Translating preclinical insights into early psychopharmacology trials: application of the IB-Derisk analyser tool

Dijkstra, F.M.

Translating preclinical insights into early psycho-pharmacology trials: application of the IB-Derisk analyser tool

FRANCIS DIJKSTRA

TRANSLATING PRECLINICAL INSIGHTS INTO EARLY PSYCHOPHARMACOLOGY TRIALS: APPLICATION OF THE IB-DERISK ANALYSER TOOL

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof. dr. ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op donderdag 4 december 2025
klokke 13:00 uur

door
Francis Marijke Dijkstra

DESIGN
Caroline de Lint, Den Haag (caro@delint.nl)

The publication of this thesis was financially supported by the foundation Centre for Human Drug Research (CHDR), Leiden, the Netherlands

PROMOTOR
Prof. dr. J.M.A. van Gerven

CO-PROMOTORES
Dr. G.E. Jacobs
Dr. R.G.J.A. Zuiker

LEESCOMMISSIE
Prof. dr. J. Burggraaf, *Leiden University*
Prof. dr. M.J.H. Kas, *University of Groningen*
Prof. dr. A. Blokland, *Maastricht University*
Dr. M. Niesters, *Leiden University*

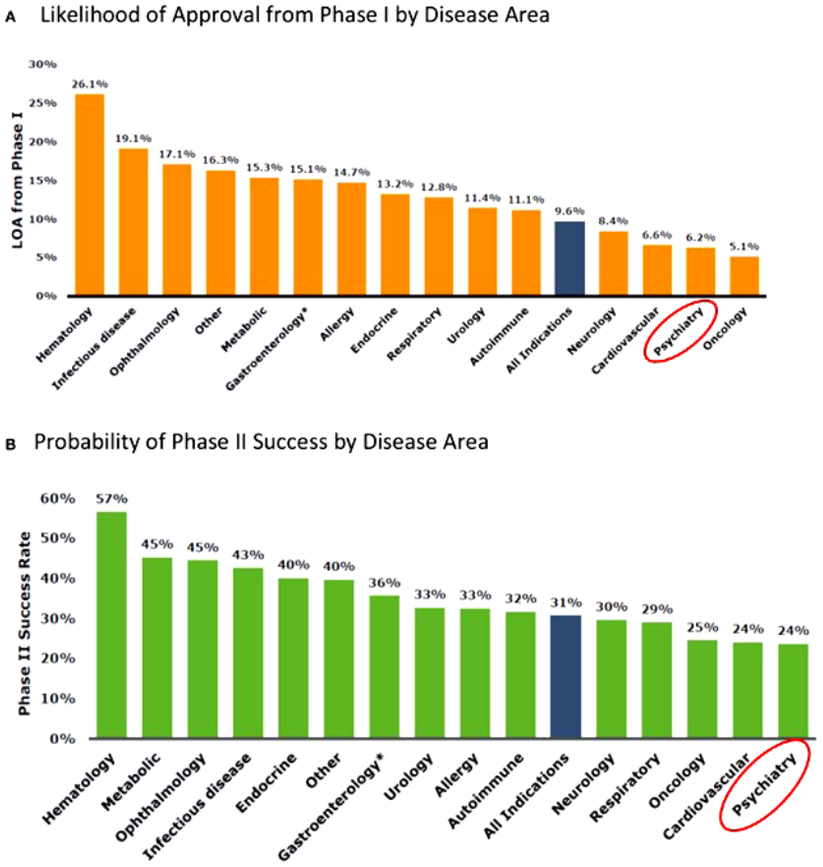
CHAPTER I

INTRODUCTION

1. CURRENT STATUS OF PSYCHIATRIC DRUG DEVELOPMENT

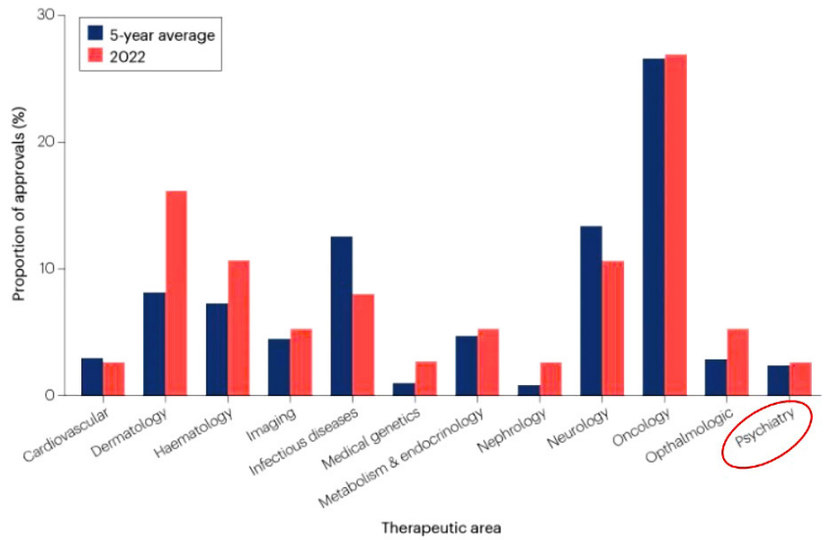
Central nervous system (CNS) drug development in general and psychiatric drug development in particular have suffered a number of important setbacks over the past two decades.[1-4] Despite heightened expectations for innovative pharmacological treatments driven by advancements in neuroscience, which have deepened our understanding of the functional processes behind CNS disruptions in psychiatric illnesses, drug development has largely failed to convert new insights into approved novel treatments in psychiatry.[5] Psychiatric drug development is characterised by higher failure rates in late stage drug development due to lack of safety and efficacy compared to other fields of drug development (Figure 1).[6-9] To illustrate, between 2011 and 2021, 12 new drugs in psychiatry were approved by the US Food and Drug Administration (FDA), while 50 new drugs in neurology and 135 new drugs in oncology were approved over the same period, respectively (Figure 2).[5,10] Moreover, of the novel pharmacological treatments for psychiatric disorders that have reached the market, only a handful include compounds featuring truly novel mechanisms of action.[11,12] As a consequence, several major pharmaceutical companies announced either reduction or discontinuation of their CNS research and development programs over the past two decades.[1-4,6,13-15] Although there have been recent successes in psychiatric drug development, such as the FDA approval of esketamine and brexanolone – two rapid-acting antidepressants with novel mechanisms of action – and while initial results from the renewed interest in psychedelic-assisted psychotherapy appear promising, further advancements are necessary to reduce the relatively high failure rates in late-stage drug development in this field.[16,17]

Figure 1 High rate of late-stage failure in psychiatry



(Reproduced with permission from 'Challenges of Psychiatry Drug Development and the Role of Human Pharmacology Models in Early Development-A Drug Developer's Perspective.' By T. Zhu. Published in *Front psychiatry*. 2020;11:562660. doi:10.3389/fpsyt.2020.562660)

Figure 2 Approval rate per drug development field



(Reproduced with permission from Mullard A. 2021 FDA approvals. *Nat Rev Drug Discov*. 2022;21 (February 2022):83-88. doi: 10.1038/d41573-023-00001-3)

2. LACK OF PRECLINICAL MODELS THAT RELIABLY TRANSLATE TO PSYCHIATRIC DISORDERS IN HUMANS

The lack of predictive preclinical models to test potential novel therapeutic compounds is often cited as a major cause for the high failure rates in the field of psychiatric drug development.[1,3,18-20] To illustrate, the forced swim test (FST) and tail suspension test (TST) continue to be widely used for nonclinical efficacy testing in drug development for Major Depressive Disorder (MDD).[18,21,22] Both tests were developed many decades ago, when antidepressants exclusively targeted monoaminergic activity, and both seem to reflect acute stress-related behaviour, rather than chronic mood disturbance.[18] In the FST, developed in 1977, a rodent (usually a mouse or rat) is placed in a container filled with water from which it cannot escape.[23] The test measures the animal's behaviour in response to the stressful situation, typically over a short period (e.g., 6 minutes).[18] A shorter duration of immobility and/or a longer latency to become immobile, compared to control groups, is interpreted as an indicator of potential antidepressant efficacy in humans.[18,23] The TST, developed in 1985, is based on the same assumption as the TST: substances with antidepressant properties will cause animals to struggle longer in physically stressful situations.[24] The TST involves administering a compound and then attaching a mouse by its tail to a suspension bar or shelf ledge using tape.[24] The experimenter records the time the mouse spends making escape-oriented movements, such as trying to reach the surrounding walls.[24] Some antidepressants have been found to increase these escape-oriented behaviours in certain strains of mice.[25] The interpretation of these tests by regarding reduced immobility, increased latency to immobility, and increased escape-related behaviours as indicators of antidepressant activity has faced criticism.[18,21,22] It is argued that this concept lacks construct, face and predictive validity.[18,22]

Construct validity refers to the extent to which the model accurately represents pathophysiological mechanisms and theoretical constructs of the human disease it aims to simulate.[22] However, especially in the field of psychiatric drug development this is challenging given the poorly understood pathophysiology of psychiatric disorders, as these result from a complex interplay of biological, psychological and social factors that are still being unravelled.[3,4,22,26,27] The construct validity of the FST and TST as a model of MDD can be assessed as poor, amongst others because depressive disorders in humans do not develop within the course of 15 minutes or even a few days.[18] Face validity implies that a model replicates essential anatomical, biochemical, neuropathological, or behavioural features of a human disease.[22] However, there are limited, if any, neurobiological abnormalities conclusively recognised as hallmarks or biomarkers for common mental illnesses.[22] Additionally, certain mainly internal and emotional dysregulations, leading to psychiatric symptoms such as hallucinations, delusions, sadness and guilt, are arguably unique to humans and cannot be definitively modelled or objectified in animals.[22] Even when there are apparent behavioural readouts in animals, like abnormal social behaviour, motivation, working memory, emotion, and executive function, the correspondence may only be approximate, and if there are plausible anthropomorphic interpretations, these have rarely been convincingly linked to pathophysiology.[21,22] Face validity of the FST and TST as models of MDD can also be assessed as poor because 'escape behaviour' is not a diagnostic feature of MDD in humans.[18] Predictive validity indicates that a model responds to treatments in a way that predicts the effects of those treatments in humans.[22] However, most models in neuropsychiatry, such as the FST and TST, developed to measure the effect of novel compounds are not mechanistic models of therapeutic activity.[18,22] Generally, these models have not been demonstrated to reflect either the pathophysiological processes of human disease, nor the therapeutic mechanism of action of existing compounds and for most new targets.[18,22] Consequently, it remains uncertain whether these models will be sensitive to the pharmacological effects of novel investigational compounds with new mechanisms of action.[18,22] Taken together, modelling human neuropsychiatric disorders in animals is highly challenging due to the

complexity and limited understanding of the pathophysiology, the heterogeneity and subjective nature of many symptoms, and the absence of biomarkers and objective diagnostic tests of psychiatric disorders.[22]

3. *How to improve translational value of psychiatric research*

Many scientists argue that to improve the translatability of preclinical experiments to therapeutic efficacy in humans, the quality of these experiments must be improved by addressing species differences, experimental environment, complications from genetically altered animals, methodology, and different forms of bias.[21] While these improvements are important, efforts to enhance translatability will remain superficial unless the scientific rationale behind the experiments is solid.[21] It is therefore argued that it would be more effective to develop preclinical models that simulate a single measurable pathological aspect of the disorder instead of attempting to model the entire disease.[21,26,28] It is recommended that preclinical models are developed to explore aspects of the disorder, in such a way that it helps to identify human-relevant biomarkers for novel compounds targeting the dysfunction of the modelled circuitry.[3,26] For example, imaging and electrophysiology biomarkers that capture neurocircuitry modulation relevant to specific disease domains in humans are being identified.[3] These biomarkers can be used in both preclinical models and clinical studies to measure target modulation by the investigational compound.[3] Furthermore, clinical research should carefully consider the results from preclinical studies to ensure that novel compounds are tested at appropriate doses and account for patient heterogeneity in psychiatric disorders by focusing on relevant subpopulations, characterised by specific disease characteristics and biomarkers that match the underlying pathophysiological and pharmacological mechanisms.[21,26,28]

While psychiatric drug development will likely benefit from advancements in preclinical research, the existing preclinical animal models for psychiatric disorders continue to play a valuable role in the drug development process. First, data from these studies can be used to assess the 'translatability' of a novel compound.[29] Within this context, translatability is defined as the property of a compound to elicit similar responses across preclinical species at equivalent ranges of exposure.[29] If a compound exhibits desired pharmacological effects at low concentrations in animal models, with undesirable effects consistently manifesting at higher levels in the same or even in other species, it raises the likelihood that this dose-responsiveness reflects a pharmacologically active range that will also be translated to humans.[29] On the contrary, the presence of significant interspecies differences in preclinical observations raises uncertainty concerning translatability to humans.[29] This can warrant further research to understand the difference between animal models, before proceeding to a human study; or influence the design of the clinical study, such as by including additional pharmacological biomarkers or safety measures.[29] Second, preclinical efficacy experiments provide insight into the dose range and/or exposure value at which the investigational compound elicits its effects or is pharmacologically active.[29] From this perspective, preclinical models should thus not be viewed as tools that directly predict therapeutic efficacy in humans but rather as resources that offer insights into the translatability of a novel compound and the dose range at which pharmacological effects can be anticipated.

4. *How to improve the design of early phase clinical drug development studies in psychopharmacology*

In order to reduce the relatively high attrition rates in CNS drug development, early-phase clinical study designs must be improved, alongside optimising the methodology and interpretation of preclinical models.[3,28,30,31] Analyses by AstraZeneca and Pfizer into their failed small-molecule drug projects categorised as efficacy failures in phase II demonstrated that in respectively 21% and 43% of studies, fundamental characteristics such as compound exposure at the site of action and/or confirmation of modulation of the pharmacological target, were not properly investigated. Consequently, it remained uncertain whether a compound had validated the mechanistic hypothesis.[30,32] For the drugs that could be advanced into phase III trials, action site penetration and target engagement had been demonstrated significantly more often than for compounds that failed earlier.[30] To improve attrition rates, it is therefore recommended that fundamental PK and pharmacodynamic (PD) properties, consisting of exposure at site of action and target modulation, must be investigated during early phases of clinical drug development.[30,32-35] This approach ensures that, in the event of a negative trial, researchers can differentiate whether this was due to inadequate drug exposure at the site of action or inadequate target modulation; or because the targeted mechanism turned out not to be relevant for the disease.[30,36] This means that, even in the case of a negative trial, there is still an advancement in knowledge about the pharmacological mechanism of the novel compound or understanding of the disease.[30,36]

According to this experience, early phase clinical drug development studies must be designed in such a manner that the essential characteristics of novel compounds, including their ability to reach the site of action and perform target modulation are thoroughly investigated.[35] This entails that biomarkers measuring pharmacological effects of investigational compounds, should be included in First-in-Human (FIH) studies.[30,33-35,37] The NeuroCart, which consists of a battery of drug-sensitive CNS tests, measuring effects on different CNS domains, such as neurophysiologic functioning, visuomotor coordination, balance and subjective feelings, provides a set of biomarkers that can easily be applied in FIH studies.[38] In recent years, the NeuroCart has

been employed to study numerous compounds with a wide variance of action mechanisms.[38] Consequently, there is a comprehensive understanding of how the NeuroCart tests are influenced by different compounds.[38] These established 'NeuroCart profiles' enable comparisons of results from investigational compounds, aiding in the assessment of whether observed effects align with the expected mechanism of action for these compounds.[38] Additionally, recent advancements in the field of neuroscience are of considerable benefit to measure fundamental pharmacological characteristics of investigational compounds.[3] Neuro-imaging techniques, such as PET-imaging with CNS-penetrating radioligands or functional Magnetic Resonance Imaging (fMRI) have enormously improved over the past years, and can also be employed to furnish evidence of a drug effectively crossing the blood brain barrier (BBB).[39] Next to that, advancements in electrophysiological techniques, such as Polysomnography (PSG) and Electroencephalography (EEG), whether used independently or in conjunction with Transcranial Magnetic Stimulation (TMS), enable non-invasive exploration of neuronal network activity in humans.[37] This facilitates the evaluation of various cortical properties, including excitability and connectivity, as well as the impact of novel drugs on these properties.[37] Taken together, an increasing variety of neurofunctional biomarkers has been shown to be able to demonstrate pharmacological effects in early-phase clinical trials in psychopharmacology, which can demonstrate target modulation either indirectly through for example NeuroCart or electrophysiological techniques, or directly through for example imaging techniques.

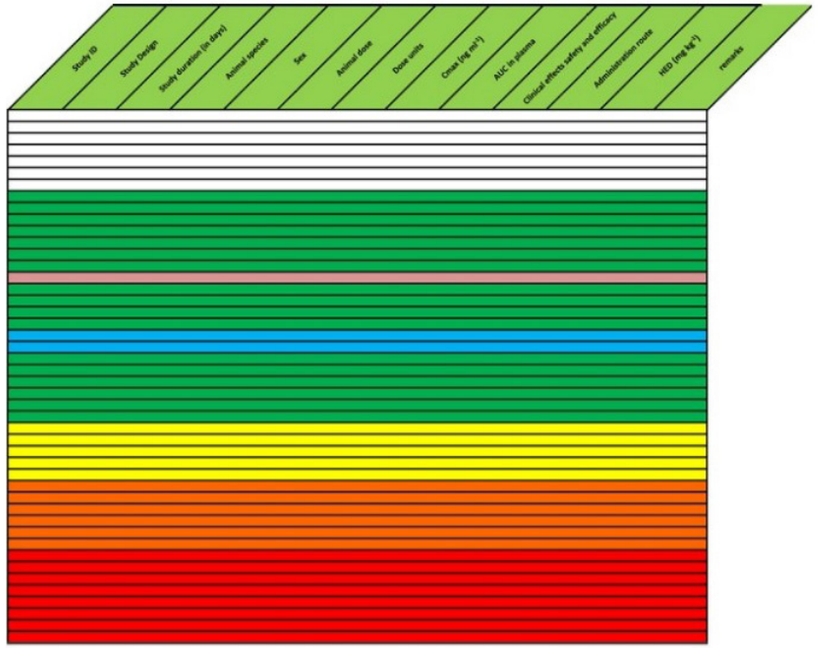
5. *THE IB-DERISK ANALYSER*

Overall, it can be stated that the relatively high failure rates in late stages of CNS drug development could be mitigated by properly interpreting preclinical data and investigating the fundamental PK and PD characteristics of novel compounds during early clinical phases.[3,30-32,35] In practice, however, predictions of clinically active dosages can be highly challenging, because of the diversity and the lack of standardisation within the preclinical development program.[29] This includes not only the absence of validated disease models discussed earlier, but also the vast amount of available preclinical data, varying reporting styles across different preclinical experiments, and incomplete data reporting of preclinical experiments, such as missing PK information from preclinical efficacy studies.[29] Furthermore, there is a communication gap between preclinical and clinical researchers.[20,28] To provide an integrated assessment of the varied and incomplete preclinical data, the IB-Derisk analyser tool was developed.[29] The IB-Derisk analyser tool can be used to summarise the often lengthy and complex preclinical data as described in the Investigator's Brochure (IB) in an organised one-page overview.[29] Additionally, the overview can be updated with emerging findings from ongoing early-phase clinical studies to contextualise and verify whether the actual outcomes align with predictions.[29] By inputting the results from all performed nonclinical and clinical experiments into the overview, a comprehensive representation of the investigated compound is generated.[29] This aids in identifying missing critical data, detecting safety issues, contextualising findings, and promoting communication among researchers.[29]

For in-depth details on the IB-Derisk analyser tool, the original publication can be consulted.[29] Essentially, the IB-Derisk analyser overview is obtained in four steps of data entry and manipulation.[29] The first step consists of selecting all single-dose PK studies and enter for all of these studies per species and per administration route, the maximum concentration after administration (C_{max}) and total exposure (area under the curve (AUC)) in the tool.[29] If different doses are given, each dose is considered a separate experiment that is individually entered in the tool.[29] In the second step single dose non-PK studies, for example disease model or safety pharmacology studies, in the same species for which separate PK experiments were entered in the first step, are entered.[29] For these kind of studies, PK values are often not reported, but the missing C_{max} and AUC values can be reasonably derived from the PK-studies in the same species.[29] The third step involves entering the multiple dose experiments, some of the acute toxicology studies, and sometimes models of special interest in a specific laboratory animal.[29] For these experiments missing PK data can often be estimated based on extrapolation of the PK studies.[29] The final step consists of colour coding the different studies based on the observed effects.[29] When the colour coding is added, the experiments can be arranged on C_{max} or another pharmacokinetic parameter, to obtain a visual impression of the dose-response curves (Figure 3).[29]

The obtained overview must then be correctly 'read'. To evaluate the translatability of a compound, it is necessary to examine the IB-Derisk overview on whether comparable effects occur at similar exposures in different species. A homogeneous distribution over different species increases the chance that humans will also fit into this pattern.[29] This is readily identifiable through the colour-coded system (Figure 3).[29] Furthermore, when selecting a starting dose and make decisions on dose escalating steps for a clinical study, the overview can be used to make an estimation of the pharmacologically active dose range.[29] When observations of pharmacological activity in early phase clinical studies deviate significantly from predictions based on preclinical data, it suggests a limited understanding of the pharmacology of the novel compound or the pathophysiology of the targeted disease.[29] Investigating this discrepancy can deliver important information on the drug target or on the pathophysiology.[29] On the other hand, when predictions of pharmacological activity based on preclinical data are met in FIH studies, our understanding of a compound and pathophysiology of a disease are strengthened.[29] Integration of all this information then provides a solid base for decisions on further development of an investigational compound.[32]

Figure 3 Schematic example of an IB-Derisk overview



(Reproduced with permission from: Gerven van, J.M.A., Cohen A.F. Integrating data from the Investigational Medicinal Product Dossier/investigator's brochure. A new tool for translational integration of preclinical effects. *Br J Clin Pharmacol.* 0(0). doi:10.1111/bcp.13529)

6. OUTLINE OF THIS THESIS

In short, over the past two decades the numbers of drug development for CNS disorders in general and psychiatric disorders in particular have been disappointing.[1-4] It is being argued that this is caused by a lack of preclinical models predicting therapeutic efficacy.[1,3,18-20] Improving this deficiency would require a thorough reconsideration of psychopathological cascades and psychiatric disease constructs, which considering the complexities is not easily accomplished.[20] However, no direct CNS-active compound can be expected to be therapeutic, if it does not exerts its intended pharmacological activity within the brain.[38] This is illustrated by empirical analyses of drug development programs, which have demonstrated that in up to almost half of studies failing due to a lack of efficacy in phase II, fundamental PK and PD characteristics, such as exposure at the site of action and target modulation, were not investigated or demonstrated in the early phases of clinical development.[30,32] It is therefore suggested that late-stage failures in CNS drug development can be reduced by designing preclinical and clinical studies and integrating their results in a manner that provides a thorough understanding of a compound's pharmacological profile during the early phases of clinical development.[30-32,35] To facilitate this the IB-Derisk analyser tool was developed.[29]

This thesis serves as an investigation of how the IB-Derisk analyser tool can be applied in early phase clinical development of neuropsychiatric drugs. First, the results of a semi-quantitative analysis are described, evaluating how accurately preclinical data, as summarised using the IB-Derisk analyser tool, can predict safe and pharmacologically active dose ranges in humans for CNS-active compounds. In the subsequent chapters, the individual results of three early phase clinical drug development studies in healthy volunteers are described. These studies involve investigational compounds with highly innovative mechanisms of action, in development for the potential treatment of psychiatric disorders. For each novel compound an IB-Derisk analyser overview was generated prior to study start and supplemented with emerging data from the clinical studies while these were ongoing. In all studies included in this thesis, the NeuroCart was performed to investigate the pharmacological characteristics of the novel compound. Each chapter contains an appendix evaluating the IB-Derisk analyser overview concerning the investigational compound. The appendices present the results obtained with the IB-Derisk analyser prior to initiation of the clinical studies, supplemented with the actual outcomes of the clinical studies. Lastly, in the final chapter the added value of using the IB-Derisk analyser tool in early phase drug development studies is discussed. This chapter includes recommendations for the future of drug development in psychiatry.

REFERENCES

1 Gribkoff VK, Kaczmarek LK. The need for new approaches in CNS drug discovery: Why drugs have failed, and what can be done to improve outcomes. *Neuropharmacology*. 2017;120:11-19. doi:10.1016/j.neuropharm.2016.03.021
2 Kesselheim AS, Hwang TJ, Franklin JM. Two decades of new drug development for central nervous system disorders. *Nat Rev Drug Discov*. 2015;14(12):815-816. doi:10.1038/nrd4793
3 O'Donnell P, Rosen L, Alexander R, Murthy V, Davies CH, Ratti E. Strategies to Address Challenges in Neuroscience Drug Discovery and Development. *Int J Neuropsychopharmacol*. 2019;22(7):445-448. doi:10.1093/ijnp/pyz027
4 Zhu T. Challenges of Psychiatry Drug Development and the Role of Human Pharmacology Models in Early Development-A Drug Developer's Perspective. *Front psychiatry*. 2020;11:562660. doi:10.3389/fpsyt.2020.562660
5 Mullard A. 2022 FDA approvals. *Nat Rev Drug Discov*. 2023;22(2):83-88. doi:10.1038/d41573-023-00001-3
6 Arrowsmith J. Phase II failures: 2008–2010. *Nat Rev Drug Discov*. 2011;10(5):328-329. doi:10.1038/nrd3439
7 Arrowsmith J. Trial watch: phase III and submission failures: 2007-2010. *Nat Rev Drug Discov*. 2011;10(2):87. doi:10.1038/nrd3375
8 Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. *Nat Biotechnol*. 2014;32(1):40-51. doi:10.1038/nbt.2786
9 Hwang TJ, Carpenter D, Lauffenburger JC, Wang B, Franklin JM, Kesselheim AS. Failure of Investigational Drugs in Late-Stage Clinical Development and Publication of Trial Results. JAMA *Intern Med*. 2016;176(12):1826-1833. doi:10.1001/jamainternmed.2016.6008
10 Mullard A. 2021 FDA approvals. *Nat Rev Drug Discov*. 2022;21(February 2022):83-88.
11 Bjornsson TD. *Progression of Modern Therapeutics*.; 2016.
12 Howes OD, Baxter L. The drug treatment deadlock in psychiatry and the route forward. *World Psychiatry*. 2023;22(1):2-3. doi:10.1002/wps.21059
13 Chandler DJ. Something's got to give: psychiatric disease on the rise and novel drug development on the decline. *Drug Discov Today*. 2013;18(3-4):202-206. doi:10.1016/j.drudis.2012.08.003
14 Craven R. The risky business of drug development in neurology. *Lancet Neurol*. 2011;10(2):116-117. doi:10.1016/S1474-4422(11)70004-7
15 van Gerven J, Cohen A. Vanishing clinical psychopharmacology. *Br J Clin Pharmacol*. 2011;72(1):1-5. doi:10.1111/j.1365-2125.2011.04021.x
16 Luscher B, Maguire JL, Rudolph U, SIBille E. GABA$_A$ receptors as targets for treating affective and cognitive symptoms of depression. *Trends Pharmacol Sci*. 2023;44(9). doi:10.1016/j.tips.2023.06.009
17 The Lancet Regional Health – Europe. Psychedelic-assisted psychotherapy: hope and dilemma. *Lancet Reg Heal - Eur*. 2023;32:100727. doi:https://doi.org/10.1016/j.lanepe.2023.100727
18 Trunnell ER, Baines J, Farghali S, et al. The need for guidance in antidepressant drug development: Revisiting the role of the forced swim test and tail suspension test. *Regul Toxicol Pharmacol*. 2024;151:105666. doi:https://doi.org/10.1016/j.yrtph.2024.105666
19 Tricklebank MD, Robbins TW, Simmons C, Wong EHF. Time to re-engage psychiatric drug discovery by strengthening confidence in preclinical psychopharmacology. *Psychopharmacology (Berl)*. 2021;238(6):1417-1436. doi:10.1007/s00213-021-05787-x
20 van der Doef TF, Zaragoza Domingo S, Jacobs GE, et al. New approaches in psychiatric drug development. *Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol*. 2018;28(9):983-993. doi:10.1016/j.euroneuro.2018.06.006
21 Stanford SC. Some Reasons Why Preclinical Studies of Psychiatric Disorders Fail to Translate: What Can Be Rescued from the Misunderstanding and Misuse of Animal 'Models'? *Altern to Lab Anim*. 2020;48(3):106-115. doi:10.1177/0261192920939876
22 Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nat Neurosci*. 2010;13(10):1161-1169. doi:10.1038/nn.2647
23 Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther*. 1977;229(2):327 – 336. http://europepmc.org/abstract/MED/596982
24 Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology (Berl)*. 1985;85(3):367-370. doi:10.1007/BF00428203
25 Cryan JF, Mombereau C, vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev*. 2005;29(4-5):571-625. doi:10.1016/j.neubiorev.2005.03.009
26 Insel T, Cuthbert B, Garvey M, et al. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry*. 2010;167(7):748-751. doi:10.1176/appi.ajp.2010.09091379
27 GrIBkoff VK, Kaczmarek LK. The Difficult Path to the Discovery of Novel Treatments in Psychiatric Disorders. *Adv Neurobiol*. 2023;30:255-285. doi:10.1007/978-3-031-21054-9_11
28 Belzung C. Innovative Drugs to Treat Depression: Did Animal Models Fail to Be Predictive or Did Clinical Trials Fail to Detect Effects? *Neuropsychopharmacology*. 2014;39(5):1041-1051. doi:10.1038/npp.2013.342
29 Gerven van, J.M.A. CAF, Adam C. Integrating data from the Investigational Medicinal Product Dossier/investigator's brochure. A new tool for translational integration of preclinical effects. *Br J Clin Pharmacol*. 0(0). doi:doi:10.1111/bcp.13529
30 Morgan P, Van Der Graaf PH, Arrowsmith J, et al. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov Today*. 2012;17(9-10):419-424. doi:10.1016/j.drudis.2011.12.020
31 Cohen AF. Developing drug prototypes: pharmacology replaces safety and tolerability? *Nat Rev Drug Discov*. 2010;9(11):856-865. doi:10.1038/nrd3227
32 Cook D, Brown D, Alexander R, et al. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. *Nat Rev Drug Discov*. 2014;13(6):419-431. doi:10.1038/nrd4309
33 Paul SM, Mytelka DS, Dunwiddie CT, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat Rev Drug Discov*. 2010;9(3):203-214. doi:10.1038/nrd3078
34 Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov*. 2004;3(8):711-716. doi:10.1038/nrd1470
35 Cohen AF, Burggraaf J, van Gerven JM, Moerland M, Groeneveld GJ. The use of biomarkers in human pharmacology (Phase I) studies. *Annu Rev Pharmacol Toxicol*. 2015;55:55-74. doi:10.1146/annurev-pharmtox-011613-135918
36 de Visser SJ, Cohen AF, Kenter MJH. Integrating scientific considerations into R&D project valuation. *Nat Biotechnol*. 2020;38(1):14-18. doi:10.1038/s41587-019-0358-x
37 Tremblay S, Rogasch NC, Premoli I, et al. Clinical utility and prospective of TMS–EEG. *Clin Neurophysiol*. 2019;130(5):802-844. doi:https://doi.org/10.1016/j.clinph.2019.01.001
38 Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood–brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol*. 2016;20:27-34. doi:http://dx.doi.org/10.1016/j.ddtec.2016.07.004
39 Howes OD, Mehta MA. Challenges in CNS drug development and the role of imaging. *Psychopharmacology (Berl)*. 2021;238(5):1229-1230. doi:10.1007/s00213-021-05838-3

CHAPTER II

TRANSLATABILITY OF PRECLINICAL TO EARLY CLINICAL TOLERABLE AND PHARMACOLOGICALLY ACTIVE DOSE RANGES FOR CENTRAL NERVOUS SYSTEM ACTIVE DRUGS

Dijkstra, F. M.[1,2*], Ferreira G.S.[3*], Veening-Griffioen D.H.[3], Boon W.P.C.[4], Schellekens H.[3], Moors E.H.M.[4], van Meer P.J.K.[3,5], Stuurman F.E.[1,2], van Gerven J.M.A.[1,2,6]
**Both authors contributed equally*

Translational Psychiatry (2023)*, 13*, Article 74.
https://doi.org/10.1038/s41398-023-02353-1

1 Centre for Human Drug Research, Leiden, the Netherlands
2 Leiden University Medical Centre, Leiden, the Netherlands
3 Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands
4 Copernicus Institute of Sustainable Development, Innovation Studies, Utrecht University, Utrecht, The Netherlands
5 Medicines Evaluation Board (CBG), Utrecht, The Netherlands
6 Central Committee on Research Involving Human Subjects (CCMO), Leiden, The Netherlands

ABSTRACT

AIM The primary purpose of this study was to assess the translatability of preclinical to early clinical tolerable and pharmacologically active dose ranges for central nervous system (CNS) active drugs.

METHODS As a part of this, IBs were reviewed on reporting quality. Investigator's Brochures (IBs) of studies performed at the Centre for Human Drug Research (CHDR) reporting statistically significant results of CNS activity related to the drug's mechanism of action were included. The quality of IBs was assessed based on the presence of a rationale for the chosen animal model, completeness of pharmacokinetic (PK) results reporting and internal validity information of the preclinical evidence. The IB-Derisk tool was used to generate preclinical and early clinical data overviews data. For each compound, the overlap between pharmacologically active dose ranges and well-tolerated levels was calculated for three pharmacokinetic (PK) parameters: human equivalent dose (HED), maximum plasma concentration (C_{max}) and area under the curve (AUC).

RESULTS Twenty-five IBs were included. In general, the quality of reporting in IBs was assessed as poor. About a third of studies did not explore the entire concentration-effect curve (pre)clinically. Single dose tolerability ranges were most accurately predicted by C_{max}. Human equivalent dose and AUC were the best predictors of pharmacologically active ranges.

CONCLUSION Tolerable and pharmacologically active dose ranges in healthy volunteers can be reasonably well predicted from preclinical data with the IB-Derisk tool. Translatability of preclinical studies can be improved by applying a higher reporting standard in IBs including comparable PK measurements across all preclinical and clinical studies.

INTRODUCTION

Drug development programs for neurological and psychiatric diseases have a high failure rate in both phase II and phase III.[1,2] Reasons include the lack of safety and efficacy in clinical stages of drug development.[1-4] Next to that, a relatively large proportion of dose reductions of novel central nervous system (CNS) drugs is needed after marketing approval due to safety concerns.[5] Lastly, poor translatability of preclinical experiments to clinical studies is often cited as a cause for these high attrition rates.[6,7]

For clinical researchers, the primary source of preclinical data for novel investigational products is the Investigator's Brochure (IB). The IB is an obligatory part of a research file for clinical studies with an investigational medicinal product (IMP), which contains all nonclinical data relevant for studies in human subjects (supplemented with subsequent clinical results).[8] According to the International Conference on Harmonisation (ICH) guideline for Good Clinical Practice (GCP), the purpose of the IB is 'to provide the investigators and others involved in the trial with the information to facilitate their understanding of the rationale for, and their compliance with, many key features of the protocol, such as the dose, dose frequency/interval, methods of administration and safety monitoring procedures'. Minimum requirements for the IB are described in this guideline.[8] Despite this guidance, the content of IBs is highly variable in practice.[9]

The European Medicines Agency (EMA) 'guideline on strategies to identify and mitigate risks for first-in-human (FIH) and early clinical trials with investigational medicinal products' provides guidance on the quality and choices of preclinical safety and efficacy studies that should be performed prior to a FIH study and on how a safe starting dose should be determined.[10] According to this guideline, the starting dose should be based on both preclinical safety studies and efficacy or pharmacodynamic (PD) experiments.[10] Safety can be quantified by the no observed adverse effect level (NOAEL) in the most sensitive relevant species, and efficacy or (pharmacological) activity by estimations of the minimal anticipated biological effect level (MABEL), the pharmacologically active dose (PAD) and/or anticipated therapeutic dose (ATD) in humans.[10] In practice, however,[10] the starting dose is often primarily based on safety considerations, as a fraction of the NOAEL.[11] This focus on NOAEL instead of a joint account of pharmacologically active levels can have disastrous consequences as observed with TGN1412 and BIA 10-2474.[12,13] When TGN1412, a CD28 superagonistic antibody in development for the treatment of chronic lymphatic leukemia and rheumatoid arthritis, was first administered to humans, it caused a cytokine release requiring intensive care treatment in all healthy individuals administered with the drug.[12] The starting dose for this study was based on the NOAEL and a factor 500 lower than the NOAEL, but at this dose cytokine release was already observed preclinically,

indicating that the starting dose should have been even lower.[12] In the clinical trial with BIA 10-2474, a fatty acid amide hydrolase (FAAH)-inhibitor in development for diseases in which elevated endocannabinoid tone might be beneficial such as pain, glaucoma and post-traumatic stress disorder, several subjects developed neurological damage and one subject died.[13] The preclinical data identified safety concerns in the form of serious irreversiblle adverse effects that were observed at varying dose levels across species.[14] There were no pharmacological measures applied in the clinical study to counter this risk and dose escalation was based solely on tolerability findings.[14] Doses were escalated to C_{max} values approximately 12 times higher than levels of maximal FAAH-inhibition, leading to the fatal consequences.[14] Not taking into account pharmacological active levels in early phase clinical studies can also have less dramatic effects, as illustrated by the example of CEP-26401.[15] The first clinical study of CEP-26401, a histamine-3 receptor antagonist developed to improve cognitive functioning in for example Alzheimer's disease, schizophrenia or attention deficit hyperactivity disorder (ADHD), demonstrated an improved cognitive functioning at the lowest dose tested.[15] Therefore a second study was required to find the dose with the best balance between wanted (improved cognition) and unwanted (sleep inhibition) effects.[15]

The aim of the current paper was to investigate the accuracy with which the IB preclinical package an predict tolerable and pharmacologically active dose ranges for CNS drugs in humans. We assessed the overlap between preclinical and clinical well-tolerated dose levels and pharmacologically active dose ranges. Furthermore, we checked whether both preclinical safety and *in vivo* pharmacology experiments were used when determining the starting dose for FIH studies as recommended by current EMA guidelines.[10] Also, we investigated the reporting quality in the IBs, and, for compounds where phase II or III clinical trials were performed in patients, we conducted an exploratory analysis of the translatability of preclinical pharmacological active ranges to therapeutic effective ranges.

METHODS

STUDY AND IB SELECTION

We performed a structural review of all IBs in CNS drug development conducted between 2003 and 2019 at the Centre for Human Drug Research (CHDR). To adhere to confidentiality agreements agreed upon with sponsors and clients of the performed clinical trials, compounds were anonymised and no individual study results are described. In order to be suitable for analysis of overlap of tolerable and pharmacologically active dose ranges, a dose range of tolerability and pharmacological activity had to be reported both preclinically and clinically. This meant that IBs were included if they included a statistically significant effect (as reported in the document) on any CNS activity related to the compound's mechanism of action for at least two dose levels across the reported preclinical studies and in the associated Clinical Study Report (CSR). Preclinical *in vitro* studies were not taken into account as we aimed to investigate the predictivity of *in vivo* pharmacology experiments in animals. Studies testing combinations of drugs and studies performed for the purpose of method development with drugs already approved by the Food and Drug Administration (FDA) or the European Medicines Agency (EMA) were excluded. For approved drugs, the Summary of Product Characteristics (SmPC) is provided to the investigator instead of an IB and animal pharmacokinetic (PK) and PD data are not systematically reported. In case phase II and III therapeutic efficacy studies were performed for a compound, an exploratory translatability assessment of therapeutic efficacy was performed.

IB-DERISK TOOL

In this paper, the IB-Derisk tool was applied to IBs of included compounds. The IB-Derisk tool (https://www.IB-Derisk.org) is a tool that can be used to integrate preclinical and clinical data reported in the IB and for the comparison of preclinical studies results with predicted or emerging human data while the clinical study is ongoing.[16] An illustration of this can be found in the publication by Cohen and colleagues, who applied the IB-Derisk tool to the BIA 10-2474 study.[14]

For detailed information on the IB-Derisk tool, we refer to the original publication.[16] In short, all reported *in vitro* findings and *in vivo* efficacy and safety findings and their according exposure parameters, such as the human equivalent dose (HED), maximum plasma concentration (C_{max}) and total exposure (AUC) are entered in a spreadsheet-like document. By doing so, missing PK parameters can be estimated by interpolation and extrapolation, taking dose duration (single or multiple dose), route and bioavailability, species and sex into account. All effects are then colour-coded. Desired pharmacological effects are indicated by green, mild manageable adverse effects in yellow, more severe adverse effects that could not be accepted in a clinical situation but without unacceptable health risks by orange, and severe irreversible adverse effects in red. The no observed adverse effect level (NOAEL) in the most sensitive species is indicated in purple, and *in vitro* experiments in light blue. By applying this colour coding, sorting the data on dose (HED), concentration or AUC provides a quick overview of dose-concentration- or exposure-response patterns of pharmacological and toxicological effects, and of deviations from predictable relationships.[16]

DATA COLLECTION

Data were collected from the source documents by the authors GSF and FMD. All *in vivo* behavioural pharmacology and safety preclinical study results reported in the included IBs were extracted, regardless of species or outcome. GSF and FMD also assessed

the quality of reporting in IB's in a similar manner as described by Wieschowski et al.[17] As a part of this it was assessed whether a rationale for the chosen animal model was provided, the completeness of reporting of pharmacokinetic results of *in vivo* pharmacology experiments (including strain, sex, route of administration) was assessed and internal validity information (randomisation, blinding) of the preclinical evidence was assessed. GSF and FMD discussed their quality assessment in case there were any unclarities and the consensus answer was incorporated in Table 1.

In addition to preclinical data retrieved from the IBs, the aims and results of the actual early human studies were extracted from the protocols and clinical study reports (CSRs). If available, these results were complemented with other clinical data provided in section 4 of the IB ('Effects in Humans'). For all included studies, information on the mechanism of action of the IMP and whether the clinical study was a FIH study was collected. For FIH studies, the section in the protocol describing the rationale for starting dose selection was assessed on whether the starting dose was primarily based on preclinical safety or also on *in vivo* pharmacological effects. As part of this, it was assessed whether the lowest preclinical and clinical pharmacologically active dose were established.

DATA ANALYSIS

Two separate analyses were performed to determine the predictability of preclinical findings to humans: one for tolerability and another for pharmacologic activity. The purpose of these analyses was to compare the tolerable and pharmacologically active ranges in laboratory animals to those found in humans. The ranges were calculated for all three exposure parameters: dose (HED), C_{max} and AUC.

For tolerability, the ratio between the starting level of the clinical study and the NOAEL, and the ratio between the highest well-tolerated level in the clinical study and the NOAEL were calculated. The starting level for the clinical study was defined as 'human lower range adverse events' (HLR_{AE}), and the highest well-tolerated level in the clinical study as 'human upper range adverse events' (HUR_{AE}).

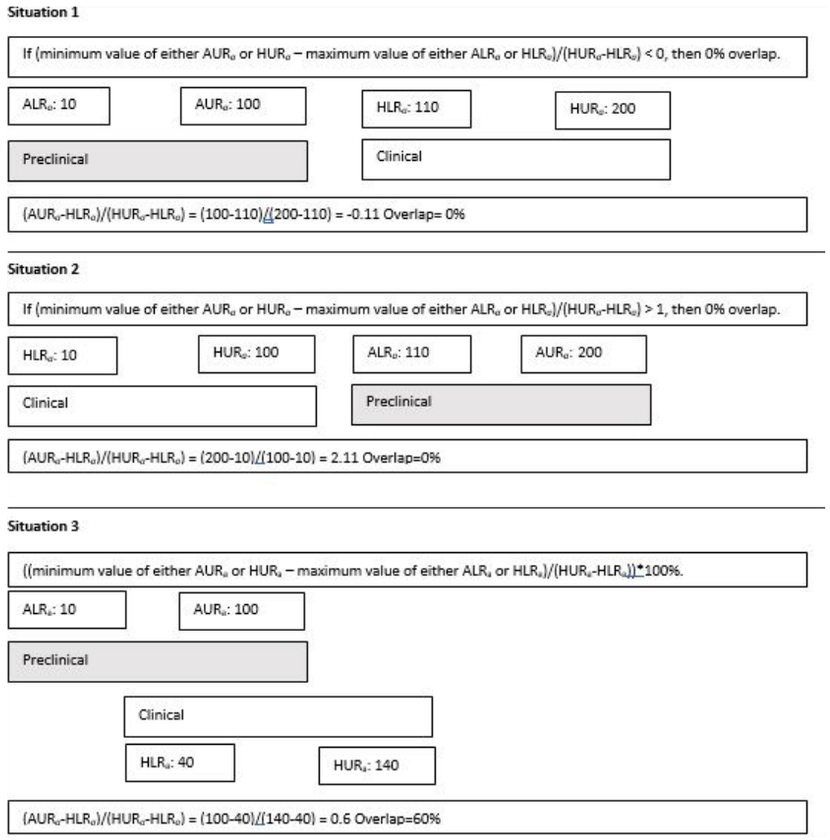
The preclinical pharmacologically active range was determined by the 'animal lowest' and 'animal highest' dose-, concentration- or exposure-level, at which any effect associated with the proposed mechanism of action of the drug was reported in any animal species. These values were called ALR_a 'animal lower range active' and AUR_a 'animal upper range active'. Preclinical safety findings were often observed at higher levels than the highest level tested in efficacy experiments. If the safety issues were judged to be related to a compound's pharmacological effects, this was used to determine the AUR_a. The human active ranges HLR_a and HUR_a were defined as the lowest and highest level, respectively, in which pharmacological effects associated with the proposed mechanism of action of the drug were reported. An illustration of the calculations of the overlap between the preclinical active dose range with the corresponding clinical dose ranges for each exposure parameter (HED, C_{max} and AUC) can be found in Figure 1. Calculations were performed according to the formula below:

If (minimum value of either AUR_a or HUR_a – maximum value of either ALR_a or HLR_a)/(HUR_a-HLR_a)< 0, then 0% overlap.

If (minimum value of either AUR_a or HUR_a – maximum value of either ALR_a or HLR_a)/(HUR_a-HLR_a)> 1, then 0% overlap.

Otherwise: ((minimum value of either AUR_a or HUR_a – maximum value of either ALR_a or HLR_a)/(HUR_a-HLR_a))*100%.

Figure 1 Overlap calculations. It illustrates how the calculations were performed.



Abbreviations: ALR_a, animal lower range active; AUR_a, animal upper range active; HLR_a, human lower range active; HUR_a, human upper range active.

RESULTS

GENERAL CHARACTERISTICS OF STUDIES

The search in CHDR's database from 2003 up to and including 2019 returned 164 finished clinical CNS drug studies in healthy volunteers with corresponding IBs. Of these, 25 studies met the inclusion criteria (Figure 2). The most common reasons for exclusion were studies being non-interventional (method development), or studies with a registered compound or patient studies (n=106), studies focusing on drug-drug interaction (n=13) and no clinical efficacy (pharmacological activity) results at two different dose levels (n=8) (Figure 2). The four studies that were excluded because there were no preclinical behavioural *in vivo* pharmacology experiments in animals performed concerned three studies in which only cell and cytokine responses were measured preclinically and a study in which only *in vitro* experiments were performed preclinically to assess the pharmacodynamics of the novel compound. The two studies that were excluded because there were no preclinical *in vivo* pharmacology results at two dose levels concerned one study in which several doses were tested in preclinical behavioural experiments, but only one dose had a statistically significant effect and one study in which only one dose was tested in a preclinical *in vivo* pharmacology experiment. These studies were not included in the analysis as the outcome could then be only 0% or 100% overlap. The two studies that were excluded because there was no efficacy (pharmacological activity) measurement in the clinical study concerned PK studies.

Of the eight studies that were excluded because there was no clinical efficacy (pharmacological activity) result at two different dose levels, there were four studies that measured a statistically significant effect in the clinical study, but only at one dose level. These studies were not included in the overlap calculations as the overlap could then only be 0% or 100%. There were four studies in which the results on the efficacy or pharmacological activity measurement in the clinical study were negative. This concerned a study measuring the effect of a single dose esketamine on driving performance compared to the effect of placebo and a positive control. A study measuring the effect of different doses of a novel compound (a selective muscarinic M1 -acetylcholine receptor agonist) on cognitive performance as measured by a battery of neurocognitive and neurophysiological tests in healthy elderly with below average cognitive functioning. A study into the effect of different doses of a novel compound (a dual enkephalinase inhibitor) on neurocognitive and neurophysiological tasks and on a nociceptive test battery, and a study measuring CNS effects of different doses of a novel compound (guanylate cyclase stimulator) in healthy volunteers measured by a battery of neurocognitive and neurophysiological tests. In all four of these studies the preclinical pharmacological activity experiments did demonstrate statistically significant effects.

Table 1 shows that 12 (48%) of the included studies concerned a First-in-Human (FIH) study. In five of these studies (42%), the starting dose was based on both safety and *in vivo* pharmacology findings. In seven (58%) studies the starting dose was only based on safety findings. In 8 of the 25 IBs (32%) a rationale for the chosen animal model was provided. The motivations included availability (commonly used models), similar phenotype (symptoms), response to effective drugs (pharmacology), histology and biomarkers. There was no explanation as to the relevance of model choice when compared to other available options. In none of the IBs pharmacokinetic reporting was complete for all *in vivo* pharmacology experiments (Table 1). In most cases PK values of behavioural or disease models in mice were not reported (Table 1). The strain and sex were frequently missing. None of preclinical experiments in the included IBs fulfilled to the criteria of internal validity as most animal experiments included a placebo arm, but blinding or randomisation was not reported in any of the experiments (Table 1).

Figure 2 Flowchart of included studies. It depicts the reasons of why studies were excluded.

