.

Profiling of a neoantigen-driven recall immune response in human skin: A randomized, double-blind, placebo-controlled study with KLH

Introduction

Novel drugs targeting the adaptive immune system are commonly initially investigated in healthy volunteers (HV). HV frequently lack constitutively expressed drug-target engagement biomarkers, complicating evaluation of pharmacological activity. This calls for an in vivo model wherein a controlled immune response is elicited. The keyhole limpet hemocyanin (KLH) neo-antigen challenge has the potential to be a model for investigating drugs targeting the adaptive immune system.

Objectives

Finetuning the KLH neo-antigen challenge model for its use in early drug development by characterizing the systemic and local immune response to KLH after repeated immunization, using an extensive battery of state-of-the-art biomarkers.

Methods

A randomized, double-blind, placebo-controlled study in HV was conducted investigating the effects of repeated immunizations with KLH on study Day 1, 15, and 29, followed by intradermal KLH administration on Day 50. Systemically, KLH-specific antibodies were evaluated. PBMCs were stimulated ex vivo to determine KLH-specific responses by measuring cytokines using ELISpot. Locally, KLH skin response was evaluated 0h-48h post intradermal administration by laser speckle contrast imaging (perfusion), multispectral imaging (erythema), suction blister fluid analysis (cytokines and immune cells), and biopsy analysis (immune cells). Descriptive analyses were performed.

Results

Twelve male HV aged 18-42 years completed the study. KLH antibody levels increased with each immunization. KLH-specific ELISpot responses demonstrated an IFN-γ response (reaching a plateau after single immunization) and an IL-13 response (increasing after each immunization) (Figure 1). Intradermal KLH injection drove a peak response 24h after administration, observed as erythema and increased perfusion (Figure 2). Blister fluid analysis showed cell influx (T cell subsets; B cells; monocytes; dendritic cells) at 24h, largely decreasing at 48h. This was confirmed by immunofluorescent staining of skin biopsies.

Conclusion

The findings of this neo-antigen challenge study demonstrate a T-cell-mediated response to intradermal KLH that is more rapid than the classical delayed type hypersensitivity response. This study highlights the importance of timing of measurements and combining objective non-invasive and invasive biomarkers. The KLH neo-antigen challenge-model presented in this study offers a framework for optimizing studies investigating the effect of novel immunomodulatory drugs on the adaptive immune system in healthy

volunteers.

Figure 1. ELISpot recall response post KLH stimulation ex vivo, mean ± sd.

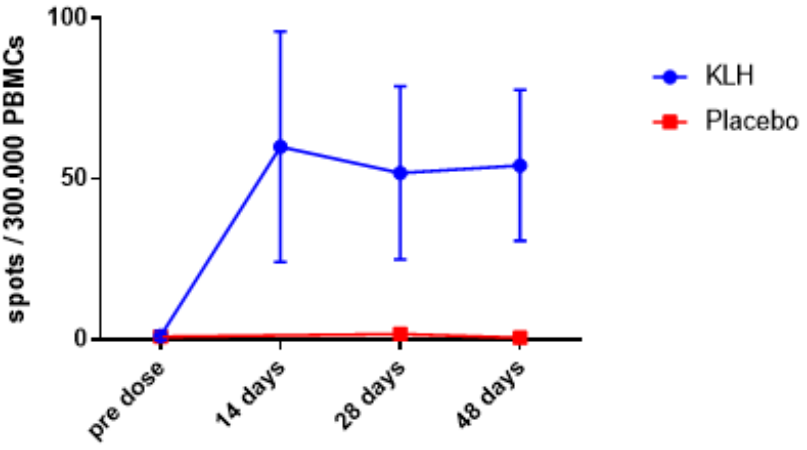
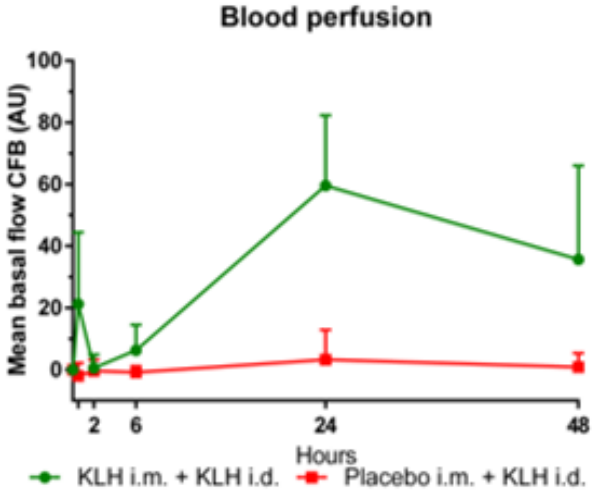


Figure 2. Laser speckle contrast imaging, mean ± sd. Study day 50 set as zero point.



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VUF26063: A second-generation photoswitchable ligand to optically control the histamine H3 receptor

Ivana Josimovic, Lars Binkhorst, Henry Vischer, Maikel Wijtmans, Rob Leurs

Biography

Ivana Josimovic is a pharmacist and researcher in the field of GPCR pharmacology, with a focus on Histamine receptors. A Vienna University graduate and a licensed pharmacist in Austria, Ivana has worked as a researcher at Vrije Universiteit Amsterdam since 2019 and currently as a PhD candidate in the group of Professor Leurs. She has so far co-authored two scientific publications and given multiple scientific talks on various conferences. Besides a passion for science, she also cares deeply about gender equality in academia and is currently active as a board member of WO&MEN, a gender equality volunteer body of the VU.

VUF26063: a second-generation photoswitchable ligand to optically control the histamine H3 receptor

Ivana Josimovic, Lars Binkhorst, Maikel Wijtmans, Henry Vischer, Rob Leurs

Photopharmacology uses light-sensitive ligands as tools to yield spatiotemporal control of protein activity and has been emerging in recent years in the field of G protein-coupled receptors (GPCRs). The histamine H3 receptor (H3R) is highly expressed in the central nervous system and has been identified as a potential target in diseases such as obesity, narcolepsy, Alzheimer's, and ADHD. Previously, our group published the first generation of azobenzene-based photoswitchable H3R antagonists. In this research, we aimed to improve their photochemical properties by replacing the classical azobenzene with an arylazopyrazole photoswitchable moiety. Compared to first generation H3R antagonists, key compound VUF26063 shows improved switching of 93% from photostationary state (PSS) PSScis to PSStrans using a less damaging wavelength of 500 nm, instead of UV light at 430 nm. Improvements of pharmacological parameters include a nanomolar H3R affinity (pKi) for VUF26063trans and a ~50 fold affinity difference between isomers. In addition, arylazopyrazole-based H3R antagonists show a ~13 fold inhibitory potency (pIC50) difference between VUF26063 isomers in a histamine-induced PKA functional assay in HEK293T cells. Furthermore, VUF26063 can be repeatedly switched back and forward between isomers in situ within this assay.

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Combined absence of APOA1 and bone marrow ABCA1 induces neutrophilic inflammation and severe atherosclerosis in LDL receptor knockout mice

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Biography

Olga Snip is currently finalizing her PhD program under supervision of professor Miranda van Eck in the division of Systems Pharmacology and Pharmacy at the Leiden Academic Centre for Drug Research (LACDR) of Leiden University. Her research focusses on the importance of macrophage ATP-binding cassette transporters A1 (ABCA1) mediated cholesterol efflux for atherosclerotic cardiovascular disease. After finalizing her PhD, Olga will continue her studies with professor Miranda van Eck as a postdoctoral researcher. Before commencing her PhD program at Leiden University, Olga obtained a bachelor and master degree in Biomedical Sciences at the University of Amsterdam, specializing in cardiovascular diseases and experimental immunology.

Combined absence of APOA1 and bone marrow ABCA1 induces neutrophilic inflammation and severe atherosclerosis in LDL receptor knockout mice

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*) These authors contributed equally to the manuscript

Background

The interaction between the macrophage ATP-binding cassette transporter A1 (ABCA1) and circulating apolipoprotein A1 (APOA1) has been consistently shown to protect mice from developing atherosclerosis. However, the dependency on this specific interaction for the anti-atherogenic capacities of the individual proteins remains unknown. Therefore, we examined the atheroprotective properties of ABCA1 on bone marrow-derived cells in the absence and presence of APOA1.

Methods

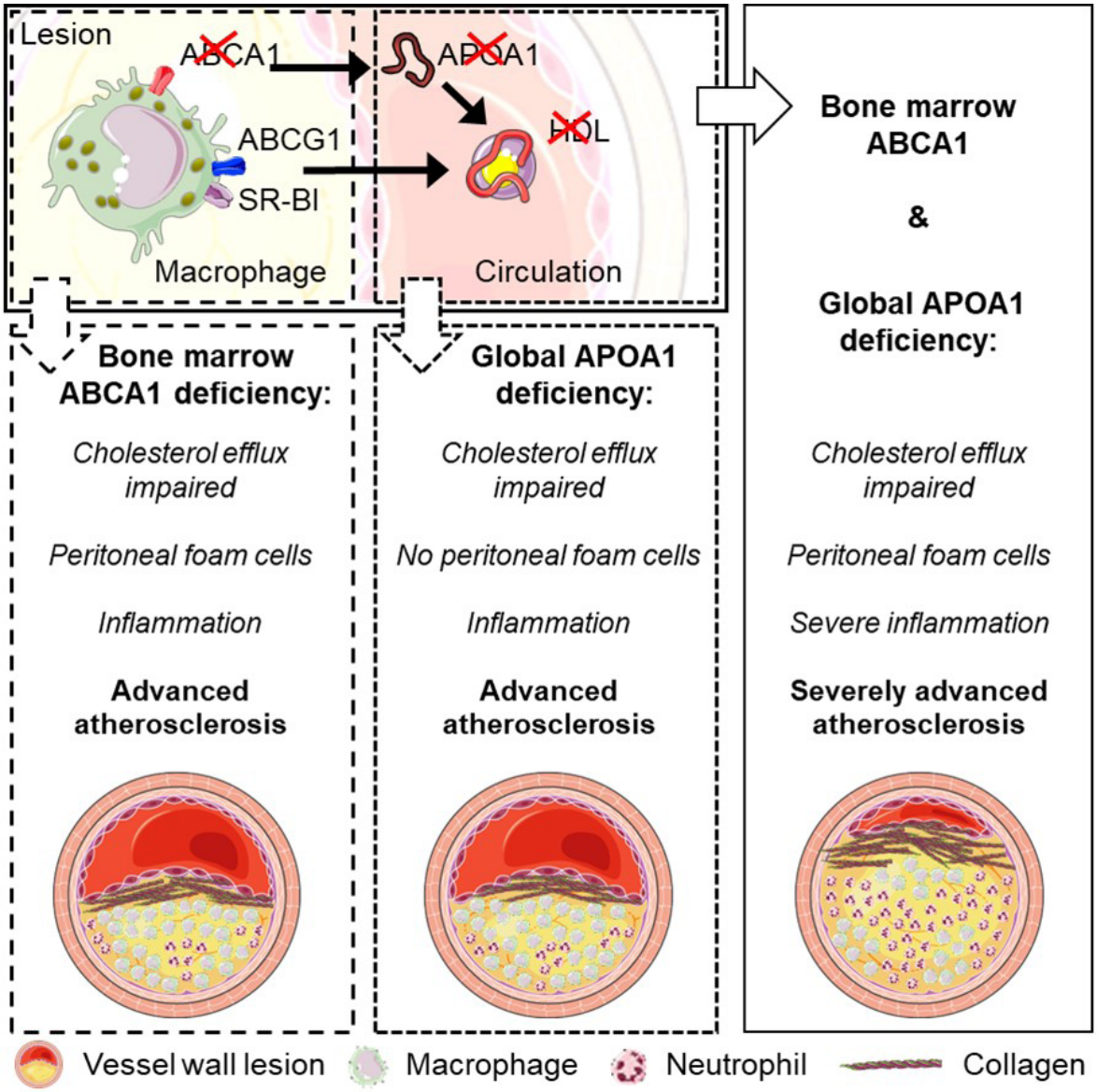
ABCA1 knockout (KO) or wild-type bone marrow was transplanted into mice that were deficient for both APOA1 and the low-density lipoprotein receptor (LDLR) or only for the LDLR. After recovery, the four groups of bone marrow transplanted mice were fed a Western-type diet for 7 weeks to stimulate atherosclerotic lesion development.

Results

Reverse cholesterol transport was equally impaired in LDLR KO mice lacking either APOA1, bone marrow ABCA1 or both. Unexpectedly, LDLR KO mice lacking APOA1 and ABCA1 in bone marrow-derived cells developed more extensive monocytosis, severe neutrophilia, and increased neutrophil infiltration into the peritoneal cavity, liver, and spleen. In addition, elevated levels of pro-inflammatory cytokines, most notably interleukin-18 (~2.4-fold increase compared to all other groups; $P<0.05$) were found. Consequently, atherosclerotic lesion development was increased ~1.7-fold ($P<0.001$) in this group of mice as compared to LDLR KO mice deficient for either bone marrow ABCA1 or APOA1. The augmented lesion size was primarily caused by an increase in collagen and neutrophils. Two-way Multivariate Analysis of Variance showed that the impact of ABCA1 on bone marrow-derived cells partially depends on APOA1 presence, but that bone marrow ABCA1 displayed the strongest effect in our model.

Conclusion

The combined absence of both APOA1 and bone marrow ABCA1 in LDLR KO mice led to more severe atherogenesis characterized by neutrophilic inflammation than when only one of the proteins was lacking. Therapeutic strategies targeting both proteins simultaneously may warrant further investigation.



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Biomarkers for Neurodegenerative Diseases in Regulatory Decision-making by the European Medicines Agency

Miss. Audrey Hermans, MSc. Elisabeth Bakker, dr. Viktoriia Starokozhko, dr. Andre Elferink, MSc Loes den Otter, dr. Lorenzo Guizzaro, dr. Falk Ehmann, prof. dr. Peter Mol, dr. Anna Pasmooij

Biography

Audrey Hermans works for the Dutch Medicines Evaluation Board as a PhD student within the IMI-EPND project. She has a background in innovation, with a special interest in healthcare, biomarkers and neurodegenerative diseases.