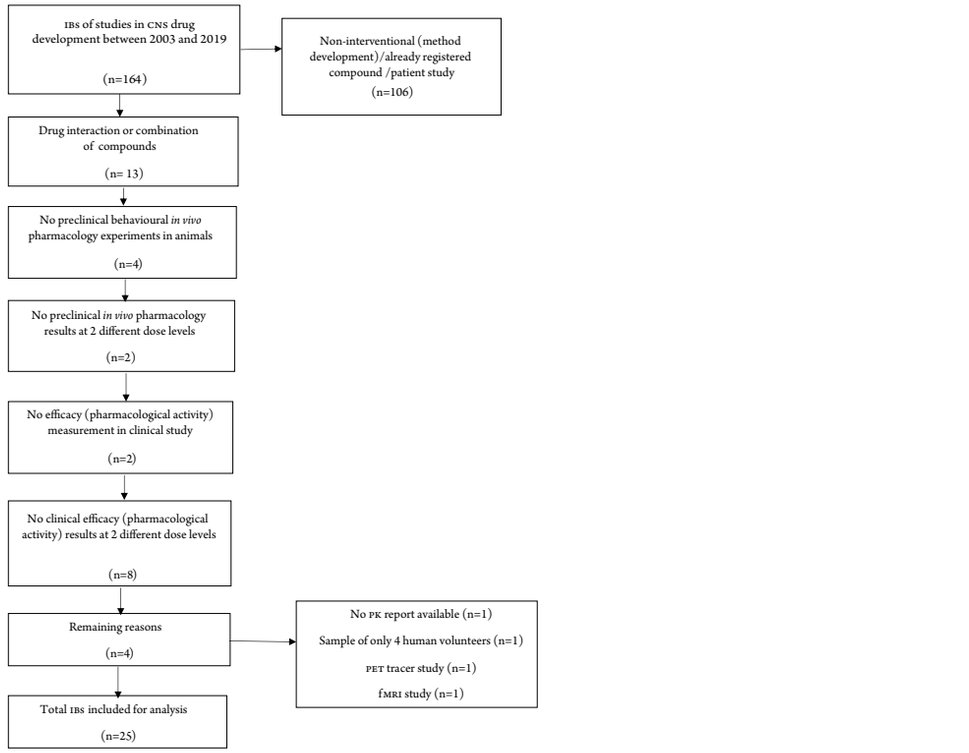


Table 1 General characteristics of included IBs

IB	Mechanism of Action	FIH	Starting dose rationale	Completeness of reporting; PK results of *in vivo* pharmacology experiments reported	Animal model justification	Internal validity
1	Cannabinoid receptor antagonist	No	*In vivo* pharmacology and safety	Dog: yes; Mouse: no; Rat: partial	None	No
2	Cannabinoid receptor agonist	Yes	Safety	Dog: partial; Micro-pig: no; Mini-pig: no; Mouse: no; Rat: partial	None	No
3	Orexin receptor antagonist	Yes	*In vivo* pharmacology and safety	Dog: partial; Guinea pig: no; Rats: partial	None	No
4	Cannabinoid receptor agonist	Yes	*In vivo* pharmacology and safety	Dogs: partial; Mini-pig: no; Mouse: no; Rat: partial	None	No
5	Cannabinoid receptor antagonist	Yes	*In vivo* pharmacology and safety	Dog: partial; Guinea-pig: no; Mouse: no; Rabbit: yes; Rat: partial	Pharmacological	No
6	Dopamine antagonist	No	Safety	Dogs: partial; Ferret: no; Guinea pig: partial; Mini-pig: yes; Monkey: yes; Mouse: partial; Rat: partial	Pharmacological	No
7	GABA receptor modulator	No	*In vivo* pharmacology and safety	Dog: yes; Guinea-pig: partial; Monkey: no; Mouse: no; Rat: partial	Pharmacological	No
8	Alpha 7 nicotinic acetylcholine receptor partial agonist	No	*In vivo* pharmacology and safety	Dog: partial; Monkey: partial; Mouse: partial; Rabbit: yes; Rat: partial	None	No
9	GABA-receptor partial agonist	Yes	Safety	Dog: partial; Mouse: no; Rat: partial	Commonly used/ pharmacological	No
10	Neublastin (GFRα3) co-receptor selective ligand	No	*In vivo* pharmacology and safety	Monkey: partial; Mouse: no; Rat: partial	Phenotypic	No
11	Orexin antagonist	Yes	Safety	Dog; partial; Guinea pig: no; Rat: partial	None	No
12	Histamine receptor agonist	Yes	Safety	Cat: partial; Dog: partial; Monkey: yes; Mouse: partial; Rat: partial	None	No
13	Histamine receptor antagonist	No	*In vivo* pharmacology and safety	Dog: yes; Monkey: partial; Mouse: no; Rat: partial	None	No
14	GABA-receptor selective positive allosteric modulator	Yes	*In vivo* pharmacology and safety	Dog: partial; Rat: partial, Mouse: partial	None	No
15	Alpha 7 nicotinic acetylcholine receptor agonist	No	*In vivo* pharmacology and safety	Dog: partial; Mouse: partial; Rat: partial	None	No
16	Orexin antagonist	No	*In vivo* pharmacology and safety	Dog: partial; Guinea pig: partial; Mouse: partial; Monkey: yes; Rat: partial	None	No
17	Trace amine associated receptor (TAAR)1 partial agonist	No	*In vivo* pharmacology and safety	Monkey: partial; Mouse: no; Rat: partial	None	No
18	Orexin receptor antagonist	Yes	Safety	Dog: partial; Guinea pig: partial; Rat: partial	None	No
19	P2X7 channel antagonist	No	*In vivo* pharmacology and safety	Rat: partial; Dog: no, Guinea pig: partial, Monkey: yes	None	No
20	Muscarinic receptor partial agonist	Yes	Safety	Dog: partial; Monkey: yes; Rat: partial	None	No
21	Nicotinic acetylcholine (NaChR) inhibitor (prodrug)	No	Safety	Dog: partial; Ferret: no; Mouse: partial; Rat: partial	Commonly used	No
22	Orexin receptor antagonist	Yes	Safety	Dog: partial; Guinea pig: partial; Mouse: partial; Rat: partial	None	No
23	Beta-glucocerebrosidase (GCase) allosteric activator	Yes	*In vivo* pharmacology and safety	Dog: yes; Monkey: yes; Mouse: partial; Rat: yes	Biomarker	No
24	GABA positive allosteric modulator	Yes	*In vivo* pharmacology and safety	Minipig: yes; Monkey: yes; Mouse: partial; Rat: partial	Symptomatology	No
25	AMPA receptor positive allosteric modulator	No	*In vivo* pharmacology and safety	Monkey: partial; Rat: partial	None	No

Abbreviations: IB, Investigator's Brochure; FIH, first in human; PK; pharmacokinetic.

TOLERABILITY ASSESSMENT

PREDICTIONS OF TOLERABILITY BASED ON HUMAN EQUIVALENT DOSE Table 2 shows that in most studies (N=22, 88%), the starting dose (HLR_{AE}) for the clinical study was at least a (rounded) factor 10 below the HED of the NOAEL (Ratio HLR/NOAEL). This is as expected, since FDA guidelines propose 10 as default safety factor.[18] Three studies that did not apply this method were the IB2, IB4 and IB21 studies. The studies of IB2 and IB4 investigated novel cannabinoid receptor agonists. In these studies, a starting dose of respectively factor 1.8 and 5.3 below the HED of the NOAEL was deemed safe by the investigators, as the NOAEL was based on transient, species specific, monitorable effects on blood pressure, and considering the safety profile of other well-known cannabinoid agonists. The study of IB21 was no FIH and the starting dose was based on results of a previous clinical study.

For HED, the human upper tolerability range HUR_{AE} surpassed the NOAEL in 9 out of 25 studies (36%): IB2, IB3, IB4, IB6, IB8, IB9, IB11, IB14 and IB20 (Table 2, Figure 3). In all except two of these studies, the compound was well tolerated up to the highest administered dose range (HUR_{AE}), probably explaining why human doses could be escalated to levels beyond the NOAEL. Studies with IB2 and IB4 both involved experimental cannabinoid receptor agonists. The preclinical NOAEL for these compounds was based on cardiovascular side effects, which can be intensely monitored in humans and therefore be used to guide dose escalation. Although such effects also occurred in humans, they were not considered dose limiting. For both cannabinoid receptor agonists, dose escalation was only halted when undesirable (reversible) psychiatric events occurred, which were in line with the action mechanism.

PREDICTIONS OF TOLERABILITY BASED ON C_{max} As shown in Table 2 and Figure 3, the C_{max} value of the HUR_{AE} surpassed the C_{max} value of the NOAEL in 8 of the 25 studies (32%; IB2, IB4, IB5, IB6, IB8, IB10, IB15 and IB21). The findings with the cannabinoid receptor agonists of IB2 and IB4 were mentioned before. In the clinical studies of IB5, IB6, IB8, IB10, IB15 and IB21 the compound was well tolerated up to the highest HUR_{AE}, allowing the dose levels to be escalated to levels beyond the NOAEL.

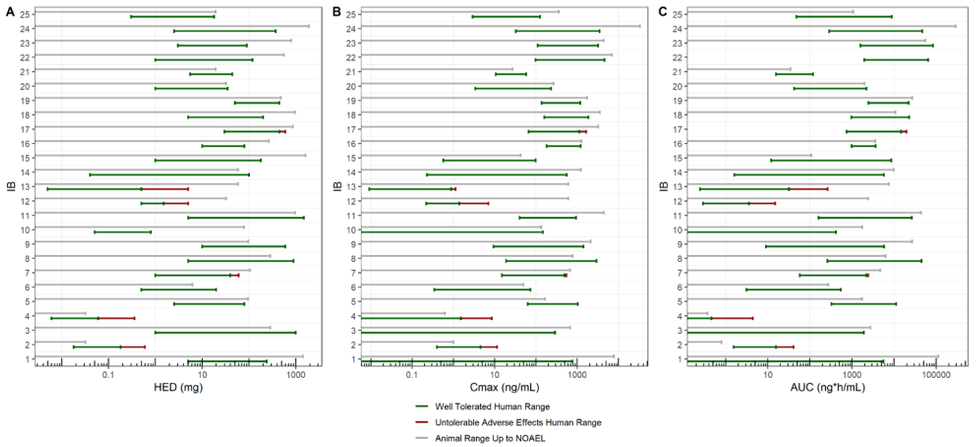
PREDICTIONS OF TOLERABILITY BASED ON AUC For AUC, Table 2 and Figure 3 show that the HUR_{AE} surpassed the AUC associated with the NOAEL in 12 out of 23 studies (52%; IB2, IB4, IB5, IB6, IB8, IB15, IB16, IB18, IB20, IB21, IB23, IB25). In all these studies (except IB2 and IB4 as explained above), the compound was tolerated well enough to be escalated to levels above the NOAEL.

Table 2 Safe and tolerable dose ranges

	Human Equivalent Dose (mg)					C_{max} (ng/ml)					AUC (ng*h/ml)				
IB	NOAEL	HLR	HUR	Ratio HLR/ NOAEL	Ratio HUR/ NOAEL	NOAEL	HLR	HUR	Ratio HLR/ NOAEL	Ratio HUR/ NOAEL	NOAEL	HLR	HUR	Ratio HLR/ NOAEL	Ratio HUR/ NOAEL
1	1440	5	240	288.0	6.0	7797	0	774	7797.0	10.1	113029	0	5594	113029.0	20.2
2	0.032	0.018	0.18	1.8	0.2	1	0.398	4.55	2.5	0.2	0.8	1.54	15.7	0.5	0.1
3	288	1	1000	288.0	0.3	677	0	291	677.0	2.3	2760	0	1910	2760.0	1.4
4	0.032	0.006	0.06	5.3	0.5	0.63	0	1.52	0.6	0.4	0.37	0	0.456	0.0	0.8
5	96	2.5	80	38.4	1.2	167	63	1030	2.7	0.2	1730	324	11200	5.3	0.2
6	6.14	0.5	20	12.3	0.3	49.3	0.343	73.3	143.7	0.7	274	3.1	542	88.4	0.5
7	105.6	1	40	105.6	2.6	674.25	15	497	45.0	1.4	4743	57.9	2182	81.9	2.2
8	288	5	900	57.6	0.3	780	18.9	2946	41.3	0.3	6336	261	45286	24.3	0.1
9	97.2	10	600	9.7	0.2	2130	9.4	1430	226.6	1.5	26800	9	5790	2977.8	4.6
10	78	0.05	0.8	1560.0	97.5	134	0	149	134.0	0.9	1763	0	417	1763.0	4.2
11	972	5	1500	194.4	0.6	4445	40.1	935.9	110.8	4.7	44200	161.4	26585.8	273.9	1.7
12	32.46	0.5	1.5	64.9	21.6	609	0.22	1.4	2768.2	435.0	2421	0.29	3.55	8348.3	682.0
13	58.3	0.005	0.5	11660.0	116.6	610	0.00913	0.885	66812.7	689.3	7584	0.245	32	30955.1	237.0
14	58.3	0.04	100	1457.5	0.6	1230	0.229	559.3	5371.2	2.2	9850	1.6	5753	6156.3	1.7
15	1623	1	180	1623.0	9.0	42.9	0.572	98.8	75.0	0.4	108	12.1	8666	8.9	0.0
16	267.8	10	80	26.8	3.3	1270	183	1208	6.9	1.1	3570	982	3648	3.6	1.0
17	874.8	30	450	29.2	1.9	3267	66.3	1130	49.3	2.9	NR	743	14600	NR	NR
18	973	5	200	194.6	4.9	3560	159.7	1868.9	22.3	1.9	10900	971.3	22906.4	11.2	0.5
19	486.9	50	450	9.7	1.1	1740	138	1180	12.6	1.5	27200	2456	22357	11.1	1.2
20	32.5	1	35	32.5	0.9	267	3.4	235	78.5	1.1	1990	42	2210	47.4	0.9
21	19.4	5.5	44	3.5	0.4	27.1	10.7	58.5	2.5	0.5	34.8	15.7	119	2.2	0.3
22	560	1	120	560.0	4.7	6976	97.4	4575	71.6	1.5	NR	1969	64642	NR	NR
23	811	3	90	270.3	9.0	4433	110	3200	40.3	1.4	55333	1600	84700	34.6	0.7
24	1920	2.5	375	768.0	5.1	33300	32.5	3520	1024.6	9.5	289000	286	46500	1010.5	6.2
25	19.44	0.3	18	64.8	1.1	359	2.93	126	122.5	2.8	1069	47.7	8882	22.4	0.1

Abbreviations: IB, Investigator's Brochure; NOAEL, no observed adverse effect level; C_{max}, maximum concentration; AUC, area under the curve; HLR, human lower range; HUR, human upper range; NR: not reported

Figure 3 Safe and tolerable dose ranges



A. The overlap ranges for HED are depicted. B. The overlap ranges for C_{max} are depicted. C. The overlap ranges for AUC are depicted. For both preclinical (animal) and clinical (human) studies the investigated ranges per pharmacokinetic parameter are depicted. The preclinical (animal) ranges are depicted in grey. The clinically (human) well-tolerated ranges are in green and the ranges that were well-tolerated preclinically, but not clinically are depicted in red. Abbreviations: HED, human equivalent dose; C_{max}, maximum concentration; AUC, area under the curve

OTHER OBSERVATIONS ON TOLERABILITY In none of the clinical studies serious adverse events or irreversible adverse events, related to the investigational compound, were reported. Four human studies (IB7, IB12, IB13 and IB17) showed unacceptable adverse events at levels below values associated with the NOAEL for all exposure parameters (Table 2, Figure 3). In the study of IB7 with a GABA receptor modulator, volunteers experienced ataxia, imbalance, tiredness and drowsiness. Symptoms of comparable nature, such as somnolence and ataxia, were also observed preclinically, albeit at higher dose levels. In the clinical study of IB12 with a histamine receptor agonist, participants reported pseudo-hallucinations and experienced hypotension. Preclinically, decreases in blood pressure were also observed, but at much higher dose levels. Pseudo-hallucinations could obviously not be observed preclinically, but behavioural changes were observed in monkeys at much higher dose levels than given in the clinical study. In the study of IB13 with a histamine receptor antagonist, subjects experienced moderate nausea and insomnia. Preclinically, increased wakefulness was also observed, but this was considered a desired effect that was observed at similar dose levels. Emesis only occurred in dogs at much higher dose levels than in the clinical study. For IB17 with a trace amine associated receptor (TAAR) partial agonist, cardiovascular AEs of tachycardia, palpitations and orthostatic hypotension were observed. Increased heart rate was also observed preclinically, but at much higher dose levels.

Taken together, C_{max} values seem to be the most accurate predictor of tolerability limits for CNS active compounds, with only 32% of clinical studies reporting well tolerated doses above the NOAEL value, compared to 36% for HED and 52% for AUC. The percentage (16%) of clinical studies reporting unacceptable side effects at values below the NOAEL was similar for all three exposure parameters.

PHARMACOLOGICAL ACTIVITY ASSESSMENT

PRECLINICAL AND CLINICAL PHARMACOLOGICALLY ACTIVE RANGES Eight (32%) IBs (IB2, IB5, IB6, IB8, IB18, IB22, IB23, IB24, IB25) reported statistically significant pharmacological effects at the lowest tested preclinical dose (Figure 4). Hence, 32% of the preclinical studies did not cover the full concentration-effect range. Nine (36%) clinical studies (IB1, IB13, IB14, IB15, IB16, IB19, IB20, IB21, IB23) showed a pharmacological effect at all administered doses (Figure 4). For one study (IB23 with a GCase modulator) both the preclinical and clinical lowest tested dose were effective, meaning that no no-effect level was defined.

PREDICTIONS OF PHARMACOLOGICAL ACTIVITY BASED ON HED On average, HED was the best predictor of pharmacologically active ranges, with 84% overlap between preclinical and clinical pharmacologically active ranges (Table 3, Figure 4). For one compound (IB20) there was no overlap between the preclinical and clinical ranges for HED or any other exposure parameter. This involved a muscarinic receptor partial agonist. Memory testing showed improvement in healthy volunteers, at lower levels than in the preclinical experiments. Memory functioning in the clinical study was tested using the NeuroCart, which consists of a battery of drug-sensitive neurophysiological and cognitive tests.[19] Possibly the NeuroCart is more sensitive for drug effects than preclinical models available to test memory functioning, explaining why drug effects in humans were observed at lower exposure levels than in animals. Further dose escalation in the clinical study was prevented by adverse events that could be expected with muscarinergic agonists, such as increased blood pressure and hypersalivation. Blood pressure increases were also described in the IB in rats, at a similar HED but with higher C_{max} and AUC values.

PREDICTIONS OF PHARMACOLOGICAL ACTIVITY BASED ON C_{max} The preclinical and clinical pharmacologically active range showed overlapping C_{max} values in 64% of studies. There was no overlap for IB2, IB3, IB4, IB11, IB19 and IB20 (Table 3, Figure 4).

In the clinical studies of IB2 and IB4, some participants experienced typical mental effects of cannabinoid receptor agonists, at C_{max} values well below preclinically active levels. The psychiatric effects were considered dose-limiting in view of the anticipated therapeutic indications (analgesia and sedation). In the clinical studies of IB3 and IB11, both with an orexin antagonist, preclinical pharmacological effects were observed at higher C_{max} levels than in the clinical study. It is possible that more sensitive PD measurements for orexin antagonists are available in humans (using the NeuroCart) than in animals, to detect (subjective) reduced alertness, attention and vigilance.

In the clinical study of IB19 with a subtype selective purine antagonist, decreased interleukine-1β release was observed in humans at a somewhat lower C_{max} value than preclinically, suggesting that humans are more sensitive to the effects of purine antagonists than animals. Pharmacological activity was measured at a lower C_{max} value in humans than in animals in the clinical study of IB20 with a muscarinic receptor partial agonist, again suggesting that memory tests are more sensitive to this class of compounds in humans than in animals.

PREDICTIONS OF PHARMACOLOGICAL ACTIVITY BASED ON AUC Pharmacologically active AUC ranges in animals and humans overlapped in 78% of studies. There was no overlap between animals and humans for AUCs of compounds in IB3, IB4, IB15 and IB2 (Table 3, Figure 4). In the clinical study of IB3 with an orexin antagonist, the preclinical effects were observed at higher AUC levels than in the clinical study. It is possible that – partly subjective – PD measurements are more sensitive to orexin antagonists in humans than in animals.[19] However, the overlap between the preclinical and clinical pharmacologically active AUC range in IB11 with an orexin antagonist as well was 97%. In general, the PK profile of this orexin antagonist was much more comparable between animals and humans than for the orexin antagonist of IB3. The same was found for the two cannabinoid receptor agonists. The AUC overlap of pharmacologically active ranges for the compound of IB2 was 100%. For IB4 there was no overlap, because in the clinical study dosing was stopped for unacceptable (albeit pharmacological) mental effects at lower AUC levels causing detectable pharmacological effects in animals.

In the clinical study of IB15 with a subtype selective nicotinic receptor agonist, the preclinical pharmacologically active range was lower than the clinical active range. However, the lowest dose in humans already showed pharmacological activity, meaning that there could be an overlap, but this was not assessed. For the muscarinic receptor partial agonist of IB20, the NeuroCart could demonstrate pharmacological activity in humans at lower AUCs, than where effects occurred in animals.

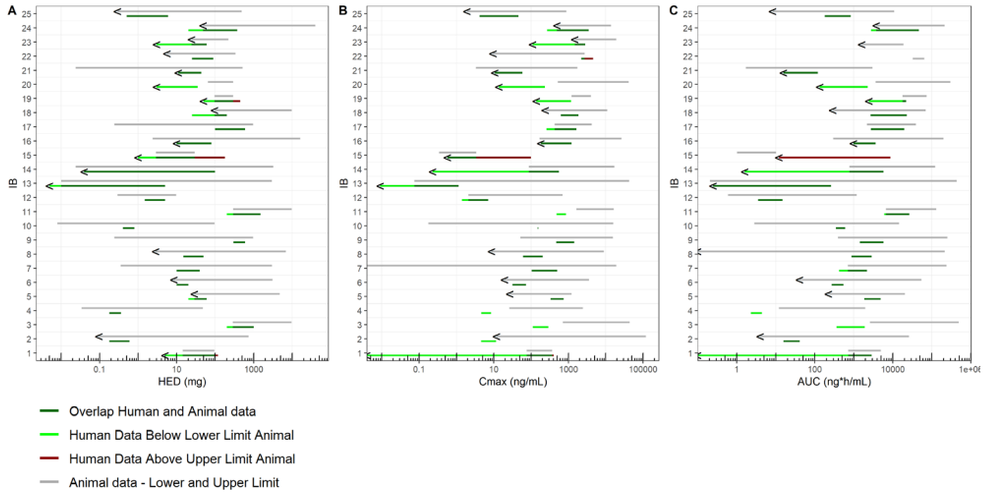
Overall, the preclinical data predicted the pharmacologically active range in humans to a high degree, as indicated by an overlap of ≥ 80% in 18 out of 25 (72%) for HED, 15 out of 25 (60%) for C_{max} and 19 out of 23 (83%) for AUC. A particularly poor preclinical prediction of the clinical active range (as indicated by ≤ 20% overlap) was shown in 2 out of 25 studies (8%) for HED, 7 of 25 (28%) for C_{max} and 4 out of 23 studies (17%) for AUC.

Table 3 Pharmacologically active dose ranges

IB	Human Equivalent Dose (mg)					C_{max} (ng/ml)					AUC (ng*h/ml)				
	ALR	AUR	HLR	HUR	% Overlap	ALR	AUR	HLR	HUR	% Overlap	ALR	AUR	HLR	HUR	% Overlap
1	14.4	96	5	120	*71%*	76.6	367	0	403	*72%*	719	4931	0	2870	*75%*
2	0.096	736	0.18	0.6	*100%*	11.9	118732	4.55	11.5	*0%*	4	26232	15.7	41.4	*100%*
3	288	9600	200	1000	*89%*	707	43324.6	111	291	*0%*	2590	492674	362	1910	*0%*
4	0.034	48	0.18	0.36	100%	26	2424	4.66	8.51	0%	12	1968	2.29	4.4	0%
5	28.8	4800	20	60	78%	27	1210	334	749	100%	225	20700	1860	4870	100%
6	8.54	3072	10	20	100%	19.4	3530	31.5	73.3	100%	40.87	54900	272	542	100%
7	0.355	3000	10	40	*100%*	0	19305	104	497	*100%*	707	238848	423	2182	*84%*
8	2.88	6912	15	50	*100%*	8.7	8990	61	205	*100%*	0	216000	875	2895	*100%*
9	0.243	972	300	600	*100%*	51.9	15619	477	1430	*100%*	396.5	250938	1440	5790	*100%*
10	0.008	97.2	0.4	0.8	*100%*	0.177	15855	149.3	156	*100%*	2.82	14523	347	606	*100%*
11	291.6	9738	200	1500	*93%*	1660	16450	479.3	868.9	*0%*	6741	130500	6113.1	26585.8	*97%*
12	0.29	9.72	1.5	5	*100%*	2.1	700	1.4	7.1	*88%*	0.6	1189	3.55	14.97	*100%*
13	0.010	2961	0.005	5	*100%*	0.074	42435	0.009	1.144	*94%*	0.205	435485	0.245	262.66	*100%*
14	0.024	3246	0.04	100	*100%*	87.2	17000	0.229	559.3	*84%*	775	122000	1.6	5753	*87%*
15	2.9	29.16	1	180	*15%*	0.342	3.42	0.572	98.8	*3%*	1.014	10.14	12.1	8666	*0%*
16	2.4	16230	10	80	*100%*	169.8	26371	183	1208	*100%*	297	200000	982	3648	*100%*
17	0.243	972	100	600	*100%*	429.7	4200	261	1660	*88%*	2180	39007	2750	19800	*100%*
18	97.2	9720	25	200	*59%*	239	11113	631.58	1868.9	*100%*	290	69700	2703.2	22906.4	*100%*
19	97.2	291.6	50	450	*49%*	1214	4013	138	1180	*0%*	17909	74330	2456	22357	*22%*
20	64.9	291.6	3	35	*0%*	523	41300	13.7	235	*0%*	3620	301000	134	2291	*0%*
21	0.024	519.4	11	44	*100%*	3.3	1740	10.7	58.5	*100%*	1.7	2990	15.7	119	*100%*
22	5.6	336	25	90	*100%*	9.6	2698	2230	4575	*20%*	NR	NR	32831	64642	*100%*
23	24.3	218.7	3	60	*63%*	1484	19286	110	2820	*49%*	NR	NR	1600	18700	*100%*
24	48.6	39600	20	375	*92%*	496	14000	271	3520	*93%*	3780	212000	2790	46500	*98%*
25	0.29	486	0.5	6	*100%*	1.9	883	4.19	45.99	*100%*	8.28	10800	181	839	*100%*
average					*84%*					*64%*					*78%*

Abbreviations: IB, Investigator's Brochure; NOAEL, no observed adverse effect level; C_{max}, maximum concentration; ALR, animal lower range; AUR, animal upper range; HLR, human lower range; HUR, human upper range; NR, not reported.

Figure 4 Pharmacologically active dose ranges



A. The overlap ranges for HED are depicted. B. The overlap ranges for C_{max} are depicted. C. The overlap ranges for AUC are depicted. For both preclinical (animal) and clinical (human) studies the investigated ranges per pharmacokinetic parameter are depicted. The preclinical (animal) ranges are depicted in grey. The clinically (human) pharmacological active dose range that was below the range in which pharmacological activity was observed preclinically (animal) is depicted as light green. The clinically (human pharmacological active range that was above the range in which pharmacological activity was observed preclinically (animal) is depicted as red. The overlap of preclinical and clinical pharmacological active dose ranges is depicted as dark green. < means that the lowest dose tested already demonstrated an effect. Abbreviations: HED, human equivalent dose; C_{max}, maximum concentration; AUC, area under the curve.

THERAPEUTIC EFFICACY ASSESSMENT

Therapeutic efficacy studies were reported for only six of the 25 included compounds in our paper. For three out of the six compounds there was a large overlap between the therapeutic effective ranges in patients, and the preclinical and human pharmacologically active ranges. For the three other compounds, the therapeutic effective ranges were lower than the preclinical pharmacologically active range, but they corresponded closely with the clinical pharmacologically active ranges in healthy volunteers.

DISCUSSION

The results show that, in general, tolerable dose ranges for clinical studies with novel CNS active compounds can be reasonably well predicted from preclinical data. Overall, C_{max} corresponding to the preclinical NOAEL was the best predictor of the tolerable range in humans, although the observed adverse effects in animals (or any other dose-limiting effect) did not occur in 32% of the healthy volunteer studies. HED and AUC predictions based on this 'default' safety level were even more conservative (with poor predictability in 36% and 52%), particularly when the effects could be readily monitored in healthy subjects and used for dose escalation (e.g., using intensive cardiovascular monitoring, or repeated NeuroCart measurements for CNS-effects).

In 4 out of 25 studies (24%), the highest tolerated (administered) doses in humans (HUR_{AE}) were much lower than expected based on the NOAEL for all three exposure parameters. This concerned the clinical study of IB7 (GABA modulator), IB12 (histamine agonist), IB13 (histamine antagonist) and IB17 (TAAR partial agonist). In three cases (IB7, IB13 and IB17) the dose limiting AEs reported by volunteers, including ataxia, hypotension, drowsiness, insomnia and nausea, were observed preclinically as well, but only at higher dose levels. Thus, a considerable proportion of CNS active agents (28%) seems to have more prominent effects in humans than animals. For some compounds, dose escalation was limited by psychiatric side effects, which are difficult to observe preclinically. This was the case for the histamine receptor agonist of IB12 at doses well below NOAEL. Mental effects also limited dosing of the two cannabinoid receptor agonists (IB2 and IB4), but here the highest tolerated dose in humans (HUR_{AE}), was higher than the values associated with the NOAEL that was based on cardiovascular effects. These results emphasise that in FIH studies with CNS active compounds, researchers should pay special attention to psychiatric effects of new compounds, as these cannot be reliably predicted from animal experiments.

In all studies included in this report, the observed adverse events were exaggerated pharmacological effects in line with the working mechanism of the compound and therefore predictable based on preclinical data. This illustrates the importance of monitoring pharmacological effects of compounds based on the mechanism of action. Monitoring based on translatable and thus predictable pharmacological mechanisms of actions can also include important off-target effects, which in the IB are presented as *ex vivo* or *in vitro* pharmacological binding studies.

The average overlap values of preclinical and clinical pharmacologically active dose ranges demonstrate that the prediction of clinical pharmacologically active dose ranges based on preclinical data of behavioural experiments is fairly reliable. With an average overlap of 84% the HED was the best predictor for the pharmacologically active dose range. Possibly, this reflects a bias in reporting as the MABEL or PAD were most often based on the HED in the IBs included in this report. When looking at the PK parameter with the highest percentage of high preclinical and clinical active dose range overlap, AUC was the best predictor with 83% of the compounds having more than 80% overlap.

In cases where no overlap between preclinical and clinical pharmacological active dose range could be observed, humans were more sensitive to the effects of the compound. In the clinical studies with cannabinoid receptor agonists, it was not possible to dose up to the levels of desired pharmacological (analgesic, sedative) effects due to unacceptable psychiatric (but still pharmacological) effects observed at lower dose levels. Not only for psychomimetic effects, but also for some other CNS effects, more sensitive methods are available in humans than in animals. Complex measures of memory or eye-hand coordination in the NeuroCart showed effects of orexin antagonists and cholinergic/muscarinergic agonists, at lower levels in humans than predicted from animal models.

Four studies were excluded from the quantitative analysis of overlapping exposure ranges, because no statistically significant effects were observed on measurements of pharmacological activity/pharmacodynamics in the clinical study. In all four of these studies statistically significant effects were observed in preclinical, behavioural experiments, but not in humans. One of the omitted studies in humans was not designed to measure pharmacodynamic effects of the compound, but to assess continuous driving performance (after t_{max}), which explains why no effect was observed. The other three studies concerned compounds that aim to modulate neuronal processes in the brain at the longer term instead of in the acute phase, which might explain why no statistically significant effect was observed in the single dose clinical studies.

The EMA guideline on how to determine the starting dose for a FIH study was updated after the TGN1412 study. In this new guideline, published in 2007, it was recommended to base the starting dose not only on the NOAEL, but also include the MABEL.[8] In our sample of FIH studies performed between 2003 and 2019, in 58% of the included studies the starting dose for the clinical study was based on preclinical safety experiments (NOAEL) only. One of these studies was performed prior to 2007. This percentage is in line with other publications reporting that the NOAEL-based approach is still the most common method to determine the starting dose for a FIH study.[11-13] In line with previous research, our data show that important details of animal studies are poorly reported in IBs.[17] In none of the IBs blinding or randomisation of the preclinical experiments was reported and most lacked important information, such as animal sex or route of administration. PK measurements were often missing for animal models of behaviour or disease. Although regulatory guidelines do not require the reporting of PK-analyses in each preclinical study, the translation to effective human dose ranges is not possible without exposure data. Since poor reporting of

study design is often associated with an overestimation of efficacy outcomes, it means that ethics committees and other regulatory bodies could be allowing first-in-humans trials to start on the basis of spurious results.[17,20]

For only six of the 25 compounds therapeutic efficacy study results were reported. For three of those, the therapeutic effective ranges in patients were lower than the preclinically pharmacologically active range, but in all cases, there was a good overlap between the pharmacological effect ranges in healthy volunteers and the therapeutic dose range. This relatively high translatability is contradictory to existing literature reporting high failure rates of translation of new investigational compounds that seem effective in preclinical experiments, but fail in clinical therapeutic studies.[21] These findings may be biased to some extent as the decisions to advance these compounds to clinical trials in patients relied on consistent results from the preclinical and human phase I studies. Next to that, phase I studies often solely focus on tolerability, safety and pharmacokinetics instead of also including relatively basic human pharmacological characteristics of new compounds such as blood brain barrier penetration, as done in included studies.[22] Another factor often cited as a cause for the high attrition rate in CNS drug development is the limited knowledge on receptor occupancy.[23] A possible solution to this problem is to perform more PET studies to study the receptor occupancy.[23] The high attrition rate in CNS drug development can also be explained by poorly understood human disease as psychiatric disorders are usually diagnosed based on a cluster of symptoms instead of a biological basis.[24] This leads to several problems, such as the animal model being a mismatch or simplification of the human disease. There are current initiatives to overcome these problems, such as the Research Domain Criteria (RDoC) initiative introduced in 2009, which aims to more precisely link treatment targets to dysfunctional mechanisms relevant to clinical manifestations.[24] By doing so, biomarkers aiming to characterise the pharmacological activity of novel compounds in early phase clinical trials are being developed as recommended in several publications.[19,22,25]

While the trends identified in our study are worth investigating, the limited number, diversity and non-randomness (only studies in our own research institute were included) of the included studies make our findings suggestive rather than confirmatory. We performed this overview solely with IBs of drugs for which at least two (pharmacodynamically) active doses were identified in phase I trials to allow a comparison between animal and human ranges. The lack of a dose range meant that we excluded six studies because preclinical efficacy was only established at one dose level or there was no preclinical *in vivo* efficacy data and ten studies because clinical pharmacodynamics was only established at one dose level or there was no clinical pharmacodynamic data (Figure 2). As such, these data cannot be used to compare the HED, C_{max} and AUC regarding their ability to predict the presence of an effect in humans. Next to that, we used linear inter- and extrapolation to determine missing pharmacokinetic parameters in animals. Although such a strategy is common practice, it might lead to prediction inaccuracies for drugs with a non-linear pharmacokinetic profile, and PK/PD-based analyses might have been more reliable (albeit unfeasible owing to the lack of data in many cases). Also, the analysis was limited to studies of unregistered compounds mostly in healthy volunteers. Despite these limitations, our sample is likely representative for IBs in practice. IBs are all investigators have at their disposal when they study the pharmacokinetics, pharmacodynamics and tolerability of a new CNS active compound.

In this report, we applied the IB-Derisk tool on a selection of 25 IBs and compared the predictions of tolerable and pharmacologically active dose ranges based on preclinical data to the results of clinical studies. The results demonstrate that tolerable and pharmacologically active dose ranges in clinical studies can be reasonably well predicted from preclinical data. Tolerability was best predicted by C_{max} and pharmacologically active ranges by HED or AUC. We noted that despite recommendations by the EMA to base the starting dose on both NOAEL and MABEL, the starting dose is often solely based on the NOAEL. In line with current literature,[17] internal validity of preclinical experiments was poor and preclinical *in vivo* CNS experiments are often performed without reporting PK results. The translation of preclinical to clinical studies would benefit from complete and comparable reporting of PK measurements of both toxicity and efficacy experiments. This report further demonstrates that an integrated presentation of the contents of the IB, such as provided by the IB-Derisk tool, can improve translatability of preclinical to clinical data. [10,14,16]

ACKNOWLEDGMENTS: We thank Michiel van Esdonk for his support in designing Figures 3 and 4. In addition, we would like to thank the reviewers for their valuable comments and suggestions, which helped us improve the readability and quality of the manuscript.

CONFLICT OF INTEREST: DVG reports personal fees from Danone Nutricia Research B.V. and MSD Animal Health outside of the submitted work. GSF reports personal fees from Merck KGaA, Curare Consulting B.V., Access to Medicine Foundation, 3D PharmXchange B.V. and Janssen Biologics B.V. outside of the submitted work. None of the other authors has any conflict of interest.

REFERENCES

1 Arrowsmith J. Phase II failures: 2008–2010. *Nat Rev Drug Discov*. 2011;10(5):328-329 doi:10.1038/nrd3439
2 Arrowsmith J. Trial watch: phase III and submission failures: 2007-2010. *Nat Rev Drug Discov*. 2011;10(2):87. doi:10.1038/nrd3375
3 Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. *Nat Biotechnol*. 2014;32(1):40-51. doi:10.1038/nbt.2786
4 Hwang TJ, Carpenter D, Lauffenburger JC, Wang B, Franklin JM, Kesselheim AS. Failure of Investigational Drugs in Late-Stage Clinical Development and Publication of Trial Results. JAMA *Intern Med*. 2016;176(12):1826-1833. doi:10.1001/jamainternmed.2016.6008
5 Cross J, Lee H, Westelinck A, Nelson J, Grudzinskas C, Peck C. Postmarketing drug dosage changes of 499 FDA-approved new molecular entities, 1980-1999. *Pharmacoepidemiol Drug Saf*. 2002;11(6):439-446. doi:10.1002/PDs.744
6 van der Doef TF, Zaragoza Domingo S, Jacobs GE, Drevets WC, Marston HM, Nathan PJ, et al. New approaches in psychiatric drug development. *Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol*. 2018;28(9):983-993. doi:10.1016/j.euroneuro.2018.06.006
7 O'Donnell P, Rosen L, Alexander R, Murthy V, Davies CH, Ratti E. Strategies to Address Challenge in Neuroscience Drug Discovery and Development. *Int J Neuropsychopharmacol*. 2019;22(7):445-448. doi:10.1093/ijnp/pyz027
8 European Medicines Agency, 2018. Guideline for good clinical practice E6 (R2). London. ema/chmp/ich/135/1995
9 Langhof H, Chin WWL, Wieschowski S, Federico C, Kimmelman J, Strech D. Preclinical efficacy in therapeutic area guidelines from the U.S. Food and Drug Administration and the European Medicines Agency: a cross-sectional study. *Br J Pharmacol*. 2018;175(22):4229-4238. doi:10.1111/bph.14485
10 European Medicines Agency, 2017. Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal compounds. London2017;emea/chmp/.
11 Baldrick P, Reichl A. Nonclinical & clinical interface - extrapolation of nonclinical data to support Phase I clinical studies. *Regul Toxicol Pharmacol*. 2021;121:104869. doi:https://doi.org/10.1016/j.yrtph.2021.104869
12 Kenter MJH, Cohen AF. Establishing risk of human experimentation with drugs: lessons from TGN1412. *Lancet*. 2006;368(9544):1387-1391. doi:https://doi.org/10.1016/S0140-6736(06)69562-7
13 Rocha J-F, Santos A, Gama H, Moser P, Falcao A, Pressman, et al. Safety, Tolerability, and Pharmacokinetics of FAAH Inhibitor BIA 10-2474: A Double-Blind, Randomized, Placebo Controlled Study in Healthy Volunteers. *Clin Pharmacol Ther*. 2022;111(2):391-403. doi:10.1002/cpt.2290
14 Cohen AF, van Smeden J, Webb DJ. De-risking Clinical Trials: The Bial Phase I Trial in Foresight. *Clin Pharmacol Ther*. 2022;111(2):362-365. doi:10.1002/cpt.2498
15 Baakman AC, Zuiker R, van Gerven JMA, Gross N, Yang R, Fetell M, et al. Central nervous system effects of the histamine-3 receptor antagonist CEP-26401, in comparison with modafinil and donepezil, after a single dose in a cross-over study in healthy volunteers. *Br J Clin Pharmacol*. 2019;85(5):970-985. doi:10.1111/bcp.13885
16 van Gerven J, Cohen A. Integrating data from the Investigational Medicinal Product Dossier/investigator's brochure. A new tool for translational integration of preclinical effects. *Br J Clin Pharmacol*. 2018;84(7):1457-1466. doi:10.1111/bcp.13529
17 Wieschowski S, Chin WWL, Federico C, Sievers S, Kimmelman J, Strech D. Preclinical efficacy studies in investigator brochures: Do they enable risk-benefit assessment? PL*oS Biol*. 2018;16(4):e2004879. doi:10.1371/journal.pbio.2004879
18 FDA. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. Published 2005. Accessed January 28, 2021. https://www.FDA.gov/media/72309/download
19 Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood–brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol*. 2016;20:27-34. doi:http://dx.doi.org/10.1016/j.ddtec.2016.07.004
20 Perel P, Roberts I, Sena E, Wheble P, Briscoe C, Sandercock S, et al. Comparison of treatment effects between animal experiments and clinical trials: systematic review. BMJ. 2007;334(7586):197. doi:10.1136/bmj.39048.407928.BE
21 van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, et al. Can animal models of disease reliably inform human studies? PL*oS Med*. 2010;7(3):e1000245. doi:10.1371/journal.pmed.1000245
22 Cohen AF, Burggraaf J, van Gerven JM, Moerland M, Groeneveld GJ. The use of biomarkers in human pharmacology (Phase I) studies. *Annu Rev Pharmacol Toxicol*. 2015;55:55-74. doi:10.1146/annurev-pharmtox-011613-135918
23 Griebel G, Holsboer F. Neuropeptide receptor ligands as drugs for psychiatric diseases: the end of the beginning? *Nat Rev Drug Discov*. 2012;11(6):462-478. doi:10.1038/nrd3702
24 Sanislow CA, Ferrante M, Pacheco J, Rudorfer MV, Morris SE. Advancing Translational Research Using NIMH Research Domain Criteria and Computational Methods. *Neuron*. 2019;101(5):779-782. doi:https://doi.org/10.1016/j.neuron.2019.02.024
25 Cai HL, Jiang P, Tan QY, Dang RL, Tang MM, Xue Y, et al. Therapeutic efficacy of atypical antipsychotic drugs by targeting multiple stress-related metabolic pathways. *Transl Psychiatry*. 2017;7(5):e1130. doi:10.1038/tp.2017 94
26 Centre for Human Drug Research. TRC Pharmacology. Published 2022. https://trc-p.nl
27 European commision health and consumers directorate-general, 2011. The rules governing medicinal products in the European Union Volume 10 - Guidance documents applying to clinical trials guidance on investigational medicinal products (IMPs) and 'non investigational medicinal products'(NIMPs) (rev. 1, March 2011). Bruxelles. https://health.ec.europa.eu/system/files/2016-11/dir_2001_20_en_0.PDf
28 Mishra A, Sarangi SC, Reeta K. First-in-human dose: current status review for better future perspectives. Eur J Clin Pharm. 2020;76:1237-1243. https://doi.org/10.1007/s00228-020-02924-x

CHAPTER III

ADMINISTRATION OF OXATHRIDINE, A FIRST-IN-CLASS HISTAMINE-3 RECEPTOR PARTIAL AGONIST IN HEALTHY MALE VOLUNTEERS: CENTRAL NERVOUS SYSTEM DEPRESSION AND PSEUDO-HALLUCINATIONS

Dijkstra, F. M.[1,2], Zuiker R.G.J.A.[1,2], Heuberger J.A.A.C.[1], Kanhai K.M.S.[1,2], de Kam M.[1], Duvauchelle T.[3], Lecomte J.M [3], Labeeuw O.4, Landais L.[4], Ligneau X.[4], Robert P.[4], Capet M.[4], Schwartz J.C.[3], van Gerven J.M.A.[1,2]

British Journal of Clinical Pharmacology (2023), 90(1), 321–335.
https://doi.org/10.1111/bcp.15910

1 Centre for Human Drug Research, the Netherlands
2 Leiden University Medical Center, Leiden, the Netherlands
3 Bioprojet Pharma, Paris, France
4 Bioprojet Biotech, Saint Gregoire, France

ABSTRACT

AIMS To characterise the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of single ascending doses of oxathridine, a first-in-class histamine-3 receptor partial agonist, in healthy male volunteers.

METHODS A randomised, double-blind, placebo-controlled study including the NeuroCart, consisting of a battery of drug sensitive neurophysiological tests, was performed. Oxathridine was administered orally as an aqueous solution. After dosing, safety and NeuroCart tests (adaptive tracking [AT], body sway [BS], saccadic peak velocity [SPV], smooth pursuit [SP] eye movements, VAS according to Bond and Lader, VAS according to Bowdle [VAS B&L, Bowdle], pharmaco-electroencephalogram [PEEG], Sustained Attention to Response Task [SART]) were performed at set times.

RESULTS Forty volunteers completed the study. Given doses were: 0.5, 2.5, 5, 0.25 and 1.5 mg. At 5 mg, unacceptable and unanticipated adverse events (AEs) of (orthostatic) hypotension and pseudo-hallucinations were reported. Statistically significant effects ([CI]; p-value) of 2.5 mg and 5 mg oxathridine were observed on AT ([-8.28, -1.60]; p=0.0048), ([-8.10, -1.51]; p=0.00530), BS ([0.6, 80.2]; p=0.0455), ([5.9, 93.1]; p=0.0205) and SPV ([-59.0, -15.9]; p=0.0011), ([-43.9, -1.09]; p=0.0399), respectively. Oxathridine 5 mg significantly increased all three VAS Bowdle subscale scores; VAS external ([0.183, 0.476]; p=<.0001), VAS internal ([0.127, 0.370]; p=0.0001) and VAS feeling high ([0.263, 0.887]; p=0.0006).

CONCLUSIONS NeuroCart tests indicated central nervous system (CNS) depressant effects. Oxathridine also unexpectedly caused pseudohallucinations. Although this led to the decision to stop further development of oxathridine, these observations suggest that the H3R system could be an interesting new target for the development of novel antipsychotics.

INTRODUCTION

Since its discovery in 1983, the histamine-3 receptor (H3R), has been a target of interest for central nervous system (CNS) drug development.[2-4] The H3R is an autoreceptor modulating histamine synthesis and release.[2,3] It also functions as a heteroreceptor regulating the release of important other neurotransmitters, such as serotonin, acetylcholine, noradrenaline and dopamine.[4] H3Rs are primarily expressed in the central nervous system (CNS) in brain regions associated with cognition, pain, sleep and homeostatic regulation, such as the cerebral cortex, hippocampal formation, basal ganglia and hypothalamus.[4] Although there have been several studies with investigational compounds targeted at the H3R, such as cipralisant, to date pitolisant, an H3R antagonist/inverse agonist, is the only drug targeted at the H3R that the EMA and FDA have approved.[5,6] Pitolisant is registered for treating excessive daytime sleepiness and cataplexy in adults with narcolepsy and to improve wakefulness and reduce excessive daytime sleepiness in adults with obstructive sleep apnoea (OSA).[5,7]

Oxathridine or 4-(1*H*-imidazol-4-ylmethyl)-pyridine sesquioxalate, is a highly selective partial agonist of the H3R with high potency (EC50=1.5nM) and intrinsic activity (0.7). Oxathridine behaves as a full agonist *in vivo*, inhibiting brain histaminergic neuron activity at low oral doses, and is therefore regarded as first-in-class. Preclinical studies demonstrated that oxathridine easily crosses the blood brain barrier (BBB) (data on file, supplementary material). In animal models of sleeping disorders, oxathridine had sleep promoting effects, without overt sedative reactions or anxiolytic properties as observed with GABAergic compounds. Preclinical safety experiments demonstrated a favourable effect profile. At high dose levels, undesired effects were observed starting with reduced arterial blood pressure and at higher doses piloerection and increased reactivity to touch and at the highest given doses initial decreased activity followed by increased activity, increased reactivity to touch and stereotypies. It was further noticed that at relatively low exposure levels monkeys demonstrated a change in behaviour with accepting and looking for human contact. The preclinical data supported further development of oxathridine, and a first in human (FIH) study was set up.

The starting dose for the FIH study was set at 0.5 mg (0.007 mg/kg for a 70 kg individual), which was more than 70 times lower than the no observed adverse effect level (NOAEL) in the most sensitive species (dogs) (data on file). This NOAEL was based on a telemetry study which demonstrated increased heart rate and decreased arterial blood pressure at a dose of 1 mg/kg with a maximum exposure (C_{max}) of 609 ng/mL/h, corresponding to a human equivalent dose (HED) of 0.54 mg/kg. Although a minimum anticipated biological effect level (MABEL) or pharmacologically active dose (PAD) was not formally established, at HED values of 0.024 mg/kg brain

histamine neuron activity in mice was significantly reduced as measured by decreased levels of the main metabolite of histamine, Nτ-methylhistamine (data on file). In cats, desirable effects on sleep were observed from C_{max} values of 30 ng/mL/h and higher. Therefore, pharmacological activity could be expected from HED values of 0.024 mg/kg (1.68 mg for a 70 kg individual) and C_{max} values of 30 ng/mL/h. A dose range was selected, which was expected to show significant pharmacological and functional effects, and to explore the large safety window that was also observed preclinically.

In addition to assessing tolerability, safety and pharmacokinetics in this FIH study, pharmacodynamics were explored using the NeuroCart.[8] The NeuroCart consists of a battery of drug-sensitive neurophysiological tests and has been applied to a broad spectrum of CNS active drugs, making it possible to compare the effect profile of a novel compound on the different NeuroCart tests, to known profiles of other compounds.[8] By doing so, the pharmacological characteristics of a novel compound can be mapped and held against predictions based on preclinical data in an early phase of clinical drug development.[8] Oxathridine was the first partial H3R agonist to be tested on the NeuroCart, so it was not yet known which NeuroCart test would be sensitive to oxathridine. Therefore NeuroCart tests sensitive to the sedative effects of GABAergic agonists were selected, such as visual analogue scales, saccadic eye movement measurements and adaptive tracking.[9-11]

Overall, this FIH study aimed to assess the pharmacokinetics, safety, tolerability and pharmacodynamic effects of single ascending doses of oxathridine in healthy male volunteers.

METHODS

GENERAL

The study was performed according to ICH GCP guidelines as laid down in the Declaration of Helsinki and its latest amendments. The Stichting Beoordeling Ethiek Biomedisch Onderzoek (BEBO), Assen, the Netherlands approved the study, and the study was registered at ToetsingOnline under number NL44541.056.13. Bioprojet Pharma sponsored the study, and the study was conducted at the Centre for Human Drug Research (CHDR), Leiden, the Netherlands. All volunteers gave written informed consent prior to the study start.

STUDY POPULATION

Healthy male volunteers between 18 and 45 years of age at screening were included. Health status was assessed by medical history, laboratory assessments and physical examination. Volunteers with a history or clinical evidence of alcohol or drug abuse within the 3 years prior to screening were excluded. Volunteers were not allowed to use any prescribed medications or over-the-counter medications within two weeks prior to the first study drug administration except for paracetamol (maximum 1g/day).

STUDY DESIGN

This was a randomised, double-blind, placebo-controlled, single ascending dose study. The study consisted of five cohorts of eight volunteers each (active/placebo ratio: 6:2). Before escalating to the next dose level, a blinded interim safety review, consisting of safety data (adverse events (AEs), ECGs, laboratory tests, vital signs), pharmacodynamic (PD) data and pharmacokinetic (PK) data of the first 24 hours after dosing was performed. In the first group, a sentinel scheme was used: two volunteers were dosed on the first day (active/placebo ratio: 1:1) and on the second day, the remaining six volunteers were dosed.

The study consisted of an inpatient study visit, a medical screening within three weeks prior to admission and a follow-up visit a week after discharge from the clinical unit. At check-in of the study visit an eligibility check consisting of concomitant medication use and AE review, body weight and height measurement, urine drug screening, alcohol breath test, 12-lead-ECG, vital signs and body temperature measurement was performed. During the dosing day (Day 1), volunteers were dosed in the morning, and throughout the day, safety, PK and PD measurements were performed at set times. Volunteers were discharged about 24 to 27 hours after dosing (Day 2).

TREATMENTS

Treatments consisted of 25 ml oxathridine solution or a matching placebo for oral administration. The solution was administered with purified water and blackcurrant syrup to a volume of 100 mL for masking purposes. Planned dose levels were 0.5, 2.5, 10, 25, 40, 60, 80 and 100 mg. Volunteers were in a fasted state from 10 hours prior to dosing and were allowed to eat from three hours after dosing. Volunteers were allowed to drink water ad libitum, except for one hour before and two hours after dosing when drinking water was not allowed.

PHARMACOKINETIC ASSESSMENTS

Blood samples for PK measurements were collected prior to dosing (1 sample) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, 12 and 24 hours after dosing. Serum oxathridine concentrations were measured using the validated analytical UPLC/MS-MS method in the Bioprojet Biotech Laboratory in accordance with the guideline EMEA/CMPH/EWP/192217/2009 Rev. 1 and the rules of Good Laboratory Practice. The lower limit of quantification (LLOQ) was 0.1 ng/mL. At the lower limit of quantification, the intraassay coefficient of variation (CV) was 10.8 % and the interassay CV was 12.4 %, respectively.

TOLERABILITY AND SAFETY ASSESSMENTS

After dosing, the following safety assessments were performed at set times throughout the study day: vital signs, physical examination, laboratory tests consisting of biochemistry, haematology and urinalysis and 12-lead ECG measurements.

PHARMACODYNAMIC ASSESSMENTS

NeuroCart measurements consisted of adaptive tracking, body sway, saccadic eye movements, smooth pursuit eye movements, VAS according to Bond and Lader, VAS according to Bowdle, and pharmaco-electroencephalogram (PEEG) and the Sustained Attention to Response Task (SART). The measurements were performed twice prior to dosing and were then repeated at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 22 hours after dosing. In addition, the Visual Verbal Learning Test (VVLT) was performed 3 hours after dosing (immediate recall) and 6 hours after dosing (recognition and delayed recall).

As part of the screening visit, volunteers underwent a NeuroCart test training to prevent learning effects during study conduct. During all test sessions, there was only one volunteer in each room, illumination settings were standardised between the rooms. When performing the tests, volunteers were comfortably seated behind a computer screen, except during body sway measurements, when they were standing.

ADAPTIVE TRACKING

The adaptive tracking test was performed as described initially by Borland and Nicholson,[12] using customised equipment and software (based on TrackerUSB hard-/software (Hobbs, 2004, Hertfordshire, UK)). During the test, a dot inside a circle is randomly moving on a screen. Volunteers are instructed to try to keep the dot inside the circle by operating a joystick. If the volunteer succeeds in this task, the speed of the moving circle increases, conversely, the speed of the circle decreases if the volunteer fails at the task. The outcome of the test is the average speed of the moving circle as a percentage of the maximum speed of the circle over a 3.5-minute period.

BODY SWAY

Body sway is a measure of postural stability, during measurements, volunteers are instructed to stand as still as possible with closed eyes. Body sway was performed as previously described by others.[13,14] The anteroposterior body sway was measured using a body sway meter based on the Wright ataxiameter.[15] All body movements over a 2-minute period were integrated and expressed as millimetres of sway and recorded.

SACCADIC EYE MOVEMENTS

The primary outcome of saccadic eye movement measurements was saccadic peak velocity (SPV) in degrees per second (deg/s). During the test, volunteers were instructed to follow a dot jumping approximately 15 degrees to either side on a computer screen with their eyes, while head movements were restrained using a fixed head support at 58 cm from the computer screen. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Saccadic eye movements were recorded using a computer-based system, customised Cambridge Electronics Design software for data sampling and analysis (Cambridge Electronics Design, Cambridge UK), and disposable surface electrodes for registration of the electro-oculographic signals (Medicotest N-00-S, Olstykke, Denmark).

SMOOTH PURSUIT EYE MOVEMENTS

For smooth pursuit eye movements, the target moves sinusoidally at frequencies ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5 degrees eyeball rotation to both sides. Four cycles for each stimulus frequency were recorded. The time during which the eyes are in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as a parameter.

PHARMACO EEG (PEEG)

Each EEG measurement duration was two minutes. EEG recordings were made using four gold electrodes, fixed with EC2 paste at Fz, Cz, Pz and Oz, with the common ground electrode for the eye movement registration (international 10/20 system). The electrode resistance was kept below 5 kOhm. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements. The signals were amplified with a time constant of 0.3 seconds and a low pass filter at 100 Hz. Data collection and analysis were performed using customised CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). Per session eight consecutive blocks of eight seconds were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK) and stored on hard disk for subsequent analysis. Data blocks containing artefacts were identified by visual inspection and excluded from the analysis. For each lead, a fast Fourier transform analysis was performed to obtain the sum of the amplitudes in the very low (0.5-2 Hz), delta- (2-4 Hz), theta (4-7.5 Hz), alpha (7.5-13.5Hz), beta (13.5-35 Hz) and gamma (35-48.9 Hz) frequency ranges.

VAS ACCORDING TO BOND AND LADER AND VAS BOWDLE

VAS in this study were used as originally described by Norris.[16] We used Dutch versions of the scales that have been frequently used at our research institute.[8] For VAS Bond and Lader, volunteers, indicate (with vertical marks) on sixteen horizontal

100-mm VAS how they feel. From these measurements, three main factors were calculated; subjective alertness' (from nine scores), 'contentedness or mood' (from five scores) and 'calmness' (from two scores).[17]

VAS Bowdle evaluates psychedelic effects with thirteen 10 cm VAS lines ranging from 0 (not at all) to 100 mm (extremely).[18] From these scores, three sum scores were calculated; 'internal perception' (reflects inner feelings that do not correspond with reality, including mistrustful feelings), 'external perception' (reflects a misperception of an external stimulus or a change in the awareness of the volunteer's surroundings) and 'feeling high.'[18]

VISUAL VERBAL LEARNING TEST

The VVLT tests the whole scope of learning behaviour, i.e. acquisition, consolidation, storage and retrieval.[19] Volunteers were presented with 30 words in three consecutive word trials. At each of these trials, an immediate recall was performed. Delayed recall was assessed six hours after dosing. Immediately after delayed recall, a recognition test was performed, consisting of 15 previously presented words and 15 new words in which the volunteer had to verbally indicate recognition of the word as quickly as possible. An operator behind a computer screen recorded the volunteer's response by clicking on the named word in a list with all 30 presented words and clicking on a bar stating 'different word' if an unlisted word was mentioned. Words mentioned twice or more were recorded as duplications. After three trials, a script automatically counted the scores per trial as: number correct, number incorrect, number double. If a correct word was mentioned twice, the overall score included 1 correct response and 1 double response. Similarly, an incorrect word, which was mentioned twice, was scored as 1 incorrect response and 1 double response

SUSTAINED ATTENTION TO RESPONSE TASK (SART)

This test was performed as a measurement of improved cognitive functioning, as H3R antagonists are hypothesised to have beneficial effects on cognition.[4] The SART is similar in many respects to a standard vigilance task, in that a single infrequent target is presented amongst a background of frequent non-targets. Unlike a traditional vigilance task, however, the volunteer is required to push the space bar to the non-target and inhibit their response to the target. To perform this task correctly, the volunteer must remain sufficiently attentive to their responses, such that at the appearance of a target they can substitute the directly antagonistic response.[20]

STATISTICAL ANALYSIS

Statistical analyses were performed using the SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA). The repeatedly measured PD endpoints were analysed separately by mixed model analyses of covariance (ANCOVA) with treatment-and-dose, time, and treatment-and-dose by time as fixed effects, subject as random effect, and with the (average) baseline value as covariate. Baseline was defined as the average of the two measurements performed prior to dosing. Measurements of VAS Bowdle, all EEG parameters, body sway and SART total omission errors were logarithmically transformed (after 0 was changed to 0.01). Log-transformed parameters were back-transformed after analysis where the results may be interpreted as percentage change.

Contrasts for all dose levels of oxathridine versus placebo were calculated within the model up to the 3-hour measurement after dosing. This timepoint was based on post hoc inspection of the time profiles of the concentrations in this clinical study, showing that for all doses a 3-hour period covered most of the exposure. Assuming that the PD effects would be closely related to the PK profile, this analysis was considered most relevant.

The VVLT endpoints were analysed separately by mixed model analyses of variance (ANOVA) with treatment-and-dose as fixed effect. Treatment effects are reported as the contrasts specified below where the average of the measurements up to last time point will be calculated within the statistical model.

For all NeuroCart tests contrasts are reported along with 95% confidence intervals and analyses are two-sided with a significance level of 0.05.

PK calculations were performed using R (v2. 12.0, R Core Team). Standard non-compartmental methods were used in the calculations. Data below the limit of quantification before t_{max} was replaced with zero. Data below the limit of quantification after t_{max} was replaced with not applicable, i.e., excluded from analysis.

RESULTS

SUBJECT DISPOSITION

In total 40 healthy male volunteers participated; all completed the study. The cohorts were comparable with regards to age, weight, height, and BMI (Table 1).

PHARMACOKINETICS

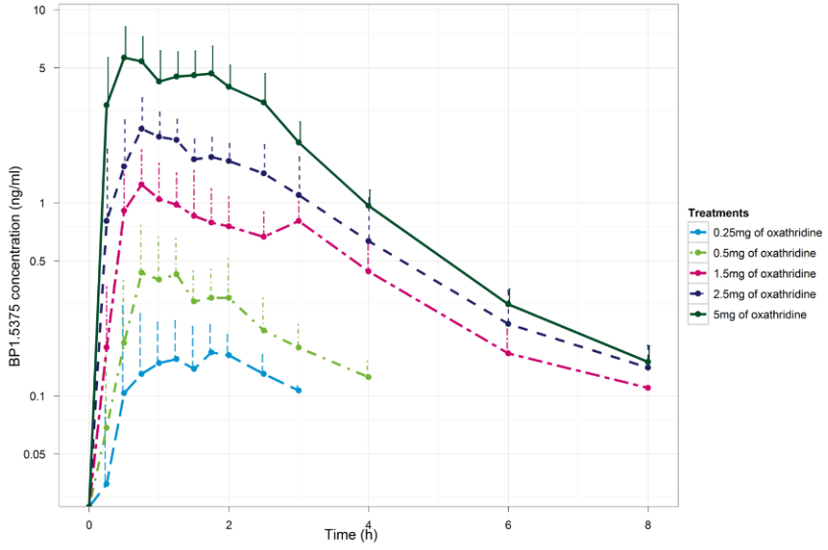
Maximum median serum concentrations were reached at about 1.00 to 1.26 hours after dosing across the dose levels (Table 2, Figure 1). The median half-life of oxathridine varied between 1.20 hours and 1.45 hours across the dose levels. Exposure to oxathridine increased more than dose proportionally in the higher dose range.

Table 1 Demographics

Demographic variables	Placebo N=10	Oxa 0.25 mg N=6	Oxa 0.5 mg N=6	Oxa 1.5 mg N=6	Oxa 2.5 mg N=6	Oxa 5 mg N=6
AGE, YEARS						
Mean	23.7	21.0	22.5	24.0	23.3	22.8
SD	3.9	3.1	2.6	4.6	3.9	2.4
Min, max	20-32	18-25	18-26	19-31	19-28	20-26
HEIGHT (CM)						
Mean	183.6	181.1	182.7	183.8	186.0	186.8
SD	5.0	9.2	6.7	8.7	11.2	4.0
Min, max	175.8 – 192.3	171.8 – 195.0	175.9 – 191.4	170.0 – 191.7	174.4 – 204.2	181.6 – 191.1
WEIGHT (KG)						
Mean	77.7	73.1	79.4	71.9	73.8	76.8
SD	8.4	10.9	11.3	9.0	11.1	5.6
Min, max	67.7 – 94.9	61.7 – 84.9	68.4 – 96.5	61.2 – 86.2	60.2 – 85.8	68.5 – 82.8
BMI (KG/M^2)						
Mean	23.1	22.4	23.7	21.3	21.2	22.0
SD	1.9	3.7	2.3	1.6	1.3	1.0
Min, max	19.9 – 26.8	18.3 – 27.2	21.5 – 26.8	19.6 – 24.1	19.8 – 22.9	20.8 – 23.0

Abbreviations: Oxa =oxathridine, SD = standard deviation, Min, max =minimum, maximum

Figure 1 Pharmacokinetics of oxathridine



Abbreviations: BP1.5375 = oxathridine

Table 2 Pharmacokinetics of oxathridine

Oxathridine													
	C_{max} (ng/mL)			AUC_{0-24} (ng/mL*h)			$AUC_{0-\infty}$(ng/mL*h)			T_{max} (h)		$T_{½}$ (h)	
Dose (mg)	Mean range	SD	Mean/ dose	Mean range	SD	Mean/ dose	Mean range	SD	Mean/ dose	Median	Range	Median	Range
0.25 (n=6)	0.22 (0.12; 0.47)	0.13	0.88	0.29 (0.04; 0.65)	0.23	1.16	0.57 (0.35; 0.79)	0.21	2.28	1.13	0.12 – 0.47	1.22	0.93 – 1.50
0.5 (n=6)	0.5 (0.23; 0.92)	0.28	1.00	0.92 (0.37; 1.78)	0.51	1.84	1.20 (0.64; 1.95)	0.46	2.40	1.26	0.75 – 1.78	1.45	0.94 – 2.33
1.5 (n=6)	1.4 (0.73; 1.81)	0.48	0.93	3.55 (2.42; 4.54)	0.77	2.37	3.83 (2.66; 4.76)	0.77	2.55	1.15	0.75 – 3.00	1.20	1.14 – 1.42
2.5 (n=6)	2.73 (1.22; 3.69)	0.87	1.09	6.54 (3.44; 9.63)	2.21	2.62	6.84 (3.69; 9.96)	2.24	2.74	1.12	0.75 – 2.00	1.29	0.83 – 1.47
5 (n=6)	7.12 (4.32; 10.05)	1.99	1.42	14.97 (12.81; 19.45)	2.33	2.99	15.26 (13.07; 19.79)	2.36	3.05	1.00	0.50 – 2.00	1.31	1.15 – 1.51

Abbreviations: C_{max}: maximum concentration, AUC: area under the curve, SD: standard deviations

TOLERABILITY AND SAFETY

An overview of AEs by dose group is provided in Table 3. All observed AEs were self-limiting. There were no serious AEs in the study. Doses could not be escalated as planned due to safety concerns about subjective effects. In cohort 1 (0.5 mg), one AE of somnolence was reported and classified as mild. As this dose was well tolerated and the next planned dose of 2.5 mg was still more than 10 times lower than the HED of the NOAEL and had a predicted C_{max} which was far below the exposure observed at the NOAEL in the most sensitive species, it was decided to escalate the dose to 2.5 mg as initially planned. In cohort 2 (2.5 mg), all volunteers who received oxathridine reported one or more AEs. The most frequently reported AEs were nausea, fatigue, somnolence, blurred vision, dizziness, and orthostatic hypotension. All these AEs were mild, whereas two AEs of balance disorder and dizziness, were reported as moderate. Because of these observations, the dose for the next cohort was escalated to 5 mg instead of the planned 10 mg.

At a dose of 5 mg, all volunteers who received oxathridine reported one or more adverse events. The most frequent events were dizziness and visual (pseudo)-hallucinations. For this study, it was chosen to use the term 'pseudo-hallucination' to describe the AEs experienced by the volunteers, as in contrast to 'typical hallucinations' the volunteers reported that they were aware that the perceived images were not real and that they could change the content of the visual phenomena.[21]The pseudo-hallucinations started on average 40 minutes after dosing and lasted 50 minutes up to two hours, in all volunteers, the effects disappeared completely without treatment and did not reoccur. One volunteer had orthostatic hypotension and fainted about 30 minutes after dosing and felt light-headed for the following three hours. Three single AEs of nausea, dizziness and syncope were reported as moderate, and the others were reported as mild.

The occurrence of these AEs led to the decision to halt further dose escalation. Two additional cohorts with low dose levels of 0.25 mg and 1.5 mg to better characterise the PD and PK of oxathridine were performed instead.

At a dose level of 0.25 mg, two of the volunteers receiving oxathridine reported AEs. AEs consisted of dizziness, somnolence, feeling of relaxation and paraesthesia, which all were of mild severity.

All volunteers receiving a dose of 1.5 mg oxathridine reported one or more AEs. The most frequent reported AEs were nausea, vision blurred, dizziness and hypotension. Four events of dizziness, two events of hypotension and one of orthostatic hypotension were reported as moderate, and the others were reported as mild. No pseudo-hallucinations were reported with these additional low doses.

As expected with AEs of dizziness, hypotension, and orthostatic hypotension, decreases in supine and standing blood pressure, mostly diastolic, were observed with dose levels of 1.5 mg oxathridine or higher (supplementary material). A compensatory increase in standing heart rate was observed.

There were no clinically relevant changes in laboratory assessments (clinical chemistry, haematology, and urinalysis). Also, there were no clinically relevant observations on any ECG parameters.

PHARMACODYNAMICS

A dose-dependent decrease in performance on adaptive tracking was observed, which was statistically significant for the two highest dose levels (Table 4). At these dose levels, a statistically significant increase in body sway was observed as well (Table 4). Additionally, a decrease in SPV and increased saccadic inaccuracy were observed with these dose levels (Table 4). In the highest dose group of 5 mg oxathridine, statistically significant effects were observed on all three VAS Bowdle subscales (VAS external, VAS internal and VAS feeling high) (Table 5, Figure 2. In the lowest dose levels (0.25 and 0.5 mg), oxathridine decreased the reaction time of correct responses of the delayed word recognition task of the VVLT (Table 5). No statistically significant effects were observed on the other NeuroCart measurements.

Table 3 AEs per dose group

System Organ Class / Preferred Term	Number (%) of subjects					
	Placebo n = 10	Cohort 4 Oxa 0.25 n = 6	Cohort 1 Oxa 0.5 n = 6	Cohort 5 Oxa 1.5 n = 6	Cohort 2 Oxa 2.5 n = 6	Cohort 3 Oxa 5.0 n = 6
Any event	2 (20)	2 (33)	1 (16.7)	6 (100)	6 (100)	6 (100)
EAR AND LABYRINTH DISORDERS						
Tinnitus					1 (16.7)	
EYE DISORDERS						
Asthenopia					1 (16.7)	1 (16.7)
GASTROINTESTINAL DISORDERS						
Nausea	1 (10.0)			3 (50.0)	2 (33.3)	2 (33.3)
Vomiting				1 (16.7)		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS						
Asthenia				1 (16.7)		
Fatigue	1 (10.0)				3 (50.0)	1 (16.7)
Feeling abnormal				1 (16.7)		1 (16.7)
Feeling of relaxation		1 (16.7)				
NERVOUS SYSTEMS DISORDERS						
Balance disorder					1 (16.7)	
Disturbance in attention				1 (16.7)		
Dizziness		1 (16.7)			1 (16.7)	
Dysarthria					1 (16.7)	
Headache	1 (10.0)			1 (16.7)	1 (16.7)	
Paraesthesia		1 (16.7)				
Somnolence	2 (20.0)	1 (16.7)	1 (16.7)	1 (16.7)	2 (33.3)	1 (16.7)
Tremor				1 (16.7)		
Vision blurred				2 (33.3)	2 (33.3)	2 (33.3)
PYCHIATRIC DISORDERS						
Disturbance in attention						1 (16.7)
Euphoric mood					1 (16.7)	2 (33.3)
Hallucination, visual						4 (66.7)
VASCULAR DISORDERS						
Dizziness		1 (16.7)		4 (66.7)	3 (50.0)	6 (100.0)
Flushing				1 (16.7)		
Hypotension				3 (50.0)		
Orthostatic hypotension				1 (16.7)	2 (33.3)	
Pallor				1 (16.7)		
Syncope						1 (16.7)

Abbreviations: Oxa: oxathridine

Table 4 PD effects

		Contrasts up to 3 hours Estimate of difference (confidence interval) p-value				
Parameter	**Treatment P-value**	**OXA-0.25 vs Placebo**	**OXA-0.5 vs Placebo**	**OXA-1.5 vs Placebo**	**OXA-2.5 vs Placebo**	**OXA-5 vs Placebo**
Adaptive tracking (%)	0.6422	-.873 (-4.16, 2.409) p=0.5939	-1.28 (-4.58, 2.017) p=0.4368	-2.51 (-5.83, 0.817) p=0.1357	-4.94 (-8.28, -1.60) p=0.0048	-4.81 (-8.10, -1.51) p=0.0053
Body sway (mm)	0.4571	-7.4% (-31.4%, 25.1%) p=0.6099	5.7% (-21.1%, 41.4%) p=0.7047	29.2% (-4.2%, 74.2%) p=0.0915	34.6% (0.6%, 80.2%) p=0.0455	43.0% (5.9%, 93.1%) p=0.0205
Saccadic inaccuracy (%)	0.1670	0.41 (-0.60, 1.42) p=0.4207	-0.19 (-1.20, 0.81) p=0.7043	0.63 (-0.54, 1.79) p=0.2877	2.00 (0.97, 3.04) p=0.0003	1.49 (0.47, 2.52) p=0.0050
Saccadic Peak Velocity (deg/s)	0.3217	3.07 (-18.3, 24.41) p=0.7733	-7.08 (-29.4, 15.25) p=0.5252	-10.8 (-33.8, 12.13) p=0.3487	-37.4 (-59.0, -15.9) p=0.0011	-22.5 (-43.9, -1.09) p=0.0399
Smooth Pursuit (%)	0.4627	2.29 (-2.23, 6.82) p=0.3113	-0.01 (-4.07, 4.04) p=0.9954	0.89 (-3.34, 5.12) p=0.6742	0.27 (-3.77, 4.31) p=0.8928	-0.77 (-4.99, 3.45) p=0.7135
VAS Alertness (mm)	0.1270	-1.89 (-8.73, 4.95) p=0.5812	2.44 (-4.36, 9.25) p=0.4738	-4.43 (-11.6, 2.77) p=0.2235	-0.70 (-8.04, 6.64) p=0.8482	-5.83 (-13.2, 1.50) p=0.1167
VAS Calmness (mm)	0.1938	6.80 (-0.37, 13.98) p=0.0626	-1.05 (-8.93, 6.84) p=0.7897	3.07 (-4.34, 10.49) p=0.4075	2.32 (-5.36, 10.00) p=0.5445	4.82 (-2.56, 12.19) p=0.1944
VAS Mood (mm)	0.2423	2.60 (-3.25, 8.46) p=0.3740	5.38 (-0.46, 11.21) p=0.0700	2.32 (-3.67, 8.31) p=0.4389	1.40 (-4.81, 7.61) p=0.6513	4.51 (-1.53, 10.55) p=0.1394
VAS External log(mm)	0.0031	-.020 (-.168, 0.128) p=0.7850	0.016 (-.127, 0.159) p=0.8213	0.096 (-.049, 0.241) p=0.1914	-.014 (-.156, 0.127) p=0.8376	0.330 (0.183, 0.476) p=<.0001
VAS Internal log(mm)	0.0226	-.006 (-.127, 0.116) p=0.9249	0.075 (-.044, 0.194) p=0.2084	0.087 (-.033, 0.207) p=0.1528	0.051 (-.064, 0.167) p=0.3751	0.249 (0.127, 0.370) p=0.0001
VAS feeling high log(mm)	0.0468	-.156 (-.471, 0.160) p=0.3249	0.049 (-.258, 0.355) p=0.7488	0.013 (-.295, 0.322) p=0.9310	0.014 (-.286, 0.314) p=0.9242	0.575 (0.263, 0.887) p=0.0006
EEG Alpha Fz-Cz (uV)	0.6320	-8.5% (-28.6%, 17.3%) p=0.4739	-14.8% (-33.4%, 9.2%) p=0.1996	0.1% (-23.5%, 31.0%) p=0.9934	-8.5% (-28.8%, 17.5%) p=0.4761	-2.2% (-24.2%, 26.1%) p=0.8581
EEG Alpha Pz-Oz (uV)	0.4700	-17.5% (-45.1%, 24.1%) p=0.3462	-35.2% (-56.4%, -3.6%) p=0.0331	-6.0% (-38.8%, 44.6%) p=0.7753	-26.9% (-51.3%, 9.6%) p=0.1255	-20.0% (-46.5%, 19.5%) p=0.2675
EEG Beta Fz-Cz (uV)	0.4216	10.0% (-14.3%, 41.2%) p=0.4424	-15.3% (-34.0%, 8.5%) p=0.1822	7.4% (-17.5%, 39.9%) p=0.5868	5.4% (-18.0%, 35.5%) p=0.6751	16.6% (-10.0%, 51.0%) p=0.2372
EEG Beta Pz-Oz (uV)	0.6551	-12.6% (-38.5%, 24.2%) p=0.4430	-29.4% (-50.1%, -0.0%) p=0.0498	-13.3% (-40.7%, 26.7%) p=0.4535	-14.2% (-39.8%, 22.3%) p=0.3873	-12.9% (-38.7%, 23.6%) p=0.4296
EEG Delta Fz-Cz (uV)	0.5773	-1.4% (-22.7%, 25.7%) p=0.9072	-10.0% (-29.3%, 14.5%) p=0.3807	13.7% (-12.1%, 47.1%) p=0.3206	-5.3% (-26.0%, 21.3%) p=0.6589	1.9% (-20.7%, 31.0%) p=0.8776
EEG Delta Pz-Oz (uV)	0.5890	-4.1% (-34.0%, 39.4%) p=0.8222	-27.6% (-50.1%, 4.9%) p=0.0858	1.6% (-31.7%, 51.1%) p=0.9366	14.0% (-21.9%, 66.4%) p=0.4887	-0.1% (-31.7%, 45.9%) p=0.9941
EEG Gamma Fz-Cz (uV)	0.6472	14.9% (-9.8%, 46.4%) p=0.2519	-10.6% (-29.8%, 13.7%) p=0.3500	5.8% (-17.8%, 36.2%) p=0.6523	6.6% (-16.5%, 36.2%) p=0.5979	9.0% (-15.2%, 40.2%) p=0.4906
EEG Gamma Pz-Oz (uV)	0.6894	2.7% (-31.3%, 53.6%) p=0.8938	-24.7% (-49.5%, 12.2%) p=0.1581	-2.8% (-36.7%, 49.3%) p=0.8945	8.2% (-27.8%, 62.2%) p=0.6946	14.6% (-24.2%, 73.2%) p=0.5092

		Contrasts up to 3 hours Estimate of difference (confidence interval) p-value				
Parameter	**Treatment P-value**	**OXA-0.25 vs Placebo**	**OXA-0.5 vs Placebo**	**OXA-1.5 vs Placebo**	**OXA-2.5 vs Placebo**	**OXA-5 vs Placebo**
EEG Theta Fz-Cz (uV)	0.6453	11.6% (-13.5%, 43.9%) p=0.3876	-11.9% (-31.6%, 13.4%) p=0.3157	11.9% (-14.3%, 46.2%) p=0.4007	5.4% (-18.3%, 36.1%) p=0.6777	10.5% (-15.3%, 44.0%) p=0.4517
EEG Theta Pz-Oz (uV)	0.5617	-13.2% (-41.5%, 28.6%) p=0.4689	-31.0% (-53.3%, 1.9%) p=0.0612	-4.3% (-36.7%, 44.9%) p=0.8332	10.9% (-25.2%, 64.3%) p=0.5978	-7.6% (-38.1%, 38.1%) p=0.6939
SART total commission errors	0.4500	-3.57 (-8.01, 0.88) p=0.1122	-0.46 (-4.64, 3.71) p=0.8228	-0.47 (-4.76, 3.82) p=0.8267	-3.68 (-8.45, 1.09) p=0.1261	-3.69 (-7.90, 0.52) p=0.0840
SART mean RT correct	0.9940	18.80 (-67.2, 104.8) p=0.6596	-0.97 (-82.2, 80.23) p=0.9807	6.87 (-77.3, 91.00) p=0.8692	53.86 (-38.4, 146.1) p=0.2434	11.66 (-70.8, 94.06) p=0.7755
SART total omission errors	0.2723	-71.9% (-95.3%, 68.9%) p=0.1609	-64.6% (-93.6%, 96.4%) p=0.2283	-7.7% (-84.1%, 436.5%) p=0.9277	-72.6% (-96.0%, 88.5%) p=0.1832	8.5% (-80.5%, 503.8%) p=0.9242
SART post error slowing	0.7256	-.052 (-.177, 0.072) p=0.4037	-.008 (-.122, 0.106) p=0.8897	-.077 (-.194, 0.040) p=0.1943	-.079 (-.212, 0.053) p=0.2332	-.038 (-.155, 0.079) p=0.5192
SART RT variability	0.4898	-8.87 (-19.1, 1.352) p=0.0869	-.664 (-10.3, 8.969) p=0.8895	4.566 (-5.27, 14.41) p=0.3532	-4.23 (-15.2, 6.787) p=0.4412	-3.83 (-13.7, 6.011) p=0.4351
SART total error score	0.6362	-6.87 (-15.5, 1.72) p=0.1135	-1.83 (-9.92, 6.25) p=0.6477	1.87 (-6.42, 10.15) p=0.6506	-6.07 (-15.4, 3.24) p=0.1941	-4.91 (-13.1, 3.26) p=0.2310

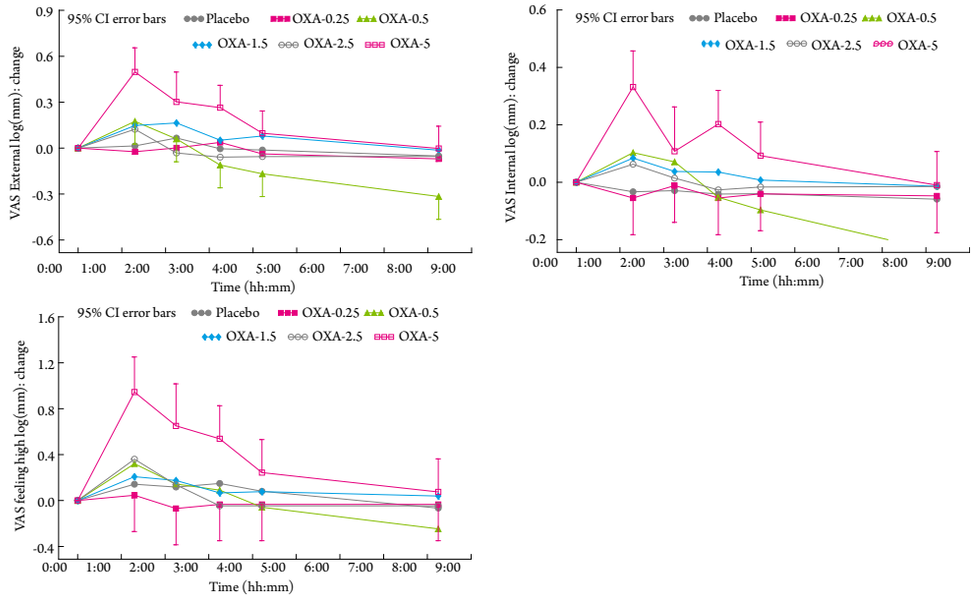
Abbreviations: EEG, electroencephalogram; Oxa, oxathridine; SART, Sustained Attention to Response Task; VAS, visual analoque scale.

Table 5 VVLT

		Contrasts all measurements				
Parameter	**Treatment P-value**	**OXA-0.25 vs Placebo**	**OXA-0.5 vs Placebo**	**OXA-1.5 vs Placebo**	**OXA-2.5 vs Placebo**	**OXA-5 vs Placebo**
Word recall correct 1	0.5126	-0.90 (-4.33, 2.53) p=0.5975	-0.73 (-4.17, 2.70) p=0.6668	-0.90 (-4.33, 2.53) p=0.5975	-1.57 (-5.00, 1.87) p=0.3601	-3.40 (-6.83, 0.03) p=0.0520
Word recall correct 2	0.6855	2.17 (-2.03, 6.37) p=0.3017	-0.50 (-4.70, 3.70) p=0.8102	1.00 (-3.20, 5.20) p=0.6315	-1.00 (-5.20, 3.20) p=0.6315	-1.17 (-5.37, 3.03) p=0.5760
Word recall correct 3	0.7095	2.53 (-2.48, 7.55) p=0.3117	-1.63 (-6.65, 3.38) p=0.5123	0.87 (-4.15, 5.88) p=0.7275	-0.63 (-5.65, 4.38) p=0.7989	-0.97 (-5.98, 4.05) p=0.6976
Delayed word recall correct	0.8847	1.93 (-3.62, 7.48) p=0.4837	0.10 (-5.45, 5.65) p=0.9710	0.77 (-4.78, 6.32) p=0.7806	-1.90 (-7.45, 3.65) p=0.4912	-0.23 (-5.78, 5.32) p=0.9324
Delayed word recognition correct	0.8967	0.33 (-2.74, 3.41) p=0.8268	-0.50 (-3.57, 2.57) p=0.7428	-0.83 (-3.91, 2.24) p=0.5850	0.83 (-2.24, 3.91) p=0.5850	-0.83 (-3.91, 2.24) p=0.5850
Delayed word recognition RT correct (msec)	0.0235	-102 (-202, -1.43) p=0.0470	-172 (-273, -71.6) p=0.0014	-24.6 (-125, 75.90) p=0.6224	-32.4 (-133, 68.07) p=0.5166	-30.1 (-131, 70.40) p=0.5471

Abbreviation: Oxa, oxathridine.

Figure 2 VAS Bowdle sum scores (internal, external, feeling high)



Abbreviations: Oxa = oxathridine, VAS = visual analoque scale

DISCUSSION

This FIH, randomised, double-blind, placebo-controlled study investigated the safety, pharmacokinetics, and pharmacodynamics of the first-in-class H_3 agonist oxathridine. As expected, the effects of 2.5 and 5 mg oxathridine on the NeuroCart tests of adaptive tracking, body sway and saccadic eye movements were indicative of CNS-depressant effects. However, the safety profile of oxathridine in healthy volunteers was different than expected based on the preclinical data, with mild to moderate orthostatic hypotension and pseudo-hallucinations at the two highest administered dose levels. This led to the decision to halt further dose escalation at 5 mg and to expand the dose range with 0.25 and 1.5 mg of oxathridine to characterise its safety, PK and PD profile more thoroughly.

In retrospect, the planned dose range of 0.5-100 mg was too large, especially when taking into account the pharmacologically active (PAD) and anticipated therapeutic doses (ATD) as recommended by current European Medicines Agency (EMA) guidelines.[1] Preclinical experiments demonstrated pharmacological effects from HED levels of 1.68 mg and C_{max} values of 30 ng/mL and higher. However, predictions of sleep-inducing doses were mainly based on cats, which is an unusual species in preclinical research. Moreover, the NOAEL was about 20-fold higher, and determined by cardiovascular effects in dogs which can be adequately monitored in humans. Since CNS-depression can also be measured accurately in healthy volunteers, a large dose range was selected for the FIH study.

The occurrence of pseudo-hallucinations was entirely unexpected. The time courses of the occurrence and resolving of pseudo-hallucinations and the increased scores on all three subscales of the VAS Bowdle (VAS internal, VAS external, VAS feeling high – Figure 2) closely followed the pharmacokinetic profile (Figure 1), providing a strong argument that pseudo-hallucinations are a pharmacological effect of oxathridine. In view of the high selectivity of oxathridine, this effect is likely related to stimulation of H_3R. However, to our knowledge there are no other reports of clinical studies with H_3R agonists, so without replication we cannot be absolutely certain that the pseudo-hallucinations are a class-effect. Due to their nature, pseudo-hallucinations could not be observed preclinically, although some non-specific behavioural symptoms such as increased reactivity to touch and monkeys accepting and looking for human contact, were observed in the animal studies, which retrospectively could have been indicative of cognitive, behavioural or perceptive dysregulations. These behavioural effects were observed at considerably higher exposure levels than for pseudo-hallucinations in humans. This could be due to different sensitivities, to detect spontaneous behavioural observations in animals, compared to subjective VAS-scores in humans. It could also be that humans are more sensitive to H_3R-agonism, since cardiovascular effects of (orthostatic) hypotension also occurred at lower exposure levels in healthy volunteers than observed preclinically, indicating that humans are more sensitive to the effects of H_3R agonists than animals.

The mechanism of action underlying pseudohallucinatory effects is uncertain. A stage of dreaming without being asleep might be an explanation. Pseudo-hallucinations have also been reported after use of drugs targeted at the GABA-ergic system, the main inhibitory neurotransmitter system in the brain.[22,23] In some healthy volunteers, but not all, zolpidem (a $GABA_A$ agonist with high α_1 subtype selectivity) caused concentration-related pseudo-hallucinations.[24,25] In the current study, all volunteers reported being able to change their visual pseudo-hallucinations intentionally. This is reminiscent of hypnagogic hallucinations which occur in narcolepsy.[26] This condition is characterised by a deficiency of orexin (hypocretin), an important neuropeptide for -among others- the regulation of the sleep-wake cycle, in which histamine plays a prominent role.[26] Within this system, H_3R pharmacology is complex and involved in the regulation of many other neurotransmitter systems such as serotoninergic (5HT), dopaminergic and cholinergic systems.[4] Several CNS-penetrating compounds affecting these systems, like antimuscarinics and 5HT2a- or D2-agonists, are well-known

psychomimetics, suggesting that the pseudo-hallucinations may have been caused by dysregulation of these systems by oxathridine. Abnormal H3R expression is observed in the brain of patients with schizophrenia, which further points in the direction of a role for the H3R in the occurrence of hallucinations.[27] It therefore seems that pharmacological interference on homeostatic brain processes of sleep can lead to a disruption of different aspects of sleep onset, leading to phenomena of pseudo-hallucinations.

From dose levels of 2.5 mg and higher, oxathridine demonstrated CNS-depressant effects on the NeuroCart. In Table 6 the NeuroCart effects of oxathridine are compared with several other sleep-promoting and (anti)histaminergic compounds: lorazepam ($GABA_A$-agonist), diphenhydramine (H1R antagonist and some antimuscarinic/anticholinergic action) and CEP-26401 (investigational H3R antagonist/inverse agonist).[9,28-30]

Table 6 Summary NeuroCart effects for different CNS active compounds

	Lorazepam 2 mg ($GABA_A$-agonist)[9,28]	Diphenhydramine 50 mg (H1R antagonist, with some antimuscarinic/ anticholinergic action)[29]	CEP-26401 125 ug (H3R antagonist/ inverse agonist)[30]	Oxathridine 5 mg (H3R partial agonist)
Adaptive tracking (%)	-9.53 (-11.9; -7.21) P<0.0001	-2.64 (-3.92; -1.13) P=0.0001	1.20 (0.42; 1.98) P=0.0029	-4.81 (-8.10, -1.51) P=0.0053
Body sway (%)	89.0 (62.8; 119.6) P<0.0001	12.25 (-2.35; 29.03) P=0.1021	-28.72 (-61.94; 4.51) P=0.0895	43.0 (5.9; 93.1) P=0.0205
Saccadic peak velocity (deg/s)	- 59.23 (- 46.05;-72.41) P<0.001	-13.8 (-21.7; -5.9) P=0.0010	16.99 (9.73; 24.24) P<0.0001	-22.5 (-43.9; -1.09) P=0.0399
Smooth pursuit (%)	-10.8 (-14.2; -7.3) P<0.0001	-0.5 (-3.1; 2.1) P=0.7149	-0.31 (-2.10; 1.48) P=0.7310	-0.77 (-4.99; 3.45) P=0.7135
pEEG	All frequencies of EEG bands were statistically significantly affected	Not reported	No effect	No effect
VAS Bond & Lader alertness (mm)	-1.80 (-3.52;-0.08) P0.041	-1.0 (-4.4; 2,3) P=0.5377	No effect	-5.83 (-13.2; 1.50) P=0.1167
VAS Bond & Lader calmness (mm)	-0.10 (-0.41; 0.22) P=0.529	1.1 (-1.0; 3.2) P=0.3066	No effect	4.82 (-2.56; 12.19) P=0.1944
VAS Bond & Lader mood (mm)	-0.24 (-0.96; 0.49) P=0.510	0.4 (-0.8; 1.7) P=0.5059	No effect	4.51 (-1.53; 10.55) P=0.1394
VAS Bowdle internal	0.07 (0.03; 0.11) P=0.0007	Not reported	No effect	0.249 (0.127; 0.370) P=0.0001
VAS Bowdle external	0.10 (0.05; 0.16) P=0.0004	Not reported	No effect	0.330 (0.183; 0.476) P<0.0001
VAS Bowdle Feeling high	0.12 (0.02; 0.22) P=0.0168	Not reported	No effect	0.575 (0.263; 0.887) P=0.0006
VVLT	Not reported	Not reported	No effect	Effect on word recall correct 1 -3.40 (-6.83; 0.03) P=0.0520

Abbreviations: pEEG, pharmaco-electroencephalogram; VAS, visual analogue scale; VVLT, visual verbal learning test

The effect size of oxathridine on adaptive tracking was approximately half the effect size of 2 mg lorazepam, and twice as large as the effect of diphenhydramine. On body sway oxathridine's effect was approximately half the effect size of 2 mg lorazepam and 4 times larger than the effect size of 50 mg diphenhydramine. The effect size of oxathridine on SPV was approximately three times smaller than the effect size of 2 mg lorazepam, but larger than the effect size of diphenhydramine. Comparable to diphenhydramine, oxathridine did not affect smooth pursuit, while lorazepam did. Overall, the NeuroCart profile of oxathridine is indicative of a CNS depressant compound with less sedative capacity than 2 mg lorazepam, but more than 50 mg diphenhydramine. Furthermore, in line with their mechanisms of action, the effects of oxathridine on the NeuroCart tests are opposite to the effects observed with the H3R-inverse agonist CEP-26401.[30] Compared with placebo, the groups with the two lowest doses of oxathridine showed lower reaction times in the delayed word recognition task of the VVLT. This might be indicative of improved cognitive functioning. However, no CNS stimulating effects were observed on the other NeuroCart tests, which in contrast to the VVLT are baseline corrected. Therefore, these effects may well have been false positive findings.

Although further development of oxathridine was ceased based on the findings of this study, important lessons can be learned from it. This study demonstrates how the NeuroCart can be used in early phase clinical trials to measure important pharmacological characteristics of novel CNS compounds and compare these to existing compounds.[8] In line with recommendations by the EMA this study demonstrates the importance of considering the predictions of MABEL, PAD and Anticipated Therapeutic Dose when designing a study, particularly for compounds with a new mechanism of action.[1] This study also shows that there is inherent uncertainty in the translation from preclinical to human studies. Although the preclinical data of oxathridine indicates an appropriate 'therapeutic window' with desired effects at lower dose levels than undesired effects, in humans there was no therapeutic window as dose levels with desired effects were associated with unacceptable AEs. This study emphasises that not all AEs are translatable between animals and humans, as some psychiatric AEs, such as hallucinations, cannot be observed in animals. However, non-specific changes in behaviour observed preclinically might indicate that animals experience disruptive cognitive or perceptive symptoms and, therefore, should make investigators aware of potential psychiatric symptoms in humans. A final intriguing lesson of the clearly drug-related and fully reversible pseudo-hallucinations in this study was, that the H3R system could be an interesting new target for the development of novel antipsychotics. Moreover, there is an increasing interest in the therapeutic effect of psychomimetic agents targeted at other receptor systems, such as psilocybin (5HT2a-partial agonist) and esketamine (NMDA-antagonist).[31,32] It could be speculated that H3R-antagonists could serve a similar role in psychomimetic assisted psychotherapies.

In conclusion, this study demonstrates how the pharmacodynamics of a novel compound can be investigated in early phase clinical trials. The findings of this study contribute to the field of knowledge about H3R pharmacology and delineate its complexity as already described by others.[33] This knowledge can be used for the future development of compounds targeted at the H3R, including potential antipsychotics or therapeutic psychomimetics.

REFERENCES

1 European MA. Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. *Stand No 07 Rev 1*. 2018;retrieved.

2 Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. *Nature*. 1983;302(5911):832-837. doi:10.1038/302832a0

3 Arrang J-M, Garbarg M, Schwartz J-C. Autoinhibition of histamine synthesis mediated by presynaptic H3-receptors. *Neuroscience*. 1987;23(1):149-157. doi:https://doi.org/10.1016/0306-4522(87)90279-X

4 Gemkow MJ, Davenport AJ, Harich S, Ellenbroek BA, Cesura A, Hallett D. The histamine H3 receptor as a therapeutic drug target for CNS disorders. *Drug Discov Today*. 2009;14(9):509-515. doi:https://doi.org/10.1016/j.drudis.2009.02.011

5 Meskill GJ, Davis CW, Zarycranski D, DolIBa M, Schwartz J-C, Dayno JM. Clinical Impact of Pitolisant on Excessive Daytime Sleepiness and Cataplexy in Adults With Narcolepsy: An Analysis of Randomized Placebo-Controlled Trials. CNS *Drugs*. 2022;36(1):61-69. doi:10.1007/s40263-021-00886-x

6 Witkin JM, Nelson DL. Selective histamine H3 receptor antagonists for treatment of cognitive deficiencies and other disorders of the central nervous system. *Pharmacol Ther*. 2004;103(1):1-20. doi:https://doi.org/10.1016/j.pharmthera.2004.05.001

7 European MA. Summary of product characteristics of Ozawade. https://www.EMA.europa.eu/en/documents/product-information/ozawade-epar-product-information_en.PDf

8 Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood–brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol*. 2016;20:27-34. doi:http://dx.doi.org/10.1016/j.ddtec.2016.07.004

9 de Haas SL, de Visser SJ, van der Post JP, et al. Pharmacodynamic and pharmacokinetic effects of TPA023, a GABA(A) alpha(2,3) subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. *J Psychopharmacol*. 2007;21(4):374-383. doi:10.1177/0269881106072343

10 de Haas SL, Schoemaker RC, van Gerven JMA, Hoever P, Cohen AF, Dingemanse J. Pharmacokinetics, pharmacodynamics and the pharmacokinetic/ pharmacodynamic relationship of zolpidem in healthy subjects. *J Psychopharmacol*. 2010;24(11):1619-1629. doi:10.1177/0269881109106898

11 Chen X, Jacobs G, de Kam ML, et al. AZD6280, a novel partial γ-aminobutyric acid A receptor modulator, demonstrates a pharmacodynamically selective effect profile in healthy male volunteers. *J Clin Psychopharmacol*. 2015;35(1):22-33. doi:10.1097/JCP.0000000000000251

12 Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. *Br J Clin Pharmacol*. 1984;18 Suppl 1(Suppl 1):69S-72S. https://www.ncbi.nlm.nih.gov/pubmed/6525331 https://www.ncbi.nlm.nih.gov/pmc/PMC1463351/

13 van Steveninck AL, Wallnofer AE, Schemaker RC, et al. A study of the effects of long-term use on individual sensitivity to temazepam and lorazepam in a clinical population. *Br J Clin Pharmacol*. 1997;44(3):267-275. doi:10.1046/j.1365-2125.1997.t01-1-00580.x

14 van Steveninck AL, Gieschke R, Schoemaker RC, et al. Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. *Br J Clin Pharmacol*. 1996;41(6):565-573. doi:10.1046/j.1365-2125.1996.38514.x

15 Wright BM. A simple mechanical aTAKia-meter. *J Physiol*. 1971;218 Suppl:27p-28p.

16 Norris H. The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology*. 1971;10(21):181-191.

17 Bond A, Lader M. The use of analogue scales in rating subjective feelings. *Br J Med Psychol*. 1974;47(3):211-218. doi:10.1111/j.2044-8341.1974.tb02285.x

18 Bowdle AT, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers relationship to steady-state plasma concentrations. *Anesthesiology*. 1998;88(1):82-88. doi:10.1097/00000542-199801000-00015

19 de Haas SL, de Visser SJ, van der Post JP, et al. Pharmacodynamic and pharmacokinetic effects of TPA023, a GABAA α2,3 subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. *J Psychopharmacol*. 2006;21(4):374-383. doi:10.1177/0269881106072343

20 Manly T, Robertson IH, Galloway M, Hawkins K. The absent mind: further investigations of sustained attention to response. *Neuropsychologia*. 1999;37(6):661-670. doi:10.1016/s0028-3932(98)00127-4

21 Wearne D, Genetti A. Pseudohallucinations versus hallucinations: wherein lies the difference? *Australas Psychiatry*. 2015;23(3):254-257. doi:10.1177/1039856215586150

22 Dundee JW. Fantasies during sedation with intravenous midazolam or diazepam. *Med Leg J*. 1990;58 (Pt 1):29-34. doi:10.1177/002581729005800105

23 Litchfield NB. Complications of Intravenous Diazepam - Adverse Psychological Reactions. (An assessment of 16,000 cases). *Anesth Prog*. 1980;27(6):175-183.

24 de Haas S, Dingemanse J, Hoever P, Cohen A, van Gerven J. Pseudohallucinations after zolpidem intake: a case report. *J Clin Psychopharmacol*. 2007;27(6):728-730. doi:10.1097/JCP.0b013e31815a5806

25 Kummer L, Rzewuska M, Sienkiewicz-Jarosz H, Mierzejewski P, Bienkowski P, Samochowiec J. Zolpidem misuse in two women with no psychiatric history: a crucial role of pleasant visual hallucinations. *J Neuropsychiatry Clin Neurosci*. 2012;24(2):E32. doi:10.1176/appi.neuropsych.11030070

26 Pizza F, Barateau L, Dauvilliers Y, Plazzi G. The orexin story, sleep and sleep disturbances. *J Sleep Res*. 2022;31(4):e13665. doi:10.1111/jsr.13665

27 Ghamari N, Zarei O, Arias-Montaño J-A, et al. Histamine H3 receptor antagonists/inverse agonists: Where do they go? *Pharmacol Ther*. 2019;200:69-84. doi:https://doi.org/10.1016/j.pharmthera.2019.04.007

28 Chen X, Jacobs G, de Kam M, et al. The central nervous system effects of the partial GABA-Aalpha2,3 -selective receptor modulator AZD7325 in comparison with lorazepam in healthy males. *Br J Clin Pharmacol*. 2014;78(6):1298-1314. doi:10.1111/bcp.12413

29 Chen X, Broeyer F, de Kam M, Baas J, Cohen A, van Gerven J. Pharmacodynamic response profiles of anxiolytic and sedative drugs. *Br J Clin Pharmacol*. 2017;83(5):1028-1038. doi:10.1111/bcp.13204

30 Baakman AC, Zuiker R, van Gerven JMA, et al. Central nervous system effects of the histamine-3 receptor antagonist CEP-26401, in comparison with modafinil and donepezil, after a single dose in a cross-over study in healthy volunteers. *Br J Clin Pharmacol*. 2019;85(5):970-985. doi:10.1111/bcp.13885

31 Thomas K, Malcolm B, Lastra D. Psilocybin-Assisted Therapy: A Review of a Novel Treatment for Psychiatric Disorders. *J Psychoactive Drugs*. 2017;49(5):446-455. doi:10.1080/02791072.2017.1320734

32 Capuzzi E, Caldiroli A, Capellazzi M, et al. Long-Term Efficacy of Intranasal Esketamine in Treatment-Resistant Major Depression: A Systematic Review. *Int J Mol Sci*. 2021;22(17). doi:10.3390/ijms22179338

33 Kuhne S, Wijtmans M, Lim HD, Leurs R, de Esch IJP. Several down, a few to go: histamine H3 receptor ligands making the final push towards the market? *Expert Opin Investig Drugs*. 2011;20(12):1629-1648. doi:10.1517/13543784.2011.625010

APPENDIX

IB-DERISK ANALYSER OVERVIEW OF OXATHRIDINE

Oxathridine or 4-(1H-imidazol-4-ylmethyl)-pyridine sesquioxalate is a first-in-class histamine-3 receptor (H3R) partial agonist, behaving as a full agonist *in vivo*. Since oxathridine was the first H3R agonist to be given to humans, the preclinical data was studied extensively before deciding to continue to a clinical study.