Biomarkers for Neurodegenerative Diseases in Regulatory Decision-making by the European Medicines Agency

Audrey M.M. Hermans1,2*, Elisabeth Bakker2*, Viktoriia Starokozhko1,2,3, Andre J.A. Elferink1,3, Loes den Otter1,4, Lorenzo Guizzaro3, Falk Ehmann3, Peter G.M. Mol1,2,3, Anna M.G. Pasmooij1,5

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Introduction

Biomarkers (BMs) can be valuable tools to facilitate earlier diagnosis of (subtypes) of diseases, improved patient selection, and detecting therapeutic effects or safety concerns in the field of Neurodegenerative Diseases (NDDs). Given the recent debate about BMs in the authorisation process of aducanumab and lecanemab, two medicines intended to treat Alzheimer's Disease, it is valuable to examine in more detail how BMs are used and discussed in interactions with the EMA. To support development in BMs, the EMA's Regulatory Science Strategy to 2025 proposed to enhance early engagement with novel BM developers to facilitate regulatory qualification and to optimise the European research infrastructure for developing personalised medicine. Several procedures exist for drug developers to interact with EMA's Committee for Medicinal Products for Human use (CHMP) prior to applying for Marketing Authorisation (MAA), including Scientific Advice (SA) and Qualification of Novel Methodologies, which can result in a Letter of Support (LoS), Qualification Advice (QA), or a Qualification Opinion (QO).

Objectives

This study aims to explore the extent to which BMs are utilized in the development of treatments for NDDs, as well as explore topics of discussion around BMs in regulatory advice- and decision-making processes.

Methods

The EMA's Scientific Advice- (SA), Qualification- (QA/QO) and Marketing Authorisation Application (MAA) procedures regarding NDDs that mention BMs were analysed. Information was extracted on intended disease area, BM type, and context of use proposed by the applicant. Each BM was assigned a category based on its function.

Results

In total, 100 procedures involving NDDs that discussed BMs were analysed; 52 SAs (Jan 2020 – Dec 2022), 19 QAs/QOs (Jan 2008 – Dec 2023), and 29 MAAs (Jan 1995 - Dec 2023) . The majority of the procedures involved Alzheimer's Disease (AD; n= 29), Parkinson's Disease (PD; n=8), and Multiple Sclerosis (MS; n=32). Imaging BMs were most predominantly used within the studied dossiers. The majority of the BMs were used as pharmacodynamic/response BMs. However, in AD diagnostic BMs, guiding patient selection were commonly discussed in regulatory procedures, in addition to PD BMs measuring efficacy. In MS MAAs BMs, especially lesions, are accepted as important supportive/secondary endpoints and drive the overall high number of efficacy BMs in regulatory procedures.

Conclusion

Despite the established role of certain imaging BMs (mainly in MS), there remains a serious need for more precise and reliable BMs to improve diagnostic accuracy and treatment monitoring for NDDs. To implement novel BMs and facilitate development of new treatments, a robust evidence base showcasing biological plausibility or clear clinical benefits is essential. Collaboration and data sharing among stakeholders will be vital in generating this evidence and enhancing the understanding and management of NDDs.

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conect4children Stichting: expert advice service to improve to your drug development programme or protocol

Fenna Mahler

Biography

Fenna Mahler is as head of operations and expert services a member of the conect4children Stichting management team.
As project lead at Radboudumc she is involved in the conect4children project.
Fenna is trained as a medical scientist. She has experience is several roles within pharmaceutical companies, CROs and academia in the field on clinical research.
She has been a board member of the Dutch Clinical Research Foundation up till 2022.

conect4children Stichting: expert advice service to improve to your drug development programme or protocol

Mahler F1, 2, Spira, C1, 2, Fernandes R, 1,3 Turner M1, 4, de Wildt S 4

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2 Radboud University Medical Centre, Nijmegen, Netherlands,
3 AIDFM, Lisbon, Portugal
4 University of Liverpool, Liverpool, United Kingdom

Introduction

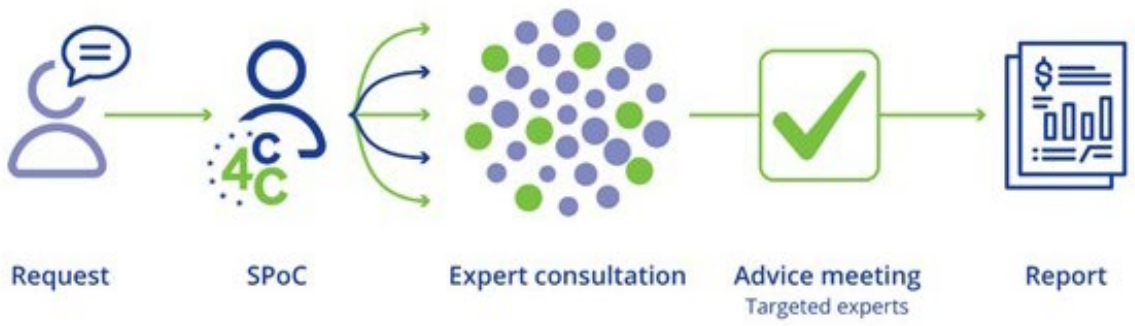
The transition from a pan-European network to the conect4children Stichting (c4c-S) represents a major step in advancing paediatric clinical trials. Through over 65 expert advices, c4c has optimized the design, setup, and conduct of trials to ensure regulatory-grade, patient-centric excellence across all paediatric ages and therapeutic areas. The insights gained during the project now guide c4c-S in refining paediatric trials, making them more feasible and tailored to the unique needs of children.

Objectives

Originally a project aimed at improving children's access to better medicines, c4c-S was established to build on and expand this mission. c4c-S aims to address the challenges of conducting paediatric trials by fostering an environment where patients, parents, and the medical community work closely together. This will speed up and facilitate the running of high quality paediatric clinical trials, ultimately contributing to the development of better medicines for babies, children and young adults.

Methods

c4c-S employs a proven methodology and efficient stepwise advice process via a Single Point of Contact (SPoC). To meet the objectives set by c4c-S, the advice service was transferred from the project to c4c-S, ensuring availability to both academic and industry sponsors.
This transfer was formalized through licensing agreements, which enable the integration of the advice service into c4c-S.



Results

Over five years the advice service has gained experience by completing over 42 advice requests, drawing on the expertise of 31 clinical and 17 methodology experts; utilizing a network of over 400 experts across different expertise's. This includes the perspectives of patients and carers, with 10 specific advice requests focused on their insights.

The establishment of a centralized contracting structure and a single point of contact has streamlined the process with a median time to convene advisory meetings of 12 weeks, and improved coordination. Scoping interviews have played a key role in clarifying sponsor needs, while expert scientific engagement and consistent reporting have enhanced the quality of the advice provided.

The experience highlights the importance of understanding various methodologies, and the involvement of experienced facilitators in patient and carer activities. c4c-S's advice service incorporated lessons learned optimizing the service.

Furthermore, collaborating with existing networks has strengthened the support infrastructure, contributing to more effective paediatric drug development.

Conclusion

The transition to c4c-S has markedly advanced the field of paediatric clinical trials, transitioning from a pan-European project to foundation dedicated to improving children's access to new and better medicines.

By fostering multinational and multidisciplinary collaboration among over 400 experts, including 21 clinical and 17 methodology specialists, the network is well positioned to make significant contributions to drug development. The creation of the non-profit foundation c4c-S, ensures a sustainable platform for providing expert advice to both academia and industry, thereby supporting ongoing advancements in paediatric health to improve paediatric development programmes and paediatric protocols.

This project has received funding from the Innovative Medicine initiative 2 joint undertaking under grant agreement number 777389. The Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA

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Specific phosphorylation barcodes modulate CCL25-mediated G protein coupling by CCR9

Thomas Lamme

Specific phosphorylation barcodes attenuate CCL25-mediated G protein coupling by CCR9

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Amsterdam Institute for Molecular and Life Sciences (AIMMS), Division of Medicinal Chemistry, Faculty of Science, Vrije Universiteit Amsterdam, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands.

Abstract

The chemokine receptor CCR9 mediates immune cell migration between the thymus and the gut through activation by its ligand CCL25. As a G protein-coupled receptor (GPCR), CCR9 activates Gi and Gq pathways to drive cell motility. Dysregulation of CCR9 or CCL25 lead to inflammatory diseases and overexpression correlates with malignant tumor metastasis. Although robust downstream G protein responses have been reported, we observe that G protein activity, determined by live-cell BRET assays, by CCR9 is severely and rapidly attenuated. What is leading to this low level of CCR9 activity is unclear. We proposed that rapid desensitization by arrestins or phosphorylation of the receptor is suppressing the G protein response in our system. Here, we test this hypothesis by observing G protein activation and signaling in cells lacking arrestins or GPCR kinases (GRKs) due to CRISPR knockout. Canonically, arrestins physically interfere with GPCR signaling, however, the absence of arrestins had no impact on CCR9 activation. Instead, a 6-fold increase in activation was observed in the absence of GRKs. Site-directed mutagenesis identified that the proximal phosphorylation sites were responsible for the attenuation. The modulation was not due to CCR9 re-localization or internalization, but rather phosphorylation appeared to interfere with G protein binding. This suggests that phosphorylation directly regulates the G protein-dependent activity of CCR9 and may have implications for targeting the receptor therapeutically and other GPCRs.

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Fragment-based design of dual-activity H1R and H4R antagonists with superior efficacy in a mouse model for allergic conjunctivitis.

Peter Weber, Rogier Smits, Herman Lim, Mabel Dekker, Tiffany van der Meer, Mounir Andaloussi, Matt J. Chapin, Paul Gomes, Andy Whitlock, Dr Maikel Wijtmans, Prof Rob Leurs, Prof Iwan de Esch

Biography:
I hold a BSc in Biological Chemistry from Johannes-Kepler University in Linz, Austria, where I developed a strong foundation in biochemistry and molecular biology. I further specialised by completing an MSc in Drug Discovery and Safety at Vrije Universiteit Amsterdam, deepening my expertise in pharmacology, medicinal chemistry, and drug development. Since July 2023, I am pursuing a PhD at Vrije Universiteit Amsterdam in the group of Prof. Iwan de Esch, focusing on the development of dual-active H1/H4 receptor compounds for potential therapeutic applications. My work bridges molecular design with medicinal chemistry, aiming to address key challenges in drug discovery.

Fragment-based design of dual-activity H1R and H4R antagonists with superior efficacy in a mouse model for allergic conjunctivitis.

Peter Weber1, Rogier Smits2, Jac Wijkmans2, Herman Lim,1,2 Mabel Dekker1, Tiffany van der Meer1,2, Mounir Andaloussi2, Henry F. Vischer1, Matt J. Chapin3, Paul Gomes3, Andy Whitlock3, Maikel Wijtmans1, Rob Leurs1,2, Iwan J.P. de Esch1,2,*

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Abstract:
Histamine receptors are G-protein-coupled receptors that play pivotal roles in health and disease and consist of four distinct subtypes (H1R, H2R, H3R and H4R). The H1R is a well-established drug target and a plethora of H1R antihistamines have been marketed to alleviate, with limited success, various pruritic and allergic conditions, including allergic rhinitis and allergic conjunctivitis. Interestingly, the H4R is also associated with inflammatory processes, but no H4R-related drugs have so far reached the clinic. The contributions of these histamine receptor subtypes on inflammation have ignited interest in polypharmacological strategies.
Starting from a quinazoline-containing fragment hit and using computer-aided drug design, synthesis, and pharmacological evaluation, we developed quinazoline-containing compounds with different and well-defined activity and polypharmacology profiles across histamine receptor subtypes. In preclinical studies, the challenges of species differences as well as various ADME-tox properties needed to be navigated, ultimately yielding several promising clinical candidates. The dual-activity H1/H4 ligands exhibit potent anti-inflammatory effects in several preclinical models. Here, we will focus on the development of GD136, a clinical candidate that shows promising in vivo efficacy for the treatment of allergic conjunctivitis and firmly establishes the clinical potential of dual-activity H1R/H4R ligands.

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Optical control of the beta2-adrenergic receptor with an azobenzene analog of Clenbuterol: from partial agonism to antagonism

Yangzhi Cao

Biography

I am currently a third-year PhD student at Vrije Universiteit Amsterdam, majoring in Medicinal Chemistry. My research focuses on photo pharmacology, with an emphasis on the study of beta receptors

Optical control of the β2-adrenergic receptor with an azobenzene analog of Clenbuterol: from partial agonism to antagonism

Yangzhi Cao, Shuang Shi, Chris0an M. L. Buzink, Simone Does, Meichun Gao, Barbara Zarzycka, Iwan J. P. de Esch, Henry F. Vischer, Maikel Wijtmans*, Rob Leurs*

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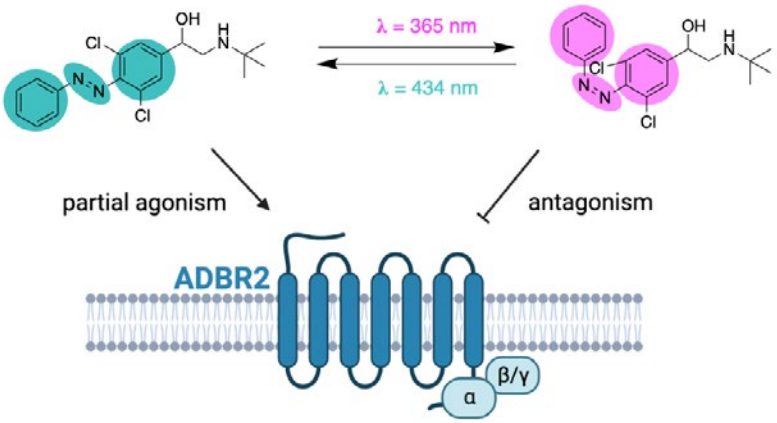
Abstract

Photopharmacology can achieve a high level of spatial and temporal control of protein signaling by using light as an external trigger. G protein-coupled receptors (GPCRs) constitute one of the most preferred families of drug targets and ~34% of the currently used drugs act on GPCRs. Applica0on of photopharmacology to GPCRs has a\racted considerable interest. 1Adrenergic receptors (ADRs) are GPCRs that recognize the endogenous signaling molecules adrenaline and noradrenaline. Among the subtypes of ADRs, the β2 adrenergic receptor (ADRB2) is an important target that mediates physiologic responses such as smooth muscle relaxation and bronchodilation. Clenbuterol is a selective β 2 par0al agonist used to treat respiratory diseases affecting, such as asthma and bronchial hyper-reactivity. We adapted an "azoextension" strategy to clenbuterol to generate a small library of azobenzene derivatives. Among them, VUF26034 shows suitable photochemical properties, good thermal stability of the cis isomer (t1/2 ~ years) and interesting photopharmacological properties (> 10-fold increase in binding affinity upon illumination from the trans to cis isomer). Furthermore, the trans and cis isomer of VUF26034 have different efficacies, enabling a switch from partial agonism to antagonism upon illumination.