The data of the preclinical package was entered in the IB-Derisk analyser tool.[1] When sorting the preclinical data on C_{max} the compound demonstrated a favourable profile, with desired effects indicated by green at relatively low C_{max} values and occurrence of undesired effects starting at higher C_{max} values (Figure 1). In general, the of occurrence of adverse events (AEs) followed a predictable pattern with occurrence of relatively mild and transient AEs indicated in yellow at lower exposures and more severe AEs indicated in orange and severe AEs indicated in red at higher exposures (Figure 1).

Desired effects are indicated in green (Figure 1). In freely moving cats, oral administration of 1-3 mg/kg oxathridine, corresponding to C_{max} values between 30-150 ng/mL, statistically significant increased deep sleep, at the expense of arousal, without any significant effect on both light and REM sleep was observed. Additionally, oxathridine decreased the levels of the main metabolite of histamine, t-methylhistamine (t-MeHA), in the brains of mice at low oral doses of 0.3 mg/kg, corresponding to an interpolated C_{max} value of 116.5 ng/mL. Furthermore, at a dose of 0.3 mg/kg, corresponding to an interpolated C_{max} value of 591 ng/mL, oxathridine almost fully suppressed the deficit in sleep elicited by zolpidem withdrawal in rats.

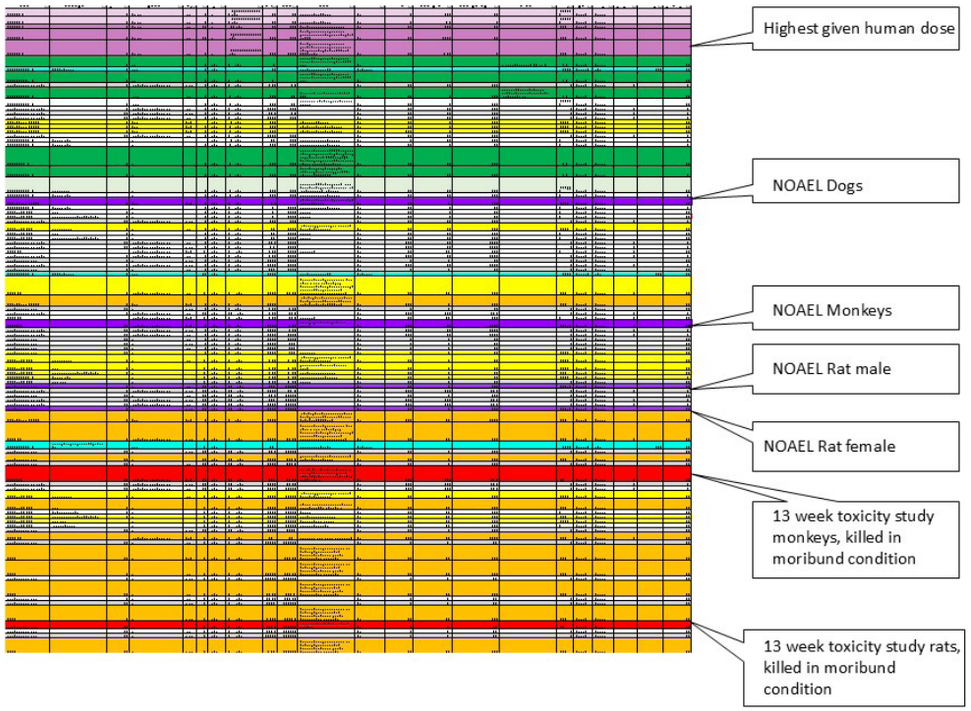
Dogs were the most sensitive species for oxathridine. The NOAEL in dogs was set at 1 mg/kg, corresponding to a C_{max} value of 609 ng/mL. At this dose a slightly reduced arterial blood pressure and slightly increased heart rate were observed. At dose levels below 1 mg/kg, some minor effects on arterial blood pressure and heart rate were observed in dogs as well, indicated in yellow in the IB-Derisk (Figure 1). At dose levels above the NOAEL, markedly increased arterial blood pressure and atrio-ventricular blocks of grade 2 and grade 3 were observed in dogs. For monkeys the NOAEL was set at 8 mg/kg, corresponding to a C_{max} of 2173 ng/mL, based on a 13-week toxicity study. At this dose, the principal clinical sign was a change of behaviour with monkeys accepting and looking for human contact. In rats the NOAEL was set at 15 mg/kg, corresponding to a C_{max} of 3440 ng/mL in males and 5385 ng/mL in females. At lower dose levels limited effects suggestive of respiratory stimulant and bronchodilatery properties, piloerection and increased reactivity to touch were observed.

The findings of the FIH study with oxathridine were entered in the IB-Derisk overview in the colour pink/purple (Figure 1). The starting dose for the FIH study of 0.5 mg was a factor 70 lower than the NOAEL in the most sensitive species (dogs). Based on the *in vivo* pharmacodynamic experiments, no pharmacological activity was expected at this dose level. In the first in human (FIH) study, this dose was well tolerated and therefore it was decided to continue to the next planned dose level of 2.5 mg. At this dose level, all volunteers reported one or more AEs, the most frequently reported being dizziness, hypotension, syncope and nausea.[2] In addition, decreases in supine and standing blood pressure were observed with a compensatory increase in heart rate. In animals slight increases in heart rate were only observed at an exposure level a factor 100 higher than the exposure associated with these effects in humans. For the next cohort, the dose was therefore escalated to 5 mg instead of the scheduled 10 mg. Even though the C_{max} value associated with 5 mg in humans was only 7.12 ng/mL, much lower than the C_{max} of approximately 273 ng/mL associated with slightly reduced arterial blood pressure in dogs (the most sensitive species), decreases in blood pressure with compensatory increases in heart rate were observed in the healthy volunteers. Furthermore, all volunteers reported the remarkable and unexpected AE of pseudo-hallucinations.[2] The volunteers reported that they were aware that the perceived images were not real and that they could change the content of the visual phenomena. Because of these AEs, it was decided to not escalate to higher dose levels, but to add two extra dose levels of 0.25 mg and 1.5 mg, to better characterise the pharmacodynamics and pharmacokinetics of oxathridine. Pharmacodynamic effects indicative of central nervous system depressant effects as measured by the NeuroCart were observed from dose levels of 2.5 mg and higher.[3]

In contrast to the preclinical findings demonstrating an appropriate 'therapeutic window' with the desired effects occurring at lower exposure levels than the undesired effects, in the clinical study the low doses at which no desired pharmacological effects were observed, were already associated with unacceptable adverse events. It seems therefore that humans are more sensitive to the effects of H3R agonism than animals as decreases in blood pressure were observed from exposure levels of 1.4 ng/mL in humans versus 273 ng/mL in animals. Due to the nature of the AEs of pseudo-hallucinations, these could not be observed preclinically. Possibly, the changes in behaviour consisting of monkeys looking for and accepting human contact from exposure levels of 2173 ng/mL and higher could be indicative of cognitive or perceptive symptoms in the animals.

This study demonstrates that not all AEs are translatable between animals and humans. This warns researchers performing FIH studies with central nervous system (CNS) active compounds, to pay special attention to psychiatric effects of new compounds, as these cannot be reliably predicted from animal experiments.

Figure 1 IB-Derisk overview oxathridine



REFERENCES

1 Gerven van, J.M.A. CAF, Adam C. Integrating data from the Investigational Medicinal Product Dossier/investigator's brochure. A new tool for translational integration of preclinical effects. *Br J Clin Pharmacol*. 0(0). doi:doi:10.1111/bcp.13529

2 Dijkstra FM, Zuiker RGJA, Heuberger JAAC, et al. Administration of oxathridine, a first-in-class histamine-3 receptor partial agonist in healthy male volunteers: Central nervous system depression and pseudo-hallucinations. *Br J Clin Pharmacol*. Published online September 2023. doi:10.1111/bcp.15910

3 Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood–brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol*. 2016;20:27-34. doi:http://dx.doi.org/10.1016/j.ddtec.2016.07.004

CHAPTER IV

PHARMACOLOGICAL PROFILE OF ALKS 7119, AN INVESTIGATIONAL COMPOUND EVALUATED FOR THE TREATMENT OF NEUROPSYCHIATRIC DISORDERS, IN HEALTHY VOLUNTEERS

Dijkstra F.M.[1,2], Zuiker R.G.J.A.[1,2], Siebenga P.S.[1], Leigh-Pemberton R.A.[3], Sun L.[3], Manthis J.D.3, de Kam M.1, Lin R.[3], von Moltke L.L.[3], Rezendes D.[3], van Gerven J.M.A.[1,2]

British Journal of Clinical Pharmacology, 88(6), 2909–2925
https://doi.org/10.1111/bcp.15229

1 Centre for Human Drug Research (CHDR), Leiden, The Netherlands
2 Leiden University Medical Center, Leiden, The Netherlands
3 Alkermes, Inc. 852, Waltham, MA 02451 US

ABSTRACT

AIM ALKS 7119 is a novel compound with *in vitro* affinity highest for the SERT, and for μ receptor, α_{1A}-adrenoceptor, α_{1B}-adrenoceptor, NMDA receptor and sigma non-opioid intracellular receptor 1. This first-in-human study evaluated safety and PK/PD effects of single ascending doses (SAD) of ALKS 7119 in healthy males and compared effects with neurotransmitter modulators with partially overlapping targets.

METHODS In 10 cohorts (n=10 subjects each), PK, safety and PD (NeuroCart tests, measuring neurophysiologic effects [pupillometry, pharmaco-EEG (pEEG)], visuomotor coordination, alertness, [sustained] attention [saccadic peak velocity, adaptive tracking], subjective drug effects [VAS Bowdle and VAS Bond and Lader] and postural stability [body sway]) were evaluated. Neuroendocrine effects (cortisol, prolactin, growth hormone) were measured. Data were analysed over the 12-hour post-dose period using mixed-effects model for repeated measure (MMRM) with baseline as covariate.

RESULTS ALKS 7119 demonstrated linear PK and was generally well tolerated. QTcF interval increases of 30-60 ms compared to baseline were observed with ALKS 7119 doses of ≥50 mg without related adverse events. Significant increases in left and right pupil/iris ratio were observed at dose levels ≥50 mg (estimate of difference [95%CI], p-value) (0.04 [0.01; 0.07], $P < 0.01$) and (0.06 [0.03; 0.09], $P = 0.01$), respectively. From dose levels ≥50 mg significant increases (% change) of serum cortisol (51.7 [8.4; 112.3], $P = 0.02$) and prolactin (77.9 [34.2; 135.8], $P < 0.01$) were observed.

CONCLUSION In line with ALKS 7119's *in vitro* pharmacological profile, the clinical profile observed in this study is most comparable to SERT inhibition.

INTRODUCTION

ALKS 7119 is an investigational compound that has been evaluated for the potential treatment of neuropsychiatric disorders. Preclinical tests using a panel of *in vitro* receptor, transporter, enzyme binding and functional assays showed that ALKS 7119 has high affinity for the SERT (K_i=0.035 μM), and lower affinities for the μ receptor (K_i=0.6 μM), α_{1A}-adrenoceptor (K_i=0.98 μM), α_{1B}-adrenoceptor (K_i=1.8 μM), NMDA receptor (K_i=7.44 μM) and sigma non-opioid intracellular receptor 1 (K_i=33.0 μM). In vivo pharmacology studies in rats demonstrated that an oral dose of 10 mg/kg ALKS 7119 completely blocked dopamine release induced by infusion of the synthetic glutamate agonist NMDA in the striatum. In preclinical pharmacokinetic studies with both dogs and rats, the median time to maximum plasma concentration (t_{max}) of ALKS 7119 was 0.5 hour after oral administration. Binding to plasma protein ranged from 11% to 28%, oral bioavailability from 43% to 76% and mean elimination half-life ($t_{½}$) from 2.9 to 5.6 hours across species.

Preclinical multiple dose toxicology studies with ALKS 7119 demonstrated a no adverse effect level (NOAEL) of 10 mg/kg in rats. At higher doses symptoms of decreased body weight and hepatocellular vacuolation and hypertrophy and changes in behaviour, such as arousal and decreased mobility in the open-field observations, were observed. The NOAEL corresponded to human equivalent dose (HED) of 96 mg, calculated per FDA guidelines (using body surface area extrapolation).[1] Preclinical multiple dose toxicology studies in dogs demonstrated a NOAEL of 3 mg/kg, corresponding to a HED of 97.2 mg.[1] At a HED of 324 mg, decreases in systolic, diastolic, and mean blood pressure as well as compensatory increases in heart rate occurred. Next to that, symptoms of recumbency and decreased activity were observed. No changes in QT intervals at any dose of ALKS 7119 were observed in the preclinical dog cardiovascular study, a finding consistent with *in vitro* hERG channel testing, showing no effects up to high concentrations (IC_{50} of 191.1 μM or 54348.8 ng/mL). ALKS 7119 showed no potential to induce neuronal abnormalities up to a HED of 3360 mg. Based on these preclinical safety data, it was decided to continue to a FIH study, starting with a dose of 3 mg (32 times lower than the NOAEL in rats, the most sensitive species).

The relatively high affinity of ALKS 7119 for several distinct receptor types offered the potential to evaluate ALKS 7119 for the treatment of various neuropsychiatric conditions, ranging from neuropathic pain and brainstem behavioural disorder to schizophrenia and depression. Further exploration of these indications would have required a large series of preclinical disease models, which all have limited predictive power for compounds with novel and complex profiles of pharmacologic action. It was therefore decided to not only characterise the PK and safety in this FIH study, but to also include a wide range of different CNS-functions, which could provide indications for BBB penetration and target engagement profiles in humans.

This approach is in line with the 'question-based drug development (QBDD)' method, which is developed to investigate novel compounds in a structured way to prevent late stage drug development failures.[2] According to QBDD, studies must be designed to answer important questions about novel compounds.[2] In case of a CNS drug, such as ALKS 7119, it was considered important to know whether the drug crosses the BBB and on which receptors it mainly acted.[2] To answer these questions, the current study utilised the NeuroCart, which consists of a battery of drug-sensitive CNS tests, measuring effects on different CNS domains, such as neurophysiologic functioning, visuomotor coordination, balance and subjective feelings.[3] Several CNS-active compounds, including compounds influencing serotonergic, opioid, GABA-ergic and glutamatergic (via NMDA-antagonism) networks, have been profiled using the NeuroCart. This allowed a comparison of the functional profile of this new pharmacologically heterogeneous compound to other known drug profiles, and consequently to obtain a better understanding of the underlying pharmacological effects.[3,4]

The preclinical pharmacological profile of ALKS 7119, with relatively high affinity for different receptor types, required NeuroCart testing at dose levels with small increments, to be able to disentangle ALKS 7119's effects on distinct receptors with different affinities. Specific NeuroCart tests were selected based on ALKS 7119's pharmacological profile. Pupil size measurements were included to measure serotonergic and μ receptor effects as opioids are known to induce pupil constriction, whilst most studies with selective serotonin reuptake inhibitors (SSRIs) show pupil dilation.[5,6] In addition, pharmaco-EEG (pEEG) was included because this is a potential biomarker for SERT engagement.[6] Ketamine, a well-known NMDA receptor antagonist, demonstrated decreased saccadic peak velocity, adaptive tracking and alertness and increased body sway and psychedelic effects as measured by visual analogue scale (VAS) Bowdle on the NeuroCart.[7] Buprenorphine, a partial μ receptor agonist decreased adaptive tracking and saccadic peak velocity and increased body sway.[8]

In addition to the NeuroCart tests, serum cortisol and prolactin levels were measured as biomarkers for serotonergic effects, as escitalopram and citalopram are known to increase levels of these hormones.[9] Although it is uncertain whether growth hormone levels are influenced by serotonergic compounds,[10] serum growth hormone levels were also measured.

There are no established CNS tests for mild adrenergic modulation. The CNS effects of strong noradrenalin release stimulators like dexamphetamine,[11] or the potent inhibitory effects of presynaptic α_2-adrenoceptor agonists and imidazoline modulators like clonidine[12] or rilmenidine,[13] can be readily shown with several NeuroCart tests. However, demonstration of more subtle noradrenergic modification does not cause spontaneous changes of NeuroCart tests in healthy subjects,[13] but requires more elaborate tests of cognition or pain.[14] The current study did not include any such specific biomarkers for modest α_{1A} or α_{1B}-adrenergic receptor modulation, other than the safety blood pressure measurements. Similarly, no specific tests for sigma non-opioid intracellular receptor 1 modulation could be identified for inclusion in the study.

The aim of this study was to profile single ascending doses of ALKS 7119 in terms of safety, tolerability, PK and PD effects in healthy male volunteers, and to compare these effects with known functional effects of different neurotransmitter modulators with partially overlapping mechanisms of action.

METHODS

GENERAL

The study was registered at ToetsingOnline under number NL155561.056.15 and approved by Foundation Beoordeling Ethiek Biomedisch Onderzoek (BEBO), Assen, the Netherlands. All subjects gave written informed consent prior to study start. The study was performed according to ICH GCP guidelines as laid down in the Declaration of Helsinki and its latest amendments. Alkermes Inc. sponsored the study, and the study was conducted from 04 January 2016 to 13 July 2016 at the Centre for Human Drug Research (CHDR), Leiden, the Netherlands.

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[15-18]

DESIGN

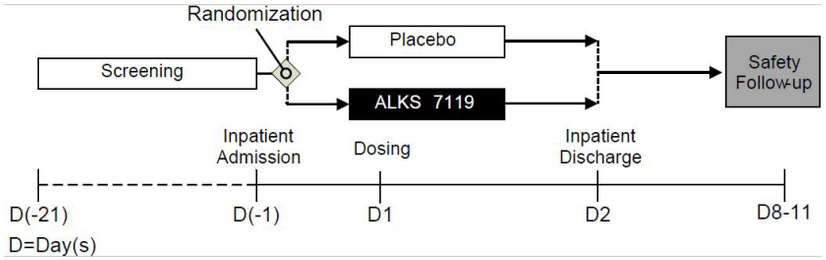
This was a single-centre, randomised, double-blind, placebo-controlled, single ascending dose study in 100 healthy male adults. Due to the exploratory character of this FIH study, the sample size was based on clinical considerations rather than power calculations. Subjects were divided over 10 cohorts (active: placebo ratio: 8:2) where each cohort represented a different dose level: 3, 10, 25, 50, 75, 100, 125, 150, 175 and 200 mg. Sentinel dosing was performed on the first 2 subjects in cohort 1. Before ascending to the next dose level, all available safety, PK and PD data of the preceding dose level(s) were reviewed.

The study consisted of a medical screening visit, an inpatient study visit and an inpatient follow up visit. Study visits consisted of 3 inpatient days; subjects arrived the day prior to dosing, were dosed the following day and were discharged the day after dosing (Figure 1). At check-in, eligibility was checked based on physical exam, including weight, laboratory testing including urinalysis, urine drug screen, electrocardiogram (ECG), breath alcohol test, concomitant medication, adverse event (AE) review and vital sign measurement including tympanic temperature measurement, pulse rate and (orthostatic) blood pressure measurements. Blood pressure and pulse

rate measurements were performed after subjects had been in supine position for 5 minutes. For orthostatic blood pressure measurements, subjects were then instructed to stand up and after 2 minutes blood pressure and pulse rate were measured again. Orthostatic hypotension was defined as ≥ 20 mmHg decrease in systolic blood pressure and ≥ 10 mmHg decrease of diastolic blood pressure. The safety measurements were repeated throughout the study at set times.

Blood samples for measurement of plasma concentrations of ALKS 7119 and serum neuro-endocrine hormone levels were collected within 1 hour pre-dose and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 16, 24, and 36 hours post-dose. NeuroCart assessments, consisting of saccadic eye movements, smooth pursuit eye movements, adaptive tracking, body sway, pupillometry, pharmaco-electroencephalography (PEEG), visual analogue scales (VAS) according to Bond and Lader and Bowdle were performed pre-dose (twice) and 0.5, 1, 1.5, 2, 3, 4, 6 and 10-hours post-dose.

Figure 1 Study design



ELECTROCARDIOGRAMS (ECGS) ACQUISITION AND ANALYSIS

At scheduled time points standard twelve-lead ECGs recordings were performed in triplicate with 1 minute in between each replicate. Recordings were made after a five minute resting period and in semi-recumbent position. The ECGs were recorded using an electrocardiograph (Marquette 800/5500/2000 or Dash 3000; General Electric Healthcare, Milwaukee, USA) and ten disposable electrodes placed in the standard anatomical position. ECG data were uploaded into the ECG warehouse, which automatically assesses interval, including QTc intervals, and amplitude data from the digital ECGs with the Marquette 12SL algorithm (Muse Cardiology Data Management System v7, General Electric Healthcare, Chicago, IL, USA). The Marquette Cubic Spline filter and Finite Residual Filter were used for artefact and noise management. A physician manually reviewed all ECGs for quality, eligibility and abnormalities.

SUBJECTS

Healthy male subjects between 18 and 45 years of age at screening were selected. Subjects were not allowed to use medication within 7 days prior to screening or inpatient admission and during the study days. Subjects were asked not to consume any alcohol, caffeine or xanthine containing beverages within 24 hours and not to use any nicotine-containing products within 30 days prior to inpatient admission and during the study days.

TREATMENTS

ALKS 7119 was provided as size 0 Swedish orange, opaque, hard gelatin capsules compounded at target strengths (i.e. 3 mg to 200 mg) for oral use. Placebo consisted of identical, empty capsules. Subjects began fasting the night before until 4 hours after study drug administration. Subjects were allowed water ad libitum except for 1 hour before and 1 hour after study drug administration.

PHARMACOKINETIC ASSESSMENTS

Plasma samples were analysed by an independent bioanalytical laboratory (Analytisch Biochemisch Laboratorium BV, Assen, The Netherlands). Concentrations of ALKS 7119 were quantified using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method with lower limit of quantification (LLOQ) of 1.00 ng/mL and coefficient of variation (CV) between 1.9 and 4.6%.

NEUROCART ASSESSMENTS

All tests were performed in a quiet room with subdued illumination with only one subject in the same room per session. A NeuroCart test training was performed during the screening visit, to prevent learning effects during study execution.

SACCADIC EYE MOVEMENT

The primary outcome of saccadic eye movement measurement is saccadic peak velocity (SPV) in degrees per second (deg/s), a sensitive parameter for numerous sedative compounds.[4,19,20] Tests were performed as described in previous publications.[19-22] Subjects were instructed to follow a dot jumping approximately 15 degrees to either side on a computer screen with their eyes, while head movements were restrained using a fixed head support at 58 cm from the computer screen. Fifteen saccades were recorded with interstimulus intervals varying randomly between 3 and 6 seconds. Average values of saccadic peak velocity were calculated for all artefact free saccades.

SMOOTH PURSUIT

Smooth pursuit was performed as described in previous publications.[20,21,23,24] In short, smooth pursuit measurements were performed using the same set-up as for saccadic eye movements, but the dot was moving continuously at a frequency ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz instead of jumping on the screen. The amplitude of target displacement corresponds to 22.5 degrees eyeball rotation to both sides. Four cycles are recorded for each stimulus frequency. The target parameter was the average percentage of smooth pursuit for all stimulus frequencies.

ADAPTIVE TRACKING

The adaptive tracking test was performed as originally described by Borland and Nicholson,[25] using customised equipment and software (based on TrackerUSB hard-/software (Hobbs, 2004, Hertfordshire, UK)). Adaptive tracking is a pursuit-tracking task that is highly sensitive to a wide range of psychoactive drugs.[19,21,26-28] During the test, a circle moves randomly on a screen and the subject is instructed to try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average speed of the moving circle as a percentage of the maximum speed of the circle over a 3.5-minute period was used for analysis.

BODY SWAY

Postural stability was assessed by body sway as previously described by others.[19,29] Anteroposterior body sway was measured with closed eyes, using a body sway meter (Celesco) based on Wright ataxiameter.[30] All body movements over a 2-minute period were integrated and expressed as millimetres of sway and recorded.

PUPILLOMETRY

Pupillometry was performed as described previously.[31] While subjects were sitting in a chair with their head resting in a head support system, a picture was taken from both eyes simultaneously. The ratio between pupil and iris diameter was measured using Qpupil (radiology department, LUMC, the Netherlands). This ratio was used to make sure that pupil size measurement was independent of distance between camera and subject.

PHARMACO-EEG

Continuous EEG recordings were made using a 40-channel recording system (Refa-40, TMSi B.V., the Netherlands). EEGs were recorded using 21 electrodes, which were placed according to the international 10-20 system, except electrodes near the mastoids replaced those on the earlobes. The scalp electrode impedance was kept below 5kΩ.

The ground electrode was placed at AFZ (Auricular Frontal midline). Additionally, to detect ocular artefacts, vertical and horizontal electro-oculo-graphic (EOG) signals were also recorded. Two Ag/AgCl electrodes were placed at the outer canthi of both eyes, and two Ag/AgCl electrodes were placed approximately 2 cm above and below the right eye. The derivations of interest for this study were medline frontal-central (Fz-Cz) and midline parietal-occipital left (Pz-O1) and right (Pz-O2).

EEGs were recorded and analysed in line with guidelines described by the international pharmaco-EEG society (IPEG).[32] Subjects were instructed not to stare, to limit their head and eye-movements, and to suppress eye-blinks. Resting-state EEG recordings with open and closed eyes for 5 minutes in each eye state were performed. All signals were sampled at a sampling rate of 1024 Hz and filtered prior to storage using a first order recursive high-pass filter with a cut-off frequency at 0.1 Hz. Digital markers were recorded by the amplifier indicating the start and end of each eye state.

Recorded channels were band-pass filtered using a third order Butterworth filter with cut-off frequencies at 0.1 and 45.0 Hz. The filtered signals were divided into four second epochs. Epochs containing ocular artifacts were removed for further analysis. A power spectrum density (PSD) was calculated for each epoch and averaged for each eye state. The resulting PSDs were subdivided into bands and the total power per band was calculated. The following parameters (all μV) were collected: Alpha-power Fz-Cz, Alpha-power Pz-Oz, Beta-power Fz-Cz, Beta-power Pz-Oz, Gamma-power Fz-Cz, Gamma-power Pz-Oz, Delta-power Fz-Cz, Delta-power Pz-Oz, Theta-power Fz-Cz, Theta-power Pz-Oz.

VISUAL ANALOGUE SCALES (VAS)

VAS in this study were used as originally described by Norris.[33] Dutch versions of the scales have been frequently employed at CHDR, for a variety of sedative agents[19] and circumstances.[20] For VAS Bond and Lader, subjects indicate (with vertical marks) on sixteen horizontal 100-mm VAS how they feel. From these measurements, three main factors are calculated as described by Bond and Lader.[34] These three factors are 'subjective alertness' (from nine scores), 'contentedness or mood' (from five scores) and 'calmness' (from two scores).[34] VAS Bowdle evaluates psychedelic effects with thirteen 10 cm VAS lines ranging from 0 (not at all) to 100 mm (extremely).[35] These scores are clustered into three distinct total sum scores: 'internal perception' (reflects inner feelings that do not correspond with reality, including mistrustful feelings), 'external perception' (reflects a misperception of an external stimulus or a change in the awareness of the subject's surroundings) and 'feeling high.'[35]

NEURO-ENDOCRINE HORMONES

Samples were analysed by an independent bioanalytical laboratory (Analytisch Biochemisch Laboratorium BV, Assen, The Netherlands). Cortisol concentration

was determined using a validated LC-MS/MS method LLOQ of 2.00 ng/mL and CV between 2.4% and 9.9% across measurements. Prolactin concentration was determined using a qualified time-resolved fluoroimmunassay with LLOQ of 0.260 ng/mL and CV between 0.9% and 1.6% across measurements. Growth hormone concentration was determined using a qualified enzyme immunoassay with LLOQ 0.550 µIU/mL and CV between-4.8% and 18.6% across measurements.

ANALYSIS

PHARMACOKINETICS

PK parameters were calculated from concentration data in mass/volume units. Parameters were calculated using noncompartmental analysis, using actual elapsed time from dosing to estimate individual plasma PK parameters. These parameters were: C_{max}, t_{max}, $t_{½}$, area under the concentration-time curve from time zero to the last quantifiable concentration timepoint (AUC_{last}), area under the concentration-time curve from time zero to infinity (AUC_{∞}). All PK data were summarised by treatment group using descriptive statistics. Values were expressed as the mean ± SD for all parameters except T_{max}, which was presented as the median (range).

STATISTICAL ANALYSIS

Statistical analyses were performed using the SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA). Placebo subjects from all cohorts were pooled together to form a placebo group. Comparisons were then made between active treatment group and the pooled placebo group. The only exception to this was VAS Bowdle as there were many 0 scores in the placebo arm. Therefore, the lowest dose of ALKS 7119 (3 mg) was used as the reference treatment to which the higher dose levels were compared. Repeatedly measured PD data were summarised by treatment group and timepoint and analysed with a mixed-effects model for repeated measure (MMRM) with treatment, timepoint, and the interaction term of treatment by time as fixed factors and subject as a random factor. The baseline measurement was included as a covariate. Baseline was defined as the last non-missing value before randomised study drug administration. MMRM was conducted for the change from baseline over the 12-hour post-dose period as the dependent variable. No adjustment for multiple testing was performed. Treatment effects of each ALKS 7119 dose against placebo were reported using least squares means (LSM), least squares mean difference, 95% confidence interval (CI) and the *P*-value.

Body sway (antero-posterior sway in mm/2 minutes) and pharmaco-EEG endpoints were natural log transformed before entering the MMRM. For these endpoints, LS mean, LS mean difference and 95% CI were transformed back to their original scale (ie, to geometric mean and geometric mean ratio).

Neuro-endocrine hormones underwent natural log transformation before entering the same MMRM model as was used for the NeuroCart analyses, with the only difference that analyses were performed with the 2 hours post-dose data, the expected t_{max}, instead of the post-dose data over 12 hours. To represent results the neuro-endocrine hormone data were transformed back to their original scale.

RESULTS

SUBJECTS

One hundred healthy male subjects between 18 and 45 years of age were included (Table 1). All except one subject completed the study. This subject discontinued the study due to personal reasons unrelated to the study and did not perform the 36-hour post dose assessments. Data obtained for this subject were included in the analysis. (Table 1).

PHARMACOKINETICS

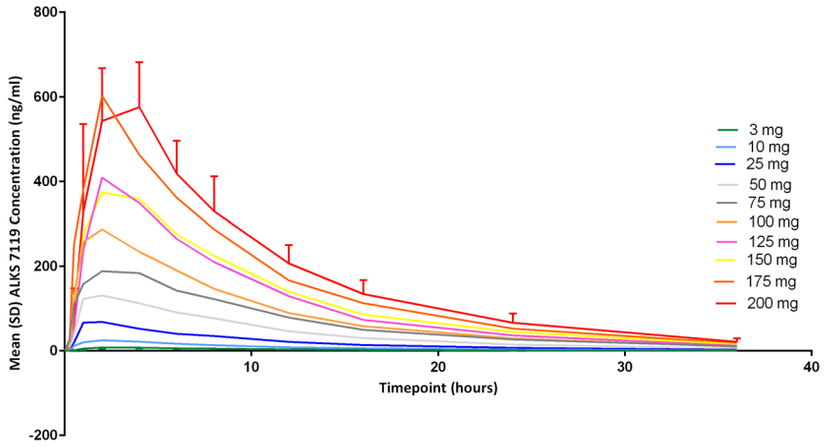
Peak plasma concentrations were reached between 0.5 and 4 hours and mean $t_{½}$ ranged from 7.0 to 8.5 hours across the dose range of 3 mg to 200 mg. Systemic exposure to ALKS 7119 (C_{max}, AUC_{∞} and AUC_{last}) increased dose proportionally over the evaluated dose range (Table 2, Figure 2).

Table 1 Demographics

Characteristic	ALKS 7119 N=80	Placebo N=20
AGE, YEARS		
Mean (SD)	24.1 (4.6)	24.6 (5.6)
HEIGHT (CM)		
Mean (SD)	182.6 (7.5)	181.8 (8.8)
WEIGHT (KG)		
Mean (SD)	76.6 (11.8)	78.8 (12.6)
BODY MASS INDEX (KG/M^2)		
Mean (SD)	22.8 (2.8)	23.7 (3.2)
RACE, N (%)		
White	69 (86.3)	16 (80)
Other	7 (8.8)	3 (15)
Asian	2 (2.5)	0 (0)
Black or African American	2 (2.5)	1 (5.0)

Abbreviations: SD, standard deviation; n, number.

Figure 2 Mean (SD) concentration of ALKS 7119 (ng/ml) (linear scale)



Abbreviation: SD, standard deviation.

Table 2 Pharmacokinetic characteristics of ALKS 7119

Parameter Statistic		ALKS 7119 10 mg (n=8)	ALKS 7119 25 mg (n=8)	ALKS 7119 50 mg (n=8)	ALKS 7119 75 mg (n=8)	ALKS 7119 100 mg (n=8)	ALKS 7119 125 mg (n=8)	ALKS 7119 150 mg (n=8)	ALKS 7119 175 mg (n=8)	ALKS 7119 200 mg (n=8)
C_{max} (NG/ML)										
Mean (SD) Range	8.33 (2.78) 5.87- 13.20	25.33 (3.91) 20.40 – 32.30	74.51 (16.19) 51.00 – 93.00	140.00 (32.44) 103.00 – 190.00	225.50 (40.59) 190.00 – 302.00	307.63 (75.28) 224.00- 465.00	447.38 (133.80 315.00 – 745.00	413.38 (65.38) 314.00 – 540.00	660.63 (167.66) 451.00 – 893.00	656.00 (104.74) 491.00 – 795.00
C_{max}/DOSE	2.78	2.53	2.98	2.80	3.01	3.08	3.58	2.76	3.78	3.28
T_{max} (HOUR)										
Median Range	2.022.00 – 4.15	2.001.00 – 2.00	1.001.00- 2.00	1.531.00 – 4.00	1.500.50 – 4.00	1.501.00 – 4.08	2.002.00 – 4.00	2.001.00 – 4.00	2.011.02 – 4.00	3.001.00 – 4.00
AUC_{∞} (HR*NG/ML)										
Mean (SD) Range	100.03 (25.04) 73.65 – 140.04	295.38 (32.29) 233.76 – 338.34	731.29 (87.27) 640.06 – 883.94	1547.06 (196.89) 1277.55 – 1828.84	2548.51 (468.23) 1772.06 – 3367.86	3136.45 (634.60) 2418.74 – 4525.46	4187.59 (840.18) 3188.91 – 5732.16	4533.66 (862.77) 3790.65 – 6362.63	5954.09 (1047.00) 4820.27 – 7488.93	6721.82 (1227.19) 5273.49 – 9126.15
$AUC_{Infinite/dose}$	33.34	29.54	29.25	30.94	33.98	31.36	33.50	30.22	34.02	33.61
AUC_{last} (HR*NG/ML)										
Mean (SD) Range	85.88 (25.56) 57.37 – 129.02	272.60 (35.69) 198.45 – 321.20	703.56 (89.80) 608.46 – 864.96	1473. 57 (203.53) 1234.86 – 1780.97	2406.33 (373.64) 1732.82 – 2969.47	3027.23 (600.53) 2340.29 – 4326.23	4059.17 (809.98) 3120.58 – 5579.28	4361.53 (782.96) 3622.31 – 6015.06	5754.94 (1013.23) 4681.45 – 7356.86	6490.47 (1144.87) 5120.43 – 8737.25
$T_{½}$ (HOUR)										
Mean (SD) Range	6.98(1.16) 5.20 – 8.77	8.51 (0.89) 7.01 – 9.45	7.81 (0.83) 6.78 – 9.41	7.81 (0.69) 6.99 – 8.68	8.42 (1.63) 6.84 – 12.01	7.63 (0.93) 6.36 – 8.84	7.26 (0.99) 6.06 – 8.72	7.53 (0.88) 6.10 – 8.51	7.48 (0.87) 5.91 – 8.83	7.35 (0.58) 6.72 – 8.33

Abbreviations: C_{max}, maximum concentration; AUC, area under the curve; SD, standard deviation.

NEUROCART® ASSESSMENTS

A statistically significant overall treatment effect towards increased pupil/iris ratio was observed for both left and right pupil/iris ratio measurements ($P < 0.01$ and $P < 0.01$), respectively) (Table 3). In general, this effect was observed with doses of 50 mg or higher (Table 3, Figure 3). Pupil/iris ratio increases were observed from approximately 2 hours post-dose, coinciding with the time where the peak concentrations of ALKS 7119 were observed (Figure 3).

On the other NeuroCart assessments no statistically significant overall treatment effects were observed (Table 3). Individual treatment effects for VAS Bowdle could not be calculated due to too many values under placebo and 3 mg being '0', making the data unsuitable for MMRM analysis.

Of note is that body sway was only performed in cohorts 1 through 6. After completion of these cohorts, the concern was raised that the test might evoke AEs of postural dizziness in some subjects, leading to the decision not to perform this test in the remaining cohorts.

Table 3 NeuroCart treatment effects compared to placebo. Least squares mean change from baseline over the 12-hour post-dose period. Estimate of difference compared to placebo (standard error)d [95%-confidence interval], p-value

Parameter	Overall	Placebo	ALKS 7119 3 mg	ALKS 7119 10 mg	ALKS 7119 25 mg	ALKS 7119 50 mg	ALKS 7119 75mg	ALKS 7119 100 mg	ALKS 7119 125 mg	ALKS 7119 150 mg	ALKS 7119 175 mg	ALKS 7119 200 mg
Left pupil/ iris ratio	LSM[a] CFB[b]	0.01	0.01	0.04	0.04	0.05	0.04	0.04	0.04	0.03	0.05	0.08
	$P < 0.01$*		0 (0.01) [-0.02, 0.03] $P = 0.93$	0.03 (0.01) [0, 0.05] $P = 0.04$*	0.03 (0.01) [0, 0.05] $P = 0.05$*	0.04 (0.01) [0.01; 0.07] $P < 0.01$*	0.03 (0.01) [0, 0.05] $P = 0.03$ *	0.03 (0.01) [0, 0.05] $P = 0.03$ *	0.03 (0.01) [0.01, 0.06] $P = 0.01$ *	0.02 (0.01) [0, 0.05] $P = 0.08$	0.04 (0.01) [0.05, 0.1] $P < 0.01$ *	0.07 (0.01) [0.05, 0.1] $P < 0.01$ *
Right pupil/ iris ratio	LSM CFB	0.01	0.02	0.03	0.03	0.07	0.03	0.05	0.05	0.05	0.06	0.06
	$P < 0.01$*		0.01 (0.01) [-0.02, 0.04] $P = 0.43$	0.02 (0.01) [-0.01, 0.04] $P = 0.23$	0.02 (0.01) [-0.01, 0.05] $P = 0.16$	0.06 (0.01) [0.03, 0.09] $P < 0.01$ *	0.02 (0.01) [-0.01, 0.05] $P = 0.12$	0.04 (0.01) [0.01, 0.06] $P = 0.01$ *	0.03 (0.01) [0.01, 0.06] $P = 0.01$ *	0.03 (0.01) [0.01, 0.06] $P = 0.02$ *	0.05 (0.01) [0.02, 0.08] $P < 0.01$ *	0.05 (0.01) [0.02, 0.08] $P < 0.01$ *
Saccadic peak velocity (deg/s)	LSM CFB	-2.14	-16.15	1.46	6.12	2.91	-5.63	13.25	5.98	18.15	5.26	-0.64
	$P = 0.19$		-14.01 (9.2) [-32.3, 4.28] $P = 0.13$	3.6 (9.44) [-15.16, 22.37] $P = 0.70$	8.26 (9.17) [-9.96, 26.48] $P = 0.37$	5.05 (9.24) [-13.31, 23.41] $P = 0.59$	-3.49 (9.17) [-21.71, 14.73] $P = 0.70$	15.39 (9.23) [-2.95, 33.74] $P = 0.10$	8.12 (9.14) [-10.03, 26.28] $P = 0.38$	20.29 (9.14) [2.13, 38.45] $P = 0.03$ *	7.4 (9.15) [-10.77, 25.57] $P = 0.42$	1.5 (9.21) [-16.81, 19.81] $P = 0.87$
Smooth pursuit (%)	LSM CFB	-1.17	-0.26	0.06	-1.44	-0.03	-3.44	-0.49	-3.82	-1.14	-1.07	1.24
	$P = 0.37$		0.91 (1.65) [-2.37, 4.2] $P = 0.58$	1.23 (1.66) [-2.06, 4.52] $P = 0.46$	-0.27 (1.65) [-3.56, 3.02] $P = 0.87$	1.14 (1.66) [-2.16, 4.44] $P = 0.49$	-2.26 (1.68) [-5.61, 1.08] $P = 0.18$	0.68 (1.66) [-2.61, 3.97] $P = 0.68$	-2.64 (1.67) [-5.96, 0.68] $P = 0.12$	0.03 (1.66) [-3.27, 3.34] $P = 0.98$	0.1 (1.66) [-3.19, 3.39] $P = 0.95$	2.41 (1.66) [-0.89, 5.7] $P = 0.15$
Body sway (LOG mm)	LSM CFB	0.03	-0.14	0.16	-0.03	0.12	-0.06	0.08	Not done	Not done	Not done	Not done
	$P = 0.34$		-0.17 (0.13) [-0.43, 0.08] $P = 0.18$	0.13 (0.13) [-0.13, 0.39] $P = 0.33$	-0.06 (0.13) [-0.32, 0.2] $P = 0.65$	0.09 (0.13) [-0.17, 0.34] $P = 0.50$	-0.1 (0.13) [-0.35, 0.16] $P = 0.45$	0.05 (0.13) [-0.21, 0.3] $P = 0.72$				

(Continuation Table 3)

Parameter	Overall	Place-bo	ALKS 7119 3 mg	ALKS 7119 10 mg	ALKS 7119 25 mg	ALKS 7119 50 mg	ALKS 7119 75mg	ALKS 7119 100 mg	ALKS 7119 125 mg	ALKS 7119 150 mg	ALKS 7119 175 mg	ALKS 7119 200 mg
Adaptive tracking (%)	LSM CFB	1.02	0.15	2.4	0.24	2.04	0.25	1.05	0.8	0.65	0.9	0.72
	P = 0.51		-0.87 (0.88) [-2.61, 0.87] *P* = 0.32	1.38 (0.87) [-0.36, 3.12] *P* = 0.12	-0.79 (0.88) [-2.53, 0.96] *P* = 0.37	1.01 (0.88) [-0.73, 2.75] *P* = 0.25	-0.78 (0.88) [-2.52, 0.97] *P* = 0.38	0.03 (0.87) [-1.71, 1.77] *P* = 0.97	-0.22 (0.88) [-1.97, 1.52] *P* = 0.80	-0.37 (0.88) [-2.11, 1.37] *P* = 0.67	-0.12 (0.87) [-1.86, 1.61] *P* = 0.89	-0.3 (0.87) [-2.04, 1.44] *P* = 0.73
VAS Bond and Lader *'Subjective alertness'* (mm)	LSM CFB	-0.57	1.64	0.03	0.26	0.96	-1.02	0.57	-0.56	1.94	-1.17	0.09
	P = 0.50		2.21 (1.28) [-0.33, 4.75] *P* = 0.09	0.61 (1.28) [-1.93, 3.15] *P* = 0.64	0.84 (1.28) [-1.7, 3.38] *P* = 0.51	1.53 (1.31) [-1.08, 4.14] *P* = 0.25	-0.45 (1.28) [-2.99, 2.09] *P* = 0.73	1.14 (1.29) [-1.41, 3.7) *P* = 0.38	0.01 (1.28) [-2.53, 2.56] *P* = 0.99	2.51 (1.28) [-0.03, 5.06] *P* = 0.05	-0.59 (1.28) [-3.14, 1.96] *P* = 0.65	0.66 (1.3) [-1.93, 2.28] *P* = 0.61
VAS Bond and Lader *'Mood'* (mm)	LSM CFB	-0.33	6.17	-0.04	-0.14	4.47	-0.82	0.56	1.63	3.35	0.11	0.51
	P = 0.15		6.5 (2.23) [2.06, 10.93] *P* < 0.00 *	0.29 (2.23) [-4.13, 4.72] *P* = 0.90	0.19 (2.23) [-4.24, 4.62] *P* = 0.93	4.8 (2.36) [0.1, 9.59] *P* = 0.05*	-0.49 (2.22) [-4.91, 3.93] *P* = 0.83	0.89 (2.23) [-3.54, 5.32] *P* = 0.69	1.96 (2.26) [-2.53, 6.44] *P* = 0.39	3.68 (2.23) [-0.74, 8.11] *P* = 0.10	0.44 (2.23) [-3.98, 4.86] *P* = 0.84	0.84 (2.28) [-3.68, 5.36] *P* = 0.71
VAS Bond and Lader *'Calmness'* (mm)	LSM CFB	-0.58	6.65	-0.31	-0.57	3.6	0.26	0.58	0.46	2.59	0.08	0.68
	P = 0.33		7.22 (2.55) [2.16, 12.29] *P* < 0.01 *	0.27 (2.58) [-4.85, 5.38] *P* = 0.92	0.01 (2.55) [-5.07, 5.08] *P* = 1.00	4.17 (2.62) [-1.03, 9.37] *P* = 0.11	0.83 (2.55) [-4.24, 5.9] *P* = 0.75	1.15 (2.55) [-3.92, 6.22] *P* = 0.65	1.04 (2.56) [-4.06, 6.13] *P* = 0.69	3.16 (2.57) [-1.95, 8.28] *P* = 0.22	0.66 (2.61) [-4.52, 5.84] *P* = 0.80	1.25 (2.61) [-3.94, 6.45] *P* = 0.63
VAS Bowdle *'external perception'*	LSM CFB *P* = 0.56	NA[c]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
			NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
VAS Bowdle *'internal perception'*	LSM CFB	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	P = 0.73		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
VAS Bowdle *'feeling high'*	LSM CFB	NA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	P = 0.35		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Alpha-power Fz-Cz (Hz)	LSM CFB	0.06	0.01	0.09	-0.01	0.08	0.16	0.06	0.05	0.15	0.02	0.01
	P = 0.99		0 [-0.97, 0.97] *P* = 1.00	0.15 [-0.94, 1.24] *P* = 0.79	-0.33 [-1.38, 0.73] *P* = 0.54	0.13 [-0.81, 1.07] *P* = 0.79	0.32 [-0.64, 1.29] *P* =0 .51	0.57 [-0.45, 1.59] *P* = 0.27	-0.17 [-1.24,-.89] *P* = 0.75	0.99 [0, 1.99] *P* = 0.05	-0.15 [-1.19, 0.9] *P* = 0.78	-0.28 [-1.39, 0.82] *P* = 0.61
Alpha-power Pz-Oz (Hz)	LSM CFB	0.1	-0.06	0.11	-0.01	0.03	0.14	0.12	0.11	-0.07	-0.02	0.24
	P = 0.63		-0.16 [-0.41, 0.10] *P* = 0.23	0.01 [-0.25, 0.27] *P* = 0.94	-0.11 [-0.36, 0.15] *P* = 0.41	-0.06 [-0.32, 0.19] *P* = 0.62	0.04 [-0.22, 0.30] *P* = 0.75	0.02 [-0.23, 0.28] *P* = 0.85	0.01 [-0.25, 0.27] *P* = 0.93	-0.17 [-0.43, 0.09] *P* = 0.19	-0.12 [-0.37, 0.14] *P* = 0.36	0.14 [-0.11, 0.40] *P* = 0.27
Beta-power Fz-Cz (Hz)	LSM CFB	0.12	0.03	0.08	0.05	0.04	0.21	0.08	0.12	0.11	0.08	0.07
	P = 0.98		-0.09 [-0.31, 0.14] *P* = 0.45	-0.03 [-0.26, 0.19] *P* = 0.78	-0.06 [-0.28, 0.16] *P* = 0.58	-0.07 [-0.31, 0.15] *P* = 0.51	0.09 [-0.13, 0.32] *P* = 0.40	-0.03 [-0.25, 0.19] *P* = 0.77	0.01 [-0.21, 0.23] *P* = 0.93	-0.01 [-0.22, 0.22] *P* = 0.97	-0.04 [-0.26, 0.18] *P* = 0.73	-0.05 [-0.27, 0.17] *P* = 0.67
Beta-power Pz-Oz (Hz)	LSM CFB	0.13	-0.03	0.14	0.08	0.13	0.17	0.25	0.14	-0.07	0.01	0.2
	P = 0.21		-0.16 [-0.36, 0.04] *P* = 0.11	0.01 [-0.19, 0.21] *P* = 0.91	-0.04 [-0.24, 0.15] *P* = 0.66	0 [-0.20, 0,20] *P* = 1.00	0.04 [-0.16, 0.24] *P* = 0.69	0.12 [-0.07, 0.32] *P* = 0.22	0.02 [-0.18, 0.22] *P* = 0.87	-0.19 [-0.39, 0.01] *P* = 0.06	-0.12 [-0.32, 0.08] *P* = 0.24	0.07 [-0.12, 0.27] *P* = 0.46

(Continuation Table 3)

Parameter	Overall	Place-bo	ALKS 7119 3 mg	ALKS 7119 10 mg	ALKS 7119 25 mg	ALKS 7119 50 mg	ALKS 7119 75mg	ALKS 7119 100 mg	ALKS 7119 125 mg	ALKS 7119 150 mg	ALKS 7119 175 mg	ALKS 7119 200 mg
Gamma-power Fz-Cz (Hz)	LSM CFB	0.1	0.05	0.09	0.06	0.01	0.27	0.1	0.12	0.14	0.16	0.21
	P = 0.70		-0.04 [-0.25, 0.16] *P* = 0.66	-0.01 [-0.21, 0.21] *P* = 0.95	-0.04 [-0.04, 0.16] *P* = 0.69	-0.09 [-0.31, 0.11] *P* = 0.39	0.17 [-0.03, 0.37] *P* = 0.10	<-0.01 [-0.20, 0.21] *P* = 0.99	0.02 [-0.18, 0.22] *P* = 0.85	0.04 [-0.16, 0.24] *P* = 0.71	0.06 [-0.14, 0.26] *P* = 0.57	0.11 [-0.09, 0.31] *P* = 0.29
Gamma-power Pz-Oz (Hz)	LSM CFB	0.21	0.09	0.13	0.16	0.08	0.27	0.27	0.12	0.09	0.03	0.31
	P = 0.67		-0.12 [-0.37, 0.13] *P* = 0.35	-0.08 [-0.33, 0.17] *P* = 0.54	-0.05 [-0.30, 0.20] *P* = 0.70	-0.12 [-0.38, 0.13] *P* = 0.33	0.06 [-0.19, 0.32] *P* = 0.64	0.06 [-0.19, 0.32] *P* = 0.63	-0.09 [-0.35, 0.17] *P* = 0.49	-0.12 [-0.37, 0.14] *P* = 0.37	-0.18 [-0.43, 0.08] *P* = 0.17	0.11 [-0.15, 0.36] *P* = 0.40
Delta-power Fz-Cz (Hz)	LSM CFB	0.1	0	0.05	-0.04	0	0.17	-0.03	0.09	-0.08	0.09	0.02
	P = 0.68		-0.11 [-0.32, 0.10] *P* = 0.32	-0.05 [-0.26, 0.16] *P* = 0.65	-0.14 [-0.35, 0.07] *P* = 0.19	-0.10 [-0.31, 0.11] *P* = 0.34	0.06 [-0.15, 0.27] *P* = 0.55	-0.13 [-0.34, 0.08] *P* = 0.22	-0.01 [0.22, 0.20] *P* = 0.90	-0.19 [-0.41, 0.03] *P* = 0.08	-0.01 [-0.23, 0.20] *P* = 0.91	-0.08 [-0.29, 0.13] *P* = 0.45
Delta-power Pz-Oz (Hz)	LSM CFB	0.06	-0.12	0.07	0	0.08	0.1	0.08	0.22	0.02	-0.07	0.26
	P = 0.28		-0.18 [-0.41, 0.05] *P* = 0.12	0.01 [-0.23, 0.24] *P* = 0.96	-0.06 [-0.31, 0.17] *P* = 0.61	0.02 [-0.21, 0.25] *P* = 0.87	0.04 [-0.21, 0.27] *P* = 0.76	0.02 [-0.21, 0.25] *P* = 0.89	0.15 [-0.08, 0.39] *P* = 0.19	-0.04 [-0.27, 0.19] *P* = 0.71	-0.13 [-0.36, 0.11] *P* = 0.26	0.20 [-0.04, 0.43] *P* = 0.10
Theta-power Fz-Cz (Hz)	LSM CFB	0.07	-0.06	0.02	-0.06	0.02	0.18	0.03	0.1	0	0.08	0.07
			-0.14 [-0.36, 0.09] *P* = 0.23	-0.05 [-0.28, 0.18] *P* = 0.65	-0.13 [-0.36, 0.09] *P* = 0.24	-0.05 [-0.28, 0.17] *P* = 0.64	0.11 [-0.12, 0.33] *P* = 0.34	-0.04 [-0.27, 0.19] *P* = 0.72	0.03 [-0.20, 0.25] *P* = 0.82	-0.07 [-0.31, 0.16] *P* = 0.54	-0.01 [-0.22, 0.23] *P* = 0.97	<-0.01 [-0.23, 0.22] *P* = 0.97
Theta-power Pz-Oz (Hz)	LSM CFB	0.07	-0.1	0.04	-0.04	0.08	0.12	0.14	0.15	0	-0.07	0.25
	P = 0.35		-0.17 [-0.40, 0.06] *P* = 0.15	-0.03 [-0.26, 0.20] *P* = 0.79	-0.11 [-0.35, 0.12] *P* = 0.33	0.01 [-0.23, 0.24] *P* = 0.95	0.05 [-0.20, 0.28] *P* = 0.69	0.08 [-0.16, 0.30] *P* = 0.56	0.08 [-0.15, 0.31] *P* = 0.50	-0.07 [-0.31, 0.16] *P* = 0.53	-0.14 [-0.37, 0.10] *P* = 0.25	0.18 [-0.05, 0.41] *P* = 0.13

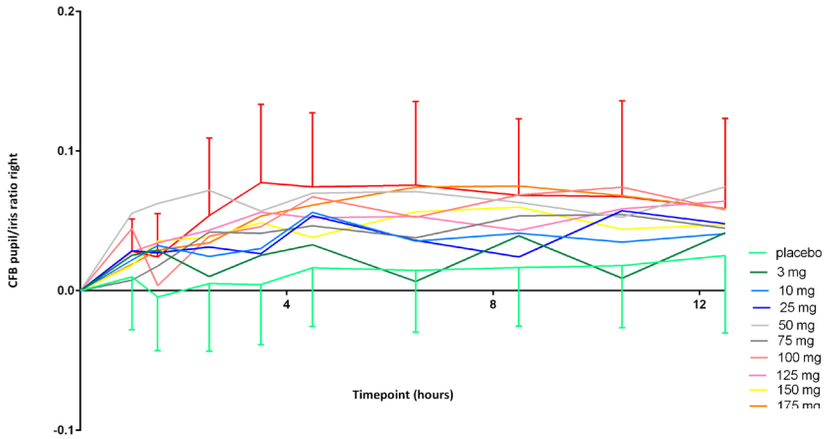
Abbreviations: [a]LSM = least squares mean, [b]CFB = change from baseline,[c]NA = not applicable,[d]for log-transformed variables no standard error was calculated, * indicates statistical significance

Table 4 Neuro-endocrine hormone levels compared to placebo at 2 hours post-dose. Estimate of difference (95% CI), p-value

	Placebo LSM[a] CFB[b]	ALKS 7119 3 mg	ALKS 7119 10 mg	ALKS 7119 25 mg	ALKS 7119 50 mg	ALKS 7119 75 mg	ALKS 7119 100 mg	ALKS 7119 125 mg	ALKS 7119 150 mg	ALKS 7119 175 mg	ALKS 7119 200 mg
Cortisol (ng/ml) (% change)	-25.7	-22.9	8.9	-20.8	12.7	36.5	70.3	52.6	84.4	53.6	64.7
		-3.6 (-31.1, 34.9) *P* = 0.83	-31.8 (-51.3;-4.5) *P* = 0.03*	-6.2 (-32.9; 31.3) *P* = 0.71	-34.1 (-52.9;-7.8) *P* = 0.02*	-45.5 (-61.1;-23.8) *P* < 0.01*	-56.4 (-68.8;-38.9) *P* < 0.01*	-51.3 (-65.2;-31.8) *P* < 0.01*	-59.7 (-71.2;-43.6) *P* < 0.01*	-51.6 (-65.4;-32.3) *P* < 0.01*	-54.9 (-67.8;-36.9) *P* < 0.01*
Prolactin (ng/ml) (% change)	-6.7	-3.6	-15.3	8.0	66.0	63.4	72.0	28.0	106.9	81.7	111.3
		-3.2 (-26.9; 28.3) *P* = 0.82	10.1 (-16.9; 45.9) *P* = 0.50	-13.6 (-34.8; 14.5) *P* = 0.31	-43.8 (-57.6;-25.5) *P* < 0.01*	-42.9 (-56.9;-24.3) *P* < 0.01*	-45.8 (-59.1;-28.1) *P* < 0.01*	-27.1 (-45.1;-3.3) *P* = 0.03*	-54.9 (-66.0;-40.2) *P* < 0.01*	-48.7 (-61.3;-32.0) *P* < 0.01*	-55.8 (-66.7;-41.5) *P* < 0.01*

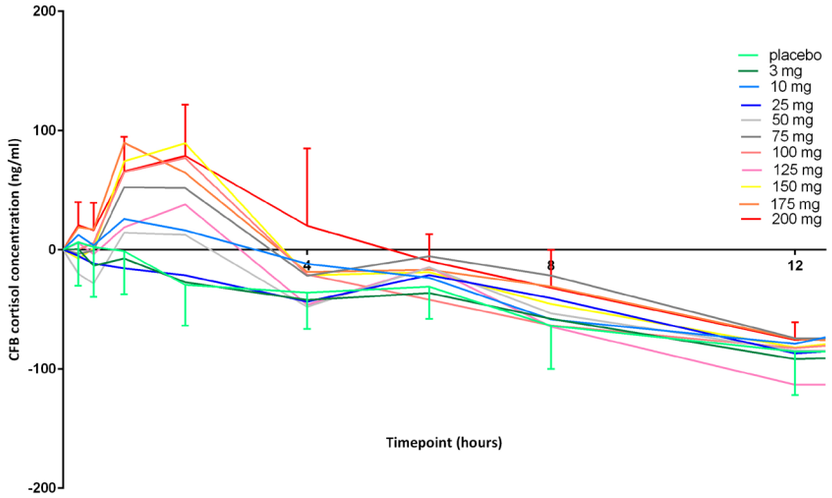
Abbreviations: LSM = least squares mean, [b]CFB = change from baseline, * indicates statistical significance

Figure 3 Mean (SD) CFB right pupil/iris ratio



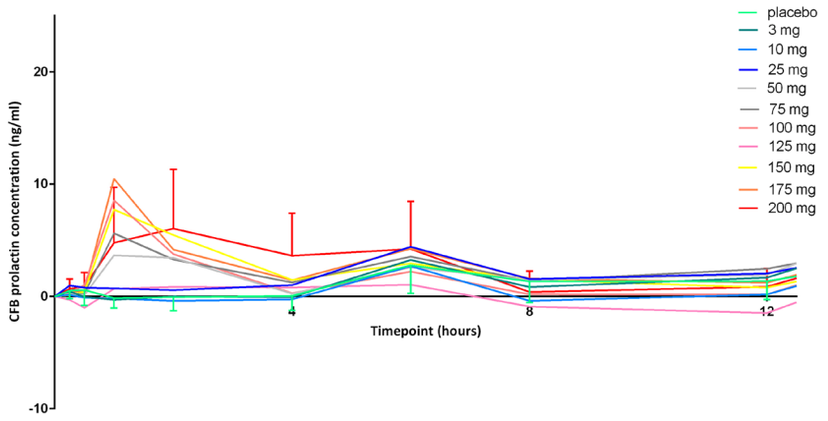
Abbreviations: CFB, change from baseline; SD, standard deviation.

Figure 4 Mean (SD) CFB cortisol concentration (ng/ml)



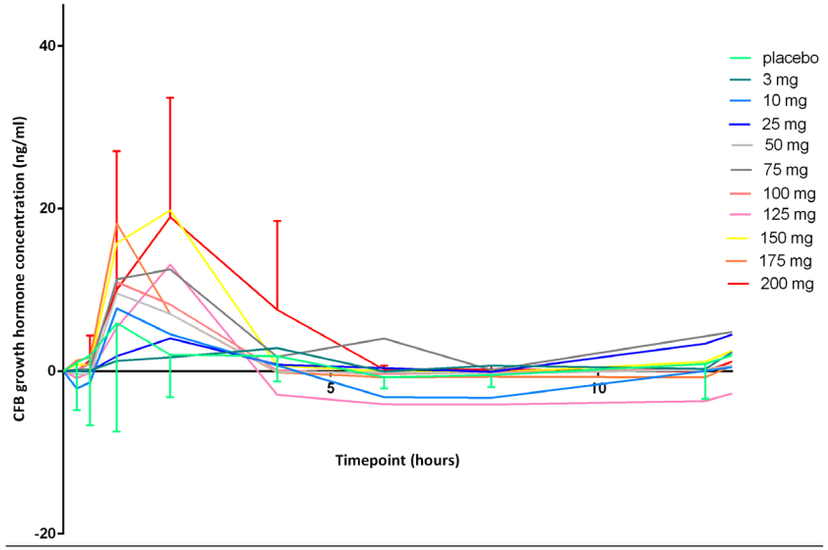
Abbreviations: CFB, change from baseline; SD, standard deviation.

Figure 5 Mean (SD) CFB prolactin concentration (ng/ml)



Abbreviations: CFB, change from baseline; SD, standard deviation.

Figure 6 Mean (SD) CFB growth hormone concentration (ng/ml)



Abbreviations: CFB, change from baseline; SD, standard deviation.

NEURO-ENDOCRINE HORMONES

A statistically significant treatment effect for serum cortisol ($P = 0.02$) and serum prolactin ($P < 0.01$) levels was observed at 2 hours post-dose, approximately the t_{max} of ALKS 7119, from dose level 50 mg and higher (Table 4, Figures 4 and 5). Growth hormone demonstrated a similar pattern as cortisol and prolactin, but this was not tested for statistical significance due to many values being below the limit of quantification (Figure 6).

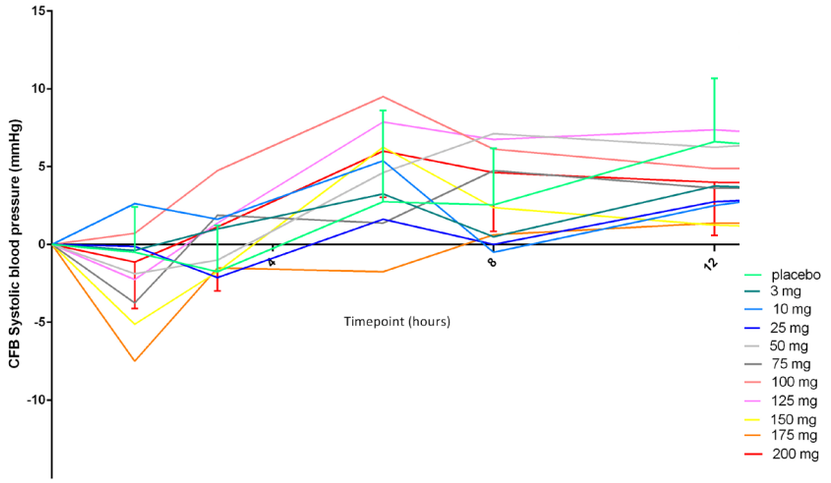
SAFETY

Treatment emergent adverse events (TEAEs) were reported in 51 (64%) subjects in the ALKS 7119 group and 8 (40%) subjects in the placebo group. The most common reported TEAEs were nausea, presyncope, somnolence, dizziness, and vomiting. Nausea and presyncope followed a dose proportional trend with greater incidence in the higher dosing groups and occurrence at or around t_{max} (Table 5). Most TEAEs were of mild severity and none were considered severe. There were no serious adverse events in the study. In general, there were no clinically meaningful findings or trends in changes from baseline for the safety laboratory parameters, urinalysis, or vital signs. For systolic blood pressure, a general trend of a mean increase from baseline was observed at all timepoints, except at 1.5 hours post-dose, where most of the ALKS 7119 treatment groups, especially the higher dose groups, demonstrated decreases from baseline (Figure 7). On diastolic blood pressure, a trend towards decrease was observed for all treatment groups, which was largest at 1.5 hours post-dose (Figure 8).

Table 5 Incidence of treatment emergent adverse events per treatment group

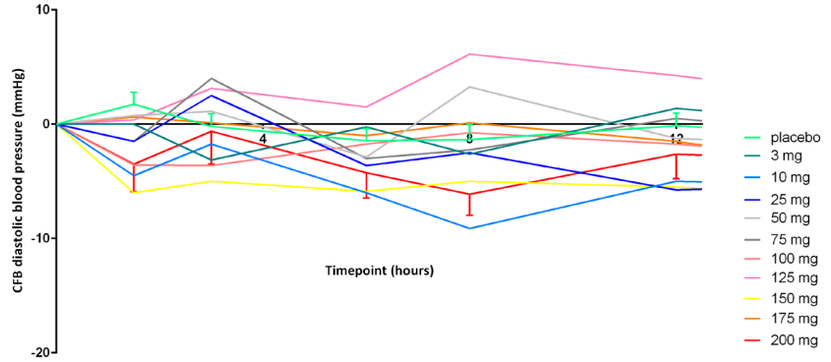
TEAE	Placebo (n=20)	ALKS 7119 3 mg (n=8)	ALKS 7119 10 mg (n=8)	ALKS 7119 25 mg (n=8)	ALKS 7119 50 mg (n=8)	ALKS 7119 75 mg (n=8)	ALKS 7119 100 mg (n=8)	ALKS 7119 125 mg (n=8)	ALKS 7119 150 mg (n=8)	ALKS 7119 175 mg (n=8)	ALKS 7119 200 mg (n=8)
Nausea	0	0	0	0	1	3	3	2	5	5	3
Vomiting	0	0	0	0	0	2	1	0	1	0	0
Fatigue	2	3	1	2	0	0	0	0	1	0	1
Headache	6	0	1	0	2	3	3	2	2	0	3
Presyncope	0	0	1	1	1	3	1	0	3	4	1
Somnolence	2	0	1	0	2	1	2	0	1	1	1
Dizziness	0	0	0	0	0	0	0	1	1	0	2
Dizziness postural	0	0	0	0	0	0	2	0	0	0	0
Oropha-ryngeal pain	0	0	0	0	0	0	0	0	0	0	0
Pruritus	0	0	0	0	0	0	0	1	0	0	2

Figure 7 Mean (±Standard Error) CFB systolic blood pressure (mmHg)



Abbreviations: CFB, change from baseline; SD, standard deviation.

Figure 8 Mean (±Standard Error) CFB diastolic blood pressure (mmHg)

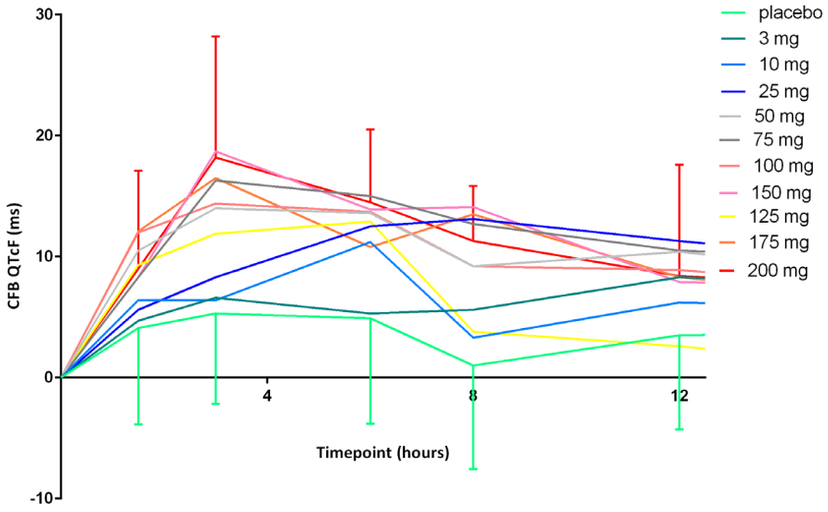


Abbreviations: CFB, change from baseline; SD, standard deviation.

The incidence of orthostatic hypotension was assessed as this could be an underlying cause of AEs of postural dizziness that were observed after the body sway test in the first six cohorts. The incidence of orthostatic hypotension was comparable between the placebo group (15%) and all ALKS 7119 dose groups (17.5%). Therefore, there did not seem to be a dose-dependent effect on orthostatic hypotension, with the possible exception of the 200 mg dosing group in which 3 (37.5%) subjects met the criteria for orthostatic hypotension.

Differences between ALKS 7119 treatment groups and placebo were observed on QT interval corrected according to Fridericia (QTcF). In 8.8% of subjects in the ALKS 7119 treatment groups, QTcF increases from baseline of 30-60 ms were observed, with the largest differences at 3, 6-and 8-hours post-dose, compared to none in the placebo group (Figure 9). Although there were no consistent dose-dependent findings, these changes were only seen at dose levels of 50 mg or higher. The highest individual QTcF value measured was 475 ms, which represented an increase of 33 ms compared to baseline in a subject treated with 200 mg of ALKS 7119. There were no TEAEs related to the changes in QTcF. No clinically meaningful trends were observed on the other ECG parameters (PR interval, QRS duration and RR interval).

Figure 9 Mean (SD) CFB QTcF



Abbreviations: CFB, change from baseline; SD, standard deviation.

DISCUSSION

From dose levels of 50 mg and higher, ALKS 7119 significantly increased pupil size and dose-dependently increased serum levels of cortisol and prolactin at 2 hours post-dose, coinciding with the t_{max} of ALKS 7119. No statistically significant overall treatment effects on the other NeuroCart tests were observed. This profile is most compatible with SERT engagement and suggestive of BBB penetration.

Therapeutic dosages of SSRIs are found to increase pupil size,[6] and to not affect other NeuroCart parameters.[3] It is hypothesised that SSRIs exert their effect on pupil size via serotonergic CNS pathways in the locus coeruleus,[36] but it cannot be completely ruled out that the pupil effects are peripherally mediated as serotonin (5-HT_7 subtype) receptors are also present on the sphincter of the pupil.[37] Binding of serotonin to these receptors leads to pupillary sphincter relaxation and thereby mydriasis.[38] The neuro-endocrine findings also point in the direction of SERT binding, as other serotonergic compounds such as fenfluramine and escitalopram are known to respectively increase cortisol and cortisol and prolactin levels in healthy volunteers.[9,11] It could be argued that the endocrine effects can be caused by pituitary stimulation, outside the BBB and CNS. However, the effects of ALKS 7119 involved several hormones concomitantly. This is difficult to attribute to a simultaneous effect on different cell populations in the pituitary, which are highly specialised and pharmacologically diverse. A hypothalamic site of action is functionally more plausible if several hormones simultaneously respond to a CNS-active compound, because of the integrative role of the hypothalamus. Since the hypothalamus is the most important autonomic command centre that governs the concerted activity of many autonomic and neuro-endocrine processes, the same argument could also be used for a central (hypothalamic) localisation of ALKS 7119-induced pupillary dilation.

Of note, no overall treatment effects of ALKS 7119 on pEEG were observed, whereas in a scientific review, it was reported that 100% of studies into the effects of SSRIs on pEEG reported an increase of total EEG power with low doses SSRIs, whilst high doses SSRIs increased delta and theta power in 33% of the studies.[6] These apparent complex dose-response relations for SSRIs seem to contrast with the lack of effects of ALKS 7119. This might reflect methodological differences in pEEG recording (for example different number of leads and analysis methods),[6] but also limitations of the literature review (for instance due to publication bias).

In theory, the effects on pupil size and neuroendocrine stimulation can also be caused by NMDA receptor antagonism.[7] However, ALKS 7119 didn't match the complete effect profile of NMDA receptor activation, which would also include decrease of saccadic peak velocity and adaptive tracking and a variety of other neurophysiological, behavioural and subjective sedative effects.[3,7,39] It is also less likely that the effects

were anti-glutamatergic rather than serotonergic, because ALKS 7119 shows a 200-fold lower affinity for the NMDA-receptor than for SERT.

The acute PD effects of mild selective sigma non-opioid intracellular receptor 1 modulation are unknown, so no PD biomarkers for this receptor could be included in our study. However, it is unlikely that the observed effects of ALKS 7119 were caused by sigma non-opioid intracellular receptor 1 modulation since ALKS 7119's affinity for this receptor is 900-fold lower than for the SERT and there was no clear indication for NMDA or even μ receptor activation, with affinities of respectively 200 and 17-fold lower than for the SERT.

A wide dose range was explored in this study, but it was not possible to escalate the dose of ALKS 7119 to levels expected to influence NMDA-receptors or sigma non-opioid intracellular receptor 1. Initially, the maximum planned dose was 100 mg based on the NOAEL in rats. This was increased based on the results of an interim analysis demonstrating linear PK with dose proportional increase in exposure and no safety or tolerability findings precluding further dose escalation to 200 mg. In the higher dose level groups, a dose-dependent trend towards a decrease in supine systolic and diastolic blood pressure at t_{max} was observed, which most likely was the result of activation of the $\alpha_{1A,B}$ adrenoceptors. SERT-inhibition might explain the occurrence of nausea and presyncope at higher dosages.

The effects of ALKS 7119 on QTcF duration were unexpected as no effects on QT intervals were observed in preclinical cardiovascular toxicity studies in dogs up to the highest given dose of 10 mg/kg corresponding to a HED of 324 mg. Next to that, *in vitro* hERG channel testing, showed no effects up to high concentrations (IC_{50} of 191.1 μM or 54348.8 ng/mL). The mechanism underlying the QTcF prolongation observed in this study remains therefore unknown. QTcF prolongation occurred in only a small number of subjects in this study, which is reminiscent of the mild prolongations that are reported for most SSRIs.[40]

Taken together, this study demonstrated a CNS effect pattern for ALKS 7119 that is in line with the drug's pharmacological binding profile. These results illustrate how biomarkers, such as the NeuroCart and serum neuro-endocrine hormone levels, can provide important information in early phase drug development to obtain a comprehensive overview of a new compound's clinical pharmacological profile. This knowledge can be used to make rational decisions in early phase clinical trials on dose escalation steps and on the further development of a compound as suggested by the conceptual framework of QBDD.[2]

REFERENCES

1 FDA guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. Published 2005. Accessed January 28, 2021. https://www.FDA.gov/media/72309/download

2 Cohen AF, Burggraaf J, van Gerven JM, Moerland M, Groeneveld GJ. The use of biomarkers in human pharmacology (Phase I) studies. *Annu Rev Pharmacol Toxicol*. 2015;55:55-74. doi:10.1146/annurev-pharmtox-011613-135918

3 Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood–brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol*. 2016;20:27-34. doi:http://dx.doi.org/10.1016/j.ddtec.2016.07.004

4 van Gerven JMAJ. Translational Medicine in CNS Drug Development. In: Nomikos, George. Feltner D, ed. *Translational Medicine in CNS Drug Development*. 1st ed. Elsevier Academic Press 2019; 2019. https://www.elsevier.com/books/translational-medicine-in-cns-drug-development/nomikos/978-0-12-803161-2

5 Murray RB, Adler MW, Korczyn AD. The pupillary effects of opioids. *Life Sci*. 1983;33(6):495-509. doi:https://doi.org/10.1016/0024-3205(83)90123-6

6 Dumont GJ, de Visser SJ, Cohen AF, van Gerven JM. Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects. *Br J Clin Pharmacol*. 2005;59(5):495-510. doi:10.1111/j.1365-2125.2005.02342.x

7 Kleinloog D. Drug-induced psychomimetic effects as a model for psychosis. Published online 2014.

8 Okkerse P, Alvarez-Jimenez R, Hay JL, et al. No evidence of potentiation of buprenorphine by milnacipran in healthy subjects using a nociceptive test battery. *Eur J Pain*. 2017;21(3):494-506. doi:10.1002/ejp.943

9 Hawken ER, Owen JA, Hudson RW, Delva NJ. Specific effects of escitalopram on neuroendocrine response. *Psychopharmacology (Berl)*. 2009;207(1):27. doi:10.1007/s00213-009-1633-1

10 Lewis DA, Sherman BM. Serotonergic regulation of prolactin and growth hormone secretion in man. *Acta Endocrinol*. 1985;110(2):152-157. internal-PDF://250.239.225.58/lewis.pdf

11 Recourt K, van der Aart J, Jacobs G, et al. Characterisation of the pharmacodynamic effects of the P2X7 receptor antagonist JNJ-54175446 using an oral dexamphetamine challenge model in healthy males in a randomised, double-blind, placebo-controlled, multiple ascending dose trial. *J Psychopharmacol*. 2020;34(9):1030-1042. doi:10.1177/0269881120914206

12 Hysek CM, Brugger R, Simmler LD, et al. Effects of the α_2-adrenergic agonist clonidine on the pharmacodynamics and pharmacokinetics of 3,4-methylenedioxymethamphetamine in healthy volunteers. *J Pharmacol Exp Ther*. 2012;340(2):286-294. doi:10.1124/jpet.111.188425

13 Jepma M, Te Beek E, Wagenmakers E-J, Van Gerven J, Nieuwenhuis S. The role of the noradrenergic system in the exploration-exploitation trade-off: a pharmacological study. *Front Hum Neurosci*. 2010;4:170. doi:10.3389/fnhum.2010.00170

14 Okkerse P, Hay JL, Sitsen E, et al. Pharmacokinetics and pharmacodynamics of intrathecally administered Xen2174, a synthetic conopeptide with norepinephrine reuptake inhibitor and analgesic properties. *Br J Clin Pharmacol*. 2017;83(4):751-763. doi:10.1111/bcp.13176

15 Alexander SPH, Christopoulos A, Davenport AP, et al. The concise guide to pharmacology 2019/20: G protein-coupled receptors. *Br J Pharmacol*. 2019;176 Suppl 1(Suppl 1):S21-S141. doi:10.1111/bph.14748

16 Alexander SPH, Kelly E, Mathie A, et al. The concise guide to pharmacology 2019/20: Transporters. *Br J Pharmacol*. 2019;176 Suppl 1(Suppl 1):S397-S493. doi:10.1111/bph.14753

17 Alexander SPH, Cidlowski JA, Kelly E, et al. The concise guide to pharmacology 2019/20: Nuclear hormone receptors. *Br J Pharmacol*. 2019;176 Suppl 1(Suppl 1):S229-S246. doi:10.1111/bph.14750

18 Alexander SPH, Mathie A, PETers JA, et al. The concise guide to pharmacology 2019/20: Ion channels. *Br J Pharmacol*. 2019;176 Suppl 1(Suppl 1):S142-S228. doi:10.1111/bph.14749

19 van Steveninck AL, Gieschke R, Schemaker RC, et al. Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. *Br J Clin Pharmacol*. 1996;41(6):565-573. doi:10.1046/j.1365-2125.1996.38514.x

20 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. *J Psychopharmacol*. 1999;13(1):10-17. doi:10.1177/026988119901300102

21 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther*. 1991;50(2):172-180.

22 de Haas SL, de Visser SJ, van der Post JP, et al. Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_A$ α2,3 subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. *J Psychopharmacol*. 2006;21(4):374-383. doi:10.1177/0269881106072343

23 Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. *Neurology*. 1975;25(11):1065-1070. doi:10.1212/wnl.25.11.1065

24 Bittencourt PR, Wade P, Smith AT, Richens A. Benzodiazepines impair smooth pursuit eye movements. *Br J Clin Pharmacol*. 1983;15(2):259-262. doi:10.1111/j.1365-2125.1983.tb01495.x

25 Borland RG, Nicholson AN. Comparison of the residual effects of two benzodiazepines (nitrazepam and flurazepam hydrochloride) and pentobarbitone sodium on human performance. *Br J Clin Pharmacol*. 1975;2(1):9-17.

26 de Haas SL, de Visser SJ, van der Post JP, et al. Pharmacodynamic and pharmacokinetic effects of MK-0343, a GABA(A) alpha2,3 subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. *J Psychopharmacol*. 2008;22(1):24-32. doi:10.1177/0269881107082108

27 Gijsman HJ, Van Gerven JM, Tieleman MC, et al. Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol*. 1998;18(4):289-295. doi:10.1097/00004714-199808000-00007

28 Cohen AF, Ashby L, Crowley D, Land G, Peck AW, Miller AA. Lamotrigine (BW430C), a potential anticonvulsant. Effects on the central nervous system in comparison with phenytoin and diazepam. *Br J Clin Pharmacol*. 1985;20(6):619-629. doi:10.1111/j.1365-2125.1985.tb05120.x

29. van Steveninck AL, Wallnofer AE, Schoemaker RC, et al. A study of the effects of long-term use on individual sensitivity to temazepam and lorazepam in a clinical population. *Br J Clin Pharmacol*. 1997;44(3):267-275. doi:10.1046/j.1365-2125.1997.t01-1-00580.x

30 Wright BM. A simple mechanical ataxia-meter. *J Physiol*. 1971;218 Suppl:27p-28p.

31 Twa MD, Bailey MD, Hayes J, Bullimore M. Estimation of pupil size by digital photography. *J Cataract Refract Surg*. 2004;30(2):381-389. doi:10.1016/S0886-3350(03)00619-9

32 Jobert M, Wilson FJ, Ruigt GS, Brunovsky M, Prichep LS, Drinkenburg WH. Guidelines for the recording and evaluation of pharmaco-EEG data in man: the International Pharmaco-EEG Society (IPEG). *Neuropsychobiology*. 2012;66(4):201-220. doi:10.1159/000343478

33 Norris H. The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology*. 1971;10(21):181-191.

34 Bond A, Lader M. The use of analogue scales in rating subjective feelings. *Br J Med Psychol*. 1974;47(3):211-218. doi:10.1111/j.2044-8341.1974.tb02285.x

35 Bowdle AT, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers relationship to steady-state plasma concentrations. *Anesthesiology*. 1998;88(1):82-88. doi:10.1097/00000542-199801000-00015

36 Yu Y, Ramage AG, Koss MC. Pharmacological studies of 8-OH-DPAT-induced pupillary dilation in anesthetized rats. *Eur J Pharmacol*.

2004;489(3):207-213. doi:10.1016/j.ejphar.2004.03.007
37 Costagliola C, Parmeggiani F, Semeraro F, Sebastiani A. Selective serotonin reuptake inhibitors: a review of its effects on intraocular pressure. *Curr Neuropharmacol*. 2008;6(4):293-310. doi:10.2174/157015908787386104
38 Gündüz GU, Parmak Yener N, Kılınçel O, Gündüz C. Effects of selective serotonin reuptake inhibitors on intraocular pressure and anterior segment parameters in open angle eyes. *Cutan Ocul Toxicol*. 2018;37(1):36-40. doi:10.1080/15569527.2017.1330270
39 Krystal JH, Karper LP, SeIByl JP, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch Gen Psychiatry*. 1994;51:199-214. http://dx.doi.org/10.1001/archpsyc.1994.03950030035004
40 Beach SR, Kostis WJ, Celano CM, et al. Meta-analysis of selective serotonin reuptake inhibitor-associated QTc prolongation. *J Clin Psychiatry*. 2014;75(5):e441-9. doi:10.4088/JCP.13r08672

APPENDIX

IB-DERISK ANALYSER OVERVIEW OF ALKS 7119

For ALKS 7119 the IB-Derisk analyser tool was applied to obtain an integrated overview of the preclinical findings (Figure 1).[1] From this overview, it became immediately apparent that only one *in vivo* pharmacology experiment was performed. In this experiment, dopamine levels in the striatum of male Wistar rats were increased by perfusion of the synthetic glutamate agonist NMDA through a microdialysis probe. Concurrent perfusion of ALKS 7119 (1mM) through the microdialysis probe completely blocked local NMDA-induced dopamine release. Oral administration of ALKS 7119 (10 mg/kg) also blocked the elevation of extracellular dopamine. In addition to this *in vivo* pharmacology experiment, predictions of pharmacological effects of ALKS 7119 were based on its *in vitro* binding affinities for different receptors. The *in vitro* profile of ALKS 7119 demonstrated a high affinity for the SERT receptor and affinities of about a factor 20 lower for the μ-receptor, a factor 30 lower for the α_{1A}-adrenoceptor, a factor 50 lower for the α_{1B}-adrenoceptor, a factor 200 lower for the NMDA receptor and a factor 900 lower for the sigma non-opioid intracellular receptor 1.

To sketch an overview of the pharmacological characteristics of ALKS 7119, its *in vitro* binding affinities for the different receptors were converted into plasma concentrations and entered in the IB- Derisk analyser tool, depicted in light blue (Figure 1). Even though there was no data on the efficacy of ALKS 7119 on the different receptors, and converting K_i values to plasma concentrations requires the debatable assumption that brain levels of ALKS 7119 equal both plasma levels and *in vitro* concentration, doing so made it possible to determine the positions of the different receptor binding levels relative to each other. Therefore, with these caveats, this overview could be used to monitor ALKS 7119's effects on the different receptors and place them in perspective.

When sorting the data on C_{max}, ALKS 7119 demonstrated a favourable profile on the IB-Derisk tool supporting the decision to progress the development of ALKS 7119 to a first-in-human study. Blockage of NMDA agonist induced dopamine release in the striatum of male Wistar rats by ALKS 7119, indicated as desired effect in green, was observed at a C_{max} value lower than C_{max} values associated with more severe adverse effects. Only in a multiple dose toxicity study in dogs of 42 days undesirable but reversible and manageable effects were observed in dogs at a similar maximum exposure as measured with at the desired effect. Undesired effects were observed in an exposure-related manner with acceptable and reversible (yellow) effects at lower C_{max} values, followed by more severe (orange) effects at higher exposures. Serious toxicity and death, indicated by the colour red, were only observed with the highest

exposure value. Next to that, a comparable pattern of the occurrence of undesired effects was observed in all the studied species in preclinical experiments. ALKS 7119 had a comparable pharmacokinetic profile across all species tested preclinically, which was reassuring for the clinical study.

In the clinical study ALKS 7119 demonstrated a pharmacological (NeuroCart) profile which was most resembling SERT inhibition.[2] ALKS 7119 significantly increased pupil size and dose-dependently increased serum levels of cortisol and prolactin. Growth hormone demonstrated a similar pattern as cortisol and prolactin, but this was not tested for statistical significance due to many values being below the limit of quantification. The neuroendocrine effects of increased cortisol and prolactin levels are also observed with known serotonin reuptake inhibitors (SSRI's).[3] Acute administration of SSRI's has also been shown to increase growth hormone levels.[4] Theoretically, these effects are difficult to separate from those of μ- opioid modulation. Opioid μ-receptor agonists are well known to decrease pupil size,[5] but the literature on the pupillary effects of opioid antagonists is contradictory, with some reporting miosis and others mydriasis.[6] It is fairly well established hat acute administration of μ-opioid agonists increases growth hormone levels in humans, this effect was attenuated by administration of an opioid-antagonist.[7] Next to that, acute administration of opioids has been found to increase prolactin levels in humans.[7] The reports on the effects of μ-receptor antagonists on prolactin release are more inconsistent, with some studies reporting no effect on prolactin release and others reporting a reduction of prolactin increase with administration of an opioid antagonist after injection of buprenorphine, a partial agonist of the μ and κ receptor.[7] ACTH levels in healthy volunteers were suppressed by opioid agonists, while acute administration of opioid antagonists increased ACTH levels.[7] Considering the 20-fold difference in affinity of ALKS 7119 for serotonergic and μ-opioid receptors, it cannot be ruled out that the effects observed in our study reflect a combination of (primarily) serotonergic agonism and (partially) μ-opioid modulation.

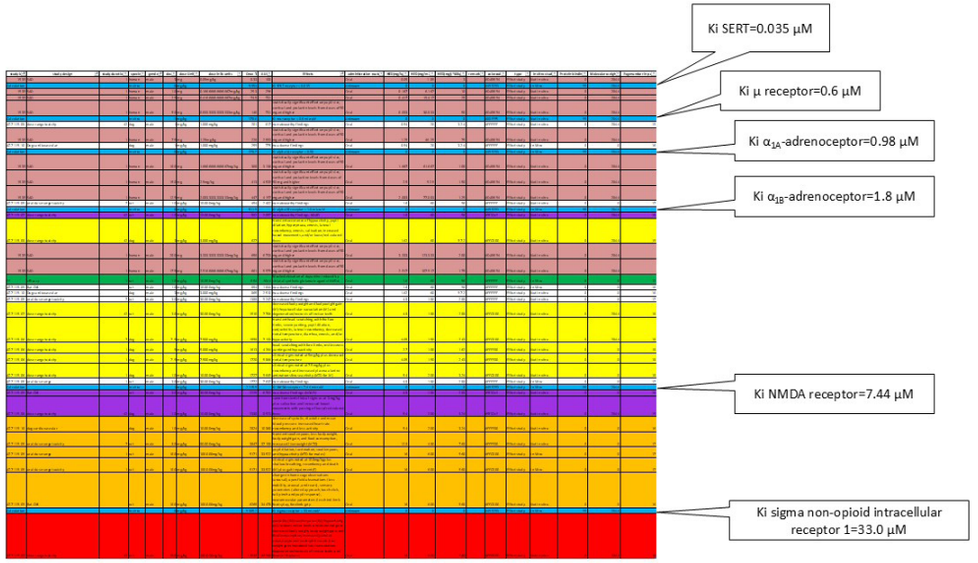
ALKS 7119 did not induce psychomimetic effects and did not affect saccadic peak velocity, adaptive tracking, alertness, body sway, all NeuroCart effects that are observed after administration of a well-known NMDA-receptor antagonist, ketamine.[8] When taking in consideration that the relative affinity for the NMDA receptor was approximately 200-fold lower than for the SERT, this is as expected based on preclinical findings (Figure 1).

In the clinical study, a dose-dependent trend towards a decrease in supine systolic and diastolic blood pressure was observed.[2] The most common observed AEs were nausea, presyncope, somnolence, dizziness and vomiting. These AEs were observed with greater incidence in the higher dose groups and started to arise from dose level 75 mg. Based on the *in vitro* affinity data, at these dose levels ALKS 7119 could be binding to the αsubscribt$_{1A}$-adrenoceptor and α_{1B}-adrenoceptor, which play an important role in blood pressure regulation,[9] possibly explaining the effects on blood pressure and adverse effects of dizziness, presyncope and nausea.

An unexpected finding in the clinical study was the increase of QTcF duration observed with dose levels of 50 mg and higher.[2] In preclinical cardiovascular toxicity studies in dogs no effects on QT intervals were observed up to the highest given dose. Next to that, *in vitro* cardiac hERG/IKr channel testing showed no effects up to high concentrations. Possibly, these effects are also caused by adrenergic inhibition, as the betablocker sotalol which inhibits adrenergic effects in the heart is associated with QTc prolongation as well.[10]

For ALKS 7119 the predictions about pharmacological effects including pharmacologically active dose ranges and safety findings based on preclinical data were well met in the clinical study, with the exception of the QTcF findings, which were not observed preclinically. In line with *in vitro* affinity findings, ALKS 7119's effects were most reminiscent of SERT inhibition, perhaps partly influenced by μ-receptor modulation. These findings illustrate how the IB-Derisk tool can be applied to integrate preclinical data and how this overview can be used during a clinical study to place observed effects in a context and make rational decisions about dose escalation and a compounds' further development.

Figure 1 IB-Derisk overview ALKS 7119



Abbreviation: K_i, inhibition constant

REFERENCES

1 Gerven van, J.M.A. CAF, Adam C. Integrating data from the Investigational Medicinal Product Dossier/investigator's brochure. A new tool for translational integration of preclinical effects. *Br J Clin Pharmacol*. 0(0). doi:doi:10.1111/bcp.13529

2 Dijkstra FM, Zuiker RGJA, Siebenga PS, et al. Pharmacological profile of ALKS 7119, an investigational compound evaluated for the treatment of neuropsychiatric disorders, in healthy volunteers. *Br J Clin Pharmacol*. 2022;n/a(n/a). doi:https://doi.org/10.1111/bcp.15229

3 Dumont GJ, de Visser SJ, Cohen AF, van Gerven JM. Biomarkers for the effects of selective serotonin reuptake inhibitors (SSRIs) in healthy subjects. *Br J Clin Pharmacol*. 2005;59(5):495-510. doi:10.1111/j.1365-2125.2005.02342.x

4 Raap D, Van de Kar LD. Selective serotonin reuptake inhibitors and neuroendocrine function. *Life Sci*. 1999;65(12):1217-1235. doi:https://doi.org/10.1016/S0024-3205(99)00169-1

5 Murray RB, Adler MW, Korczyn AD. The pupillary effects of opioids. *Life Sci*. 1983;33(6):495-509. doi:10.1016/0024-3205(83)90123-6

6 Loimer N, Schmid R, Grünberger J, Linzmayer L. Naloxone induces miosis in normal subjects. *Psychopharmacology (Berl)*. 1990;101(2):282-283. doi:10.1007/BF02244141

7 Vuong C, Van Uum SHM, O'Dell LE, Lutfy K, Friedman TC. The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev*. 2010;31(1):98-132. doi:10.1210/er.2009-0009

8 Kleinloog D. Drug-induced psychomimetic effects as a model for psychosis. Published online 2014.

9 Reid JL. Alpha-adrenergic receptors and blood pressure control. *Am J Cardiol*. 1986;57(9):6E-12E. doi:10.1016/0002-9149(86)90716-2

10 Poluzzi E, Raschi E, Moretti U, De Ponti F. Drug-induced torsades de pointes: data mining of the public version of the FDA Adverse Event Reporting System (AERS). *Pharmacoepidemiol Drug Saf*. 2009;18(6):512-518. doi:10.1002/PDs.1746

CHAPTER V

TRANSCRANIAL MAGNETIC STIMULATION AS A TRANSLATIONAL BIOMARKER FOR AMPA RECEPTOR MODULATION

Dijkstra, F. M.[1,2*], O'Donnell P.[3,4*], Damar U.[5*], Quanhong L.[3], de Goede A.A.[1], Xu L.[3], Pascual-Leone A.[5], Buhl D.L.[3], Zuiker R.G. J.A.[1], Ruijs T.Q.[1], Heuberger J.A.A.C.[1], MacMullin P.5, Lubell M.[3], Asgharnejad M.[3], Murthy V.[3], Rotenberg A.[5], Jacobs G.E.[1,2], Rosen L.[3]

**Both authors contributed equally*

Translational Psychiatry, 11(1), 325.
https://doi.org/10.1038/s41398-021-01451-2

1 Centre for Human Drug Research (CHDR), Leiden, The Netherlands
2 Department of Psychiatry, Leiden University Medical Center, Leiden, The Netherlands
3 Takeda Pharmaceuticals International, Inc., Cambridge, MA, USA
4 McLean Hospital, Department of Psychiatry, Harvard Medical School, Belmont, MA, USA
5 Neuromodulation Program, Department of Neurology and F.M. Kirby Center for Neurobiology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

ABSTRACT

AIMS TAK-653 is a novel α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)-positive allosteric modulator being developed as a potential therapeutic for major depressive disorder (MDD). Currently, there are no translational biomarkers that evaluate physiological responses to the activation of glutamatergic brain circuits available. Here, we tested whether noninvasive neurostimulation, specifically single- pulse or paired-pulse motor cortex transcranial magnetic stimulation (spTMS and ppTMS, respectively), coupled with measures of evoked motor response captures the pharmacodynamic effects of TAK-653 in rats and healthy humans.

METHODS In the rat study, five escalating TAK-653 doses (0.1–50 mg/kg) or vehicle were administered to 31 adult male rats, while measures of cortical excitability were obtained by spTMS coupled with mechanomyography. Twenty additional rats were used to measure brain and plasma TAK-653 concentrations. The human study was conducted in 24 healthy volunteers (23 males, 1 female) to assess the impact on cortical excitability of 0.5 and 6 mg TAK-653 compared with placebo, measured by spTMS and ppTMS coupled with electromyography in a double-blind crossover design. Plasma TAK-653 levels were also measured.

RESULTS TAK-653 increased both the mechanomyographic response to spTMS in rats and the amplitude of motor-evoked potentials in humans at doses yielding similar plasma concentrations. TAK-653 did not affect resting motor threshold or paired-pulse responses in humans.

CONCLUSION This is the first report of a translational functional biomarker for AMPA receptor potentiation and indicates that TMS may be a useful translational platform to assess the pharmacodynamic profile of glutamate receptor modulators.

INTRODUCTION

According to the Global Burden of Diseases, Injuries, and Risk Factors study in 2017[1] depressive disorders such as major depressive disorder (MDD) are amongst the leading causes for years lived with disability (YLD) worldwide. In 2017 depressive disorders were estimated to affect over 264 million people worldwide.[1] Furthermore, depressive disorders are associated with an increased risk of mortality.[2] Pharmacological treatments targeting monoaminergic neurotransmission are available, but these fail to achieve an adequate response in up to 50% of MDD patients.[3] This illustrates the need for the development of novel pharmacological therapeutics for the treatment of MDD.

In 2000 it was demonstrated that subanesthetic doses of ketamine, a N-methyl-D-aspartate-receptor (NMDAR)-antagonist had antidepressant effects in patients with depression.[4] Since then, many studies have replicated these findings.[5,6] Although the mechanisms by which ketamine exerts its antidepressant effects are not yet fully understood, it has been demonstrated that NMDAR blockade leads to a selective reduction in γ-aminobutyric acid (GABA) interneuron function that enhances glutamate function and increases α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor -(AMPAR)- mediated signalling.[7] This leads to a release of brain derived neurotrophic factor (BDNF) and stimulation of mammalian target of rapamycin (mTOR) signalling, which are both hypothesised to play a role in the pathophysiology of depressive disorders.[7] The importance of AMPA receptors for the antidepressant effects of ketamine was further demonstrated by the finding that pretreatment with an AMPA antagonist completely blocked the antidepressant effects of ketamine.[8] It is therefore hypothesised that direct AMPA receptor potentiation might lead to similar antidepressant efficacy as ketamine, without causing the psychotomimetic side effects commonly observed after ketamine administration.[9]

TAK-653, also known as NBI-1065845, is a central nervous system (CNS)-penetrant, selective AMPA receptor positive allosteric modulator that is being developed as a potential adjunctive therapeutic agent for patients with MDD.[10] It is intended to enhance or reproduce ketamine-driven AMPAR potentiation. In cognitive and depression-related behavioural assays TAK-653 exhibited antidepressant-like effects at low exposures in rodents (Haruride Kimuru, Takeda Pharmaceuticals, unpublished data), but evidence of immediate pharmacodynamic (PD) effects that could be translated to human studies was missing. A first-in-human dose-escalating study in healthy volunteers established the safety and tolerability of TAK-653, but there was no established methodology to assess CNS-target engagement or PD effects.[11] To continue TAK-653 development with confidence, we needed a neurocircuit-based translational PD biomarker that captures the modulation of glutamatergic synapses.

The paucity of reliable translational biomarkers that capture functional modulation of brain circuitry is a key challenge in the field of neuropsychiatric drug development.[12] With the exception of evoked potentials sensitive to N-methyl-D-aspartate (NMDA) receptor function, such as mismatch negativity and auditory steady state responses,[13-15] there has been little progress toward translatable glutamate-sensitive circuitry function biomarkers. Reliable (PD) biomarkers are needed to evaluate the functional impact of novel glutamatergic drugs on defined neurocircuits in order to guide dose selection in clinical studies and to support go/no-go decisions during drug development.[12,16]

Here, we explored whether transcranial magnetic stimulation (TMS) could be applied to produce a translational neurocircuitry biomarker for the development of a novel glutamatergic compound. TMS is a non-invasive neurostimulation method based on the principles of electromagnetic induction, in which a fluctuating magnetic field generates a localised intracranial electric current that can be sufficient to depolarize cortical neurons and activate neuronal circuits.[17] When delivered over the motor cortex, TMS leads to reliable limb muscle activation that can be quantified by surface electromyography (EMG) in humans or by accelerometer-based mechanomyography (MMG) in rats. Motor cortex TMS thus enables measures of input-output relationships between the strength of the cortical electrical stimulus and the magnitude of muscle activation. Using various stimulation paradigms that include single or paired pulses (spTMS and ppTMS, respectively), cortical signals involving glutamate or GABA signalling can be isolated.[18-20]The motor responses to TMS have been well characterised and used to demonstrate that such evoked responses are sensitive to pharmacological manipulation of CNS targets.[21,22] We therefore utilised TMS to assess corticospinal and intracortical excitability, allowing determination of a functional outcome of AMPA receptor activation.