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Discovery of small-molecule ACKR3 (CXCR7) inverse agonists

Laura Wijffelaars, Rick Riemens, Reggie Bosma, Desislava Nesheva, Sebastiaan de Jager, Wessel Sinnige, Barbara Zarzycka, Mirjam Zimmerman, Susanne Roth, Nadine Dobberstein, Aurélien Rizk, Henry Vischer, Maikel Wijtmans, Rob Leurs, Iwan de Esch

Biography

Laura Wijffelaars is a PhD student in medicinal chemistry at VU Amsterdam, where she focuses on the design and synthesis of new bioactive compounds for a chemokine receptor. She holds a bachelor's degree in chemistry with a minor in pharmaceutical sciences from Utrecht University and a master's degree in drug discovery and safety, specializing in drug design and synthesis, from VU Amsterdam.

Discovery of small-molecule ACKR3 (CXCR7) inverse agonists

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Abstract

The atypical chemokine receptor 3 (ACKR3, also known as CXCR7) is a class A G-protein-coupled receptor that is activated by the chemokines CXCL11 and CXCL12. Unlike conventional chemokine receptors, activation of this receptor leads to phosphorylation, β-arrestin recruitment and internalisation. The receptor plays a role in the immune system by directing leukocytes to the site of inflammation through regulation of the chemokine gradient and is involved in inflammatory diseases like multiple sclerosis (MS). Therefore, targeting ACKR3 is of therapeutic interest. Moreover, small-molecule ligands targeting ACKR3 are efficacious in vivo in the treatment of multiple inflammatory diseases as well as in several cancer models. A potent ACKR3 small-molecule antagonist (ACT-1004-1239) was patented in 2018 and is currently being evaluated in clinical trials for treating MS. We have developed ACKR3 structure-activity relationships with the specific aim of obtaining potent ACKR3 ligands with a lower molecular complexity (e.g., avoiding two chiral centers). This work has resulted in the identification of two novel, high-affinity inverse agonists for ACKR3: VUF25661 and VUF25983.

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ChemoPar-db: A Structural Chemogenomics Database for Chemokines and their Binding Partners

Bas de Boer, Albert J. Kooistra, Iwan J.P. de Esch, Barbara A. Zarzycka

Biography

Bas de Boer is a PhD Student in the Department of Medicinal Chemistry at VU Amsterdam, specialising in computational methods for drug discovery.

ChemoPar-db: A Structural Chemogenomics Database for Chemokines and their Binding Partners

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Abstract

Chemokines play a central role in the organization of the immune system in both homeostatic and inflammatory processes.1 As highly versatile scaffolding proteins, chemokines bind a diverse range of partners. This includes binding to chemokine receptors, other chemokines, glycosaminoglycans, antibodies, and various chemokine binding proteins, indicating their important role in highly specific and complicated cellular communication.2,3 Considering that chemokines engage with a multitude of other interaction partners, targeting the diverse scaffolding properties of these chemokines could lead to the discovery of drugs that have unprecedented control over their physiological action. Here we present ChemoPar-db, a database in which we compiled all publicly available structural data on chemokines. By using a Python-based workflow, ChemoPar-db collects and processes chemokine 3D structures from the Protein Data Bank. This workflow involves the alignment of sequences to a reference multiple sequence alignment, preparing structures by protonation and separation of structural elements, structural alignment onto a common template, and calculation of chemokine-partner interactions. Utilizing the Django framework, we integrated all this information into an organized database which is freely accessible at https://chemopar-db.net. By providing a focused structural overview of chemokines and their molecular interactions, we expect ChemoPar-db to aid in efforts targeting the chemokine signaling axis. Specifically, the database can help structure-based drug discovery efforts, leading to more small-molecule modulators of chemokines.

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Photopharmacology for GPCR receptor proteins: 1st and 2nd generation chemical biology tools

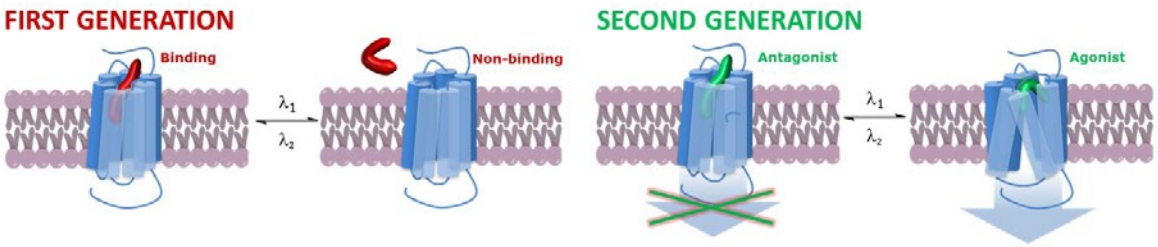
Lars Binkhorst, Niels Hauwert, Xavier Gómez-Santacana, Tamara Mocking, Henry Vischer, Maikel Wijtmans, Rob Leurs

Biography

Lars Binkhorst completed his Bachelor's degree in Pharmaceutical Sciences and Master's degree in Drug Discovery and Safety at the Vrije Universiteit Amsterdam. He is currently a PhD student in Medicinal Chemistry at the same institution, specializing in photopharmacology. His research focuses on the design and synthesis of light-activated drugs.

Abstract

Photopharmacology is a discipline that uses photoswitchable ligands as pharmacological tool compounds to yield spatiotemporal control of protein activity with light. Photopharmacology in the G protein-coupled receptor (GPCR) field has been emerging in recent years. In this poster, our general approach towards GPCR photopharmacology will be discussed as well as exemplary contributions from our laboratory including several series of photoisomerisable azobenzene-based GPCR ligands. First-generation series involve photoswitchable antagonist or agonist ligands that shift affinity/potency for an aminergic GPCR. In a second-generation series, we incorporated a change in the ligand efficacy for a peptidergic GPCR upon illumination, thus establishing a photoswitch from antagonism to agonism. Our contributions deliver a toolbox of compounds capable of photomodulating GPCR signaling in complementary ways.



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De-risking clinical trials: forecasting and preventing disasters by pursuing an IB-derisk approach

Dr. Jeroen Van Smeden, Drs. Francis M. Dijkstra, Prof. Dr. Adam F. Cohen, Prof. Dr. Joop M.A. van Gerven, Prof. Dr. David J. Webb, Prof. Dr. Jacobus Burggraaf

Biography

Jeroen van Smeden performed his PhD at the University of Leiden, and defended his PhD on "A breached barrier: analysis of stratum corneum lipids and their role in eczematous patients" in 2013". Afterwards, he became an assistant professor at the "Drug Development Technology" department of the Leiden Academic Centre for Drug Research (LACDR). In 2017, he became Education Director of the Centre for Human Drug Research (CHDR). Where he is responsible for all internal scientific education (mainly PhD and Clinical Pharmacology related), as well as external educaction (predominantly academic pharmacology and pharmacotherapy and drug development education, as well as the TRC-p.nl pharmacology platform). Jeroen won the 'Teacher of the Year' award in 2015 and was nominated again in 2023. He is member of the Education Committee (COZ) of the Dutch Society for Clinical Pharmacology & Biopharmacy, and is chairman of PScribe-society, the e-learning platform for students to develop pharmacotherapeutic skills.

Abstract

In early-phase drug trials, accurate translation of non-clinical data to human predictions is crucial for safe dosing, as evidenced by first-in-man studies with TGN1412 (2006, UK) and BIA-2474 (2016, France). In both cases, incorrect dosage translation led to severe reactions: cytokine storm, organ failure, and in the latter trial, one death. These incidents highlight the challenge researchers face when relying on non-clinical data from the investigator's brochure (IB) in first-in-human trials. Human predictions depend on in vitro and animal studies, but the IB's complexity (including 10s-100s of pages of text and tables) makes it daunting to fully capture potential therapeutic ranges and safety concerns of a drug.
Therefore, CHDR developed an integrated method called IB-derisk. This approach constructs an integrated tabular overview. It combes all (non-)clinical data (PK, PD, tox) and is color coded to the severity of findings. The table is sorted across species on relevant PK/exposure parameters like Cmax, AUC, or HED (human equivalent dose). The rationale for this approach is that compounds that translate well from experimental animal to humans, will show comparable concentration-effect relationships. Integration of all IB-data (non-clinical and clinical if available) results in a tabular overview, and assisting graphs (Table 1): low drug dosages/exposure will lead to no noticeable effects (colour-coded white); at higher drug plasma concentrations, positive PD effects (green) or mild side effects (yellow) may occur; at even higher dosages, this transposes into severe, non-tolerable side effects (orange) or even dead animals (red). The resulting table contains tens or even >100 color-coded rows, which shape a (hopefully) consistent picture that translates well into humans, or otherwise should enable further discussion when colours do not align – as appeared in case of BIA-2474 and TGN1412.

Table 1:(Pre)clinical data sorted on relevant PK-parameter to predict safe dose range in humans

Low Dose/AUC/Cmax		No (pre)clinical observations (side-effects/effects)
↓		Desirable (Pharmacological) effects
		Mild (Tolerable) side effects
		More severe side effects (not tolerable)
High Dose/AUC/Cmax		Serious irreversible side effects / death

CHDR monitors the IB-derisk approach for predictability. In this study, the tool was applied to first-in-human studies with 25 CNS-active compounds. Predictions of tolerable and pharmacologically active dose ranges based on non-clinical data were compared to actual outcomes of clinical studies. The results demonstrate that non-clinical data can reasonably predict clinical active dose ranges. With an average overlap of 84%, HED was the best predictor for pharmacologically active dose range whereas tolerability

was best predicted by Cmax.
Beyond its application in clinical trial settings, IB-derisk is valuable for education: BSc/MSc students of various disciplines were asked to review pseudonymised IBs including TGN1412 and BIA-2474. Results demonstrate that students unfamiliar with IBs benefit from the IB-derisk approach, providing relevant questions for proper dose rationale. Regarding BIA-2474, the large majority of all student cohorts (82-94%) identified major safety concerns using the IB-derisk approach. Many of these concerns were prohibitive for human dosing of BIA-2474, and align with conclusions of the formal post-mortem reports. In conclusion, the IB-derisk approach provides an integrated tabular overview that aids in meaningful integration of preclinical to clinical data, gives rise to relevant questions about translatability, facilitates communication amongst researchers and regulators, and serves as teaching tool for future professionals who may be involved in drug development.

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Modelling asthma treatment trajectories using the parametric g-formula: predicting subgroup differences in switching behavior

Irene Mommers, Dr. Job van Boven, Jens Bos, Dr. Sumaira Mubarik, Prof. Dr. Eelko Hak, Dr. Maarten Bijlsma

Biography

Hi, my name is Irene and I am a third-year PhD-candidate at the department of PharmacoTherapy, -Epidemiology, & -Economics (PTEE) at the University of Groningen. The goal of my project is to improve the understanding of asthma treatment trajectories, identify relevant subgroups and treatment effect heterogeneity, and to improve methodology for assessing complex, longitudinal treatment trajectories. My previous education mainly consists of a Bachelor's degree in Biomedical Sciences and a Master's degree in Epidemiology.

Modelling asthma treatment trajectories using the parametric g-formula: predicting subgroup differences in switching behavior

Irene Mommers1, Job F M van Boven2,3, Jens H J Bos1, Sumaira Mubarik1, Eelko Hak1, Maarten J Bijlsma1,4

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3 Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;
4 Laboratory of Population Health, Max Planck Institute for Demographic Research, Rostock, Germany

Introduction

Treatment of asthma is a dynamic process, entailing multiple treatment steps with the possibility of switching up and down between these steps whenever needed based on (lack of) disease control. The g-formula offers a promising approach to analyze the complex, dynamic trajectories of asthma treatment over time.

Objectives

This study aims to investigate whether the parametric g-formula could be used to approximate empirical asthma treatment trajectories over time and aims to predict subgroup differences in switching behavior within asthma treatment trajectories.

Methods

This retrospective cohort study, using data from 1994 to 2021 from the IADB.nl community pharmacy dispensing database, included 16- to 45-year-olds initiating inhaled asthma medication in the Netherlands. We used multinomial logistic regression models with the parametric g-formula to predict asthma treatment trajectories and their association with various patient characteristics (age, sex, chronic drug treatment for atopic diseases (ATD), cardiovascular diseases (CVD), thyroid diseases, arthritis, diabetes, gastroesophageal reflux disease (GERD), mental health problems (MHP), and immunosuppressants)

Results

The simulations predicted 76% of individuals to switch treatment steps, on average 2.3 times, with a mean duration till first switch of 8.3 months, which aligned closely with the actual rates (77%, 2.3 times and 7.9 months, respectively). Comparatively, fewer 45-year-olds than 16-year-olds switched treatments (74% vs. 78%, $p < 0.001$), but earlier (8.1 vs. 8.6 months, $p < 0.001$) and more frequently (2.4 vs. 2.3 times,

$p < 0.001$). Males switched less frequently. ATD, CVD, MHP, or GERD showed significantly less switching (fewer switchers and after longer duration: $p < 0.05$ for ATC, CVD, MHP, and GERD; lower number of switches among switchers: $p < 0.05$ for CVD and MHP).