The overall aim of our study was to evaluate TMS-evoked motor responses as potential translational neurocircuitry biomarkers for AMPA receptor modulation by TAK-653. To obtain measures of cortical excitability, we coupled TMS with MMG in rats and EMG in humans. We hypothesised that TMS-evoked motor responses would be amplified by positive allosteric modulation of AMPA receptors by TAK-653. As there was no precedent of TMS use to test the effects of agents that increase glutamate function, we included an open-label ketamine period intended to establish assay sensitivity, based on a report of ketamine effects on TMS in a small sample of healthy volunteers;[23] however, a subsequent report did not show the same effect.[24] Given these mixed results of ketamine on TMS, we did not intend to compare the ketamine results with placebo or TAK-653, so ketamine pharmacokinetic (PK) and TMS results are not be included in this report. The primary goal of the study was to assess neurostimulation with TMS as a translational biomarker for the modulation of excitatory neural circuits.

MATERIALS/SUBJECTS AND METHODS

ANIMALS

Adult male Sprague Dawley rats were housed in standard cages in a temperature-controlled facility with a 12-h light/dark cycle and a continuous supply of water and food *ad libitum*. All procedures were approved by, and in accordance with the guidelines of, the Institutional Animal Care and Use Committee at Boston Children's Hospital and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimise the number of rats used in the present experiments.

DOSING AND PHARMACOKINETIC ASSESSMENT IN RATS

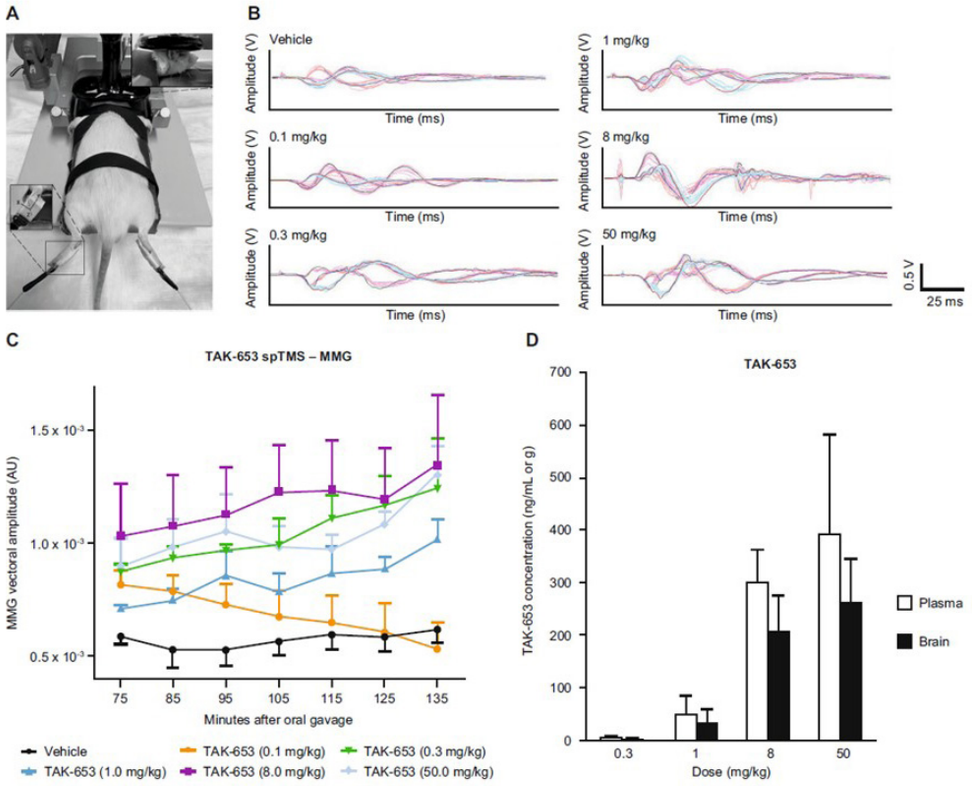
TAK-653 was provided by Takeda Pharmaceutical Company Limited (Japan) and prepared in a vehicle formulation consisting of 0.5% methylcellulose in double-distilled water. All dosing was performed *per os* via oral gavage in 10 ml/kg. Animals in the vehicle group received an equal volume per weight of the vehicle solution.

We used 20 rats to assess plasma and brain levels of TAK-653 (n = 5 per TAK-653 dose). Two hours after TAK-653 administration (0.3, 1, 8, and 50 mg/kg, oral gavage), we collected plasma (intracardiac-blood sampling) and brain (decapitation) specimens.

TMS IN RATS

We tested whether TAK-653 augments corticospinal excitability using spTMS in 31 rats. MMG was chosen instead of needle EMG to allow for a lighter anaesthesia level and avoid pain that could confound motor-evoked potential MEP responses. Changes in MMG amplitude were captured using three-axis accelerometers attached to the rats' hind paws.[25] Rats received vehicle (n = 6) or TAK-653 (0.1, 0.3, 1, 8, or 50 mg/kg; n = 6, 4, 5, 5, and 5, respectively) before being anesthetised with pentobarbital (25 + 15 mg/kg intraperitoneally, doses spaced 30 min apart to maintain stable anaesthesia). Because of the oral dosing required for TAK-653, no baseline TMS values were obtained. After appropriate depth of anaesthesia was confirmed, rats were placed on a platform and restrained using Velcro straps, and three-axis accelerometers were attached to the soles of the hindlimbs to record MMG (Figure 1A). spTMS was delivered with a figure-eight coil (25 mm diameter; Magstim, Eden Prairie, MN, USA) centred over the midsagittal plane at the interaural line at which similar bilateral hindlimb activation can be reliably produced. TMS-MMG took place 75-135 min after TAK-653 administration (seven time points, 10-min intervals). Ten single pulses at 80% of the maximum machine-output intensity were applied at each time point.

Figure 1 TAK-653 enhanced TMS-evoked motor responses in rats



A. Example of an animal placement on the stereotaxic apparatus, a small figure-8 coil, and an accelerometer attached to the hind paws. B. Representative waveforms in three dimensions (pictured in different colours) used for MMG calculation with vehicle or five different doses of TAK-653. C. Summary graph illustrating the MMG vectoral amplitude over time for all six treatment groups (Mean ± SEM). All TAK-653 doses except for 0.1 mg/kg increased MMG amplitude. No dose-response effect was observed. D. Plasma and brain TAK-653 levels 2 hours after administration via oral gavage in rats with the four effective doses. MMG, mechanomyography; spTMS, single-pulse transcranial magnetic stimulation; TMS, transcranial magnetic stimulation.

STATISTICAL ANALYSIS IN THE RAT STUDY

The MMG signals were converted to voltage values, and three-dimensional vector amplitudes were calculated *post hoc* [$\sqrt{(x^2 + y^2 + z^2)}$]. Data from animals with 6/10 or more MMG signals with acceptable quality (obtained during rest, in absence of baseline muscle activity) at all seven time points in at least one hind limb were used for analysis. Data were analysed in Graphpad Prism (Version 8.0.3, Graphpad Software, San Diego, CA). A mixed-model repeated-measures 2-way analysis of variance (ANOVA) was used to compare the effects of different TAK-653 doses and vehicle on TMS measures. Time and dose were fixed factors and animal was a random factor.

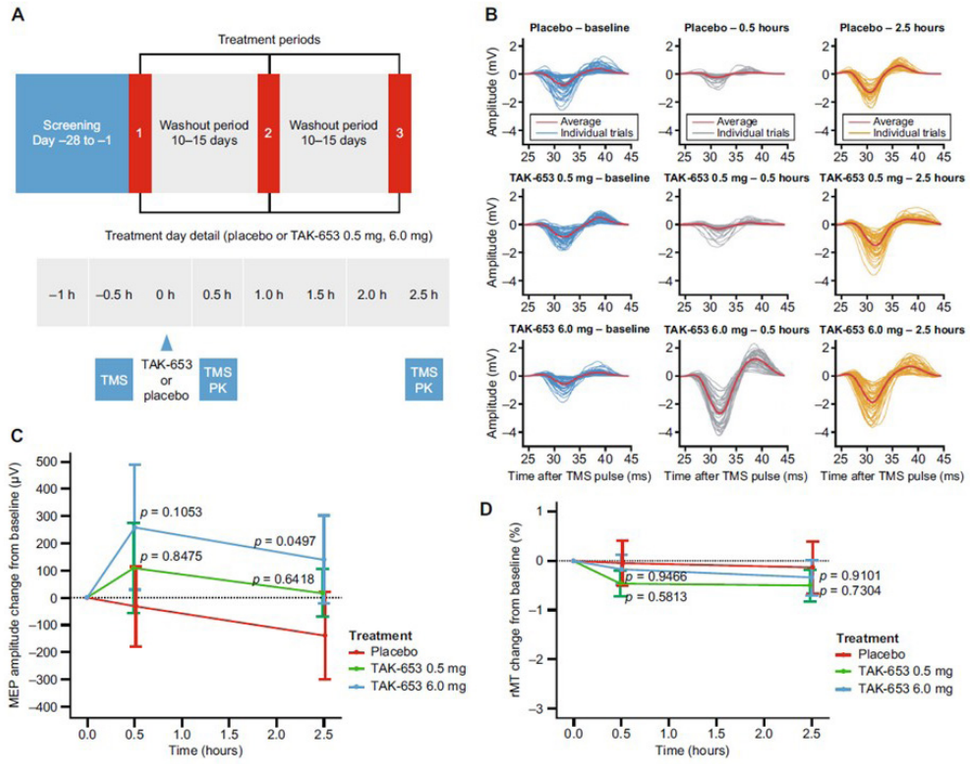
HUMAN STUDY PARTICIPANTS AND DESIGN

This study was approved by the Medical Ethics Committee, Foundation Beoordeling Ethiek Biomedisch Onderzoek (BEBO) and was registered at clinicaltrials.gov (NCT03792672). Written informed consent was obtained from all participants before study start. The study was performed according to International Conference on Harmonisation guidelines on Good Clinical Practice guidelines, as laid down in the Declaration of Helsinki and its latest amendments. The study was sponsored by Takeda Pharmaceuticals and conducted at the Centre for Human Drug Research, Leiden, The Netherlands, from January 23, 2019, to June 18, 2019. The study was registered at Clinicaltrials.gov; http://ClinicalTrials.gov; NCT03792672.

The study consisted of an initial randomised, double-blind, placebo-controlled, three-period crossover phase, followed by an open-label ketamine period. The crossover phase included three treatments (oral placebo, TAK-653 0.5 mg, and TAK-653 6 mg), each 1 day in duration, with washout periods of 10–15 days (Figure 2A). During treatment days, participants reported at the research centre in the morning. Prior to dosing, safety assessments were performed, consisting of physical examination, urine drug screen, urinalysis, vital signs, electrocardiogram, and safety chemistry and haematology laboratory assessments. TMS–EMG assessments were performed 40 min prior to dosing (baseline), 30 min after dosing, and at expected t_{max} (2.5 h after dosing). Participants were discharged by a physician 6 hours after dosing.

Healthy males and females (of non-childbearing potential) between 18 and 55 years of age were selected. Participants with contraindications for TMS procedures based on the TMS safety questionnaire[26] (such as having metal objects in the brain or having a family history of epilepsy, seizures, or convulsions) were excluded. Individuals with a resting motor threshold (RMT) higher than 75% of the maximum stimulator output (MSO) were excluded, as stimulation at 120% of this value would be very close to the MSO. Participants having a clinically significant previous or current psychiatric disorder according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) or a history of alcohol consumption exceeding two standard drinks per day on average were also excluded. Participants were not allowed to use concomitant medications from 7 days before administration of the first dose of study drug throughout the study. Use of alcohol was not allowed from 7 days before the screening visit and 7 days before dosing until the last treatment period. Participants refrained from using caffeine from 24 h before the screening visit, 24 h before each dosing, and during each treatment period. In between visits, participants were allowed up to six servings of caffeine per day. From 48 h before each dosing until the end of the treatment period, participants were not allowed to smoke. In between visits, participants were allowed to smoke up to five cigarettes a day.

Figure 2 6 mg TAK-653 enhanced MEPs in healthy volunteers



A. Study schematic (top) and detail of treatment day events (bottom). B. Representative MEP waveforms from one participant at baseline, 30 minutes, and 2.5 hours post-dose for all three treatment periods. C. Changes from baseline in MEP amplitude for placebo, 0.5 mg TAK-653, and 6 mg TAK-653 periods. *p* = Dunnett's adjusted *p* value. D. Changes from baseline in rMT for all three periods. MEP, motor-evoked potentials; PK, pharmacokinetic; rMT, resting motor threshold; TMS, transcranial magnetic stimulation.

HUMAN PHARMACOKINETIC ASSESSMENT

Pharmacokinetic assessment times for TAK-653 were matched to the times of TMS procedures. For TAK-653, samples were collected pre-dose, and 30 min and 2.5 h post-dose. Plasma concentrations of TAK-653 were measured by a validated high-performance liquid chromatography with tandem mass spectrometry assay, and the lower limit of quantitation was 0.1 ng/ml.

HUMAN TMS

TMS measurements were conducted using a MagPro R30 with MagOption stimulator and an MCF-B65 butterfly coil (2 x 75 mm; both MagVenture GmbH, Hueckelhoven, Germany). The motor cortex of the dominant hemisphere, as assessed by the Edinburgh Handedness Questionnaire,[27] was stimulated to elicit a motor response. The coil was placed tangentially to the skull and at an angle of 45˚ from the midline and held in place by a frame. Participants were lying in a semi-recumbent position and were instructed not to move their heads and to keep their eyes open. (MEPs) were measured from the *abductor digiti minimi* muscle by placing two surface Ag/AgCl electrodes in a belly-tendon montage. The active electrode was placed on top of the muscle and the reference electrode on the little finger.

rMT was determined according to established procedures.[28,29] The target area was manually stimulated using single pulses starting at an intensity of 40% of MSO. When there was no MEP, stimulation intensity was increased in steps of 5%. Once the motor hotspot was located, stimulation intensity was decreased in steps of 1% to determine the intensity at which at least 5 out of 10 TMS pulses elicited a MEP with a peak-to-peak amplitude of at least 50 μV. After determination of rMT, spTMS and ppTMS protocols were applied.

spTMS consisted of 50 single pulses at 120% of baseline rMT (defined as the rMT measured in the morning of each treatment period prior to dosing) with a randomised interval between 3.5 s and 4.5 s. The spTMS protocol was followed by 50 pairs of pulses in randomised order with inter-stimulus intervals (ISI) of 2, 5, 50, 100, 200, and 300 ms. For ISIs of 2 ms and 5 ms, conditioning pulses were delivered at 80% of baseline rMT. For all other intervals, conditioning and test pulses were given at an intensity of 120% of baseline rMT.

EMG was measured and recorded with an electroencephalogram (EEG) amplifier (TMSi, Oldenzaal, The Netherlands) with a sample frequency of 2048 Hz. A ground electrode was located between EEG electrode positions Fz and Fpz, as EEG signals were also collected (not analysed for the current publication). EMG recordings were checked for muscle pre-activation and responses were excluded when muscle activity was greater than 50 μV in the 50 ms prior to the single or conditioning pulse. Customised MATLAB (version R2015a, MathWorks, Natick, MA, USA) routines were used for all analyses. MEPs within 20–45 ms post-spTMS intervals were analysed post hoc. Peak-to-peak EMG amplitudes were calculated and averaged over 50 repetitions.

For short-interval intracortical inhibition (SICI; 2 and 5 ms ISI), mean peak-to-peak amplitudes of the responses to the 50 unconditioned and 50 conditioned test pulses were calculated. For the unconditioned response, single-pulse responses were evaluated. SICI was calculated as the ratio between conditioned test response (TR)

amplitude and the unconditioned response (SP_MEP) amplitude according to the following formula: 100*TR/SP_MEP (%). For long-interval intracortical inhibition (LICI; 50-300 ms ISI), the mean peak-to-peak amplitude of the responses to the 50 conditioning and 50 test pulses was calculated. LICI was calculated as the ratio between the mean test response amplitude and the mean conditioning response (CR) using the following formula: 100*TR/CR (%).

STATISTICAL ANALYSIS OF THE HUMAN STUDY

Peak-to-peak MEP amplitude, RMT, SICI, and LICI were analysed using a mixed model for repeated measures with fixed factors for treatment, period, sequence and treatment by period interaction, and subject nested in sequence as a random effect. The baseline measure for the corresponding outcome was included in the model as a covariate. Estimated treatment effects, two-sided 90% confidence intervals (CI) and *p* values were calculated for measures at 30 min and 2.5 h post-dose. Hochberg's step-up procedure was used to adjust for multiple testing and Dunnett adjusted *p* values were calculated.

RESULTS

TAK-653 INCREASED CORTICOSPINAL EXCITABILITY ASSESSED WITH TMS IN RATS

We observed a significant increase in corticospinal excitability (as reflected in larger MMG amplitude) with doses of 0.3 mg/kg TAK-653 or higher, compared to vehicle (Figure 1B,C). In satellite animals, the lowest effective dose resulted in 5.32 ± 0.94 (mean ± standard deviation [SD]) ng/ml TAK-653 in plasma, 1 mg/kg yielded 45.9 ± 35.9 ng/ml, 8 mg/kg yielded 298.2 ± 65.3 ng/ml, and 50 mg/kg resulted in 391.0 ± 190.6 ng/ml (Figure 1D). Brain concentrations of TAK-653 were 3.53 ± 0.42 ng/g for 0.3 mg/kg, 36.5 ± 24.8 ng/g for 1 mg/kg, 210.6 ± 64.8 ng/g for 8 mg/kg, and 264.2 ± 81.9 ng/g for 50 mg/kg (Figure 1D). In the animals used for TMS-MMG, a repeated-measures ANOVA revealed a significant effect of dose ($F_{(5,25)} = 4.399$; $p = 0.005$), time ($F_{(2.475,61.87)} = 4.076$; $p = 0.015$) and a time x dose interaction ($F_{(30,150)} = 1.692$; $p = 0.022$). We were unable to observe a dose-response effect with the current data set, yet all effective TAK-653 doses resulted in evoked MMG amplitudes 30–70% higher than those with vehicle.

HUMAN TMS STUDY PARTICIPANTS AND PHARMACOKINETICS

Twenty-three males and one female were included. All participants completed the first three study periods (Figure 2A). Four individuals did not participate in the open-label ketamine period. Demographics are summarised in Table 1. TAK-653 plasma levels at 30 minutes and 2.5 hours post-dose were 0.99 ± 0.94 (mean ± SD) ng/ml and 4.19 ± 0.83 ng/ml for 0.5 mg TAK-653, and 2.57 ± 3.29 ng/ml and 45.99 ± 8.84 ng/ml for 6.0 mg TAK-653, respectively.

SAFETY AND TOLERABILITY

TAK-653 was well tolerated, and all TAK-653-related adverse events (AEs) were of mild intensity. No serious AEs occurred. In the TAK-653 0.5 mg and 6 mg dose periods, 37.5% and 50.0% of participants experienced a treatment-emergent AE (TEAE), respectively, compared to 29.2% of participants in the placebo period. The most frequently reported TEAEs after TAK-653 administration were somnolence, headache, and nasopharyngitis (Table 2).

Table 1 Demographics

Individuals enrolled	***n***	**24**
AGE, YEARS	Mean (SD)	27.9 (9.0)
	Median	24.5
	Range	20–49
SEX, N	Female	1 (4.2%)
	Male	23 (95.8%)
RACE, N	White	22 (91.7%)
	Asian	1 (4.2%)
	Multiple	1 (4.2%)
WEIGHT, KG	Mean (SD)	79.12 (10.81)
	Median	78.38
	Range	63.8– 115.0
HEIGHT, CM	Mean (SD)	181.98 (9.88)
	Median	181.50
	Range	158.3–201.2
BMI, KG/M^2	Mean (SD)	23.92 (2.85)
	Median	23.35
	Range	19.5–29.2

Abbreviations: BMI, body mass index; SD, standard deviation.

Table 2 Most frequent TEAEs (≥ 5% of individuals in placebo or overall TAK-653)

Preferred Term	**Participants, n (%)**			
	Placebo (*n* = 24)	**TAK-653 0.5 mg (*n* = 24)**	**TAK-653 6 mg (*n* = 24)**	**All TAK-653 (*n* = 24)**
Any TEAE	7 (29.2)	9 (37.5)	12 (50.0)	15 (62.5)
Somnolence	2 (8.3)	3 (12.5)	3 (12.5)	6 (25.0)
Headache	2 (8.3)	1 (4.2)	4 (16.7)	4 (16.7)
nasopharyngitis	0	3 (12.5)	1 (4.2)	4 (16.7)
Oropharyngeal Pain	1 (4.2)	0	2 (8.3)	2 (8.3)
Diarrhea	0	1 (4.2)	1 (4.2)	2 (8.3)
Seasonal Allergy	0	1 (4.2)	1 (4.2)	2 (8.3)
Fatigue	2 (8.3)	0	1 (4.2)	1 (4.2)

Abbreviation: TEAE, treatment-emergent adverse event

TAK-653 INCREASED CORTICOSPINAL EXCITABILITY ASSESSED WITH TMS IN HUMANS

No significant effects on peak-to-peak MEP amplitude were observed with 0.5 mg TAK-653 compared to placebo at 30 min and 2.5 h post-dose (p = 0.6328, Dunnett adjusted p value = 0.8475 at 30 min; p = 0.4278, Dunnett adjusted p value = 0.6418 at 2.5 h; Figure 2B,C, and Tables 3,4). For 6 mg TAK-653 compared with placebo, the effect observed 30 min post-dose was not statistically significant (p = 0.0586, Dunnett adjusted p value = 0.1053); however, 2.5 h post-dose, a statistically significant increase in MEPs compared to placebo was observed (p = 0.0269, Dunnett adjusted p value = 0.0497; Figure 2B,C, and Tables 3,4). No statistically significant effects were found on change from baseline RMT for TAK-653 0.5 mg or 6 mg compared with placebo at 30 minutes or 2.5 h post-dose (Figure 2D).

ppTMS responses were evaluated in humans. The changes in magnitude from baseline compared with placebo for LICI using 50, 100, and 200 ms ISIs were not statistically significant for TAK-653 0.5 mg or 6 mg at 30 minutes or 2.5 hours post-dose. Using a 300 ms ISI, the change from baseline in magnitude of LICI was statistically significant only for TAK-653 0.5 mg at 2.5 h post-dose (an increase revealed by the estimate of difference in least-squares means: 17.2% [90% CI: 5.06%, 29.4%], p = 0.0220, Dunnett adjusted p value = 0.0406; Table 5). The changes from baseline in the magnitude of SICI were not statistically significant.

Table 3 Single-pulse peak-to-peak Amplitude

	Placebo		TAK-653 0.5 mg		TAK-653 6 mg	
	0.5 h post-dose	2.5 h post-dose	0.5 h post-dose	2.5 h post-dose	0.5 h post-dose	2.5 h post-dose
n	24	24	24	24	24	23[a]
Change from pre-dose baseline mean (SD) µV	−32.81 (756.65)	−139.12 (829.60)	110.32 (845.96)	17.27 (466.26)	260.38 (1132.49)	139.93 (813.97)
Estimate of difference in LS means	NA	NA	99.7	115	411	338
90% CI for difference in LS means	NA	NA	−249, 499	−128, 359	55.5, 766	90.5, 585
p value, Dunnett adjusted p value for TAK-653 vs. placebo	NA	NA	0.633, 0.848	0.428, 0.642	0.059, 0.105	0.027[b], 0.05[b]

Abbreviations: CI, confidence interval; h, hour; LS, least-squares; NA, not applicable; SD, standard deviation.
a. One measurement is missing due to a technical error. b. Statistically significant compared to placebo.

Table 4 Single-pulse peak-to-peak amplitude raw values

Mean (SD) single pulse peak-to-peak amplitude µV	Placebo	TAK-653 0.5 mg	TAK-653 6 mg
Predose (baseline)	898.93 (693.15)	841.07 (591.14)	1004.13 (574.60)
0.5 hours postdose	866.02 (798.62)	951.39 (542.72)	1230.41 (1057.11)
2.5 hours postdose	759.71 (537.31)	858.33 (516.76)	1101.58 (839.55)

Abbreviation: SD, standard deviation

Table 5 Paired-pulse TMS was not affected by TAK-653 (n=24)

		Placebo		TAK-653 0.5 mg		TAK-653 6 mg	
		0.5 hours post-dose	2.5 hours post-dose	0.5 hours post-dose	2.5 hours post-dose	0.5 hours post-dose	2.5 hours post-dose
SICI 2 ms	Change from predose baseline mean (SD) %	18.35 (57.04)	5.05 (47.57)	0.87 (33.33)	1.77 (35.92)	2.56 (57.34)	-14.58 (37.73)
	Estimate of difference in LS means			-19.7	-5.65	-13.6	-16.1
	90% CI for difference in LS means			-40.6 - 1.26	-34.8 - 7.64	-34.8 - 7.64	-32.5 - 0.242
	P value, Dunnet adjusted p value for TAK-653 vs. placebo			0.121 (0.210)	0.559 (0.782)	0.288 (0.461)	0.105 (0.183)
SICI 5 ms	Change from predose baseline mean (SD) %	28.02 (115.12)	14.30 (81.61)	1.57 (33.19)	4.27 (30.90)	1.36 (81.23)	-7.25 (52.92)
	Estimate of difference in LS means			-33.9	-16.7	-25.8	-18.3
	90% CI for difference in LS means			-69.3 - 1.36	-39.4 - 6.06	-61.4 - 9.8	-41.3 - 4.56
	P value, Dunnet adjusted p value for TAK-653 vs. placebo			0.113 (0.197)	0.224 (0.369)	0.229 (0.377)	0.185 (0.310)
LICI 50 ms	Change from predose baseline mean (SD) %	19.12 (68.54)	0.68 (54.57)	10.93 (56.56)	18.32 (81.44)	16.71 (59.26)	19.40 (110.09)
	Estimate of difference in LS means			-4.67	14.0	1.12	15.1
	90% CI for difference in LS means			-33.3 - 24.0	-14.8 - 42.8	-27.6 - 29.8	13.7 - 43.9
	P value, (Dunnet adjusted p value) for TAK-653 vs. placebo			0.787 (0.947)	0.417 (0.626)	0.948 (0.997)	0.383 (0.584)
LICI 100 ms	Change from predose baseline mean (SD) %	19.37 (98.14)	0.15 (29.16)	12.31 (28.81)	5.91 (18.89)	-8.53 (58.03)	-28.64 (142.56)
	Estimate of difference in LS means			-9.50	-0.326	-14.9	3.71
	90% CI for difference in LS means			-38.6 - 19.6	-7.23 - 6.58	-44.3 - 14.6	-3.32 - 10.7
	P value, Dunnet adjusted p value for TAK-653 vs. placebo			0.582 (0.802)	0.937 (0.995)	0.398 (0.604)	0.379 (0.582)
LICI 200 ms	Change from predose baseline mean (SD) %	7.44 (30.26)	2.88 (21.25)	15.01 (47.77)	9.90 (42.58)	4.64 (32.12)	11.54 (30.16)
	Estimate of difference in LS means			7.67	7.36	-2.59	9.36
	90% CI for difference in LS means			-3.96 - 19.3	-3.44 - 18.2	-14.2 - 9.04	-1.45 - 20.2
	P value, Dunnet adjusted p value for TAK-653 vs. placebo			0.274 (0.439)	0.258 (0.417)	0.709 (0.909)	0.152 (0.259)
LICI 300 ms	Change from predose baseline mean (SD) %	3.25 (46.44)	-10.0 (27.72)	6.51 (44.03)	8.62 (36.05)	0.05 (35.65)	-18.03 (33.48)
	Estimate of difference in LS means			1.15	17.2	-6.28	9.58
	90% CI for difference in LS means			-16.3 - 18.6	5.06 - 29.4	-23.7 - 11.2	-2.59 - 21.8
	P value, Dunnet adjusted p value for TAK-653 vs. placebo			0.912 (0.991)	0.022 (0.041)	0.547 (0.769)	0.193 (0.321)

Abbreviations: SICI, short interval intracortical inhibition; LICI, long interval intracortical inhibition; LS, least-squares; SD, standard deviation; CI, confidence interval.

DISCUSSION

TMS-evoked motor responses were enhanced by TAK-653 in both rats and humans at similar plasma concentrations. In rats, we observed no effect with a dose of 0.1 mg/kg and an increase in MMG amplitude with doses of 0.3 mg/kg or higher, corresponding to 5.32 ng/ml or higher in plasma. TAK-653 was detected in the brains after the procedures, indicating the compound crossed the blood-brain barrier. In healthy humans, a single dose of TAK-653 6 mg, corresponding to a mean plasma level of 45.99 ng/ml at expected t_{max}, significantly increased MEP amplitude from baseline compared to placebo. TAK-653 0.5 mg, corresponding to a mean plasma level of 4.19 ng/ml at expected t_{max}, did not elicit an effect on MEP amplitude. There was no change in RMT with either dose of TAK-653. In addition, the only ppTMS assay that revealed a difference in TAK-653 from placebo in humans was with LICI at ISI 300 ms, but the difference was with the low dose that did not induce changes in spTMS.

These results indicate that non-invasive brain stimulation can be used to generate translational neurocircuitry biomarkers that capture subtle modulation of glutamate synaptic activity. TMS of the primary motor cortex is likely activating a cortical column and its projection to the spinal cord motoneurons that drive the response in the activated muscle. As neuromuscular junction synapses utilise acetylcholine as their neurotransmitter and TAK-653 has demonstrated *in vitro* selectivity for AMPA receptors, the change in MEP by TAK-653 should be driven by CNS effects. Further in support of a glutamate receptor-mediated effect, only evoked responses elicited by spTMS rather than the ppTMS metrics that capture modulation of cortical inhibition[21] were altered by TAK-653. Thus, our data reveal that TAK-653 modulates corticospinal excitability in a healthy brain and indicate that neurostimulation approaches, such as TMS, can be applied as biomarkers to capture modulation of glutamate synaptic activity. Our ppTMS studies should be interpreted with caution, however. In order to minimise the duration of the procedure in the human study and driven by technical limitations in the rat study, we chose to test SICI and LICI, excluding intra-cortical facilitation (ICF). SICI and LICI are thought to capture intracortical inhibitory processes and ICF is related to glutamate activity,[21] therefore we chose to focus ppTMS on inhibition-related measures.

The effect of TAK-653 on corticospinal excitability assessed with MEPs supports the use of neurostimulation as a biomarker but does not necessarily mean that this compound will restore circuitry function in depression. The motor cortex is neither anatomically nor functionally involved in the regulation of emotional behaviour in humans. However, a large body of data implicates the frontal-striatal circuitry that includes the dorsolateral prefrontal cortex (DL-PFC), subgenual anterior cingulate cortex, amygdala and ventral striatum in mood disorders.[30] Furthermore, impaired functional connectivity of the DL-PFC with emotion-related circuits has been identified in MDD patients,[31] and EEG signals evoked by DL-PFC TMS differ in MDD patients from controls.[20] In line with these observations, devices for repeated TMS of the DL-PFC have been approved and a range of TMS protocols are rapidly expanding as therapeutic options for treatment-resistant depression.[32,33] Thus, DL-PFC TMS, perhaps coupled with EEG to record TMS-evoked regional potentials, can be used to assess the effect of ketamine, TAK-653 or any novel glutamate-based antidepressant on disease-related circuitry dysfunction in MDD patients.

The study was performed with great caution as both TMS and AMPA receptor potentiation might theoretically increase the risk of seizures by potentiating glutamatergic synapses.[26] We did not observe any evidence of seizures or convulsions and there were no dose-related AEs and no serious AEs. TAK-653 was generally well tolerated by the participants, supporting further development of TAK-653.

Some caveats with our study are worth addressing. We tried to reproduce stimulation parameters and data collection as much as possible in rats and humans. However, in order to minimise stress and movement artifacts while allowing reliable muscle response in rats, we aimed for light anaesthesia combined with a non-invasive method to record muscle responses. Thus, TMS-MMG was employed given it is a reliable surrogate for TMS-EMG.[25] Baseline TMS-MMG could not be obtained in rats because of the need of oral dosing 2 h prior to the measures of interest. Therefore, the comparisons were made between TAK-653 and vehicle and any difference in baseline responses could not be identified. TAK-653 increased MMG amplitude, and any effect of the aesthetic was controlled by inclusion of vehicle-treated animals. Another potential concern is the high variability of human TMS–EMG data; however, this variability was within the expected range. The fact that a significant difference from placebo was observed with our higher dose despite such variability reinforces the conclusion that TAK-653 increased circuitry excitability. In addition, we used baseline RMT to guide stimulation intensity for all measurements. Because adjusting the TMS intensity to compensate for post-drug changes in RMT can change the outcome,[34] and because the rat TMS study used the same stimulation intensity in all treatment groups, we chose not to adjust the TMS strength. We did not observe changes in RMT with treatment, so it is unlikely that any adjustment would have revealed a different outcome. Lastly, we omitted testing ICF to minimise patient burden. This decision resulted in not having a pTMS paradigm related to intracortical glutamate function[21] and MEP data being the only direct assessment of excitatory neurotransmission.

In conclusion, our data represent an important step forward because they provide evidence of a non-invasive, translational modulation of physiological outcomes of a glutamate-based neural circuit in a healthy brain. Methodologies such as quantitative EEG or magnetic resonance spectroscopy could be considered to measure subtle

AMPA receptor modulation in humans, but they miss the detection of functional outcomes of brain circuit activation. Our data show that TMS-evoked motor responses can detect discrete changes in cortical excitability in a defined neural circuit, enabling pharmacological assessments of glutamatergic CNS activity in early drug development. To our knowledge, this is the first demonstration of a circuitry biomarker sensitive to direct positive modulation of AMPA receptors being modulated in a similar manner in rodents and humans.

REFERENCES

1 Global Burden of Disease Study. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **392**, 1789-1858, doi:10.1016/S0140-6736(18)32279-7 (2018).

2 Walker, E. R., McGee, R. E. & Druss, B. G. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. JAMA *Psychiatry* **72**, 334-341, doi:10.1001/jamapsychiatry.2014.2502 (2015).

3 Fava, M. Diagnosis and definition of treatment-resistant depression. *Biol Psychiatry* **53**, 649-659, doi:10.1016/s0006-3223(03)00231-2 (2003).

4 Berman, R. M. *et al.* Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* **47**, 351-354, doi:10.1016/s0006-3223(99)00230-9 (2000).

5 Zarate, C. A., Jr. *et al.* A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry* **63**, 856-864, doi:10.1001/archpsyc.63.8.856 (2006).

6 DeWilde, K. E., Levitch, C. F., Murrough, J. W., Mathew, S. J. & Iosifescu, D. V. The promise of ketamine for treatment-resistant depression: current evidence and future directions. *Ann N Y Acad Sci* **1345**, 47-58, doi:10.1111/nyas.12646 (2015).

7 Duman, R. S., Shinohara, R., Fogaca, M. V. & Hare, B. Neurobiology of rapid-acting antidepressants: convergent effects on GluA1-synaptic function. *Mol Psychiatry*, doi:10.1038/s41380-019-0400-x (2019).

8 Maeng, S. *et al.* Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiatry* **63**, 349-352, doi:S0006-3223(07)00547-1 [pii] 10.1016/j.biopsych.2007.05.028 (2008).

9 Short, B., Fong, J., Galvez, V., Shelker, W. & Loo, C. K. Side-effects associated with ketamine use in depression: a systematic review. *Lancet Psychiatry* **5**, 65-78, doi:10.1016/S2215-0366(17)30272-9 (2018).

10 Witkin, J. M., Martin, A. E., Golani, L. K., Xu, N. Z. & Smith, J. L. Rapid-acting antidepressants. *Adv Pharmacol* **86**, 47-96, doi:10.1016/bs.apha.2019.03.002 (2019).

11 Asgharnejad, M. *et al.* in *American College of Neuropsychopharmacology Annual Meeting* (Hollywood, FL, 2018).

12 O'Donnell, P. *et al.* Strategies to Address Challenges in Neuroscience Drug Discovery and Development. *Int J Neuropsychopharmacol* **22**, 445-448, doi:10.1093/ijnp/pyz027 (2019).

13 Joshi, Y. B. & Light, G. A. Using EEG-Guided Basket and Umbrella Trials in Psychiatry: A Precision Medicine Approach for Cognitive Impairment in Schizophrenia. *Front Psychiatry* **9**, 554, doi:10.3389/fpsyt.2018.00554 (2018).

14 Tada, M. *et al.* Mismatch negativity (MMN) as a tool for translational investigations into early psychosis: A review. *Int J Psychophysiol* **145**, 5-14, doi:10.1016/j.ijpsycho.2019.02.009 (2019).

15 Sullivan, E. M., Timi, P., Hong, L. E. & O'Donnell, P. Effects of NMDA and GABA-A Receptor Antagonism on Auditory Steady-State Synchronization in Awake Behaving Rats. *Int J Neuropsychopharmacol* **18**, pyu118, doi:10.1093/ijnp/pyu118 (2015).

16 Cohen, A. F., Burggraaf, J., van Gerven, J. M., Moerland, M. & Groeneveld, G. J. The use of biomarkers in human pharmacology (Phase I) studies. *Annu Rev Pharmacol Toxicol* **55**, 55-74, doi:10.1146/annurev-pharmtox-011613-135918 (2015).

17 Cohen, D. & Cuffin, B. N. Developing a more focal magnetic stimulator. Part I: Some basic principles. *J Clin Neurophysiol* **8**, 102-111, doi:10.1097/00004691-199101000-00013 (1991).

18 Premoli, I. *et al.* TMS-EEG signatures of GABAergic neurotransmission in the human cortex. *J Neurosci* **34**, 5603-5612, doi:10.1523/JNEUROSCI.5089-13.2014 (2014).

19 Cash, R. F. *et al.* Characterization of Glutamatergic and GABAA-Mediated Neurotransmission in Motor and Dorsolateral Prefrontal Cortex Using Paired-Pulse TMS-EEG. *Neuropsychopharmacology* **42**, 502-511, doi:10.1038/npp.2016.133 (2017).

20 Voineskos, D. *et al.* Altered Transcranial Magnetic Stimulation-Electroencephalographic Markers of Inhibition and Excitation in the Dorsolateral Prefrontal Cortex in Major Depressive Disorder. *Biol Psychiatry* **85**, 477-486, doi:10.1016/j.biopsych.2018.09.032 (2019).

21 Ziemann, U. in *Handbook of Clinical Neurology* Vol. 116 *Handbook of Clinical Neurology* (eds A.M. Lozano & M. Hallett) Ch. 32, 387-397 (Elsevier, 2013).

22 Kobayashi, M. & Pascual-Leone, A. Transcranial magnetic stimulation in neurology. *Lancet Neurol* **2**, 145-156, doi:10.1016/s1474-4422(03)00321-1 (2003).

23 Di Lazzaro, V. *et al.* Ketamine increases human motor cortex excitability to transcranial magnetic stimulation. *J Physiol* **547**, 485-496, doi:10.1113/jphysiol.2002.030486 (2003).

24 Sorel, M. *et al.* A reappraisal of the mechanisms of action of ketamine to treat complex regional pain syndrome in the light of cortical excitability changes. *Clin Neurophysiol* **129**, 990-1000, doi:10.1016/j.clinph.2018.02.124 (2018).

25 Hsieh, T. H. *et al.* A new measure of cortical inhibition by mechanomyography and paired-pulse transcranial magnetic stimulation in unanesthetized rats. *J Neurophysiol* **107**, 966-972 (2012).

26 Rossi, S., Hallett, M., Rossini, P. M. & Pascual-Leone, A. Screening questionnaire before TMS: an update. *Clin Neurophysiol* **122**, 1686, doi:10.1016/j.clinph.2010.12.037 (2011).

27 Oldfield, R. C. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**, 97-113, doi:10.1016/0028-3932(71)90067-4 (1971).

28 Groppa, S. *et al.* A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol* **123**, 858-882, doi:10.1016/j.clinph.2012.01.010 (2012).

29 Rossini, P. M. *et al.* Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol* **126**, 1071-1107, doi:10.1016/j.clinph.2015.02.001 (2015).

30 Hamilton, J. P. *et al.* Functional neuroimaging of major depressive disorder: a meta-analysis and new integration of base line activation and neural response data. *Am J Psychiatry* **169**, 693-703, doi:10.1176/appi.ajp.2012.11071105 (2012).

31 Rolle, C. E. *et al.* Cortical Connectivity Moderators of Antidepressant vs Placebo Treatment Response in Major Depressive Disorder: Secondary Analysis of a Randomized Clinical Trial. JAMA *Psychiatry*, doi:10.1001/jamapsychiatry.2019.3867 (2020).

32 Kelin, E. *et al.* Therapeutic efficacy of right prefrontal slow rePETitive transcranial magnetic stimulation in major depression. *Arch. Gen. Psychiat.* **56**, 315-320 (1999).

33 George, M. S. Whither TMS: A One-Trick Pony or the Beginning of a Neuroscientific Revolution? *Am J Psychiatry* **176**, 904-910, doi:10.1176/appi.ajp.2019.19090957 (2019).

34 Premoli, I., Costantini, A., Rivolta, D., Biondi, A. & Richardson, M. P. The Effect of Lamotrigine and Levetiracetam on TMS-Evoked EEG Responses Depends on Stimulation Intensity. *Front Neurosci* **11**, 585, doi:10.3389/fnins.2017.00585 (2017).

CHAPTER VI

CENTRAL NERVOUS SYSTEM EFFECTS OF TAK-653, AN INVESTIGATIONAL ALPHA-AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLE RECEPTOR (AMPAR) POSITIVE ALLOSTERIC MODULATOR IN HEALTHY VOLUNTEERS

Dijkstra, F.[1,2], O'Donnell P.[3,4], Klaassen E.[1], Buhl D.[3], Asgharnejad M.[5], Rosen L.[3], Zuiker R.G.J.A.[1], van Gerven J.M.A.[1,2], Jacobs G.E.[1,2]

Transl Psychiatry. 2022;12(1):408.
doi:10.1038/s41398-022-02148-w

1 Centre for Human Drug Research (CHDR), Leiden, the Netherlands
2 Department of Psychiatry, Leiden University Medical Center (LUMC), Leiden, the Netherlands
3 Neuroscience Translational Medicine, Takeda Pharmaceutical Company Ltd, Cambridge, MA, USA
4 McLean Hospital, Department of Psychiatry, Harvard Medical School, Belmont, MA, USA
5 Neuroscience Therapeutic Area Unit, Takeda Pharmaceutical Company Ltd, Cambridge, MA, USA

ABSTRACT

AIMS TAK-653 is a novel AMPA receptor positive allosteric modulator in clinical development for the treatment of major depressive disorder (MDD). This study aimed to measure the functional pharmacodynamic central nervous system (CNS) effects of TAK-653.

METHODS A randomised, double-blind, placebo-controlled, three-way crossover (placebo, TAK-653 0.5 mg and 6 mg) study with 24 healthy volunteers was performed. NeuroCart tests consisting of body sway (BS), saccadic peak velocity (SPV), smooth pursuit eye movements (SP), adaptive tracking (AT), Bowdle and Bond and Lader Visual Analogue Scales (B-VAS and BL-VAS) and Stroop test were performed pre-dose and 3.5 and 4 h post-dose. Data were analysed using a mixed model analysis of covariance with baseline as covariate.

RESULTS It was found that TAK-653 did not affect BS and subjective drug effects as measured by B-VAS and BL-VAS at either dose level. TAK-653 0.5 mg increased SPV (degrees/second) (19.49 [5.98, 32.99], $P = 0.02$) and affected Stroop difference in reaction time between correct congruent and correct incongruent answers and number of correct responses in incongruent trials (22.0 [4.0, 40.0], $P = 0.05$ and −0.3 [−0.5, −0.1], $P = 0.02$, respectively). TAK-653 6 mg improved AT (%) (1.68 [0.51, 2.84], $P = 0.02$) and increased SPV (degrees/s) (15.40 [1.91, 28.90], $P = 0.06$) and SP (%) (2.32 [0.37, 4.27], $P = 0.05$).

CONCLUSION Based on these findings it can be concluded that TAK-653 demonstrated a psychostimulant-like pharmacodynamic profile on the NeuroCart consistent with previously reported increase of cortical excitability following Transcranial Magnetic Stimulation (TMS) of the human motor cortex.

INTRODUCTION

Since ketamine, a N-methyl-D-aspartate (NMDA) receptor antagonist, has been shown to have rapid occurring antidepressant effects,[1-3] there is growing interest in the NMDA receptor as potential novel target for the pharmacological treatment of depressive disorders. Studies into the mechanisms underlying the antidepressant effects of NMDA receptor antagonism have demonstrated an important role for alpha-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) receptor-mediated signalling.[4-7] Blocking NMDA receptors and thereby indirectly stimulating AMPA receptors, leads to a shift towards predominantly stimulatory glutamate-mediated neurotransmission.[4-7] This is believed to affect molecular processes implicated in the pathophysiology of (chronic) mood disorders related to synaptic plasticity and/or cellular resilience,[8] including the enhanced production of brain-derived neurotrophic factor and triggering of the mammalian target of rapamycin (mTOR) signalling.[6] The importance of AMPA receptor mediated signalling is further supported by the finding that the preclinical antidepressant-like effects of ketamine and related compounds are opposed by AMPA receptor antagonists.[9] These findings support the development of novel antidepressants that target AMPA receptors. The novel AMPA receptor positive allosteric modulator (PAM) TAK-653 (9-[4-(cyclohexyloxy)phenyl]-7-methyl-3,4-dihydropyrazino[2,1-*c*][1,2,4] thiadiazine 2,2-dioxide) is an investigational potential therapeutic compound in clinical development for major depressive disorder. As full functional agonism of AMPA receptors is associated with potential untoward central nervous system (CNS) stimulation, AMPA receptor PAMs have been proposed as an alternative pharmacological strategy for glutamatergic modulation.[10] In initial healthy volunteer studies with oral doses of TAK-653 0.3 mg to 18 mg, the compound was well tolerated and, in contrast to ketamine, did not cause dissociative adverse effects.[11] Maximum plasma concentrations were attained within 1.25 hours to 5 hours after dosing, the terminal half-life varied from 33.1 hours to 47.8 hours and cerebrospinal fluid concentrations were suggestive of rapid brain penetration.[11] These pharmacokinetic (PK) and safety profiles in healthy volunteers were promising for further clinical development, but the pharmacodynamic (PD) properties of TAK-653 had not been systematically assessed.

In early phases of clinical drug development a full characterisation of both the PK and PD properties of innovative compounds is crucial to rationally guide drug development.[12,13] The question-based clinical development (QBCD) concept has previously been proposed as a conceptual framework for characterising drugs in early clinical development.[7] Specific to CNS drug development, QBCD allows for systematic investigation of crucial issues such as blood–brain barrier (BBB) penetration, intended target engagement and off-target effects.[12] By explicitly incorporating methodologies to address these issues when designing early-phase CNS studies, findings may support go/no-go decisions in subsequent development phases.[12]

In order to characterise TAK 653's PD profile, we applied transcranial magnetic stimulation (TMS) as a potential biomarker for cortical excitability and we performed a test battery of extensively validated, drug-sensitive neurophysiological and neurocognitive CNS tests, the Neurocart.[14] Based on its *in vitro* profile and preclinical effects, TAK-653 was hypothesised to yield stimulatory CNS effects in healthy clinical populations. As TAK-653 was the first AMPA receptor PAM to be tested using the NeuroCart it was decided to compare the NeuroCart profile of TAK-653 to the profiles of both excitatory or CNS-stimulant (e.g. dopamine releasers)[15] and inhibitory or CNS-depressant (e.g. $GABA_A$-agonists) compounds.[16-21] Although the mechanism of action of these compounds differs from TAK-653's mechanism of action, their PD profiles were expected to be relevant for the 'pharmacological benchmarking' of TAK-653's functional PD effects in healthy humans.

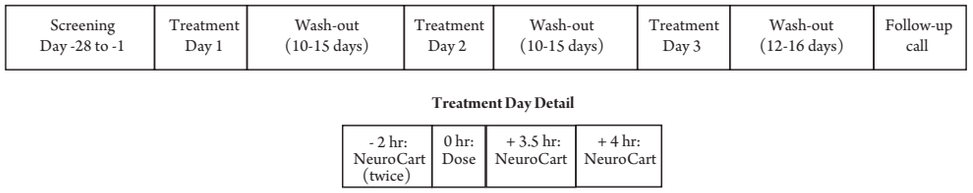
Our previous paper reported the effects of TAK-653 0.5 mg and 6 mg on TMS motor evoked potentials following stimulation of the motor cortex.[22] It was observed that TAK-653 increased the amplitude of motor evoked potentials, indicative of increased AMPA receptor-mediated cortical excitability.[22] In the current paper, the NeuroCart data will be presented.

METHODS

STUDY DESIGN AND PARTICIPANTS

The study was a randomised, double-blind, placebo-controlled, three-period crossover study (Figure 1). A fourth, open-label period was conducted with ketamine for assay sensitivity, but the TMS and NeuroCart results were equivocal for reasons discussed previously,[22] and will not be discussed here. During the three-period crossover phase, each treatment period was one day in duration and separated by a wash-out period of 10 to 15 days. During treatment days, volunteers received one oral dose of either placebo, TAK-653 0.5 mg or TAK-653 6 mg; all treatments had the same appearance to guarantee blinding of volunteers and research staff. At 12 to 16 days after the last study visit, a telephone call was made to volunteers as part of the follow-up procedure.

Figure 1 Study design and the timing of NeuroCart assessments on treatment days



TAK-653 dose levels of 0.5 mg and 6 mg were selected as the lower dose was expected to have minimum to no effects based on the preclinical data and the higher dose to fall into the pharmacological active range.[22] Both dose levels were well tolerated in the previous healthy volunteer study.[11]

Twenty-four healthy volunteers were included in this study. To assess eligibility, volunteers were screened using the following procedures: a review of their medical and psychiatric history, a physical examination, measurement of vital signs, an electrocardiogram (ECG), blood chemistry and haematology laboratory assessments, and urinalysis. Volunteers who had a clinically significant previous or current psychiatric disorder according to Diagnostic and Statistical Manual-5 were excluded. In addition, volunteers who had a history of drinking an average of two or more alcoholic drinks per day were excluded.

During treatment days, volunteers reported at the research facility in the morning. Before a test dose was given, safety assessments were performed, including: adverse event (AE) occurrence, a physical examination, a suicidality assessment using the Columbia suicide severity rating scale,[23] measurement of vital signs, an ECG, laboratory assessments and urinalysis. The same safety assessments and AE recording were performed at set times after dosing.

During the study, volunteers were instructed to restrict the use of substances that could alter brain activity, including concomitant medication, alcoholic beverages, caffeinated products and nicotine-containing products. First, volunteers were instructed not to use concomitant medication starting from the seven days before the first test dose through to the end of the study. Second, they were instructed not to consume alcoholic beverages seven days before the screening visit and each treatment day. Third, they were instructed not to consume caffeinated products 24 hours before the screening visit and each treatment day. Outside of these restrictions, volunteers could consume up to six servings of caffeinated products a day. Finally, they were instructed not to use nicotine-containing products 48 h before the screening visit and each treatment day. Otherwise, volunteers could use up to five nicotine-containing products per day.