Conclusion
The g-formula effectively approximates asthma treatment trajectories in clinical settings and found age, sex, ATD, CVD, MHP, and GERD to be predictive of switching behavior.

Keywords
Asthma; inhaler medication; treatment steps; trajectories; g-formula; prediction

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Who actually benefits from a drug? Tools for data-driven prospective responder identification

Prof. Dr. Ton Coolen

Biography

Ton Coolen did his PhD in Theoretical Physics in Utrecht. He then moved to the United Kingdom, where after four years in Oxford he became Reader in Applied Mathematics and in 2000 Full Professor at King's College London. There he created the Institute for Mathematical and Molecular Biomedicine. In 2020 he left the UK to accept a Professorship at Radboud University. Coolen's area of expertise is the analysis of complex biomedical problems, using advanced mathematical and computational methods, with emphasis on topics in cancer medicine. His team develops novel methods for survival analysis and clinical outcome prediction from high dimensional data, and for handling competing risks and latent patient or disease heterogeneity. Coolen has published three books and around 200 papers in refereed scientific journals. He is founder/CEO of Saddle Point Science Europe, which focuses on the implementation and application in medicine of novel data analytics and Bayesian inference methods.

Who actually benefits from a drug? Tools for data-driven prospective responder identification

Ton Coolen, Radboud University and Saddle Point Science Europe

Introduction

There is renewed focus on innovating medical data analysis tools, driven by the increasing complexity of medical data and the drive towards personalized medicine. Personalized medicine requires accurate mapping and predictive use of latent heterogeneity in diseases and patients, which standard medical statistics cannot do. Moreover, especially in oncology, clinical trials have low success rates. Also this relates to the use of primitive statistics methods, which only quantify the average cohort-wide benefit of a drug. Hence, trials fail if a detrimental effect in one patient subgroup cancels the beneficial effect in another, and many drugs are licensed even if they benefit only a small patient subgroup. This causes unnecessary harm by side-effects, reduced availability of drugs, and waste of healthcare resources.

Objectives

Data-driven prospective responder identification (PRI) seeks to infer from individual baseline patient characteristics how likely they are to respond to a given treatment, and benefit from a given drug. If successful, PRI can lead to improved treatment outcomes, prevention of overtreatment, fewer side effects, higher drug response rates, and fewer trial failures. It should impact on clinical trial design and ethical considerations. PRI requires more powerful mathematical and computational methods than those typically used in medicine, but these are now available and have passed their pilot study tests.

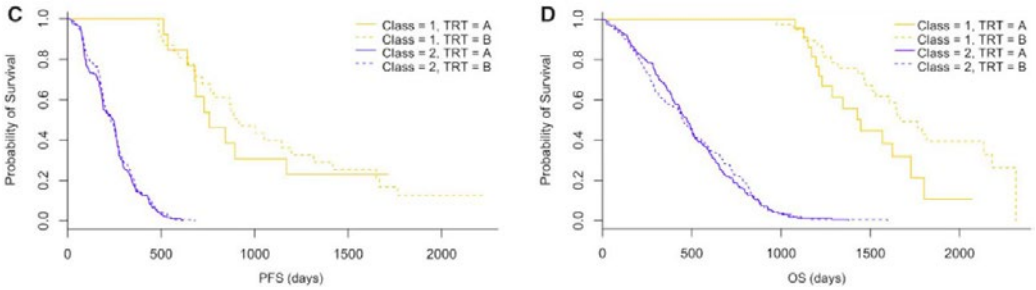
Methods

In failed but stratifiable trial cohorts, the association between treatment and clinical outcome is heterogeneous. Our Bayesian PRI method detects and characterizes latent patient subgroups by modelling the relationship between individual and cohort level outcome probabilities. It is fully interpretable (in contrast to most AI approaches), and differs from similar methods in literature by:

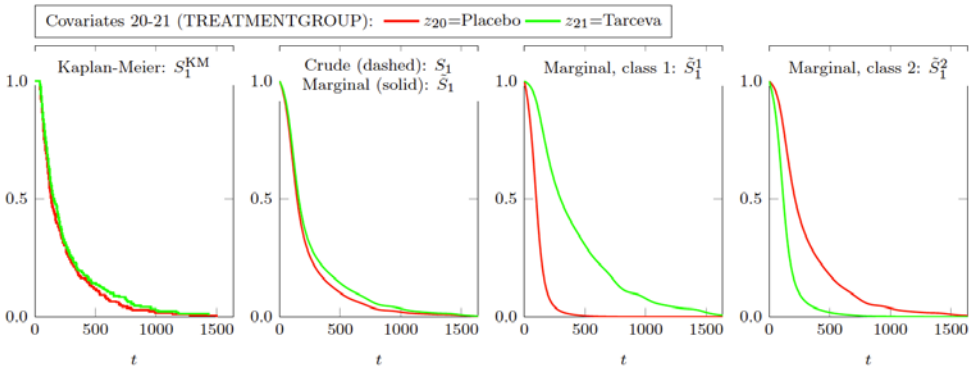
- Modelling multiple active risks, and using these to improve latent class mapping.
- Allowing for heterogeneity not only in associations but also in base hazard rates.
- Controlling model complexity via Bayesian model selection.
- Removing the impact of local minima via innovative sampling protocols.
- Reducing to standard Cox regression if latent heterogeneity is absent.
- Computing strata membership probabilities if latent heterogeneity is present.

- Quantifying the potential for prospective class prediction from the available covariates.

Results



This picture (from Barbet et al, JNCI J Natl Cancer Inst, 2020) shows the subgroups of responders (yellow) versus non-responders, detected upon application of Bayesian responder identification to the (failed) colorectal cancer trail COIN. Dashed curves: cetuximab; solid curves: placebo.



This picture (paper in preparation) shows the subgroups of responders (class 1) versus non-responders (class 2, who are actually harmed by the drug Tarceva), detected upon application of Bayesian responder identification for the (failed) non-small-cell lung cancer trial TOPICAL.

Conclusion

With the Bayesian PRI pipeline we now have a reliable and practical analysis tool for predicting (if possible) prospectively, on the basis of data alone, which individuals are likely to benefit from which treatment, enabling true personalized treatment. We now need to integrate PRI methods into standard clinical trial practice and the design of future clinical trials.

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Local target binding and internalization of large molecules in tissue interstitial space

Msc Tatiana Zasedateleva, PhD Stephan Schaller, PhD Wilhelmus de Witte

Biography

Wilbert de Witte is a Pharmacologist with a strong drive to understand complex mechanisms and the models that represent them.
Before joining ESQlabs, he worked at Ablynx NV, later Sanofi Ghent, on the preclinical and clinical development of NANOBODY® therapeutics. He developed several PBPK and PBPK-QSP models as well as traditional TMDD and PKPD models for mechanistic analysis of in vitro, in vivo, and clinical data. He accumulated in-depth knowledge on the behavior of large molecules in different modalities and with various target binding characteristics.
Wilbert de Witte obtained his Master's degree in Bio-Pharmaceutical Sciences from Leiden University (the Netherlands). For his PhD thesis, he studied the impact of drug-target binding kinetics on in vivo drug action. His PhD research was supervised by Prof. Liesbeth de Lange, Prof. Piet-Hein van der Graaf and Prof. Meindert Danhof at the department of Pharmacology at Leiden University.

Local target binding and internalization of large molecules in tissue interstitial space

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Introduction

Large molecule drugs bind to their targets with high affinity and specificity and have long pharmacokinetic half-lives. These properties increase their therapeutic effects but also introduce a propensity to non-linear pharmacokinetics due to extensive target binding, known as Target-Mediated Drug Disposition (TMDD) [1–3]. TMDD describes that drug-target binding may impact a drug's pharmacokinetics [4]. However, many targets are expressed in tissues rather than in plasma, implying a much lower impact of target binding on drug plasma concentrations than drug-target binding in the circulation.

Objectives

This study aimed to collect physiological values for TMDD parameters in tissues, encompassing various targets from different sources and species. Based on these obtained values, we sought to enhance our understanding of each parameter's significance in this system and identify the likelihood of local depletion of drug concentrations for biologics whose target is expressed in tissues.

Methods

Literature studies were conducted to gather typical values for target concentration, target degradation (kdeg), and drug-target complex internalization rate constants (kint). Simulations were performed using a whole-body Physiologically-Based Pharmacokinetic model (PK-Sim v11.0), with the target expressed in the interstitial space of the heart or muscle and the drug simulated as a large molecule in the large molecule model of PK-Sim [5].

Results

The interquartile ranges of collected target concentrations and target turnovers are limited to two orders of magnitude (Figure 1). This provides a good starting point for projects lacking quantification of the in vivo target concentration. Across various species, target concentrations have a similar distribution, with a median of around 10 nM. Moreover, the soluble targets typically exhibit lower concentrations than targets expressed in tissue cell membranes and intracellularly. The free target internalization rate constants generally exceed the internalization rate constants of the drug-target complex in most species, resulting

in ratios of drug-target complex versus free target internalization (k¬¬int/kdeg) below 1. Additionally, the complex internalizaion rate constants (kdeg) are higher for soluble targets, while membrane-bound targets expressed in tissues show higher internalization rates. The kint/kdeg ratios for soluble targets are predominantly below 1, while these ratios are distributed around 1 for membrane targets.

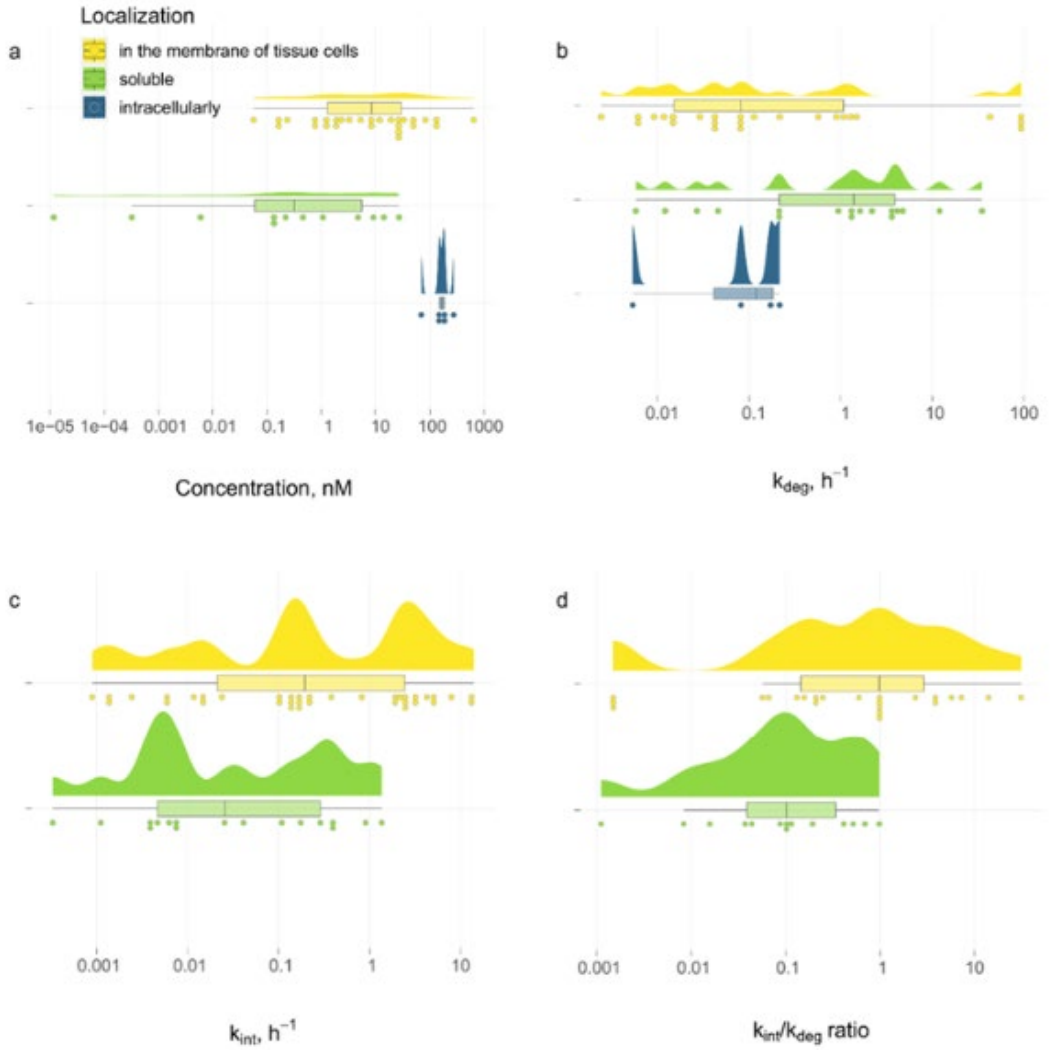

Figure 1. Distribution of target concentration, kdeg, kint, and kint/kdeg ratio by target localization. Shaded areas represent density distributions, dots represent individual data points, and boxplots indicate the three quartiles with whiskers extending up to 1.5 * IQR (Inter-Quartile Range, i.e., from the 25th to the 75th percentiles).

In many simulated scenarios, local drug depletion was observed, as evidenced by a significant decrease in interstitial concentrations and, consequently, in target occupancy, with increasing target concentrations and turnover (Figure 2). However, plasma concentrations remained minimally affected (left column).

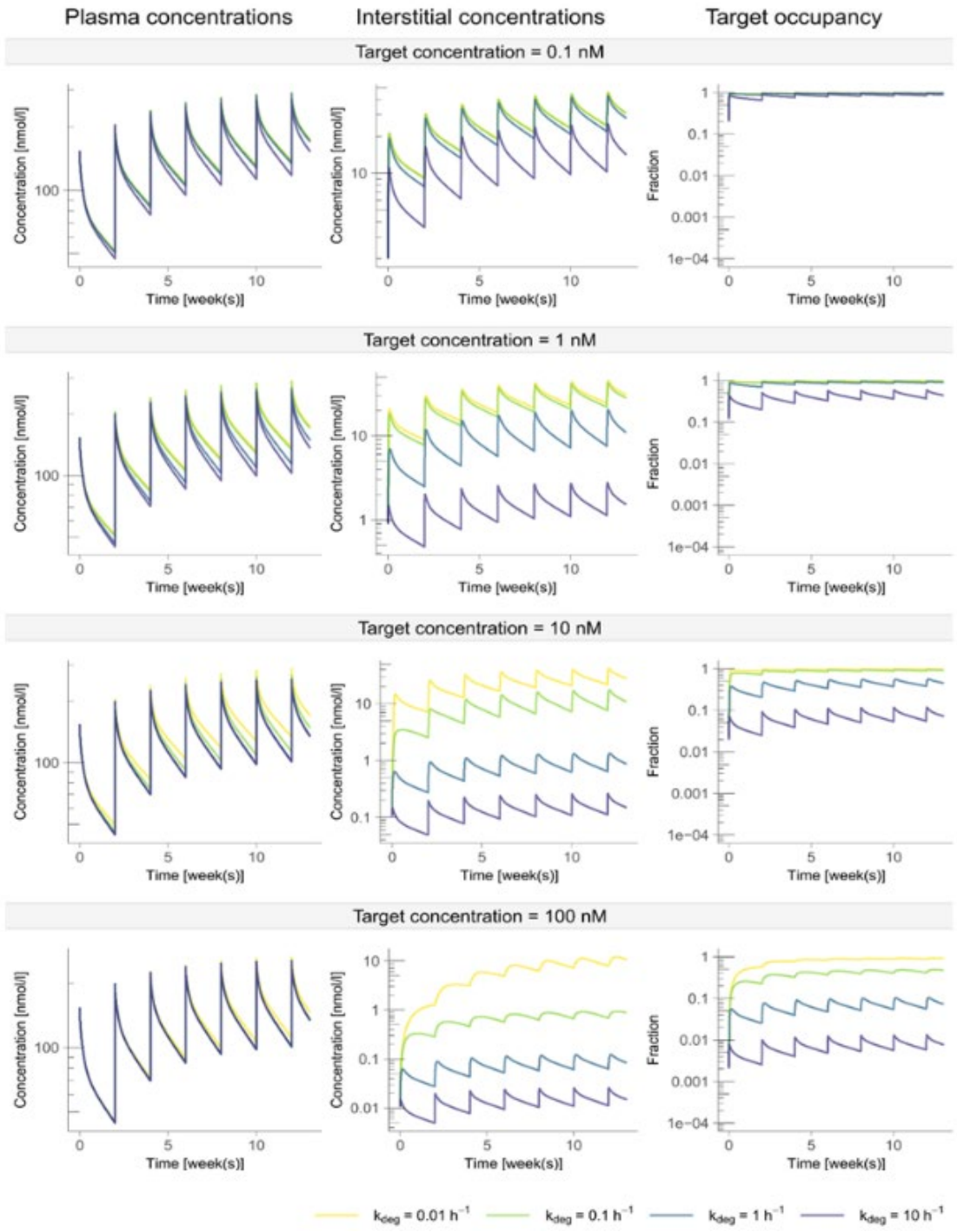

Figure 2. Comparative plasma concentrations, interstitial concentrations, and target occupancy simulations with an integrated large molecule PBPK – TMDD model for a target expressed in the heart interstitial space. A standard 150 kDa mAb was simulated with an affinity of 1 nM and a dose of 1 mg/kg. The kint/kdeg ratio was kept constant at 0.1 in all simulations.

Conclusion

The study sheds light on the impact of target concentration and target turnover on local drug concentrations and target occupancy within the tissue interstitial space in physiologically realistic scenarios. These findings emphasize the importance of evaluating TMDD parameters and raise questions regarding the sufficiency of relying on conventional plasma measurements in cases with a potential of localized TMDD since this approach might lead to developing suboptimal dosing regimens. Implementing TMDD in a whole-body PBPK model allows us to explore drug-target binding in various organs and species for molecules of different sizes, making it a versatile tool for studying drug-target interactions in tissue interstitial spaces.

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From human in vivo co-expression modules to cross-species comparisons: a comprehensive analysis of toxicity models.

Imke Bruns, Dr. Lukas Wijaya, Hugo van Kessel, Dr. Steven Kunnen, Dr. Jesper Kers, Dr. Giulia Callegaro, Prof.dr. Bob van de Water

Biography

Imke Bruns is a dedicated bioinformatician with an academic background in Bio-Pharmaceutical Sciences, having completed both her Bachelor's and Master's degrees at Leiden University. During her Master's studies, Imke developed a keen interest in bioinformatics, which led her to undertake two internships in the field. These experiences paved the way for her current role in Bob van de Water's research group at the LACDR. Currently, Imke focuses on Weighted Gene Co-expression Network Analysis (WGCNA), applying this methodology to human in vivo kidney data. Her work aims to compare co-expression patterns in humans with in vitro test systems to assess the human relevance of these test systems.

From human in vivo co-expression modules to cross-species comparisons: a comprehensive analysis of toxicity models.

I.B. Bruns1, L.S. Wijaya1, H. van Kessel1, S.J. Kunnen1, J. Kers2,3, B. van de Water1.

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3 Amsterdam Medical Center (AMC), Amsterdam, the Netherlands

Advancing to a next-generation risk assessment necessitates the integration of mechanistic insights following exposure to chemicals. Such integration not only enhances informed decision-making but also strengthens the determination of the applicability domain and the identification of uncertainties inherent in in vitro human toxicity models. Here, we present a system toxicology model of an exceptionally extensive transcriptomic dataset of human kidney pathologies. Our objective is to deepen the understanding of the molecular characteristics linked to various kidney adversities and subsequently define the applicability domain of renal in vitro models.
A total of 678 human kidney allograft formalin fixed paraffin embedded biopsy samples were collected from the biobanks in Leiden University Medical Center (LUMC) and the Amsterdam Medical Center (AMC). Every biopsy classified as a pathology sample was compared to kidney samples scored with no pathological diagnosis to derive differentially expressed genes. From the entire dataset a Weighted Gene Co-regulation Network Analysis (WGCNA) was applied to generate co-expression modules corresponding to mechanisms and processes typical of kidney adversities.
We initially validated the generated modules using predefined gene sets from the Molecular Microscope Diagnostic System (MMDx) database, confirming the relevance of our modules. Upon discovering significant associations between some modules and cell-type specific transcripts, we expanded our analysis to deconvolute cell-type specific signatures hidden in the bulk data but recognized by the co-expression model. Using the MuSiC2 package, we examined associations between cell-type proportions and module eigengene scores to determine if observed patterns in module behavior could be attributed to variations in cell-type proportions. Finally, a comparative analysis was performed, comparing expression profiles observed in human in vivo settings with those in an in vivo rat setting and an in vitro human RPTEC/TERT1 cell line, thereby elucidating the human relevance of these measured responses.

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Lasso Logistic Regression and Cluster Analysis in Predicting Adherence and Drug Patterns among New Users of Monotherapy for Antihypertensive Drugs

Xuechun Li, Mutiara Tia, Jens Bos, Catharina Schuiling-Veninga, Eelko Hak, Sumaira Mubarik

Biography

Xuechun Li is in her 3rd year of the Ph.D. program in PharmacoTherapy, -Epidemiology and -Economics, Groningen Research Institute of Pharmacy, University of Groningen. Her research interests are in Pharmacoepidemiology. Her current research mainly focuses on adherence and drug patterns of anti-hypertensive and anti-hyperlipidemic drug monotherapy in the real world.

Lasso Logistic Regression and Cluster Analysis in Predicting Adherence and Drug Patterns among New Users of Monotherapy for Antihypertensive Drugs

Xuechun Li 1, Mutiara Djayanis Tia 1, Jens H J Bos 1, Catharina C. M. Schuiling-Veninga 1, Eelko Hak 1, Sumaira Mubarik 1

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Introduction

In hypertension management, patient drug adherence is essential for successful monotherapy. However, not much is known about real-world long-term adherence and drug patterns in antihypertensive monotherapy.

Objectives

Our study aims to assess adherence rates and drug patterns among patients after start with anti-hypertensive monotherapy.

Methods

We designed a retrospective inception cohort study using pharmacy records from the University of Groningen IADB.nl dispensing database from January 1, 1996, to December 31, 2020. Among the 33,427 adult starters with antihypertensive drug monotherapy, Cohort 1 contained 13,155 patients with a follow-up period exceeding 3 years (1080 days), while Cohort 2 comprised 2,086 patients with a follow-up duration surpassing 10 years (3600 days). Exposures were anti-hypertensive drug monotherapy including angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, beta-blockers, calcium channel blockers, and thiazides. Primary outcomes included adherence rates, calculated as the proportion of days covered by antihypertensive monotherapy during follow-up. Binary adherence classified levels as high (≥0.8) or low (<0.8). Secondary outcomes encompassed drug patterns, such as discontinuation, continuation, switch, and add-on. Adherence and drug patterns were expressed using descriptive statistics. Cluster analysis was performed to categorize patients with similar risk factors. Lasso logistic regression was then employed to construct prediction models.

Results

Adherence rates were 84.2%, 92.1%, and 94.9% in all patients, cohort 1, and cohort 2 patients receiving antihypertensive drug monotherapy, respectively. In cohort 1, drug pattern rates were 45.4% for discontinuation, 37.8% for continuation, 11.6% for switch, and 31.1% for add-on. Thiazides exhibited the highest rates in discontinuation, switch and add-on, while CCBs showed the highest continuation rate. In cohort 2, drug pattern rates were 4.1%, 92.1%, 0.6%, and 4.1%, respectively. CCBs had the highest rates of continuation, switch, and add-on, while ARBs showed the highest discontinuation rate. ACEIs, ARBs, and Thiazides demonstrated a lower likelihood of low adherence compared to BBs in cohort 1, while most monotherapies displayed greater ease in terms of discontinuation, switching, and adding compared

to BBs in cohort 1 or 2. Similar results were observed across different distinct risk clusters within both cohorts.

Conclusions

The statistical clustering analysis of adherence and drug patterns among patients on monotherapy offers valuable insights into medication options.

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CO2 inhalation challenge: optimization for early anxiolytic drug development

Drs Asso Safai Pour

Biography

Dr. Safai Pour obtained his MD at Leiden University in the Netherlands in 2019. He is currently performing his PhD research in psychopharmacology and training in clinical pharmacology at CHDR (Centre for Human Drug Research) and Leiden University in Leiden, the Netherlands. His work focusses on challenge models for fear and anxiety in early psychiatric drug development.

Introduction

Panic attacks (PAs) in humans are acute episodes characterized by intense fear, cognitive distortions, and physical symptoms driven by autonomic nervous system activation. Traditional retrospective assessments of PAs suffer from limitations like recall bias and subjective variability, complicating the accurate evaluation of anxiolytic interventions. To address these challenges, experimental models such as the 35% CO2 inhalation challenge have been developed. This model induces panic-like responses in a controlled environment, enabling real-time quantification of fear-related biomarkers and offering a more objective measure of anxiolytic efficacy. The 35% CO2 model has demonstrated the ability to replicate real-life panic symptoms, with several CNS-active compounds showing consistent panicolytic effects. However, variability in CO2 administration protocols and potential habituation effects over repeated exposures have raised questions about the model's consistency, reliability, and overall translational value in early drug development.

Objectives

This study aimed to enhance the 35% CO2 challenge model by investigating the effects of single versus double vital capacity inhalations in healthy volunteers. The study also explored the potential for habituation across repeated exposures.

Methods

Twenty healthy participants aged 18-65, previously identified as CO2-sensitive, were included in a randomized, open-label, two-way crossover design. The first two visits involved randomized single and double CO2 inhalations, followed by three non-randomized double inhalations over subsequent visits. Each visit was separated by seven days to allow for recovery and ensure consistent physiological conditions. Subjective fear ratings, cardiovascular parameters, and neuroendocrine biomarkers were measured before, during, and after each challenge. Subjective fear responses were captured using the Panic Symptom List (PSL-IV), State-Trait Anxiety Inventory (STAI-Y1), and Visual Analog Scale (VAS) for Fear and Discomfort. Additionally, a continuous Visual Analog Scale (cVAS) was employed to measure real-time fear intensity throughout the challenge. Cardiovascular responses, including beat-to-beat blood pressure and heart rate, were continuously monitored. Neuroendocrine markers, such as plasma ACTH, cortisol, and prolactin, alongside salivary cortisol, cortisone, and alpha-amylase, were assessed at multiple time points pre- and post-challenge.

Results

Both single and double CO2 inhalations effectively induced panic-like responses, with double inhalations leading to more pronounced effects across key pharmacodynamic (PD) markers. Notably, double inhalations resulted in higher PSL scores, increased cVAS fear ratings, and elevated cardiovascular and neuroendocrine responses. Habituation effects were observed in approximately half of the PD markers across repeated exposures, particularly in subjective fear ratings and neuroendocrine measures, suggesting that habituation should be accounted for in crossover study designs.

Conclusion

The 35% CO2 inhalation challenge remains a valuable tool for evaluating novel anxiolytic compounds, particularly in early-phase clinical trials. However, the study highlights the need for methodological refinements to maximize the model's translational value. Standardizing administration protocols, incorporating real-time assessments, and addressing habituation effects are critical to enhancing the reliability of the CO2 challenge. By optimizing these factors, the model can better support the generation of proof-of-mechanism data, ultimately facilitating the development of effective therapies for panic and anxiety disorders.