PHARMACOKINETIC ASSESSMENTS

The PK sample collection times were aligned with timings of the TMS assessments, as these were the assessments of primary interest in this study; PK samples were collected before drug administration, and 0.5 h and 2.5 h after administration. Based on initial healthy volunteer studies, it was expected that the mean maximal plasma concentration (C_{max}) would be reached at 2.5 h post-dose and due to the relatively long terminal half-life ($t_{½}$) TAK-653 of 33.1 to 47.8 hours, plasma levels were then expected to remain stable over a few hours.[11] A validated high-performance liquid chromatography with tandem mass spectrometry assay with a lower limit of quantification of

0.1 ng/mL and coefficient of variation between 1.41% and 5.22% was used to measure TAK-653's plasma concentrations.

FUNCTIONAL PHARMACODYNAMIC NEUROCART ASSESSMENTS

NeuroCart tests that have been shown sensitive to CNS depressant and/or CNS stimulant compounds were selected for this study.[14] These were: body sway, smooth pursuit eye movements, saccadic eye movements, adaptive tracking test, Stroop coloured word test, and Bond and Lader and Bowdle Visual Analogue Scales (VAS). In Table 1 the effects of different CNS depressant and CNS stimulant compounds on the NeuroCart tests performed in this study are summarised. The tests were performed twice prior to dosing as well as at the time of expected maximum plasma concentrations, namely 3.5 hours and 4 hours post-dose (Figure 1). During all tests, lighting conditions were standardised and volunteers were comfortably seated in front of a computer screen, except for body sway measurements, for which volunteers were standing.

Table 1 Summary of effects of CNS depressant and CNS stimulant compounds on selected NeuroCart tests

Test	CNS depressant		CNS stimulant			
	Diazepam 10 mg	Benzo-diazepines (dose unspecified)	Modafinil 200 mg	Dexam-phetamine 20 mg	Methyl-phenidate (average 20 mg)	Caffeine (60 mg)
Body sway (%)	+[1]	NR[3]	-	-	-	NR
Smooth pursuit (%)	-[2]	NR	NR	NR	+	NR
Saccadic peak velocity (deg/s)	NR	-	+	+	NR	+
Adaptive tracking (%)	NR	-	+	+	+	+
Stroop coloured word test	NR	-	NR	NR	NR	NR
Bond and Lader VAS alertness	NR	-	+	+	+	+
Bowdle VAS Feeling high	No effect	No effect	No effect	+	No effect	No effect

1. + indicates improvement or increase; 2. - indicates deterioration or decrease; 3. NR: not reported

BODY SWAY.

Body sway measurements are used to assess postural stability and are often used in pharmacologic studies.[24-26] Measurements of movements in the anteroposterior direction were performed as in previously published studies,[26,27] with a string similar to the Wright ataxiameter [28] attached to the waist of participants. Volunteers were instructed to stand comfortably on a firm surface with their feet slightly apart and eyes closed for 2 minutes. In previous studies, CNS-stimulant compounds demonstrated reductions in body sway; for example, modafinil (200 mg), dexamphetamine (20 mg) and clinical doses (average 20 mg) of methylphenidate reduced body sway by approximately 35% [29], 19.4% [15] and 36.8%, respectively.[30] Conversely, CNS-depressant compounds such as benzodiazepines are associated with increased body sway; for example, diazepam (10 mg) increased body sway by 119%.[31]

SMOOTH PURSUIT EYE MOVEMENTS.

The computerised smooth pursuit measurement was performed as described previously [32] and used in many studies to assess drug effects.[14] During this test, participants followed a light source with their eyes, that moved continuously in a horizontal direction on a screen placed 58 cm away. The outcome of smooth pursuit was defined as the percentage of time the participant's eyes were in smooth pursuit of the target for each stimulus velocity and frequency. In a previous study, the velocity of smooth pursuit eye movements was impaired by diazepam (10 mg).[32] Improvements in smooth pursuit of approximately 6% have been reported with CNS stimulants such as methylphenidate (average 20 mg).[30]

SACCADIC EYE MOVEMENTS.

The computerised measurement of saccadic eye movements was performed as described previously [33] and used in many pharmacological studies.[14] Briefly, to measure saccadic eye movements, participants were positioned identically to when performing the smooth pursuit measurement and instructed to follow a light source that jumped from side to side.[33] The parameter collected was saccadic peak velocity (SPV) in degrees/second (deg/s). Previous studies have demonstrated that CNS-stimulant compounds such as caffeine (60 mg), modafinil (200 mg) and dexamphetamine (20 mg) increased the average SPV by 11.6 deg/s, [34] 24.6 deg/s, [29] and 12.7 deg/s, [15] respectively. CNS-depressant compounds such as benzodiazepines have been shown to decrease SPV.[35]

ADAPTIVE TRACKING TEST

Adaptive tracking tests have been used in many pharmacological studies to evaluate visuomotor coordination and vigilance.[14] In this study, we used an adaptive tracking test according to specifications from Borland and Nicholson.[36] During the test, a circle moved randomly on a screen. Participants were given a joystick and instructed to use it to keep a dot within the moving circle. When an effort was successful, the speed of the moving circle increased. Conversely, the speed decreased if the participant was not able to maintain the dot within the circle, resulting in a constant and individually adapted challenge throughout the procedure. The outcome of the test is the average speed of the moving circle as a percentage of the maximum speed of the circle. In previous studies, CNS-stimulant compounds such as caffeine (60 mg), modafinil (200 mg), dexamphetamine (20 mg) and methylphenidate (average 20 mg) improved

average adaptive tracking by approximately 1.6%,[34] 1.8%,[29] 4.2%,[15] and 2.2%,[30] respectively. For CNS-depressant compounds, such as benzodiazepines, an impairment of adaptive tracking has been demonstrated.[14]

STROOP COLOURED WORD TEST.

The Stroop effect test involves identifying the colour of coloured words,[37] many CNS-active compounds have an effect on this test. [38-40] In this study, we used a computer-adapted version from the Psychology Software Tools website (https://pstnet.com/products/e-prime/), comprising two subtests as described in a previous publication.[41] In the first subtest, six coloured items were presented at random. The possible colours were green, red and blue, and each colour corresponded to a number key on the numpad section of the keyboard; green corresponded with 1, red with 2 and blue with 3. Participants were instructed to place the index, middle and ring fingers of their dominant hand on keys 1, 2 and 3. When a coloured item appeared on the screen, participants were to press the corresponding key as quickly as possible. In the second subtest, which immediately followed the first, 34 colour and word pairs were presented randomly. The words that were used were 'red', 'green' and 'blue', and the colour and word pairs were either congruent or incongruent matches. Again, the participants were asked to identify the correct colour as quickly as possible by pressing either keys 1, 2 or 3 on the numpad. Each item or word was shown for 4 seconds, and there was a 0.5 s pause after every response. Two parameters were derived from this test: Stroop 1 is the difference in reaction time between correct congruent and correct incongruent answers (ms) and Stroop 2 is the number of correct responses in incongruent trials. Previous studies demonstrated that benzodiazepines impair performance on this test.[38]

BOND AND LADER AND BOWDLE VISUAL ANALOGUE SCALES.

VAS, as originally described by Norris, have commonly been used to quantify the subjective effects of sedative agents.[35,42] Subjects were instructed to use the computer mouse to select their response to each VAS item. The Bond and Lader VAS involved collecting scores from 16 horizontal scales related to how a person feels. From these measurements, three main factors, namely 'alertness', 'mood' and 'calmness', were calculated as described in previous publications.[34,43] Benzodiazepines have consistently shown reductions on VAS alertness,[44] whereas variable but consistent increases are observed with caffeine,[34] dexamphetamine,[15] modafinil,[29] and methylphenidate.[30] Psychedelic effects were measured using the Bowdle VAS as previously described.[45] This scale consists of 13 items on which three summary scales (internal perception, external perception and 'feeling high') are calculated using log transformation as described in previous publications.[46] Dexamphetamine (20 mg) has been shown to increase the summary scale 'feeling high',[15] in contrast to the other CNS stimulants mentioned earlier.

STATISTICAL ANALYSIS

Analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA). Residual Q-Q plots were produced for all NeuroCart parameters to check the assumption of normality of the error term in the mixed effects models. This was done by visual inspection and the Shapiro–Wilk test statistic. To assess the treatment effects, data for each parameter were analysed with a mixed model analysis of covariance. We defined treatment, time, period and treatment by time as fixed factors; subject, subject by treatment and subject by time as random factors; and the (average) baseline measurement per study period as a covariate. The Kenward–Roger approximation was used to estimate denominator degrees of freedom and the model parameters were estimated using the restricted maximum likelihood method. Individual treatment effects over the 4-hour post-dose time period for the different doses were reported with the least squares mean estimated difference, the two-sided 90% confidence interval (CI) and the *P*-value. Owing to the exploratory nature of this study, a 90% CI instead of 95% CI was deemed sufficient. Next to that, no correction for multiple comparisons was performed as due to the exploratory nature of this study, hypothesis testing was not used in the strict way, but to guide the direction of future research.

RESULTS

DEMOGRAPHICS

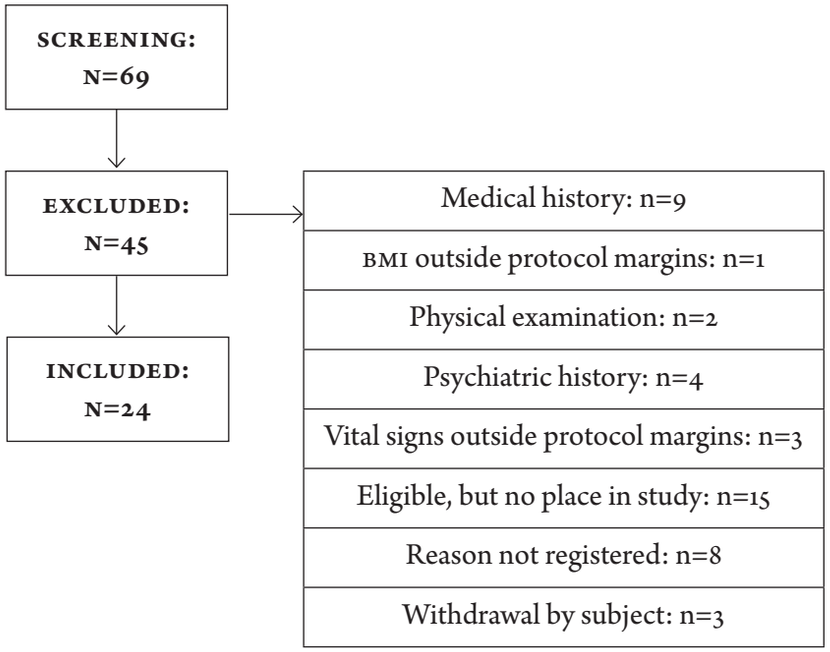
In total, 69 volunteers were screened, of which 24 healthy volunteers (23 male and 1 female of non-childbearing potential) between 18 and 55 years of age were included (Table 2). Subject disposition can be found in Figure 2. All participants completed the three study periods.

Table 2 Demographic characteristics of study participants

Characteristic	Subjects enrolled (N = 24)
AGE (YEARS)	
mean (SD[1])	27.9 (9.0)
SEX, N (%)	
Female	1 (4.2%)
Male	23 (95.8%)
WEIGHT (KG)	
mean (SD)	79.12 (10.81)
HEIGHT (CM)	
mean (SD)	181.98 (9.88)
BMI (KG/M^2)	
mean (SD)	23.92 (2.85)

1. SD: standard deviation; 2. BMI: body mass index

Figure 2 Subject disposition



PHARMACOKINETIC ASSESSMENTS

As reported in our previous publication, mean (SD) TAK-653 plasma levels for the 0.5 mg dose were 0.99 (0.94) ng/ml at 0.5 h post-dose and 4.19 (0.83) ng/ml at 2.5 h post-dose.[22] Plasma levels for the 6 mg dose were 2.57 (3.29) ng/ml at 0.5 h post-dose and 45.99 (8.84) ng/ml at 2.5 h post-dose.[22]

FUNCTIONAL PHARMACODYNAMIC NEUROCART ASSESSMENTS

All NeuroCart parameters were normally distributed, except body sway measurements, which were log-normal distributed and therefore natural log transformation was applied for their analysis. For interpretation back transformation was applied. To calculate summary scores for VAS Bowdle, log transformation was performed as well as described in previous publications.[46] Results are summarised in Table 3. On smooth pursuit eye movements (%), a clear statistically significant improvement was observed with the TAK-653 6 mg dose (Figure 3). At the same dose level, a similar improvement was observed for adaptive tracking (%) (Figure 4). Both doses of TAK-653 increased SPV (deg/s) to a similar extent (Figure 5).

Table 3 Least squares mean overall treatment effects and individual treatment effects of TAK-653 0.5 mg and 6 mg over the 4-hour post-dose period.

	Least squares mean			Contrasts (90% CI) *p*-value	
	Placebo	TAK-653 0.5 mg	TAK-653 6 mg	TAK-653 0.5 mg vs. placebo Estimate of difference, 90% CI[1], p-value	TAK-653 6 mg vs. placebo Estimate of difference, 90% CI[1], p-value
Body sway, log (mm) (N=24)	202.3	199.6	185.7	−1.3% (−13.4%, 12.4%) *P* = 0.86	−8.2% (−19.4%, 4.6%) *P* = 0.28
Smooth pursuit (%) (N=24)	44.5	44.8	46.8	0.26 (−1.69, 2.21) *P* = 0.82	2.32 (0.37, 4.27) *P* = 0.05*
Saccadic peak velocity (deg/s) (N=24)	475.5	495.0	490.9	19.49 (5.98, 32.99) *P*= 0.02*	15.40 (1.91, 28.90) *P* = 0.06*
Adaptive tracking (%) (N=24)	30.8	31.2	32.5	0.41 (−0.73, 1.56) *P* = 0.55	1.68 (0.51, 2.84) *P* = 0.02*
Stroop 1[3] (ms) (N=24)	71.4	93.4	71.0	22.0 (4.0, 40.0) *P* = 0.05*	−0.5 (−18.3, 17.3) *P* = 0.96
Stroop 2[4] (ms) (N=24)	16.7	16.4	16.6	−0.3 (−0.5, −0.1) *P* = 0.02*	−0.1 (−0.3, 0.2) *P* = 0.61
VAS[2] alertness (mm) (N=24)	49.9	50.5	50.7	0.65 (−0.38, 1.67) *P* = 0.30	0.77 (−0.24, 1.79) *P* = 0.21
VAS[2] *calmness* (mm) (N=24)	52.4	52.4	52.2	−0.03 (−1.65, 1.60) *P* = 0.98	−0.25 (−1.88, 1.38) *P* = 0.80
VAS[2] *mood* (mm) (N=24)	51.5	51.8	52.0	0.28 (−0.29, 0.85) *P* = 0.41	0.48 (−0.10, 1.05) *P* = 0.17
VAS[2] *external*, log (mm) (N=24)	0.35	0.35	0.35	−0.01 (−0.03, 0.01) *P* = 0.63	−0.01 (−0.03, 0.01) *P* = 0.58
VAS[2] *internal*, log (mm) (N=24)	0.35	0.35	0.35	< 0.00 (−0.01, 0.01) *P* = 0.92	< 0.00 (−0.01, 0.02) *P* = 0.76
VAS[2] *'feeling high'*, log (mm) (N=24)	0.37	0.35	0.34	−0.02 (−0.05, 0.01) *P* = 0.25	−0.03 (−0.06, −0.01) *P* = 0.05*

1. CI: confidence interval; 2. VAS: Visual Analogue Scale; 3. Stroop 1 is the difference in reaction time between correct congruent and correct incongruent answers; 4. Stroop 2 is the number of correct responses in incongruent trials.
*Indicates a statistically significant effect.

Figure 3 Smooth pursuit eye movements: change from baseline time effect profile of the least square (LS) mean 90% confidence interval (CI)

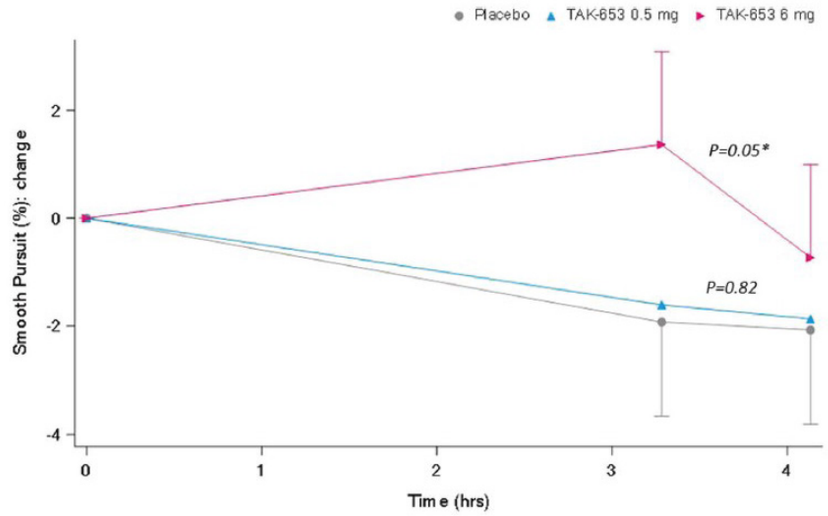


Figure 4 Adaptive tracking: change from baseline time effect profile of the least square (LS) mean 90% confidence interval (CI)

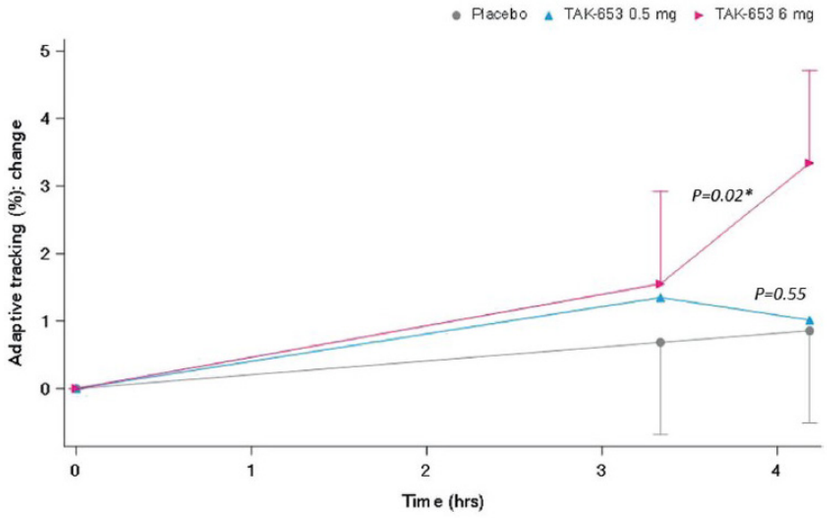
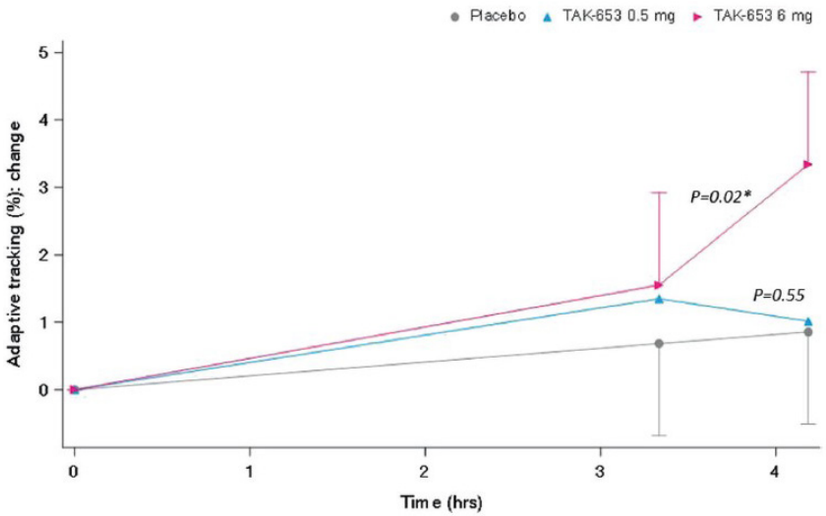


Figure 5 Saccadic peak velocity: change from baseline time effect profile of the least square (LS) mean 90% confidence interval (CI)



The VAS Bowdle subscale 'feeling high' remained stable under TAK-653 0.5 mg and 6 mg; however, average VAS-high increased with placebo, resulting in a statistically significant reduction with TAK-653 6 mg. A review of the raw data revealed that this effect was caused by one subject who indicated a 20 mm (large) increase in VAS-high after placebo. This entry was judged to be an artefact, given 'feeling high' does not occur spontaneously or under placebo and the subject did not have AEs indicating subjective drug effects such as 'feeling abnormal', 'feeling drunk' or 'feeling high'.

On the Stroop coloured word test TAK-653 0.5 mg increased the difference in reaction time between correct congruent and correct incongruent answers and decreased the number of correct responses in incongruent trials compared to placebo whereas TAK-653 6 mg did not affect any parameter of the Stroop coloured word test.

No significant effects were observed on body sway (%), other VAS Bowdle subscales (mm) or any of the Bond and Lader VAS subscales (mm) (Table 3).

SAFETY AND TOLERABILITY

For details on TAK-653's safety and tolerability in this study, please refer to previous reported results.[22] In summary, TAK-653 was well tolerated, no serious AEs were observed and there were no withdrawals related to an AE. The most frequently reported AEs after administration of TAK-653 were somnolence (TAK-653 0.5 mg: 3 of 24 subjects [12.5%], TAK-653 6 mg: 3 of 24 subjects [12.5%]), headache (TAK-653 0.5 mg: 1

of 24 subjects [4.2%], TAK-653 6 mg: 4 of 24 subjects [16.7%]) and nasopharyngitis (TAK-653 0.5 mg: 3 of 24 subjects [12.5%], TAK-653 6 mg 1 of 24 subjects [4.2%]). Of these AEs, somnolence and headache were reported after administration of placebo as well (2 of 24 subjects [8.3%] each). No clinically significant effects on vital signs, ECGs or laboratory measurements were observed. Of note is that no AEs of seizure, dissociative effects or euphoria were observed.

DISCUSSION

Similar to CNS stimulant compounds TAK-653 increased SPV, SP and adaptive tracking at the time maximum plasma concentration was reached. These effects were more pronounced with 6 mg than with 0.5 mg TAK-653. TAK-653 increased SPV at both 0.5 mg and 6 mg, while smooth pursuit eye movements and adaptive tracking increased at 6 mg but not at 0.5 mg. The effects of TAK-653 on the NeuroCart tests contrasted with the effects of CNS-depressant compounds such as benzodiazepines, which have been shown to decrease smooth pursuit eye movements,[32] SPV,[44] and adaptive tracking.[14] The absence of an effect of TAK-653 on any of the VAS subscales supports the finding that TAK-653 is devoid of subjective mood-related derangements observed with other CNS-stimulant compounds such as dexamphetamine.[15] When comparing the acute pharmacodynamic NeuroCart profile of TAK-653 to known profiles of CNS stimulant and CNS depressant compounds, TAK-653's profile is suggestive of stimulatory CNS effects. This is consistent with the TMS-EMG (electromyography) findings demonstrating increased cortical excitability with the 6 mg dose.[22] While one could argue that the observed effects on the NeuroCart tests are due to TMS itself, this can be ruled out for this study as a placebo arm was included and the effects of the different doses of TAK-653 on the NeuroCart were observed compared to placebo.

Compared to clinical doses of psychostimulants previously characterised using Neurocart test, TAK-653's stimulatory CNS effects appear more limited. TAK-653 increased SPV by 15.5 to 19.5 deg/s, which is larger than caffeine 60 mg (11.6 deg/s) [34] and dexamphetamine 20 mg (12.7 deg/s),[15] but smaller than modafinil 200 mg (24.6 deg/s).[29] Increases in smooth pursuit eye movements represented only roughly one-third of those induced by methylphenidate.[30] The increase in adaptive tracking of 1.6% with TAK-653 6 mg was comparable to modafinil (1.8%),[29] caffeine (1.6%),[34] and methylphenidate (2.2%),[30] but smaller than dexamphetamine (4.2%).[15] Next to that, decreases in BS have been observed for other CNS stimulant compounds, but no effect of both dose levels TAK-653 on BS was observed. Although direct comparisons should be made for an unequivocal interpretation of our findings, TAK-653 seems to have a novel stimulatory CNS profile that is generally more subtle than clinical doses of known psychostimulants, and distinguishes itself by a relatively large stimulatory effect on saccadic peak velocity but devoid of any subjective mood-related derangement such as dysphoria, anxiety or feeling high.

Although TAK-653 demonstrated psychostimulant effects, its impact on different aspects of cognition was less consistent. The Stroop test was included as it can be helpful in understanding complex attention, perception and elements of executive function.[47] TAK-653 0.5 mg but not 6 mg increased the difference in reaction time between correct congruent and correct incongruent answers. Similarly, TAK-653 0.5 mg but not 6 mg decreased the number of correct responses in incongruent trials. It cannot be excluded that the lower dose may affect aspects of cognitive functioning, which are obscured at a higher dose. The overall pharmacodynamic profile however, provides no reason to assume a bell-shaped dose-response curve. Therefore, the Stroop results are currently best considered as a potential type I error of a less robust test with multiple complex endpoints.

The PK results of this study were in line with results from initial healthy volunteer studies, as mean maximal plasma concentrations for 0.5 and 6 mg TAK-653 were comparable to those observed at similar dose levels.[11] Therefore, although the area under the curve from time 0 to infinity (AUC_{∞}) was not determined in the current study, this was expected to correspond to the AUC_{∞} observed in initial healthy volunteer studies with 406 h*ng/ml and 3167 h*ng/ml for TAK-653 0.5 and 6 mg, respectively. These data support dose and concentration dependence since more pronounced effects were observed with the 6 mg dose compared to the 0.5 mg dose.

A limitation of this study is that full dose/concentration-response characterisation was precluded by safety concerns. Given both AMPA receptor PAMs and TMS are associated with an increased albeit very limited risk of convulsions,[10] 6 mg was selected as the highest dose as it was expected to yield a mean maximum plasma concentrations well below those at which partial seizures were observed in primates (Takeda internal data).

Taken together, the PD profile of TAK-653 was characterised in this study according to recommendations by the 'QBDD' framework.[12] As hypothesised based on its mechanism of action of AMPA receptor PAM, TAK-653 demonstrated an acute functional PD profile of CNS stimulatory effects on the NeuroCart. This confirms BBB penetration and, moreover, target engagement that is consistent with the previously reported TMS results of increased cortical excitability.[22] No undesired pharmacological effects associated with AMPA receptor stimulation, such as seizures or euphoria, off-target effects or unexpected AEs, were observed in this acute dosing study. The insights obtained in this study, can be used to design future studies in both healthy individuals and selected patient populations that are hypothesised to benefit from AMPA receptor-mediated stimulatory CNS effects.

REFERENCES

1 Berman RM, Cappiello A, Anand A, Oren DA, Heninger GR, Charney DS, Krystal JH. Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry*. 2000;47(4):351-354.

2 Murrough JW, Iosifescu DV, Chang LC, Al Jurdi RK, Green CE, Perez AM, et al. Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *Am J Psychiatry*. 2013;170(10):1134-1142. doi:10.1176/appi.ajp.2013.13030392

3 Zarate Jr. CA, Singh JB, Carlson PJ, Brutsche NE, Ameli R, Luckenbaugh DA, et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry*. 2006;63(8):856-864. doi:10.1001/archpsyc.63.8.856

4 Collo G, Cavalleri L, Chiamulera C, Merlo Pich E. (2R,6R)-Hydroxynorketamine promotes dendrite outgrowth in human inducible pluripotent stem cell-derived neurons through AMPA receptor with timing and exposure compatible with ketamine infusion pharmacokinetics in humans. *Neuroreport*. 2018;29(16):1425-1430. doi:10.1097/WNR.0000000000001131

5 Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, et al. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*. 2016;533(7604):481-486. doi:10.1038/nature17998

6 Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science (80-)*. 2010;329(5994):959-964. doi:10.1126/science.1190287

7 Shaffer CL, Dutra JK, Tseng WC, Weber ML, Bogart LJ, Hales K, et al. Pharmacological evaluation of clinically relevant concentrations of (2R,6R)-hydroxynorketamine. *Neuropharmacology*. 2019;153:73-81. doi:10.1016/j.neuropharm.2019.04.019

8 Duman RS, Li N, Liu RJ, Duric V, Aghajanian G. Signaling pathways underlying the rapid antidepressant actions of ketamine. *Neuropharmacology*. 2012;62(1):35-41. doi:https://doi.org/10.1016/j.neuropharm.2011.08.044

9 Maeng S, Zarate Jr. CA, Du J, Schloesser RJ, McCammon J, Chen G, et al. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiatry*. 2008;63(4):349-352. doi:10.1016/j.biopsych.2007.05.028

10 Leo A, Giovannini G, Russo E, Meletti S. The role of AMPA receptors and their antagonists in status epilepticus. *Epilepsia*. 2018;59(6):1098-1108. doi:10.1111/epi.14082

11 Asgharnejad Xu, Dong, Iadevaia, Dorner A, Murthy V, Ratti E, et al. Pharmacokinetic and Pharmacodynamic Properties of the Investigational AMPA Receptor Positive Allosteric Modulator TAK-653 After Single and Multiple Rising Doses in Healthy Volunteers. Neuropsychopharmacology. 43, S157 (2018).

12 Cohen AF, Burggraaf J, van Gerven JM, Moerland M, Groeneveld GJ. The use of biomarkers in human pharmacology (Phase I) studies. *Annu Rev Pharmacol Toxicol*. 2015;55:55-74. doi:10.1146/annurev-pharmtox-011613-135918

13 Morgan P, Van Der Graaf PH, Arrowsmith J, Feltner DE, Drummond KS, Wegner CD et al. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov Today*. 2012;17(9-10):419-424. doi:10.1016/j.drudis.2011.12.020

14 Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood–brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol*. 2016;20:27-34. doi:http://dx.doi.org/10.1016/j.ddtec.2016.07.004

15 Recourt K, van der Aart J, Jacobs G, de Kam M, Drevets W, van Nueten L, et al. Characterisation of the pharmacodynamic effects of the P2X7 receptor antagonist JNJ-54175446 using an oral dexamphetamine challenge model in healthy males in a randomised, double-blind, placebo-controlled, multiple ascending dose trial. *J Psychopharmacol*. 2020;34(9):1030-1042. doi:10.1177/0269881120914206

16 Chen X, Broeyer F, de Kam M, Baas J, Cohen A, van Gerven J. Pharmacodynamic response profiles of anxiolytic and sedative drugs. *Br J Clin Pharmacol*. 2017;83(5):1028-1038. doi:10.1111/bcp.13204

17 Chen X, Jacobs G, de Kam ML, et al. AZD6280, a novel partial γ-aminobutyric acid A receptor modulator, demonstrates a pharmacodynamically selective effect profile in healthy male volunteers. *J Clin Psychopharmacol*. 2015;35(1):22-33. doi:10.1097/JCP.0000000000000251

18 Chen X, Jacobs G, de Kam, Jaeger J, Lappalainen J, Maruff P, et al. The central nervous system effects of the partial GABA-Aalpha2,3 -selective receptor modulator AZD7325 in comparison with lorazepam in healthy males. *Br J Clin Pharmacol*. 2014;78(6):1298-1314. doi:10.1111/bcp.12413

19 de Haas SL, de Visser SJ, van der Post JP, de Smet M, Schoemaker RC, Rijnbeek B, et al. Pharmacodynamic and pharmacokinetic effects of TPA023, a $GABA_A$ α2,3 subtype-selective agonist, compared to lorazepam and placebo in healthy volunteers. *J Psychopharmacol*. 2006;21(4):374-383. doi:10.1177/0269881106072343

20 de Haas SL, Franson KL, Schmitt JA, Cohen AF, Fau JB, Dubruc C, van Gerven JMA. The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A alpha2,3 selective agonist, in comparison with lorazepam in healthy volunteers. *J Psychopharmacol*. 2009;23(6):625-632. doi:10.1177/0269881108092595

21 de Haas SL, de Visser SJ, van der Post JP, Schoemaker RC, van Dyck K, Murphy MG, et al. Pharmacodynamic and pharmacokinetic effects of MK-0343, a GABA(A) alpha2,3 subtype selective agonist, compared to lorazepam and placebo in healthy male volunteers. *J Psychopharmacol*. 2008;22(1):24-32. doi:10.1177/0269881107082108

22 O'Donnell P, Dijkstra FM, Damar U, Quanhong L, de Goede A, Xu L, et al. Transcranial magnetic stimulation as a translational biomarker for AMPA receptor modulation. *Transl Psychiatry*. 2021;11(1):325. doi:10.1038/s41398-021-01451-2

23 Posner K, Brown GK, Stanley B, Posner, Brent DA, Yershova KV, et al. The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. *Am J Psychiatry*. 2011;168(12):1266-1277. doi:10.1176/appi.ajp.2011.10111704

24 van Steveninck AL, Schoemaker HC, Pieters MS, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther*. 1991;50(2):172-180.

25 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. *J Psychopharmacol*. 1999;13(1):10-17. doi:10.1177/026988119901300102

26 van Steveninck AL, Wallnofer AE, Schoemaker RC, Pieters MS, Danhof M, van Gerven JMA, et al. A study of the effects of long-term use on individual sensitivity to temazepam and lorazepam in a clinical population. *Br J Clin Pharmacol*. 1997;44(3):267-275. doi:10.1046/j.1365-2125.1997.t01-1-00580.x

27 van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MS, Kroon JM, Breimer DD, et al. Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. *Psychopharmacol*. 1993;110(4):471-478. doi:10.1007/bf02244655

28 Wright BM. A simple mechanical ataxia-meter. *J Physiol*. 1971;218 Suppl:27p-28p.

29 Baakman AC, Zuiker R, van Gerven JMA, Gross N, Yang R, Fetell M, et al. Central nervous system effects of the histamine-3 receptor antagonist CEP-26401, in comparison with modafinil and donepezil, after a single dose in a cross-over study in healthy volunteers. *Br J Clin Pharmacol*. 2019;85(5):970-985. doi:10.1111/bcp.13885

30 Klein RH. Minimally invasive methodology for pharmacological research involving children. Published online 2013.

31 van Steveninck AL, Gieschke R, Schoemaker RC, Roncari G, Tuk B, Pieters MS, et al. Pharmacokinetic and pharmacodynamic interactions of bretazenil and diazepam with alcohol. *Br J Clin Pharmacol*. 1996;41(6):565-573. doi:10.1046/j.1365-2125.1996.38514.x

32 Bittencourt PR, Wade P, Smith AT, Richens A. Benzodiazepines impair smooth pursuit eye movements. *Br J Clin Pharmacol*. 1983;15(2):259-262. doi:10.1111/j.1365-2125.1983.tb01495.x

33 Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. *Neurology*. 1975;25(11):1065-1070. doi:10.1212/wnl.25.11.1065

34 Wilhelmus MM, Hay JL, Zuiker RG, Okkerse P, Perdrieu C, Sauser J, et al. Effects of a single, oral 60 mg caffeine dose on attention in healthy adult subjects. *J Psychopharmacol*. 2017;31(2):222-232. doi:10.1177/0269881116668593

35 de Visser SJ, van der Post J, Pieters MS, Cohen AF, van Gerven JM. Biomarkers for the effects of antipsychotic drugs in healthy volunteers. *Br J Clin Pharmacol*. 2001;51(2):119-132.

36 Borland RG, Nicholson AN. Visual motor co-ordination and dynamic visual acuity. *Br J Clin Pharmacol*. 1984;18 Suppl 1(Suppl 1):69S-72S. https://www.ncbi.nlm.nih.gov/pubmed/6525331 https://www.ncbi.nlm.nih.gov/pmc/PMC1463351/

37 Stroop JR. Studies of interference in serial verbal reactions. *J Exp Psychol*. 1935;18(6):643-662. doi:10.1037/h0054651

38 Griffiths AN, Jones DM, Richens A. Zopiclone produces effects on human performance similar to flurazepam, lormetazepam and triazolam. *Br J Clin Pharmacol*. 1986;21(6):647-653. doi:10.1111/j.1365-2125.1986.tb05229.x

39 Bohnen NI, Kaufer DI, Hendrickson R, Ivanco LS, Lopresti BJ, Koeppe RA, et al. Degree of inhibition of cortical acetylcholinesterase activity and cognitive effects by donepezil treatment in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2005;76(3):315-319. doi:10.1136/jnnp.2004.038729

40 Rose AK, Duka T. The influence of alcohol on basic motoric and cognitive disinhibition. *Alcohol Alcohol*. 2007;42(6):544-551. doi:10.1093/alcalc/agm073

41 Liem-Moolenaar M, te Beek ET, de Kam ML, Franson KL, Kahn RS, Hijman R, et al. Central nervous system effects of haloperidol on THC in healthy male volunteers. *J Psychopharmacol*. 2010;24(11):1697-1708. doi:10.1177/0269881109358200

42 Norris H. The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology*. 1971;10(21):181-191.

43 Hoever P, de Haas S, Winkler J, Schoemaker RC, Chiossi E, van Gerven J, Dingemanse J, et al. Orexin receptor antagonism, a new sleep-promoting paradigm: an ascending single-dose study with almorexant. *Clin Pharmacol Ther*. 2010;87(5):593-600. doi:10.1038/clpt.2010.19

44 De Visser SJ. A question based approach to drug development. 2003;PhD.

45 Bowdle AT, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers relationship to steady-state plasma concentrations. *Anesthesiology*. 1998;88(1):82-88. doi:10.1097/00000542-199801000-00015

46 Zuurman L, Roy C, Schoemaker RC, Hazekamp A, den Hartigh J, Bender JCME, et al. Effect of intrapulmonary tetrahydrocannabinol administration in humans. *J Psychopharmacol*. 2008;22(7):707-716. doi:10.1177/0269881108089581

47 Gerstadt CL, Hong YJ, Diamond A. The relationship between cognition and action: performance of children 3 ½-7 years old on a Stroop-like day-night test. *Cognition*. 1994;53(2):129-153. doi:10.1016/0010-0277(94)90068-x

APPENDIX

IB-DERISK ANALYSER OVERVIEW OF TAK-653

TAK-653 is an alpha-amino-3-hydroxy-5-methyl-4-isoxazole receptor (AMPAR) positive allosteric modulator (PAM) in clinical development for the treatment of major depressive disorder (MDD). When sorted on C_{max} values in the IB-Derisk analyser tool, TAK-653 demonstrated an overall favourable profile, with desired green pharmacological effects at lower exposures followed by a range of acceptable yellow side effects, and only at higher exposures unacceptable orange and severe red side effects emerged.[1] It was noticeable however, that at dose levels above the NOAEL, the dose-response curve for adverse effects was relatively steep. This indicates that severe side effects (red), such as tonic-clonic seizures, occurred at exposure levels following exposure levels where only mild side effects (yellow), such as tremors, had been observed. In this respect, the IB-Derisk analyser overview did not show the optimal pattern of side effects gradually increasing in severity, where mild (yellow) adverse effects are followed by more severe (orange) adverse effects and ultimately severe (red) adverse effects.

In line with the excitatory/stimulatory effects expected from an AMPA-PAM, in preclinical experiments in rats, monkeys and mice convulsions were observed at exposure levels at least a factor 10 higher than levels associated with desired pharmacological effects. In rabbits, which were used for reproductive and developmental toxicity studies, no convulsions were observed. Desired pharmacological effects consisted of positive results observed on *in vivo* behavioural experiments testing memory and antidepressant-like effects in rats.

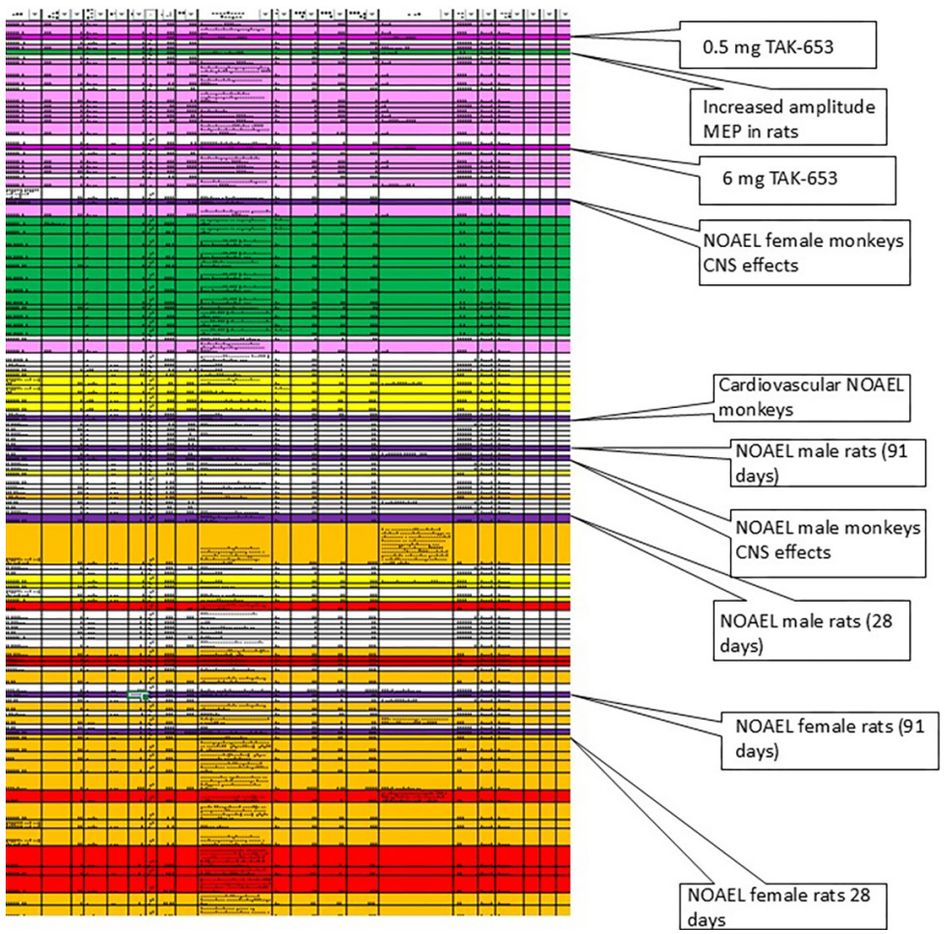
A dose range between 0.3 and 18 mg was tested in a first-in-human (FIH) study with TAK-653.[2] The exposure level associated with the highest dose of 18 mg was still a factor 13 below the values associated with convulsions in preclinical experiments, while it fell in the range associated with desired pharmacological effects. As expected based on the preclinical data, the dose range tested in the FIH study was well tolerated, specifically no tremors or convulsions were observed.

Since the FIH study with TAK-653 was primarily geared towards characterising safety and PK, a second clinical study was set up to assess the pharmacodynamics of TAK-653. In rats TAK-653 had shown to increase mechanomyography (MMG) amplitude elicited by Transcranial Magnetic Stimulation (TMS) of the motor cortex. It was therefore decided to perform a comparable measurement in humans, namely motor evoked potential (MEP) amplitude after TMS stimulation of the motor cortex. Because of the theoretical increased risk of seizures by potentiating glutamatergic synapses by both TMS and AMPA receptor potentiation, dose selection for the clinical study was performed with great caution.[3] Doses selected for the human study were 0.5 and 6 mg, the estimated C_{max} values of these doses were expected to be within the anticipated dose range to be used in a phase 2 study of TAK-653 to obtain maximum treatment response while maintaining sufficient margins to the observed convulsions in preclinical studies. The results of the study demonstrated that increases in the amplitude of MMG in rats and MEPs in humans arose at comparable exposure levels (Figure 1).[4] Thereby demonstrating that MMG or MEPs as elicited by TMS can be used as a translational biomarker for AMPA receptor modulation.[4]

In addition to measuring TMS effects in humans, the Neurocart was performed as well with the 0.5 and 6 mg dose.[5] In line with TAK-653's mechanism of action and the findings of the TMS assessments, TAK-653 demonstrated a psychostimulant-like profile on the NeuroCart without unwanted effects of for example euphoria often observed with other psychostimulant compounds such as amphetamine.[5] While some small psychostimulant effects consisting of increased saccadic peak velocity, were observed with the 0.5 mg dose, the 6 mg dose demonstrated a more pronounced psychostimulant profile consisting of improved adaptive tracking, increased saccadic peak velocity and improved smooth pursuit eye movements.[5]

Taken together, these studies demonstrate how the overview of the available preclinical and clinical data on a compound obtained with the IB-Derisk analyser tool, can be used to guide decisions for early phase clinical studies. TAK-653 demonstrated consistency in pharmacokinetics and exposure-effect relations across species, which was reassuring for the clinical study. Furthermore, desired pharmacological effects were observed at much lower exposure levels than undesired effects. Based on the overview obtained with the IB Derisk analyser tool, we were able to select dose levels to study TAK-653's pharmacodynamics that were expected to sort out an effect, but still had sufficient margins to avoid seizures.

Figure 1 IB-Derisk analyser overview TAK-653



Abbreviations: MEP, motor evoked potential; NOAEL, no observed adverse effect level; CNS, central nervous system

REFERENCES

1 van Gerven J, Cohen A. Integrating data from the Investigational Medicinal Product Dossier/investigator's brochure. A new tool for translational integration of preclinical effects. *Br J Clin Pharmacol*. 2018;84(7):1457-1466. doi:10.1111/bcp.13529

2 Asgharnejad M, Zicha S, Lin X, Cheng D, Iadevaia S, Dorner A, Murthy V RE. Pharmacokinetic and pharmacodynamic properties of the investigational AMPA receptor positive allosteric modulator TAK-653 after single and multiple rising doses in healthy volunteers. *Poster Present Am Coll Neuropsychopharmacol 57th Annu Meet 9-13 Dec, 2018; Hollywood, Florida*. Published online 2018.

3 Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol*. 2009;120(12):2008-2039. doi:10.1016/j.clinph.2009.08.016

4 O'Donnell P, Dijkstra FM, Damar U, et al. Transcranial magnetic stimulation as a translational biomarker for AMPA receptor modulation. *Transl Psychiatry*. 2021;11(1):325. doi:10.1038/s41398-021-01451-2

5 Dijkstra F, O'Donnell P, Klaassen E, et al. Central nervous system effects of TAK-653, an investigational alpha-amino-3-hydroxy-5-methyl-4-isoxazole receptor (AMPAR) positive allosteric modulator in healthy volunteers. *Transl Psychiatry*. 2022;12(1):408. doi:10.1038/s41398-022-02148-w

CHAPTER VII

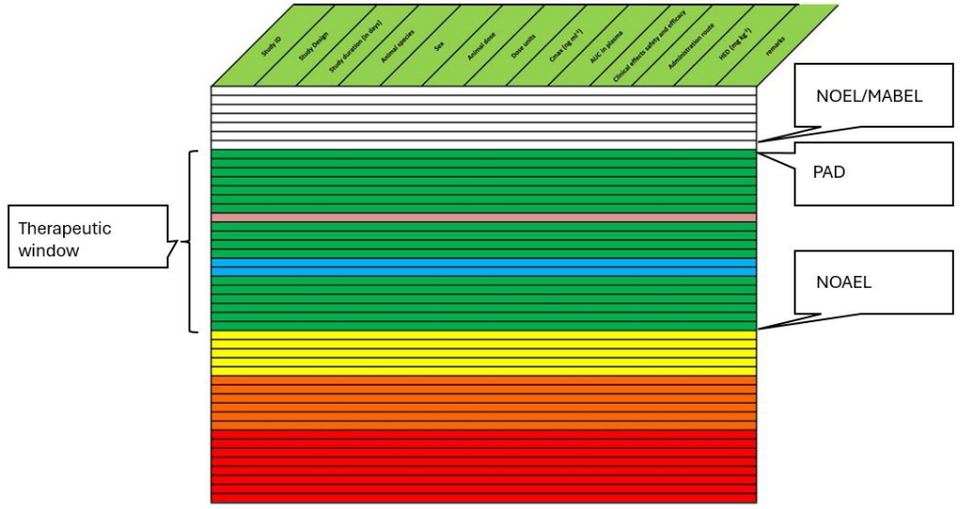
GENERAL DISCUSSION

The IB-Derisk analyser tool has been developed to enable investigators to obtain an integrated assessment of the often unstructured, varied and incomplete preclinical data reported in the investigator's brochure (IB).[1] It achieves this by organising findings across species based on drug exposures and/or human equivalent dose (HED), allowing for an intuitive assessment of all available (pre)clinical data in a single page, colour coded overview.[1] The aim of this overview is to support investigators in efficiently and accurately identifying missing critical data, detecting potential safety issues, predicting potential clinical effects, selecting relevant clinical biomarkers, contextualising findings of ongoing clinical studies, and enhancing communication within research teams[1] In this way, the IB-Derisk analyser tool facilitates a 'pharmacological understanding' of novel compounds prior to, during and following completion of early phase clinical trials.[1] Previous analyses have shown that to reduce the relatively high attrition rates in late phases of development, it is important that a thorough understanding of a novel compound's pharmacological characteristics is obtained in the early phases of clinical development.[2-7] The IB-Derisk overview's colour coding provides insight into the complete exposure-effect relationship, from subeffective dose/concentrations (white), through pharmacologically active doses (green), up to manageable -pharmacological- (yellow/orange) adverse and toxic (red) dose ranges.

In this thesis, the application of the IB-Derisk analyser tool in early phase clinical development of neuropsychiatric drugs was investigated. **Chapter II** evaluated how accurately available preclinical data, summarised using the IB-Derisk analyser tool, can predict tolerable and pharmacologically active dose ranges for Central Nervous System (CNS) drugs in humans. **Chapters III**, **IV**, **V**, and **VI** describe the results of three early-phase clinical development studies involving investigational CNS active compounds with highly innovative mechanisms of action. For each of these compounds the accuracy and utility of the predictions based on preclinical data, summarised using the IB-Derisk analyser tool, were evaluated.

The General discussion in this final chapter of the thesis evaluates the practical application of the IB-Derisk analyser tool in early phase clinical trials involving CNS active compounds based on the reported studies in **Chapters III to VI.** As illustrated in Figure 1, the IB-Derisk overview's colour coding profile reveals essential drug characteristics such as minimal pharmacologically active level (MABEL, PAD); anticipated therapeutic dose (ATD, EC_{50}); no observable adverse effect level (NOAEL) and expected maximum tolerated dose (MTD); and the resulting therapeutic window (Figure 1). Moreover, descriptions of the effects provide indications for biomarker selection and clinical monitoring in clinical studies. An important aspect of the colour coding profile is that it explicitly demonstrates the consistency of exposure-effect relationships across different species, which is an indication of translatability to humans.[1] This General Discussion follows the sequence of the IB-Derisk analyser overview assessment, beginning with a review of compound translatability, and then assessing the onset of pharmacological effects through the range of desired effects, up to the adverse effect levels. This chapter concludes with an outlook towards the future of drug development in psychopharmacology and the potential role of the IB-Derisk tool therein.