Parameter	Panic Response: Double vs. Single CO2 Inhalation (Estimate, 95% CI, p-value)	Tolerance to Repeated Double CO2 Inhalation (Slope, p-value)
Subjective Measures		
Panic Symptoms List-IV Total Score	2.9 (0.9; 5.0), p=0.0066	-0.90, p=0.0248
VAS Discomfort (Not at all - extremely, mm)	7.7 (-0.6; 16.1), p=0.0671	-2.53, p=0.0799
VAS Fear (Not at all - extremely, mm)	6.4 (-1.5; 14.3), p=0.1100	-2.86, p=0.0300
VAS Fear Continuous Measures		
Area Under the Curve (%/s)	426 (44; 808), p=0.0296	-53.81, p=0.3036
Maximum Score (%)	8.59 (-0.06; 17.25), p=0.0516	-0.29, p=0.8534
Duration (s)	9.401 (-0.886; 19.688), p=0.0718	-3.87, p=0.0035
Physiological Measures		
AUC Diastolic Blood Pressure (mmHg*sec)	268.4 (23; 513.2), p=0.0324	-68.75, p=0.1688
AUC Heart Rate (BPM*sec)	-34.7 (-320.6; 251.1), p=0.8070	-2.95, p=0.9526
AUC Systolic Blood Pressure (mmHg*sec)	444.2 (65.9; 822.5), p=0.0226	-139.36, p=0.0813
Peak Diastolic Blood Pressure (mmHg)	7.2 (2.2; 12.2), p=0.0062	-1.10, p=0.2955
Peak Heart Rate (BPM)	-5.6 (-9.4; -1.7), p=0.0051	1.94, p=0.0284
Peak Systolic Blood Pressure (mmHg)	11.8 (3.5; 20.1), p=0.0063	-2.05, p=0.2000
Blood/Saliva Measures		
Total Plasma Cortisol (nmol/L)	55.8 (22.9; 88.8), p=0.0015	-16.85, p=0.0196
Total Plasma Prolactin (ug/L)	0.990 (0.190; 1.789), p=0.0167	-0.24, p=0.1438
Adenocorticotropic Hormone (ng/L)	2.841 (-0.214; 5.896), p=0.0673	-0.74, p=0.2294
Alpha-Amylase Concentration in Saliva (U/L)	7.5% (-4.9%; 21.5%), p=0.2401	4540.57, p=0.3301
Cortisol Concentration in Saliva (nmol/L)	20.3% (2.6%; 41.1%), p=0.0242	-0.64, p=0.0097
Cortisone Concentration in Saliva (nmol/L)	2.71 (-0.35; 5.77), p=0.0809	-1.93, p=0.0109

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Isoniazid pharmacodynamics in three wild-type zebrafish lines of the zebrafish tuberculosis disease model

Dina Berlina, Prof. Dr. Herman Spaink, Dr. Rob Van Wijk

Biography

Dina Berlina is a first-year Ph.D. student in the Translational Immuno-Pharmacology group of Dr. R.C. van Wijk at Leiden University. She graduated with a Master's degree in Biomedical Sciences from the Russian National Research Medical University in 2021. During her master's project, she gained research experience in molecular biology, focusing on microRNA research related to HPV-associated cervical cancer. From 2021 to 2023, Dina worked at a Contract Research Organization, where she conducted preclinical studies. Currently, her research focuses on the translational aspects of pharmacology, specifically how findings from zebrafish tuberculosis models can be applied to develop host-directed therapies for tuberculosis in humans. Dina is dedicated to advancing the field of translational pharmacology, with the aim of contributing to more effective treatments for tuberculosis.

Isoniazid pharmacodynamics in three wild-type zebrafish lines of the zebrafish tuberculosis disease model

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Introduction

Tuberculosis (TB) causes 1.3 million deaths annually and treatment with antibiotics takes 4-6 months. New treatment strategies and faster drug development are necessary. Recently, the zebrafish (Danio rerio) has been suggested as a model for studying anti-TB medication due to its high-throughput screening potential, while sufficiently genetic and (patho)physiological similar to humans. [1] Specifically, Mycobacterium marinum, a close relative of Mycobacterium tuberculosis, causes a TB-like granulomatous infection in zebrafish. As zebrafish have high genetic diversity which may impact pharmacology, testing drugs in multiple lines is important to ensure robust and generalizable drug efficacy results.

Objectives

Our objective is a proof-of-concept of the zebrafish as model for TB drug efficacy, by quantifying the response of 3 different wild-type zebrafish lines infected with Mycobacterium marinum to isoniazid as cornerstone antibiotic against TB. Secondary, we establish isoniazid as a reliable control drug for future anti-TB drug efficacy studies.

Methods

Three wild-type zebrafish lines, ABTL, AB, and VUmc [1] , were infected with ~200 colony forming units of fluorescently labelled M. marinum at 1 day post fertilization and changes in the bacterial burden were observed longitudinally in individual larvae using stereo fluorescent microscopy and automated image analysis.
Isoniazid treatment was initiated at 2 days post-infection (dpi) at an aqueous concentration of 75 mg/L, which corresponds to 5 times the minimum inhibitory concentration (5xMIC). Each control and treatment group contained n= 15-20 larvae. Statistical analysis of the data was performed using log-linear regression in R (v4.2.1).

Results

In the control groups of all three zebrafish lines (ABTL, AB, and VUmc), M. marinum exhibited exponential

growth, indicating uncontrolled bacterial proliferation in the absence of treatment. Exponential growth rate constant ranged from 0.35 /d for ABTL to 0.95 /d for VUmc and 1.07 /d for AB. In the isoniazid- treated groups a robust treatment effect was observed within 48h of treatment, showing isoniazid efficacy in this zebrafish TB disease model (Fig 1). In the AB and ABTL lines, isoniazid showed a similar treatment effect of 4.4-fold reduction of the growth rate, while in the VUMC line it showed a bactericidal decline from 0.95 to -0.072 /d.

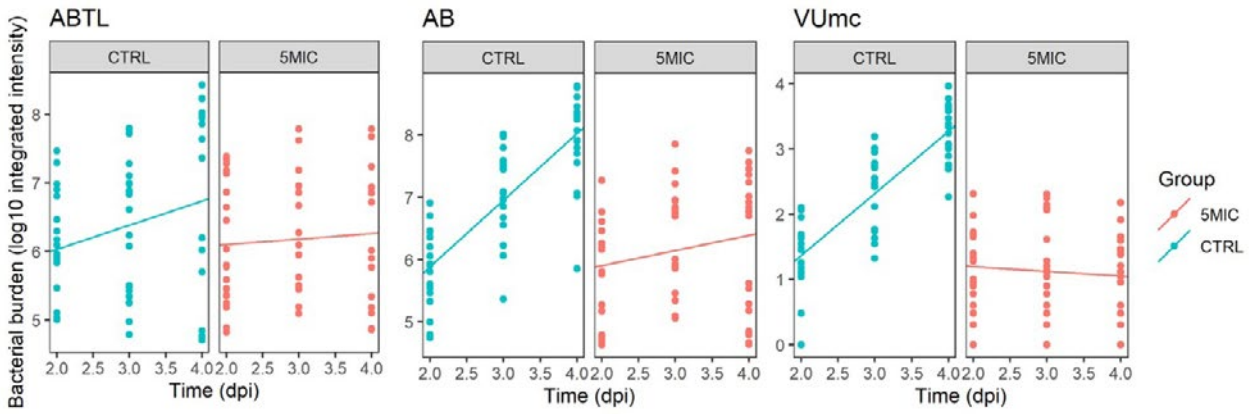

Figure 1. Bacterial burden in fluorescent pixel counts quantified by automated image analysis over time in dpi for 3 different zebrafish strains (AB, ABTL and VUmc) shown as symbols. CTRL – untreated control group, 5MIC – group treated with 5x minimum inhibitory concentration of isoniazid. Linear regression lines are shown on the graphs.

Conclusion

We show the proof-of-concept of determining drug efficacy in the zebrafish TB model through the robustness of quantifying isoniazid efficacy across three wildtype lines. The zebrafish TB model, with isoniazid as control drug, can be utilized for future drug efficacy studies.

Reference

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Predicting Early Efficacy of Host-Directed Combination Therapy for MRSA Through Mathematical Modelling of Host-Pathogen-Drug Dynamics

Msc. Bart van Lieshout, Dr. Robin van den Biggelaar, Prof. dr. Coen van Hasselt, Dr. Anno Saris, Dr. Rob van Wijk

Biography

Bart van Lieshout is a PhD student in translational pharmacometrics at the LACDR of Leiden University. With a background in biopharmaceutical sciences and having worked previously on projects on antibiotic resistance and pediatric pharmacokinetics, he is now dedicated to advancing the understanding of the optimal treatments for infectious diseases. Currently, Bart's research focuses on exploring novel host-directed therapies, which seek to boost the host's response to a pathogen rather than the pathogen itself. By combining computational models and experimental data, he aims to optimize drug development, treatment strategies, and patient outcomes.

Introduction

Pathogens that infect host cells are difficult to treat due to sub-optimal intracellular antibiotic exposures. Host-directed therapies (HDT) offer a potential strategy to treat these infections by targeting the host. Previously, the kinase inhibitor GW296115X was identified as an HDT candidate that clears intracellular methicillin-resistant Staphylococcus aureus (MRSA) (Figure 1). However, methods to evaluate the complex effect of HDT compounds are lacking, impeding their development. Mathematical models can aid in the understanding of host-pathogen-drug interactions, allow predictions, and thereby determine the potential of an HDT candidate. Nevertheless, they have not yet been developed for HDTs.

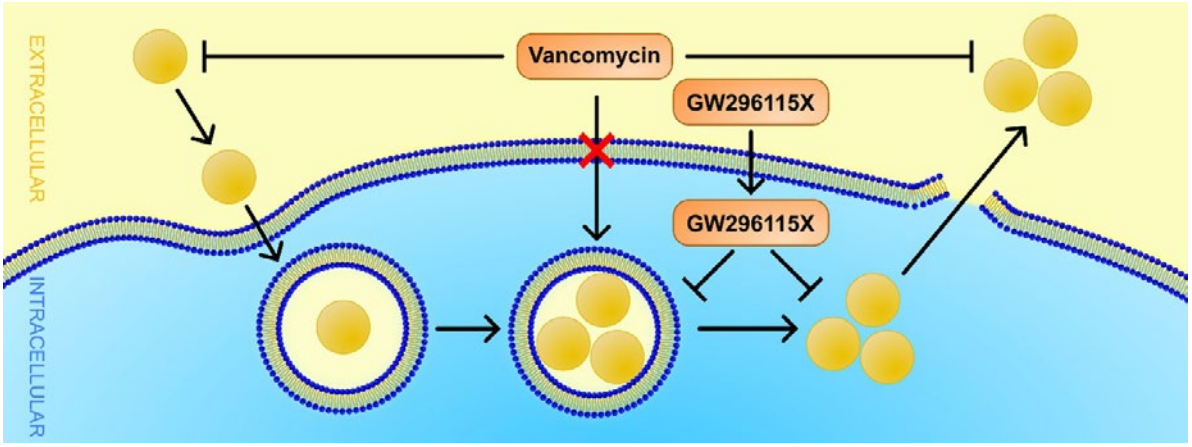

Figure 1. Overview of host-pathogen-drug dynamics.

Objectives

To establish a mathematical model that is able to quantify the host-pathogen-drug dynamics of HDT therapy, in addition to antibiotic therapy.

Methods

Existing host-pathogen models for viruses, specifically target cell-limited models [1], were used as a starting point. Variations of these models were adapted with bacterial pharmacodynamic models to encompass possible mechanisms for infection, escape from host cells, extracellular growth, and effects of compounds. Additionally, different model structures were considered to account for intracellular growth, keeping track of both the number of infected cells and the number of bacteria within.

Determining the correct type of model and evaluating its applicability was achieved with in vitro data. Data were obtained by co-culturing HeLa cells with a luminescent MRSA strain (USA300 LAC JE2) for 1 hour, followed by a 15-minute gentamicin wash, removing remaining extracellular bacteria. Subsequently, GW296115X and vancomycin were administered (both in concentrations from 0 to 10,000 nM) and the luminescence was measured continuously for 16 hours.

Results

The in vitro data showed several key dynamics of intracellular HDT processes that the model needed to capture. Firstly, intracellular growth after infection was briefly delayed and inhibited in the presence of GW296115X. Secondly, bacteria escaped from the host after 5-6 hours of intracellular growth, coinciding with the extracellular effect of vancomycin (a maximum inhibition of ~94%).

The time delay before intracellular growth was included in the model by an eclipse phase after infection (Figure 2). Intracellular growth with a distinct timepoint of escape from the host was incorporated by separating the infected cells into stages which reflected the number of intracellular bacteria. The transition between stages approximated exponential growth, with an estimated inhibitory effect of GW296115X of ~60% at 10,000 nM.

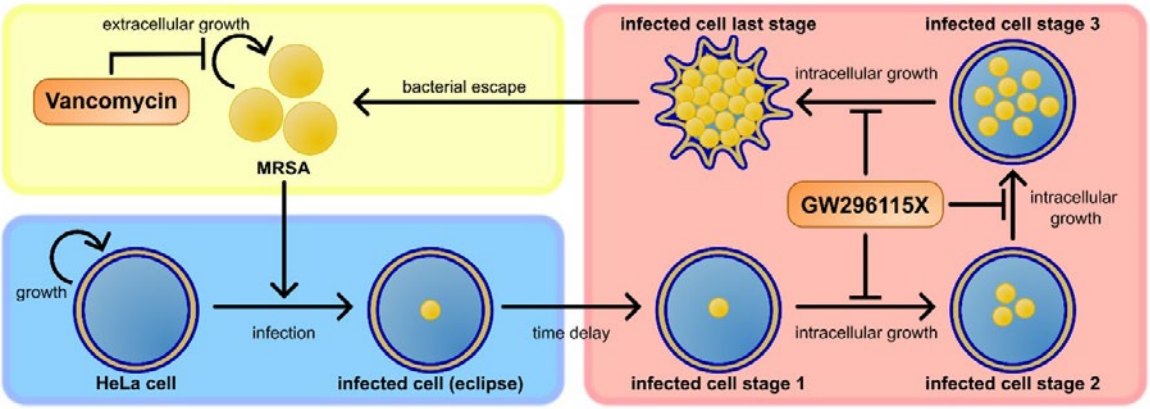

Figure 2. The general model structure of infection, extracellular growth, and intracellular growth, which in this example is separated into four stages.

Conclusions

A mathematical bacterial infection model was developed that can explain the host-pathogen dynamics of MRSA and HeLa with the capacity to incorporate and quantify the effects of both antibiotic and HDT therapy. This model has the potential to expedite the development of GW296115X treatment for MRSA. Additionally, it could serve as a framework that can be applied to other HDTs, pathogens, and hosts.

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External validation of models for assessing eligibility for referral for device-aided therapies in Parkinson's disease

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Biography

Harmen Moes (1988) earned Bachelor's and Master's degrees in Medicine and a Bachelor's degree (Honours) in Philosophy from the University of Groningen. After obtaining his medical degree in 2015, he worked on the scientific editorial board of the Dutch Journal of Medicine. In 2016, he joined the Department of Neurology at the University Medical Center Groningen. Since 2018, he has been pursuing a dual track, combining his residency in Neurology with a PhD trajectory. His research aims to develop a clinical decision rule for timely referral of patients with Parkinson's for device-aided therapy. In 2022, he completed a clinical internship at Karolinska University Hospital in Stockholm, Sweden. In 2023, he was awarded the ParkinsonNL Talent Prize for the best scientific publication. Furthermore, he pursues the training program in clinical pharmacology (NVKFB). In early 2025, he will start working as a neurologist at the Martini Hospital in Groningen.

External validation of models for assessing eligibility for referral for device-aided therapies in Parkinson's disease

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Background

The symptomatic treatment of patients with Parkinson's disease (PD) entails the administration of dopaminergic medications, with oral levodopa as the preferred initial option. As PD progresses, a narrowing therapeutic window gives rise to the emergence of dyskinesias and response fluctuations, which in turn necessitates adjustments to the oral drug regimen (e.g., increasing dosing frequency). In cases where oral dopaminergic medication cannot be further optimized, device-aided therapy (DAT) may be considered. DAT options include deep brain stimulation and continuous infusion of apomorphine (subcutaneous) or levodopa (intrajejunal). For general neurologists, timely identification of PD patients who may be eligible for DAT is important but challenging. Several screening tools have been proposed for this purpose, including the user-friendly Dutch Device-Aided Therapy Screening tool (D-DATS) (Moes et al. 2023). D-DATS is based on the presence of response fluctuations and troublesome dyskinesias, and

the levodopa equivalent daily dose. The aim of the current study was to externally validate D-DATS and to compare it with the 5-2-1 criteria.

Methods

We performed a multicenter prospective, cross-sectional diagnostic study in DAT-naive PD patients attending a routine visit in a secondary care setting in the Netherlands. Clinical characteristics of consecutive patients were collected by the attending physician. Anonymized patient vignettes were assessed by a panel of 5 movement disorder experts with expertise in DAT. The outcome (reference test) was eligibility for referral for DAT as assessed by the expert panel. The diagnostic accuracy of the D-DATS and 5-2-1 criteria was compared with the reference test.

Results

The cohort of 250 patients included 32 patients who were eligible for DAT referral (12.4%). The D-DATS had excellent discrimination in assessing eligibility for DAT referral (ROC AUC = 0.95; 95% CI: 0.91-0.98), while the 5-2-1 criteria had acceptable discrimination (ROC AUC = 0.77; 95% CI: 0.70-0.84). The diagnostic accuracy of the D-DATS (cut-off value 5.8) compared to the 5-2-1 criteria was 88% vs 97% for sensitivity, and 91% vs 57% for specificity respectively. The positive predictive value was 58% for D-DATS and 25% for the 5-2-1 criteria.

Discussion

The results of this external validation study confirm the validity of the D-DATS in identifying PD patients eligible for referral for DAT. Compared to the 5-2-1 criteria, the D-DATS appears to be slightly less sensitive but more specific, resulting in a superior positive predictive value.

Reference:

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Evaluating CXCL12 scaffolding interactions

Natalia Janowiak

Biography

I am a second-year PhD candidate in molecular pharmacology, specializing in the scaffolding interactions of the chemokine CXCL12. I hold a degree in Pharmacology from King's College London and a Master's degree in Drug Discovery and Safety with a specialization in Molecular Pharmacology from VU Amsterdam. During my studies, I conducted research at MDC in Berlin involving human-induced pluripotent stem cells. After completing my studies, I joined Martine Smit's lab at VU Amsterdam as a PhD candidate. My current research focuses on chemokine pharmacology and structure-activity relationships with chemokine receptors, particularly in the context of cancer.

Evaluating CXCL12 scaffolding interactions

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Background

CXCL12 (stromal cell-derived factor-1, SDF-1) belongs to the family of chemokines, small, secreted proteins of molecular weight ranging from 8-12 kDa. CXCL12 exerts its biological effect by interacting with two chemokine receptors, namely the CXC chemokine receptor (CXCR4) and an atypical chemokine receptor 3 (ACKR3). To achieve their specific effects, extracellular distribution of chemokines is essential and regulated by binding to glycosaminoglycans (GAGs), which are prominently expressed on cell surfaces. Chemokines play critical roles in regulating immune responses, wound healing, angiogenesis, haematopoiesis, and embryogenesis. However, they are also implicated in the development and progression of various pathological conditions, such as cancer metastasis. Although targeting chemokine receptors has been a widely explored approach in drug discovery, targeting chemokines directly has been underexplored. In this research we aim to explore the interactions of CXCL12 with known interacting molecules and to assess the therapeutic potential of CXCL12 binding ligands.

Methods

We established a framework of biochemical techniques to explore CXCL12 function. Initially, to explore the functional effects of CXCL12 binding ligands, (inhibitory) potencies on CXCL12 function were determined using Nano-Bioluminescence Energy Resonance Transfer (Nano BRET) and reporter gene assays. Moreover, we produced CXCL12 (variants) using E.coli, which are used to further characterize the CXCL12 ligands. To assess the therapeutic potential of CXCL12 ligands we are also exploring new relevant cell models. As endothelial cells are known to contain various CXCL12 binding molecules (CXCR4, ACKR3 and heparan sulfate) we are characterizing endothelial cells, derived from human-induced pluripotent stem cells, as a model system for the effects of CXCL12 binding ligands.

Results

We validated the obtained methodology using molecules that are known to bind the CXCL12 and its receptors. The methods seem to be suitable for characterizing CXCL12 ligands, however, the tested CXCL12 ligands seem to have a low inhibitory potency on CXCL12 function.

Conclusions

We established a methodological framework to characterize CXCL12 ligands. Using the new methodology, we are now exploring new CXCL12 ligands which we aim to characterize for their therapeutic potential.

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New Chemical Biology Tools for Atypical Chemokine Receptors (ACKRs)

Maurice Buzink

Biography

Maurice Buzink has completed his MD in Drug discovery at the Vrije Universiteit Amsterdam. He is currently a researcher in Prof. Leurs' lab, where he develops assays to study GPCR modulation. His research focuses on collaborative drug discovery and repurposing efforts, particularly targeting the atypical chemokine receptors.

New Chemical Biology Tools for Atypical Chemokine Receptors (ACKRs)

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Atypical chemokine receptors (ACKRs) play a crucial role in modulating the activity of both chemokine and non-chemokine receptors, positioning them as intriguing therapeutic targets1. Nevertheless, investigating their pharmacology presents significant challenges due to the intricate chemokine-receptor interactions, the lack of conventional G protein signalling, the absence of arrestin-mediated pathways in some cases, as well as their constitutive receptor internalization and chemokine ligand scavenging. In this study, we employed a comprehensive array of bioluminescence- and imaging-based methodologies to precisely monitor the interactions between ACKR1-5 and their cognate chemokine ligands in real time. Additionally, we assessed the recruitment of effector proteins by ACKRs and the receptor-mediated ligand scavenging process. Utilising these advanced tools, we conducted a high-throughput screening of over 2,000 FDA-approved compounds, successfully identifying novel non-chemokine ligands that provide valuable insights and tools for advancing research on atypical chemokine receptors.

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Factor XII contact activation can be prevented by targeting 2 unique patches in its EGF-1 domain with a nanobody

Rowan Frunt

Biography

Rowan Frunt is a fourt-year PhD candidate in the field of thrombosis and haemostasis at UMC Utrecht. In his research he focusses on surface induced blood coagulation in the context of medical device-related thrombosis. Studying the biochemical properties of Factor XII, he tries to understand the FXII surface interaction in order to prevent this specifically.

Factor XII contact activation can be prevented by targeting 2 unique patches in its epidermal growth factor-like 1 domain with a nanobody

Rowan Frunt, Hinde El Otmani, Simone Smits, Chantal C. Clark and Coen Maas

Background

Medical devices are essential to patient care, but their negatively-charged, blood-contacting surfaces induce medical device-induced thrombosis. The inherent use of anticoagulants also increases bleeding risks in patients. Factor XII (FXII) initiates contact activation upon binding to foreign surfaces. Its EGF-1 domain was identified as the main surface-binding domain. Therapeutically targeting this domain might prevent undesired FXII contact activation.

Objectives

To unravel and prevent EGF-1–mediated FXII surface-binding with a variable domain of heavy chain–only antibody (V^HH).

Methods

The by AlphaFold predicted tertiary structure of FXII was used to identify outward-oriented positively-charged amino acids in its EGF-1 domain. Glutamine substitutions of two unique, positively-charged patches in the FXII EGF-1 domain were generated. The role of these patches in FXII contact activation and FXII zymogen quiescence were studied in pull-down, chromogenic substrate, and clotting assays. FXII EGF-1 domain–specific VHHs were raised to inhibit EGF-1–mediated FXII contact activation. Their affinity to the two unique, positively-charged patches was examined using surface plasmon resonance and their functional effect on FXII contact activation was examined in the abovementioned assays.

Results

Two unique, positively-charged patches were identified in FXII EGF-1 (upstream: 73K74K76K78H81K82H; downstream: 87K113K). Neutralizing both patches cooperatively decreased kaolin binding with 99% and subsequent FXII activity with 94%. Consequently, clot formation in activated partial thromboplastin time assays was prolonged from 36 (±2) to 223 (±13) seconds. Nonetheless, its activation by plasma kallikrein in solution was unaffected, indicating these patches do not play a role in preserving zymogen conformation and are thus suitable as a druggable target. Three FXII EGF-1 specific VHHs were raised that can inhibit FXII-kaolin binding and subsequent FXII contact activation in plasma. The most effective VHH "F2" binds the positively-charged patches and thereby dose-dependently extends activated partial thromboplastin time clotting times from 29 (±2) to 43 (±3) seconds without disrupting FXII quiescence.

Conclusion

Two unique, positively-charged patches in FXII EGF-1 were identified that cooperatively mediate FXII surface-binding making both patches crucial for contact activation. Targeting FXII EGF-1 with specific VHHs can exclusively decrease FXII surface-binding and subsequent contact activation, while preserving zymogen quiescence. These patches thus have potential as druggable target in preventing medical device-induced thrombosis, without increasing the risk of bleeding.

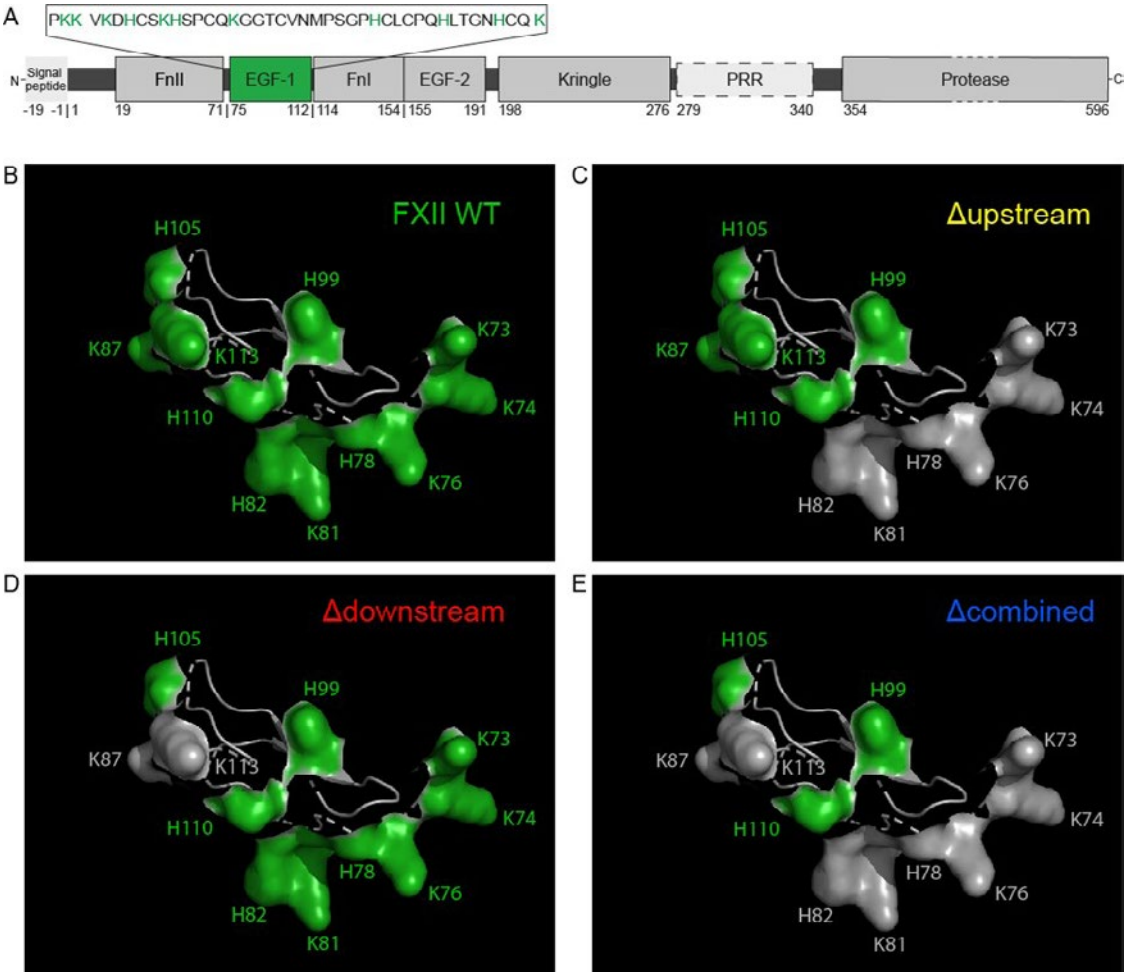

Figure 1 Comparison of the hepatocyte growth factor activator (HGFA) epidermal growth factor-like 1 (EGF-1) and factor (F)XII EGF-1 domains reveals 2 positively charged amino acid patches unique to the FXII EGF-1 domain.

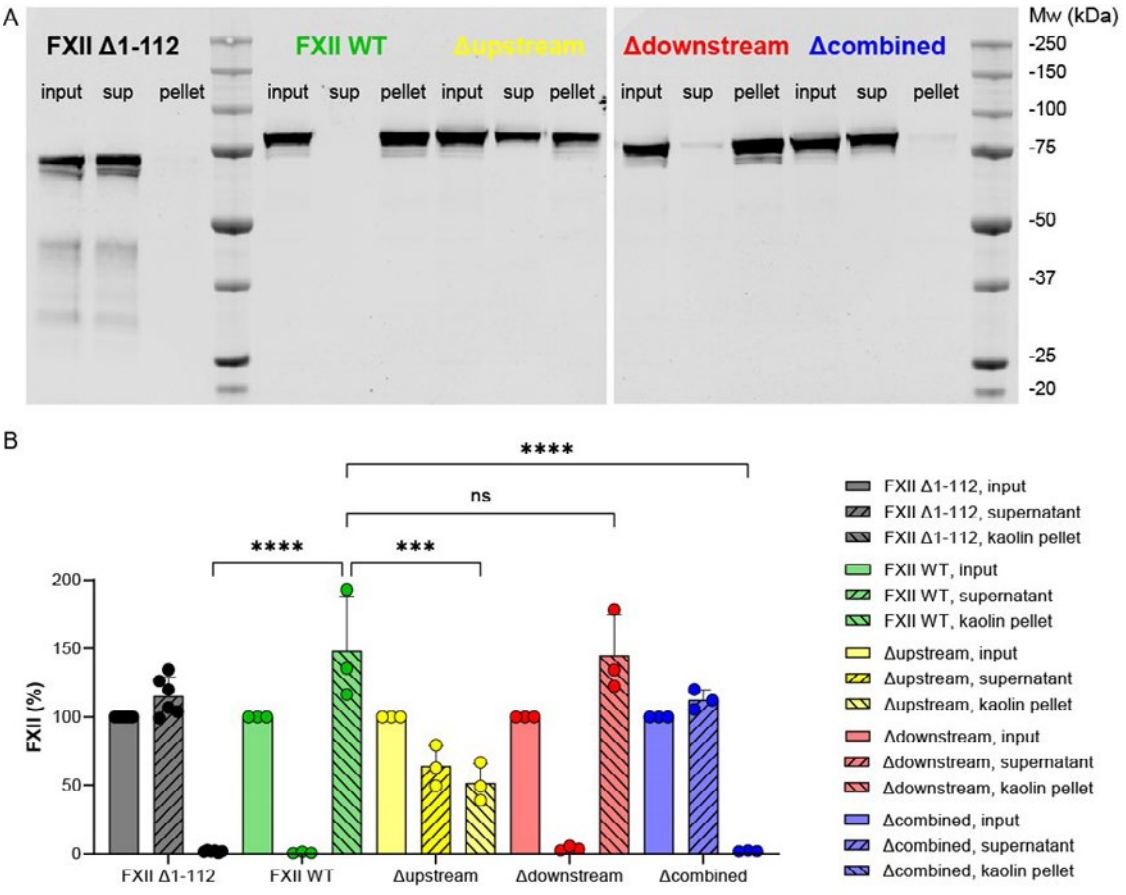

Figure 2 Factor XII surface-binding is cooperatively affected by neutralization of the upstream patch in combination with the downstream patch.

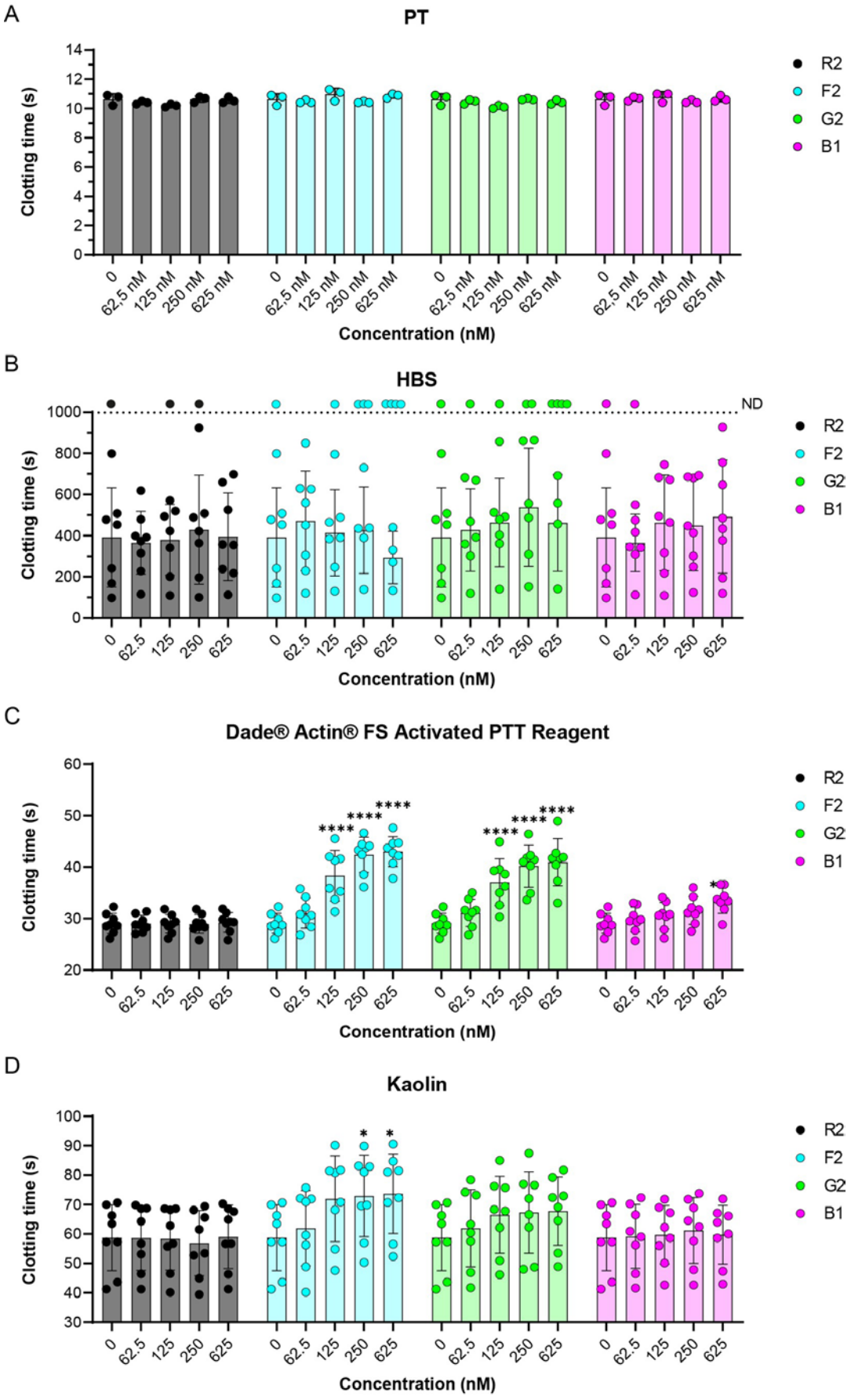

Figure 6 Factor XII EGF-1–specific VHHs prolong the activated partial thromboplastin time clotting times of healthy donor plasmas.

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Drug pricing models, no 'one-size-fits-all' approach: A systematic review and critical evaluation of pricing models in an evolving pharmaceutical landscape

Evert Manders

Biography

PhD student at 'Medicine for Society', a platform dedicated to the sustainable and affordable availability of medications for rare diseases, at the Amsterdam Medical Centre. My research focuses on innovative pricing methods for new drugs and new approaches that balance sustainable pharmaceutical innovation with affordability and accessibility for patients.

Abstract

Access to new medicines is crucial for patients but increasingly sparks discussion due to high prices. Simultaneously, a growing emphasis on specialized products and uncertainty surrounding the long-term effectiveness of new drug classes brought to the market underscore the need for innovative pricing approaches. A systematic literature review of pharmaceutical pricing models was conducted, accompanied by a critical appraisal, aiming to offer insights contributing to novel approaches that balance sustainable pharmaceutical innovation with affordability and accessibility for patients.

Six different pricing models, each with unique assumptions and areas of application, ranging from generic medicines to highly innovative drugs such as precision medicines, were identified: value-based pricing, basic cost-based pricing, and four more comprehensive pricing models incorporating numerous elements: the cancer-drug-pricing model, AIM model, Nuijtens discounted cash flow, and the real-option rate of return method. Although there are many similarities among the models, each has unique assumptions for implementation. For instance, all models except for the standard incremental cost-effectiveness ratio and basic cost-based pricing consider the number of eligible patients and incorporate production costs. However, only the AIM model and Nuijtens discounted cash flow use lump sums. The latter and the real-option rate of return method also explicitly include the cost of capital as a major cost-based component.

Recognizing the diverse applications of each model emphasizes the need for a more differentiated and dynamic pricing framework tailored to the characteristics and therapeutic area of each drug. Consequently, the findings of this study can aid stakeholders in paving the way for sustainable, affordable drug pricing mechanisms that accommodate the complexities of the ever-changing pharmaceutical landscape.

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Enhancing Sustainable Drug Use: Overcoming Barriers to Effective SSRI Tapering Practices

Chaimae Mahtour, MSc Lisa Heltzel, PhD Anne Loeber, PhD Anita Volkers

Biography

Chaimae Mahtour is a dedicated VU student currently in the graduation phase of her Master's program in Management, Policy Analysis, and Entrepreneurship in the Health & Life Sciences. She has completed a research internship at the Medicine Evaluation Board and has previous experience as a research intern at both the RIVM and Lygature. Chaimae has a passion for conducting research and enjoys exploring health-related topics, continually seeking to expand her knowledge and contribute to the field.

Abstract

The frequent and continuous use of SSRIs increases the need for sustainable drug use and has brought attention to the long-term risks and cost-effectiveness of these treatments. The Dutch Summaries of Product Characteristics (SmPC), the official product information for medicines managed by the Dutch Medicines Evaluation Board (MEB), serves as key reference for Health Care Professionals (HCPs) and provide warnings about abrupt discontinuation and recommend gradual tapering, based on clinical evidence. Additionally, there is the multidisciplinary document for tapering SSRIs that provides more detailed guidance compared to the SmPC. However, adherence to these documents vary among HCPs. This variation necessitates an investigation into the reasons behind non-adherence to optimize patient care and promote sustainable drug use.

This study, conducted by the Dutch MEB in collaboration with the Vrije Universiteit (VU), aims to identify barriers faced by HCPs in adhering to guidance and to enhance the support provided for safe and effective tapering practices. Given the limited awareness of the SmPC, the study's scope was expanded to encompass adherence to clinical guidance documents more broadly. A qualitative research design was employed to explore barriers for adherence. Fourteen stakeholders, including general practitioners (GPs), pharmacists, psychiatrists, and MEB board members, were interviewed using purposive and snowball sampling strategies. Data collection involved semi-structured interviews, guided by the socio-behavioral Cabana framework. Ethical considerations were strictly followed.

The study uncovered several barriers to successful SSRI tapering, highlighting the need for enhanced collaboration among GPs, psychiatrists, and pharmacists. Key obstacles identified were: time constraints for GPs, inadequate communication among HCPs, and a lack of awareness regarding tapering methods and available services. The motivation and understanding of the tapering process among patients also significantly influence outcomes, with expectation management often lacking from initial prescribers and GPs who take over patient care. According to HCPs, successful tapering also depends on the individual patient (severity, of disease, pregnancy, and duration of SSRI use).

Improving HCPs' knowledge may ensure the success of the tapering process and better support patients. There is a critical overarching need for clear, concrete, and consistent recommendations on how and when to taper SSRIs, which are currently insufficiently provided in the SmPC and clinical guidelines. To optimize sustainable drug use in the Netherlands, this study recommends improving consistency of guidance, enhancing interprofessional communication, and providing detailed, user-friendly tapering schedules, based on scientific evidence. Information that is easily and digitally accessible during patient consultations is also preferred, this could be provided through an electronic product information. Future research should confirm above mentioned findings from the patient perspective. Despite the identified barriers, numerous ongoing initiatives favor tapering and align with the improvements this research aims to bring about. By addressing these challenges and implementing these recommendations, we can improve the tapering process for SSRI users, ensuring safer, more effective, and sustainable drug use practices.

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