Figure 1 Translatable colour profile of occurrence of pharmacological effects in IB-Derisk analyser overview



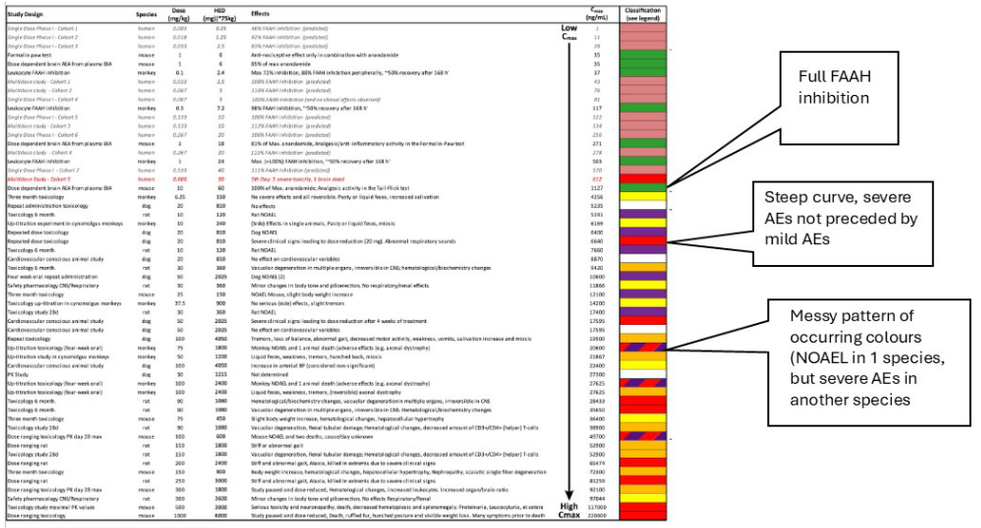
White: Studies without clinical observations / Blue: *In vitro* studies / Green: Safe or desirable effects (including doses that show pharmacological activity or beneficial effects on disease models) / Pink: Human observations inserted during first clinical studies / Yellow: Undesirable effects that could be acceptable in a clinical study if monitored adequately and are reversible / Orange: More severe adverse effects that could not be accepted in a clinical situation but without unacceptable health risks / Red: Serious irreversible toxicity and death (Reproduced with permission from: Gerven van, J.M.A., Cohen A.F.. Integrating data from the Investigational Medicinal Product Dossier/investigator's brochure. A new tool for translational integration of preclinical effects. *Br J Clin Pharmacol.* 0(0). doi:10.1111/bcp.13529)

1. TRANSLATABILITY

The initial step review of the IB-Derisk analyser overview is to evaluate the translatability of the compound concerned. A key characteristic of a reliably translatable compound is its ability to produce similar responses at comparable exposure levels across different species.[1] The likelihood that preclinical findings are translatable to humans increases if a compound demonstrates a consistent pattern of translatability across different preclinical studies.[1] For all three individual investigational compounds included in this thesis translatability was assessed as good. The importance of careful assessment of effect consistency across species – including the risk of poor

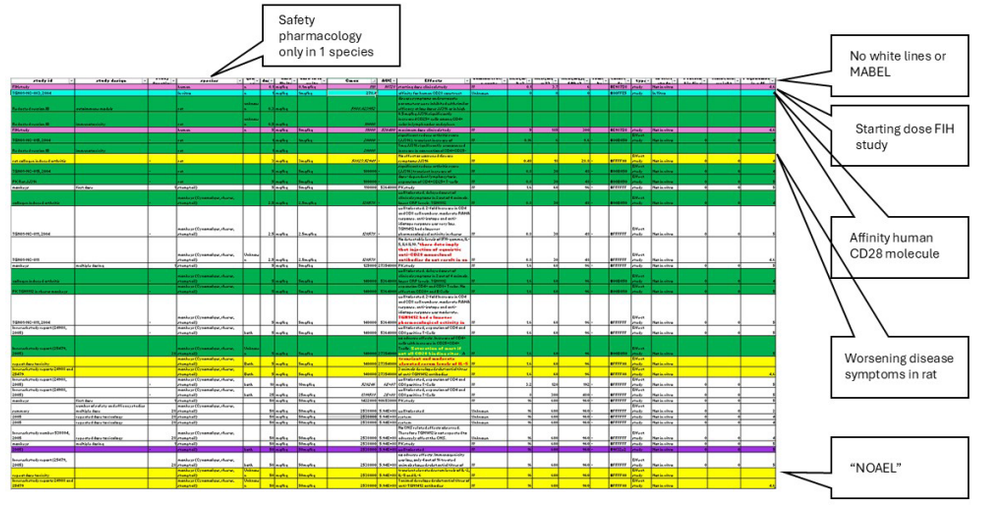
translatability – is dramatically illustrated by the case of BIA 10-2474.[1,8] In the first-in-human (FIH) trial with BIA-10-2472, a Fatty Acid Amide Hydrolase (FAAH) inhibitor, several healthy volunteers experienced neurological damage, and one volunteer died as a result of escalating the doses up to toxic levels at which off-target inhibition of other hydrolases occurred.[9-11] Interestingly, the IB-Derisk analyser overview for BIA 10-2474 clearly demonstrated poor translatability in the dose range planned for the human studies (Figure 2).[9] For example, dogs experienced serious irreversible side effects at concentrations well tolerated by rats and monkeys.[9] Even within individual species, severe effects were not always preceded by manageable adverse effects that could act as warning for impending toxicity. Moreover, in some cases the C_{max} values for the NOAEL overlapped with those associated with severe 'red' findings (mortality).[9] During the FIH study, dose escalations were not guided by quantification of FAAH inhibition, leading to dose escalation far beyond what was sufficient to achieve maximal human FAAH inhibition. The combination of these factors resulted in after all preventable and unintended, serious adverse effects and death.[9] The importance of assessing a compound's potential animal to human translatability before commencing a clinical trial is further underlined by the case of TGN1412 (Figure 3).[12,13] TGN1412 is a potent CD28 superagonistic monoclonal antibody, designed to stimulate regulatory T-cell activity to control a host of autoimmune diseases.[12,13] After administration of the initial dose to six healthy volunteers in the FIH study, all volunteers developed a cytokine storm, leading to life-threatening multi-organ failure, requiring intensive care unit (ICU) admission.[12,13] In an attempt to investigate whether such dramatic undesired clinical effects could have been predicted based on the preclinical data, we composed an IB-Derisk analyser overview using the same Investigator's Brochure (IB) that was available to the researchers and regulators prior to the FIH study (Figure 3). When assessing the translatability of TGN1412, it was immediately noticeable that preclinical studies had only been conducted in two preclinical species, rats and non-human primates. This is an unusually small number of species investigated preclinically, making it difficult to properly assess the compound's translatability, particularly because TGN1412 specifically targets primate CD28. Within these two species, the IB-Derisk overview demonstrated limited variation in terms of preclinical experiments performed, when considering the relative mechanistic novelty and potential range of proinflammatory effects that might be hypothetically associated with CD28 superagonism. While the results of *in vivo* activity studies with JJ316, an agonistic anti-CD28 monoclonal antibody homologous to TGN1412 binding to CD28 in rats, were described in the TGN1412 IB, no safety pharmacology experiments were performed with this compound in rats. In addition, only one exposure level of JJ316 in rats was reported, further impeding the assessment of the pharmacologically active dose range in rats, and as a result, potential translatability to humans.

Figure 2 IB-Derisk analyser overview of BIA 10-2474



Abbreviations: FAAH, fatty acid amide hydrolase; AE, adverse event; NOAEL, no observed adverse effect level

Figure 3 IB-Derisk analyser overview of TGN1412



Abbreviations: MABEL, minimum anticipated biological effect level; FIH, first in human; NOAEL, no observed adverse effect level

When a TGN1412 starting dose was selected for the FIH clinical study, the inability to reliably assess the compound's translatability was not adequately recognised. In the conducted preclinical studies, TGN1412 was not associated with undesired effects, even at the highest administered dose, since the explored dose range remained well below toxic levels, even though at least some toxicity would have been expected from CD28 superagonism.[13] Consequently, the risk profile of TGN1412 was significantly underestimated, which may have contributed to administering an excessively high starting dose in the clinical study. Perhaps in hindsight, based on the IB-Derisk overview of preclinical studies with TGN1412, questions could have been raised by the lack of any moderate or severe toxicity, considering its high potential to activate T cells without specific engagement of the T-cell receptor with an antigen-presenting cell.[12,13] But even if this were falsely assumed to indicate safety for humans, it should have been noticed that the variation of preclinical experiments was unusually small.[13] More studies across multiple species would have been needed to be conducted including toxicology studies, which might have revealed a limited understanding of CD28's role in the immune system, or a too varied, non-selective role, to proceed with a FIH trial for a compound targeting this receptor. Ultimately, such a systematic approach could have prevented the disastrous outcome of the clinical study.

In total, the individual studies presented in this thesis (along with the BIA 10-2472 and TGN1412 cases), emphasise the importance of evaluating the potential translatability of novel compounds before initiating clinical trials. An important lesson is that to determine translatability, the IB-Derisk analysis must cover the entire range of target binding and pharmacological activity, from levels with low binding and no effects, to full target saturation and exaggerated pharmacological activity, including secondary non-specific targets. An IB-Derisk overview that does not show the full spectrum of white-green-yellow-orange-red studies should be considered somewhat suspiciously. Unexpected 'safety' as observed in the IB-Derisk overview of TGN1412 may be due to not only to a limited dose range, but also when the included animal species are not representative enough or insensitive to the particular compound's mechanism of action, or if inappropriate effect markers are applied in preclinical experiments. In cases of poor translatability, further investigation is warranted to understand the differences in sensitivity among species, and which animals are most predictive of humans (or which not - and why).[1] If translatability remains uncertain, moving forward with a clinical study could still be considered if sudden occurrence of severe toxicity can be excluded – provided that additional pharmacological biomarkers or safety measures are in place to monitor the anticipated pharmacological effects of the investigational compound. This approach minimises the risk of escalating doses to toxic levels.[1] The remainder of this chapter is structured according to the different colour codes as presented in an IB-Derisk overview of a 'well-behaved' compound.

2. ABSENCE OF PHARMACOLOGICAL EFFECTS: THE IB DERISK OVERVIEW 'WHITE CATEGORY' AND THE FIH STARTING DOSE

Once the translatability of a novel compound has been evaluated and the decision is taken to proceed with a clinical study, a safe starting dose for the study must be established.[1] For this, it is important to focus on the part of the IB-Derisk overview which covers the low exposures that still cause no detectable effect in preclinical models. In principle, this is represented in the IB-Derisk overview by a range of white lines (Figure 1). There may be different reasons why no effects are reported in the IB. First, it is possible that effects were observed but not reported, because this was outside the scope of the study – for instance mild behavioural changes in dedicated pharmacokinetic (PK)-study. Second, and more reliably, the study report (in the IB) can specifically confirm that no effects were observed. This again can have different causes: lack of sufficient action site exposure (*eg* subactive doses or inadequate tissue penetration); or true absence of observed effects (*eg* because no 'clinical' effects were detected, and no pharmacodynamic measurements were performed). This 'no-observed effect level' (NOEL) is covered in the IB-Derisk overview by the highest dose with a white line, just below the first dose level with a green line (Figure 1). If it is clear that the NOEL line truly represents a dose with some target activity, but still without the effects that are observed at the next higher dose in the same species, this highest 'white' NOEL is likely to capture the Minimum Anticipated Biological Effect Level (MABEL), and the lowest 'green' level represents the Pharmacologically Active Dose (PAD) (Figure 1).[1] Establishing these levels is considered a sound basis for considerations of the starting dose.[14] The concept of MABEL was introduced into the guidelines after the incident with TGN1412 in 2006, where the starting dose was far above the MABEL of this CD28-superagonist antibody.[12,15,16,] In the IB-Derisk overview of TGN1412, no NOEL or MABEL could be identified, as indicated by the absence of white lines at the lowest doses/exposures. This means that it was impossible to determine the onset of pharmacological effects, from the animal studies published in the IB. As discussed in the next sections, this was one of the reasons for the miscalculation of the lowest pharmacologically/biologically active dose of TGN1412. Although MABEL was formally introduced in the European first-in-man guidelines in 2007, many IB's still contain no 'white' no-observable effect levels.[16] The semi-quantitative analysis of IB's for CNS-active compounds described in **Chapter II** demonstrated that in 32% of studies, the lowest tested preclinical dose was already pharmacologically active, meaning that the full dose range was not evaluated. This analysis also revealed that in 58% of studies, the starting dose was selected without considering MABEL or pharmacologically active dose ranges. An important reason is that the starting dose for FIH-studies is still often calculated from a fraction (often 10%) of the highest 'safe' dose – the No Observable Adverse Effect Level (NOAEL) in the most sensitive animal species.[17]

The critical analysis of the TGN1412 case demonstrates how the IB-Derisk analyser overview could have been used to establish a safe(r) clinical starting dose, relatively independently of the mechanism of action. Since the overview demonstrated no white no-observed effect levels (NOEL), information about the minimum pharmacological activity was estimated from pharmacological activity reported from *in vitro* cellular experiments. Although formal physiologically based/ pharmacokinetic/ pharmacodynamic (PB/PK-PD)-modelling would have been more precise, some simplified translations from receptor binding to human cells to pharmacological activity in humans, indicated that considerable pharmacological activity might already be expected at the planned starting dose, since the expected concentrations at this dose would be about equal to the dissociation constant K_d of CD28 receptors – so roughly around EC50. Thus, this simple suppletion of available *in vitro* pharmacological characteristics of the compound to the IB-Derisk analyser overview, already suggests that the starting dose of TGN1412 should be considerably lower than the actual administered FIH dose. Receptor occupancy (RO) calculations performed following the clinical study indeed showed that the RO at the starting dose was approximately 90% (EC90) – resulting in the described cytokine storm.[12,13,18] This simplified approach of using the IB-Derisk tool to roughly estimate the expected pharmacological activity based on a compound's receptor binding affinity is presented here not to suggest that *in vitro* data can replace missing animal studies. Instead, it serves to demonstrate how the IB-Derisk overview can be used to explore dose-response information described within the IB. Additional peculiarities of BIA 10-2474 and TGN 1412 will be discussed in relation to the other colour-coded parts of their respective IB-Derisk overviews.

For all three individual investigational compounds described in this thesis, the IB-Derisk analyser overview was applied as an aid in establishing safe clinical starting doses. In **Chapter III**, the overview indicated that the lowest oxathridine dose tested preclinically already demonstrated pharmacological activity. Consequently, a starting dose for the clinical study was selected to ensure that exposures would remain below those which elicited pharmacological (rather than adverse) effects in preclinical experiments. As predicted, no (pharmacological or clinical) effects were observed at the starting dose in the FIH-study.

For ALKS 7119 described in **Chapter IV**, only one *in vivo* preclinical pharmacology experiment was performed with only one dose level, implying that no pharmacologically active dose range could be established preclinically. This resulted from the development strategy for this compound heavily relying on comparisons with receptor binding profiles of similar registered congeners. Therefore, predictions of ALKS 7119's pharmacological effects were also based on its *in vitro* binding affinities for different receptors, which were converted to expected plasma concentrations and entered in the IB-Derisk analyser overview. ALKS 7119 demonstrated binding affinity for a wide range of receptors, including the serotonin transporter (SERT), μ-receptor, and NMDA receptor (NMDAR). Its binding affinity for SERT was highest and comparable to that of registered selective serotonin reuptake inhibitors (SSRIs). ALKS 7119 had a 100-fold lower binding affinity for the μ-receptor than opioids and a 35-fold lower affinity for the NMDAR compared to ketamine. Based on the IB-Derisk analyser overview, the starting dose for the clinical study was expected to have no pharmacological activity, as the anticipated exposure levels were below the estimated threshold required to significantly engage SERT or other receptors. Again, in line with IB-Derisk-predictions, no effects were demonstrated at the lowest doses in the FIH-study.

For TAK-653, described in **Chapters V** and **VI** the lowest pharmacologically active dose level was established preclinically as demonstrated by 'white' NOEL-exposures. Additionally, a FIH-study had already been performed with TAK-653, but without employing reliable PD biomarkers. To address this, a dedicated follow-up study was carried out to investigate TAK-653's clinical PD profile, which is presented in this thesis. TAK-653's IB-Derisk analyser overview provided indications for effects on neuronal excitability in rats using transcranial magnetic stimulation (TMS) combined with mechanomyography (MMG). A NOEL as indicated by white lines in the IB-Derisk overview was established as well. The preclinically active dose range and methodology were adapted to design a study and select a relevant dose range in healthy volunteers, where the primary endpoint was transcranial magnetic stimulation combined with electromyography (TMS-EMG). For the clinical study, two dose levels were selected, with the expectation that the 'low' dose would not affect TMS-EMG while the 'high' dose would. As predicted, the lowest dose of TAK-653 resulted in an exposure level below that associated with increased motor responses observed in preclinical studies, which also did not increase motor responses in humans, while the higher dose statistically significantly increased the motor response after TMS.

Overall, the TGN1412 case and the individual studies described in this thesis demonstrate how the IB-Derisk overview can be applied to select a safe and meaningful starting dose for a clinical study. This is achieved by carefully considering the 'white' no-observed-effect levels, which encompass the MABEL. This dose level represents the lower end of the exposure-effect range, where pharmacological activity is often still too limited to cause detectable clinical responses. For TGN1412 the tool accurately highlighted that no NOEL was determined, precluding reliable estimations of a sub-effective starting dose in humans. Rough estimations of pharmacologically active concentrations from the *in vitro* characteristics of the compound, suggested that the planned (and administered) starting dose would already lead to high receptor occupancy. All these suspicions were confirmed after careful *post hoc* analyses of the disastrous outcomes of the FIH-study with TGN1412.[18] In all three individual studies described in this thesis, the predictions derived from the IB-Derisk analyser overview regarding pharmacologi-

cally inactive and safe starting doses were accurate. This highlights the importance of a NOEL-zone, where absence of effects is confirmed, and it encourages the use of the IB-Derisk analyser tool in determining a starting dose for a clinical trial with at least the same rigour as the NOAEL or other parts of the dose response curve.

3. *DESIRED PHARMACOLOGICAL EFFECTS: THE IB DERISK OVERVIEW 'GREEN CATEGORY'*

For compounds with desirable broad therapeutic windows, the IB-Derisk analyser overview starts with white rows, indicating no pharmacological effects, followed by green rows, representing desired pharmacological effects, when the overview is sorted by lowest to highest exposure (Figure 1).[1] It is important to note in this context however, that pharmacological effects should not be confused with therapeutic effects, although these are obviously dependent on pharmacological activity. As outlined in the introduction of this thesis and in the Translatability section (§1) of the current discussion, data from pharmacologically relevant functional experiments in animals (and humans if available) should first be compiled to establish a pharmacologically active dose range. This analysis should guide the design of the clinical study by defining the intended pharmacological activity, identifying the (green) pharmacologically active dose range where this activity occurs, and importantly, selecting relevant PD biomarkers to demonstrate such effects in humans. Doses within the pharmacologically active dose range identified using the IB-Derisk overview, can also be expected to largely cover therapeutic activity, as a CNS-active compound cannot be therapeutic if it does not produce its intended pharmacological effects within the CNS, at least, provided that the indicated disease does not cause major shifts in dose-response relationships for the particular compound in question.[4,19-21] Biomarker-based assessments of intended pharmacological effects can therefore be performed during clinical study conduct to guide dosing escalation steps. Furthermore, these assessments enhance the understanding of a novel compound's clinical pharmacology, which can inform decisions about its further development, particularly when similar biomarker-based assessments are also obtained in early studies in patients.

The importance of including both relevant and reliable PD biomarkers to quantify intended pharmacological effects in a FIH study, is also illustrated by the BIA 10-2474 case, presented in the Translatability section (§1) of this discussion.[9] In the FIH study with BIA 10-2474 PD measurements were performed to a limited extent, consisting of the determination of anandamide concentrations in blood plasma.[9] However, since PD results were not available when determining dose escalation steps, these therefore relied solely on safety and tolerability outcomes.[9] As a result, dose levels were unintendedly escalated to levels approximately 12 times higher than necessary for maximal human FAAH inhibition (Figure 2).[9,10] At this dose level, healthy volunteers developed the described serious adverse effects,[8] probably as a result of inhibition of non-specific hydrolases in the CNS,[11] If the FIH BIA 10-2474 trial had included PD measurements that were reviewed to inform dose-escalation decisions together with PK and safety data, doses escalating well above maximum FAAH inhibition could have been prevented. Continuously updating the IB-Derisk analyser overview with emerging PK, PD, and safety data from the ongoing clinical study could potentially have helped prevent the extremely unfavourable outcome for both the compound and the healthy volunteers involved in the study.

Chapter II presents a semi-quantitative analysis aimed at evaluating whether preclinical data can predict the dose range in which desired pharmacological effects, indicative of target modulation, are observed in clinical studies. This was achieved by calculating the overlap between observed preclinical and clinical pharmacologically active dose ranges. The preclinically pharmacologically active dose range was defined as the dose range covering both primary (desired) and secondary (undesired) pharmacological effects, provided these effects were related to the compound's mechanism of action. The same definition was applied to the human (clinically) pharmacological active dose range. This analysis demonstrated an overlap of 84% between the HED of preclinical and observed human pharmacologically active dose ranges, implying that preclinical models can predict the dose levels needed for pharmacological activity in clinical studies. This overlap of 84% consists of both desired and undesired pharmacological effects and is therefore likely an overestimation. In the semi-quantitative analysis, the overlap between the dose ranges specifically linked to desired pharmacological effects preclinically and clinically was not investigated. However, in the vast majority of included studies, the investigational compound was well tolerated up to the highest administered dose, suggesting that the clinically pharmacologically active dose range consisted predominantly of intended pharmacological effects related to the compound's mechanism of action. The observed overlap of 84% therefore primarily demonstrates the overlap of intended pharmacological effects across different species, which is an indication of the overall translatability of the compounds. In cases where overlap between preclinical and human pharmacologically active dose levels was low, pharmacological activity in humans was generally found at lower exposures than those reported preclinically. This appeared to be particularly the case for compounds sorting psychomimetic effects, such as cannabinoids. Additionally, this seemed to apply to compounds targeting the orexin system and muscarinic compounds. A possible explanation is that more sensitive measuring methodologies for assessing CNS functions are available in humans compared with preclinical methods. For example, the Neurocart CNS test battery allows for the detection of more subtle effects on for example memory and eye-hand coordination in humans while that might not be the case in animals, or alternatively, humans may report subjective drug effects which are

per definition not evaluable in preclinical species. In summary the semi-quantitative analysis demonstrates that, although preclinical models often fall short in accurately predicting therapeutic effects in patients,[22-25] they still provide a good indication of the pharmacologically active dose range in healthy humans. It is essential to establish the pharmacologically active dose range of a new drug, as pharmacological activity is a prerequisite for therapeutic efficacy.

Concerning the individual studies discussed in this thesis, the predictions based on the IB-Derisk analyser overview of doses at which desired effects were observed in humans, proved to be accurate for ALKS 7119, as detailed in **Chapter IV**. More interestingly, this case illustrates how the IB-Derisk analyser overview offers insight into the selectivity of the investigational compound for its pharmacological target. ALKS 7119 was designed as an NMDAR antagonist but had a higher binding affinity for other targets such as the SERT and μ-receptor. The K_i or EC50 values of ALKS 7119 for the different receptors were entered in the IB-Derisk analyser overview as plasma concentrations. This is a very plain manner of 'modelling' basic pharmacological characteristics, and although there are some caveats when doing so (as described in the individual chapter), it offers an indication of how the affinity or potency values for the different receptors compare to one another. As argued earlier, inclusion of hypothetical *in vitro* characteristics might have given second thoughts about the dosing decisions for TGN1412 and BIA 10-2474. In the case of ALKS 7119, the IB-Derisk analyser overview indicated that within the full clinically active dose range, receptor modulation was anticipated not only for the specific desired modes of action, but also for other secondary pharmacological effects. From this, it was also possible to predict a selective dose-concentration range, expected to predominantly affect the targeted specific receptor. Indeed, when comparing the combined NeuroCart and neuroendocrine profile of ALKS 7119 to previously determined PD fingerprints of other related compounds, the PD profile of the selected doses most resembled SERT inhibition. This study therefore illustrates how the IB-Derisk analyser overview can support predictions and interpretations of the pharmacological effects of investigational compounds in FIH-studies. Importantly, the studies with ALKS 7119 but also with BIA 10-2474 illustrate the benefits of updating the IB-Derisk analyser overview with emerging clinical data to place these into 'pharmacological perspective', as an add-on to tolerability-based dose selection in ascending dose design.

For TAK-653, discussed in **Chapter V** and **Chapter VI**, exposure values at which desired pharmacological effects occurred could be directly compared between preclinical species and humans due to the use of a nearly identical biomarker of pharmacological activity in rats and humans. In line with predictions based on the IB-Derisk analyser overview the 'high' dose of TAK-653 increased the motor response in humans elicited by TMS, while the 'low' dose did not. This indicates that TAK-653 increases cortical excitability, as would be expected from a glutamatergic stimulant compound.[26] Additionally, consistent with its mechanism of action, TAK-653's NeuroCart profile , as described in **Chapter VI**, was similarly indicative of stimulatory CNS effects that were distinct from the dopamine-mediated psychostimulant profile of dexamphetamine. In this case, the IB-Derisk analyser overview facilitated identifying comparable effects at similar exposures in both preclinical and clinical settings.

In summary, for a compound to produce therapeutic effects, it must reach its target site and exert its intended pharmacological action.[4,19-21] Thus, incorporating measurements of the intended pharmacological activity in FIH studies is crucial .[4,19-21] Moreover, information about intended pharmacological activity can be used during the study to guide dose escalation steps and to contextualise the findings pharmacologically. The semi-quantitative analysis described in **Chapter II** demonstrates that data from preclinical models generally predict the pharmacologically active dose ranges and the dose range of desired pharmacological effects well. Furthermore, the individual studies described in this thesis demonstrate how the IB-Derisk analyser overview can be used to predict pharmacological effects of novel compounds in humans. Additionally, this thesis illustrates how the IB-Derisk analyser tool can be used to contextualise clinical findings, providing a 'pharmacological understanding' of novel compounds both during study conduct to support dose-escalation decisions and retrospectively after study completion.

4. UNDESIRED PHARMACOLOGICAL EFFECTS: THE IB DERISK OVERVIEW 'YELLOW, ORANGE AND RED CATEGORIES'

When sorting the IB-Derisk analyser overview by a measure of exposure (HED, C_{max} or AUC), the green lines that depict desirable pharmacological effects typically give way to increasing yellow, orange, and red lines, which represent undesired mild to severe adverse effects observed in preclinical (safety) pharmacological or toxicity experiments (Figure 1).[1] Detailed understanding and accurate interpretation of preclinical toxicity is required for establishing a safe dose range for a clinical study and to guide decisions on which safety measures to include.[1,27]

To investigate whether the IB-Derisk tool can accurately predict safe dose ranges in humans, the semi-quantitative analysis described in **Chapter II** calculated the ratio between the highest well-tolerated dose levels in the conducted clinical studies and the NOAELs determined in the preclinical studies. The analysis revealed that in a minority of studies (4 out of 25 [16%]), dose-limiting adverse effects in humans occurred at exposures lower than the NOAELs established preclinically. The dose-limiting AEs in these studies with a $GABA_A$ modulator, two histaminergic compounds and a Trace Amine-Associated Receptor (TAAR) partial agonist were in line with the mechanisms of action of the investigated compounds and consisted of ataxia, hypotension,

drowsiness, insomnia and nausea. It was noteworthy that both histaminergic compounds were not tolerated clinically at dose levels that were well tolerated in preclinical species. One of the histaminergic compounds concerned oxathridine described in **Chapter III**, which caused pseudo-hallucinations in healthy volunteers. Preclinically, monkeys had demonstrated remarkable behaviour, such as unexpectedly seeking and accepting human contact, but this occurred at exposure levels 300 times higher than those associated with pseudo-hallucinations in humans. Preclinically, these behavioural abnormalities were attributed to cerebral lesions found during pathological examination of the brains of monkeys given high doses of oxathridine, rather than being indicative of psychomimetic effects occurring in animals. These unexpected psychomimetic effects possibly reflect the complexity of the histaminergic system which is involved in the regulation of various other neurotransmitter systems, such as serotonin, acetylcholine, noradrenaline and dopamine, some of which are also implicated in the psychomimetic or psychotic phenomena associated with drugs like MDMA, psilocybin or Amanita mushrooms, and amphetamine or cocaine.[28]

In five out of 25 studies (20%), doses in the clinical studies were escalated to exposure values exceeding the NOAEL. In these studies, expected adverse effects could be closely monitored using intensive cardiovascular monitoring or NeuroCart measurements, which explains why doses could be escalated to levels above the NOAEL. However, in two of these studies, both with a cannabinoid agonist, further dose escalation was limited due to reversible psychiatric side effects including derealisation, auditory and visual hallucinations and anxiety that were not observed in animals in preclinical studies. For both compounds however, the NOAEL was based on cardiovascular effects, which in humans could be monitored well enough to stop dosing while they were still limited.

For all individual compounds described in this thesis the IB-Derisk analyser overview demonstrated similar favourable preclinical profiles with desired effects occurring at lower exposures than undesired effects. For ALKS 7119 and TAK-653 described in **Chapter IV** and **Chapter V**, and **Chapter VI**, respectively, this was also the case in healthy human volunteers. For oxathridine described in **Chapter III**, this was not the case as in the healthy volunteer study as doses could not be escalated to exposure values preclinically associated with desired pharmacological effects due to the occurrence of psychotomimetic effects. Altogether, the findings of the semi-quantitative analysis and individual studies show that preclinical data can reliably predict well-tolerated dose levels in humans. However, the studies involving oxathridine and cannabinoid agonists emphasise the need for investigators to remain cautious of potential psychiatric side effects when evaluating novel CNS-active compounds, as these effects are not readily predicted by preclinical data. In most cases, such effects can be anticipated from activators of specific pharmacological mechanisms, such as 5HT2, DA2,

CB1 and – as shown in **Chapter III.** Moreover, some adverse effects that determine the NOAEL in animals are due to reversible pharmacological mechanisms that are well understood and can be accurately measured in humans. In such cases, cautious dose escalation beyond the NOAEL may be possible in clinical studies, provided there is intensive monitoring and other safety precautions in place.

5. IB DERISK OVERVIEW 'COLOUR PROFILES'

In the first section (§1) of this discussion, it was argued that translatability of a compound from preclinical animals to humans, is most persuasively demonstrated by a full IB-Derisk analyser overview profile that covers the entire colour range across various representative preclinical species. Therefore the colour profile can be considered a relevant indicator of a compound's safety profile in humans. Ideally, the emergence of effects follows a predictable exposure-related colour pattern, starting with increasingly consistent desirable pharmacological effects (green) followed by mild unwanted effects (yellow), and subsequently by increasingly severe effects (orange), progressing to toxic effects or death (red) (Figure 1).[1] This ensures that in clinical studies, adverse effects are predictable and can be closely monitored as more severe effects are anticipated to be preceded by less severe ones, ensuring that dose escalation can be discontinued in time to guarantee volunteer safety. The IB-Derisk analyser overviews of both BIA 10-2474 and TGN1412 did not follow this preferred pattern of onset of effects.[9] For BIA 10-2474, serious irreversible side effects were observed in dogs at concentrations that were still well tolerated by rats and monkeys (Figure 2).[9] Additionally, in some instances, C_{max} values for the NOAEL overlapped with preclinical effects classified as red (Figure 2), implying poor translatability of BIA 10-2472 across preclinical species. Preclinical observation of poor translatability of a new compound reduces the predictability of that compound's effects in humans.[1]The observation of poor translatability across preclinical species should ideally lead to additional research to understand why different species respond so differently to the compound.[1] Once this is understood, predictions of the compound's effects in humans can be made with greater confidence.[1] .[9] For TGN1412, almost no preclinical adverse effects were observed which was remarkable given that based on the mechanism of action, adverse effects would have been expected at (beyond) maximal receptor occupancy of CD28 receptors (Figure 3). In both cases, a comparison of the obtained IB-Derisk overview colour pattern to the IB-Derisk overview colour pattern of a hypothetical 'well-behaved [white-green-yellow-orange-red]' compound could have pointed to the unusual patterns of BIA 10-2474 (largely red, chaotic – Figure 2) and TGN1412 (unexpectedly absent subpharmacological white or toxic orange/red – Figure 3). This insight could have prompted additional investigation of the pharmacology of these compounds before proceeding with studies in healthy volunteers, potentially preventing the resulting disastrous outcomes.

The pattern of effect onset depicted in the IB-Derisk analyser overview was assessed for all the individual compounds discussed in this thesis. The IB-Derisk analyser overview for oxathridine in **Chapter III** and ALKS 7119 in **Chapter IV** demonstrated the preferable pattern of effect occurrence, i.e. starting with white lines indicative of absence of any effect to increasingly consistent desirable pharmacological effects (green) followed by mild unwanted effects (yellow), and subsequently by increasingly severe effects (orange), progressing to toxic effects or death (red). However, this was not the case for TAK-653 (**Chapters V** and **VI**) , since the dose-response curve for adverse effects was relatively steep at dose levels beyond the NOAEL. Specifically, the IB-Derisk overview revealed severe side effects (red), such as tonic-clonic seizures, occurring at TAK-653 exposures higher than those associated with mild undesired effects (yellow), such as tremors. Nonetheless, it was considered safe to initiate a clinical study, aiming to show 'green' pharmacological effects, while avoiding the undesirable 'yellow' effect range. Importantly, both of the selected dose levels for the clinical TMS-EEG and Neurocart study with TAK-653 described in this thesis, were not only below the well tolerated highest dose level administered in the FIH study, but also well below the preclinically established threshold for increased risk of convulsions. This approach ensured that the selected dose levels for the clinical PD study with TAK-653 remained well below the threshold for risk of inducing seizures, which otherwise in the absence of the IB-Derisk analyser overview could have resulted in a potentially unsafe dose being selected. The example of TAK-653 therefore illustrates how findings from the IB-Derisk analyser overview can inform the design and conduct of an early phase clinical study, for compounds that have a pharmacologically optimal effect profile over a limited exposure range.

The studies described in this thesis demonstrate the application of the IB-Derisk analyser overview to facilitate the prediction of both desired and unwanted PD effects consistent with the compound's mechanism of action or exaggerated pharmacological activity. Within this context, exaggerated pharmacology can refer to both on-target and off-site effects. On-target effects refer to the compound producing effects through modulation of the intended target (i.e., antipsychotic effect of low-dose or extrapyramidal motor symptoms by high-dose haloperidol by binding to dopamine D2 receptors in the mesolimbic and nigrostriatal pathways, respectively), while off-site effects refer to the compound producing effects by modulating a receptor or target beyond the primary target (*e.g.,* sedative effects of high-dose haloperidol by interacting with adrenergic and/or histamine receptors).This is particularly evident in the clinical study of ALKS 7119, described in **Chapter IV**, where the most commonly observed adverse effects – nausea, presyncope, and somnolence – were consistent with its preclinical receptor binding profile of SERT inhibition, as similar adverse effects are commonly associated with SSRIs. Lastly, it is important to realise that using the IB-Derisk analyser tool cannot prevent the occurrence of idiosyncratic AEs, which per definition are unrelated to the known pharmacological actions of the investigational compound.[29]

Overall, the studies in this thesis together with the BIA 10-2474 and TGN1412 cases, demonstrate several ways in which the IB-Derisk analyser can facilitate and support assessment and prediction of the pharmacological effect range and the safety window of novel compounds, with a wide variety of pharmacological mechanisms of action, in early phase clinical pharmacology studies. Examples of the use of IB-Derisk analyser overviews cover determining the margin between desired and adverse effects in preclinical experiments. Additionally, it includes evaluating the colour profile of (adverse) effect onset as reflected by the IB-Derisk analyser overview, to support designing a safe and informative dose escalation and monitoring schedule, and moreover, to facilitate timely dose adaptation and/or discontinuation of dose escalation to prevent volunteers from being unnecessarily exposed to adverse drug effects in clinical studies. Furthermore, the studies presented in this thesis highlight the importance of evaluating the affinity of novel compounds for both on-target (on- and off-site) and off-target receptors. This assessment is essential for predicting potential undesired pharmacological effects that may arise from exaggerated pharmacology, in line with a compound's mechanism of action.[11] A final example is the use of the IB-Derisk in pharmacology-guided effect optimisation of compounds with a specifically desired pharmacological effect profile, where the IB-Derisk can be continuously updated with PK-and PD-data that emerge during a dose escalation study.

6. CURRENT DEVELOPMENT STATUS OF INVESTIGATED COMPOUNDS IN THIS THESIS

This thesis describes studies conducted in accordance with current regulatory and scientific recommendations to investigate fundamental pharmacological properties of new compounds, such as exposure at the target site and target modulation, in the early stages of clinical development.[3,4,20] It is therefore of interest to examine how the obtained insights were applied in further development of these compounds and whether this approach indeed leads to lower attrition rates in the later stages of clinical development. Of the 25 compounds included in the semi-quantitative analysis described in **Chapter II**, eleven are currently still in clinical development – which for early phase I-studies seems a relatively large proportion. The development status of one of the compounds could not be traced, and development of the remaining thirteen compounds was ceased. When comparing the IB-Derisk analyser overviews of the currently discontinued studies and studies that progressed to a further development stage, it was noticed that the degree of overlap in pharmacological activity between preclinical and clinical studies was similar between the discontinued studies and the

ongoing studies. However, the percentage of IB-Derisk analyser overviews with a preferred colour-coded pattern was higher in the currently ongoing studies (64%), than in the discontinued studies (38%). While the number of compounds analysed in the semi-quantitative overview is too limited to draw definitive conclusions, this finding suggests that the IB-Derisk analyser overview offers valuable insight into the likelihood of successful development of novel compounds. Of the compounds that were no longer in development, the reasons for discontinuation were unclear for three compounds, the remaining ten compounds were discontinued due to safety or efficacy issues, of which six compounds were discontinued due to the occurrence of psychotropic side effects such as mood alterations and perceptual changes. Psychotropic side effects, such as paraesthesia, delusional perception, derealisation, auditory and visual hallucinations and anxiety were observed with all cannabinoid compounds, oxathridine (described in **Chapter III**) and a compound targeted at the GABAergic system.[4,20,30] Due to their nature, psychotomimetic effects are difficult to predict based on preclinical studies. Therefore, it is crucial to be alert to unexpected or otherwise remarkable behavioural changes in preclinical studies that may indicate the possible occurrence of psychotomimetic effects in humans. Upon reviewing the four individual IB-Derisk analyser overviews of the compounds that failed due to safety or efficacy reasons not related to psychotropic effects, only one IB Derisk overview demonstrated the preferred colour coded pattern. In the other three cases the IB Derisk overview was already indicative of poor translatability or desired effects only occurring at exposure levels higher than those associated with undesired effects. In total, this demonstrates the usefulness of assessing the colour-coded pattern of the IB-Derisk analyser overview in terms of translatability and occurrence of desired and undesired pharmacological effects as an undesired colour-coded pattern is suggestive of a high failure rate. Furthermore, this demonstrates that the occurrence of psychotomimetic effects in humans is difficult to predict and using the IB-Derisk analyser tool does not help in recognising the potential occurrence of psychotomimetic effects in humans.

The individual compounds described in this thesis are currently in different phases of development. Development of oxathridine described in **Chapter III** was stopped after the described FIH-study due to the unacceptable AEs of pseudo-hallucinations. Development of ALKS 7119 described in **Chapter IV** was ceased as well as the results from the FIH-study made clear that further dose escalation was not expected to achieve plasma exposures needed for relevant modulation of the NMDA-receptor. A recently completed phase II study on the effectiveness of TAK-653 for the treatment of Major Depressive Disorder (MDD,) demonstrated a statistically significant reduction of depressive symptoms based on the Montgomery-Åsberg Depression Rating Scale (MADRS) total score at Day 28 and Day 56 of dosing.[31] Dose levels at which these antidepressant effects, however, were observed were not revealed which precludes

relating the reported antidepressant effects to the CNS effects reported in **Chapters V** and **VI**.[31] At any rate though, the therapeutic effectiveness of TAK-653 will now need to be further investigated in phase III studies, that ideally should involve MDD patients who are, at least theoretically, expected to benefit from a compound with CNS stimulating activity, such as MDD patients with symptoms of apathy or anhedonia, or alternatively reduced positive valence or increased negative valence according to the Research Domain Criteria (RDoC) initiative.[32]

Overall, the individual studies included in this thesis demonstrate that an intricate understanding of action site exposure and target modulation obtained in early phases of clinical drug development contributes to the reduction of late-stage drug development failures. In the cases of oxathridine and ALKS 7119, the FIH studies indicated that escalating the doses would not achieve the necessary plasma exposures for effective target modulation and therapeutic efficacy, without having an undesirable impact on secondary pharmacological targets or (psychomimetic) CNS functionality. As a result, the decision was made to halt the development of these compounds. TAK-653 is arguably the most successful example described in this thesis. For this compound findings from the human PD study indicated target modulation and provided a good 'pharmacological understanding' of the compound. It was therefore decided to continue its development to a Phase II study, in which preliminary therapeutic efficacy was demonstrated.[31]

7. OUTLOOK OF PSYCHIATRIC DRUG DEVELOPMENT

The challenge of translating preclinical findings to human studies is often cited as a reason for the largely unsuccessful development of new drugs for the treatment psychiatric disorders compared to other therapeutic areas.[2,33-35] This thesis demonstrates in which ways the IB-Derisk analyser tool can bridge the gap between preclinical findings and clinical studies involving novel compounds, with the aim to optimise the dose range to demonstrate their intended pharmacological effects in humans. Although this should be considered a necessary prerequisite for therapeutic activity in patients, it is not sufficient *per se*, since ultimately, effectiveness is determined by various other factors including pathophysiological characterisation and/or clinical heterogeneity of the intended target population, and additionally, a host of PK and PD sources of variability associated with demographics, psychiatric and somatic comorbidities, comedication and others. Although arguably all therapeutic areas face such difficulties, challenges that are relatively specific to the field of psychiatric drug development are worth consideration. These include the particularly poorly understood pathophysiology of most psychiatric disorders, leading to the widespread reliance on phenomenology-based classification systems in psychiatric drug research, and as a consequence patient heterogeneity within diagnostic categories, the general lack of

reliable pharmacological and response biomarkers for pharmacological interventions in psychiatry and the sizable placebo responses in efficacy trials for psychiatric disorders, which are strongly influenced by external psychosocial circumstances.[2,36-38] Currently, new methodologies and conceptual approaches are being explored to address these challenges. As such, the IB-Derisk analyser tool could be integrated into these approaches as further explained below.

To address the issue of patient heterogeneity, the concept of 'precision psychiatry' has been proposed.[35] According to this approach, individuals with psychiatric symptoms are clustered based on relevant biological phenotypes (so-called endophenotypes) rather than phenomenological classifications, as is currently the focus of the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5).[35] This approach aligns with the Research Domain Criteria (RDoC) framework, which is designed to serve as a basis for investigating the pathophysiology of psychiatric disorders and, ultimately, classifying psychiatric disorders based on biological constructs.[32] RDoC advocates for the development of biosignatures, comprising molecular, genetic, neurocircuitry and behavioural assessments, to classify patients and explore pathophysiology.[32] Ideally, these biosignatures could also provide biomarkers for matching drug effects.[32] In this approach, the IB-Derisk analyser tool could be utilised to explore the similarities of psychoactive compounds across preclinical, pharmacological, psychopathological, and clinical outcomes. For instance, if the IB-Derisk analyser overview reveals a harmonious pattern of an RDoC measure across these different aspects, this would suggest a possible therapeutic match between the condition characterised by RDoC, and the drug with the compatible effect profile. The IB-Derisk analyser could also provide biomarkers for patient selection and individual optimization and monitoring of drug effects.

In line with the principles of 'precision psychiatry' and RDoC, new digital measures and biomarkers (DMBs) are currently being developed. DMBs may address several challenges: patient heterogeneity, the lack of objective biomarkers for therapeutic effects of pharmacological interventions and placebo effect in clinical trials with novel compounds for psychiatric disorders.[35,39-41] Examples of DMBs include everyday wearable sensors that could track sleeping and activity patterns.[35,39] Firstly, in line with the 'precision psychiatry' approach, DMBs may address patient heterogeneity by identifying predictive DMBs for treatment response and targeting drugs to specific psychiatric subtypes.[35,39,40] Secondly, by objectively and continuously measuring various aspects of a patient's disease, DMBs could provide clinical trial endpoints that are more sensitive to treatment effects compared to traditional clinician-reported outcomes.[35,39] Lastly, DMBs may address the issue of high placebo response rates by developing predictive DMBs for placebo response, thereby facilitating more effective enrichment study designs to mitigate placebo effects.[39] Additionally, objective digital measures might be more resilient to placebo effects and offer better alternatives for study endpoints.[39]

Another notable innovative trend in psychiatric drug development is the use of quantitative systems pharmacology (QSP) models.[42] QSP models are mathematical models used to understand and predict how biological systems respond to drug interventions.[43] These models provide a detailed mechanistic representation of the underlying biology and physiology of the system of interest by integrating insights from pharmacokinetics, pharmacodynamics, physiology, and disease biology. They offer a comprehensive and quantitative representation of the interactions between drugs and biological systems.[43] Also, QSP models often incorporate artificial intelligence (AI) and machine learning techniques to analyse large datasets, identify patterns and enhance predictive accuracy.[43] QSP models therefore can support drug development decisions, such as dose selection for FIH studies and have been demonstrated to reduce development time and costs of investigational compounds.[43] It could be argued that the IB-Derisk analyser method is in fact, a very basic form of a QSP model. Aligning with this, there is a current initiative to integrate AI in the IB-Derisk method, which could replace the currently still manual procedure to generate IB-Derisk analyser overviews, and allow the integration of additional more sophisticated pharmacological analyses (quantitative pharmacophore structure-activity relationships, PK/PD-modelling), or information from related compounds.[44]

In conclusion, a number of innovative concepts are currently being developed in the field of psychiatric drug development. To capitalise on these innovations and improve the success rate of psychiatric drug development, it is crucial to design clinical studies rationally, not only from the onset in healthy volunteers but also ultimately in later stage efficacy trials. This implies however, that fundamental properties such as exposure at the target site and target modulation, should be subject to detailed investigation during the early phases of drug development. This thesis encourages the use of the IB-Derisk analyser tool in the process of rational drug development. The described studies illustrate how the comprehensive overview facilitates translation of preclinical findings to clinical studies and identification of missing data. Additionally, the studies presented in this thesis illustrate how the IB-Derisk analyser tool can be utilised to determine safe starting doses for clinical studies and to accurately evaluate the safety profiles of novel compounds. This thesis also illustrates application of the IB-Derisk analyser overview to contextualise emerging findings in ongoing early phase clinical trials, aiding in decision-making for subsequent development steps. Finally, the versatility of the IB-Derisk approach also holds promise for other future innovations in drug development – as a systematic tool to integrate pharmacological activity across preclinical *in vitro* experiments and *in vivo* studies in animals, healthy volunteers and patients, to identify biomarkers for the range of concentration-effect relationships, and optimise the dose range for desirable pharmacological effects in humans.

REFERENCES

1 Gerven van, J.M.A. CAF, Adam C. Integrating data from the Investigational Medicinal Product Dossier/investigator's brochure. A new tool for translational integration of preclinical effects. *Br J Clin Pharmacol*. 0(0). doi:doi:10.1111/bcp.13529

2 O'Donnell P, Rosen L, Alexander R, Murthy V, Davies CH, Ratti E. Strategies to Address Challenges in Neuroscience Drug Discovery and Development. *Int J Neuropsychopharmacol*. 2019;22(7):445-448. doi:10.1093/ijnp/pyz027

3 Morgan P, Brown DG, Lennard S, et al. Impact of a five-dimensional framework on R&D productivity at AstraZeneca. *Nat Rev Drug Discov*. 2018;17(3):167-181. doi:10.1038/nrd.2017.244

4 Cook D, Brown D, Alexander R, et al. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. *Nat Rev Drug Discov*. 2014;13(6):419-431. doi:10.1038/nrd4309

5 Paul SM, Potter WZ. Finding new and better treatments for psychiatric disorders. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol*. 2024;49(1):3-9. doi:10.1038/s41386-023-01690-5

6 Kola I, Landis J. Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov*. 2004;3(8):711-716. doi:10.1038/nrd1470

7 Cohen AF, Burggraaf J, van Gerven JM, Moerland M, Groeneveld GJ. The use of biomarkers in human pharmacology (Phase I) studies. *Annu Rev Pharmacol Toxicol*. 2015;55:55-74. doi:10.1146/annurev-pharmtox-011613-135918

8 Rocha J-F, Santos A, Gama H, et al. Safety, Tolerability, and Pharmacokinetics of FAAH Inhibitor BIA 10-2474: A Double-Blind, Randomized, Placebo-Controlled Study in Healthy Volunteers. *Clin Pharmacol Ther*. 2022;111(2):391-403. doi:10.1002/cpt.2290

9 Cohen AF, van Smeden J, Webb DJ. De-risking Clinical Trials: The BIAL Phase I Trial in Foresight. *Clin Pharmacol Ther*. 2022;111(2):362-365. doi:10.1002/cpt.2498

10 Peck RW, Holstein SA, van der Graaf PH. BIA 10-2474: Some Lessons are Clear but Important Questions Remain Unanswered. *Clin Pharmacol Ther*. 2022;111(2):343-345. doi:10.1002/cpt.2495

11 van Esbroeck ACM, Janssen APA, Cognetta AB, et al. Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. *Science (80-)*. 2017;356(6342):1084-1087. doi:10.1126/science.aaf7497

12 Attarwala H. TGN1412: From Discovery to DiSASter. *J Young Pharm*. 2010;2(3):332-336. doi:10.4103/0975-1483.66810

13 Kenter MJH, Cohen AF. Establishing risk of human experimentation with drugs: lessons from TGN1412. *Lancet*. 2006;368(9544):1387-1391. doi:https://doi.org/10.1016/S0140-6736(06)69562-7

14 Cohen AF. Developing drug prototypes: pharmacology replaces safety and tolerability? *Nat Rev Drug Discov*. 2010;9(11):856-865. doi:10.1038/nrd3227

15 van Gerven J, Bonelli M. Commentary on the EMA Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. *Br J Clin Pharmacol*. 2018;84(7):1401-1409. doi:10.1111/bcp.13550

16 Guideline M3 (R2) Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals - Scientific guideline. European medicines agency. Published 2009. https://www.EMA.europa.eu/en/ich-m3-r2-non-clinical-safety-studies-conduct-human-clinical-trials-pharmaceuticals-scientific-guideline

17 Baldrick P, Reichl A. Nonclinical & clinical interface - extrapolation of nonclinical data to support Phase I clinical studies. *Regul Toxicol Pharmacol*. 2021;121:104869. doi:https://doi.org/10.1016/j.yrtph.2021.104869

18 Duff GW. *Expert Group on Phase One Clinical Trials: Final Report 2006* TSO *(The Stationary Office)*.; 2006.

19 Groeneveld GJ, Hay JL, Van Gerven JM. Measuring blood–brain barrier penetration using the NeuroCart, a CNS test battery. *Drug Discov Today Technol*. 2016;20:27-34. doi:http://dx.doi.org/10.1016/j.ddtec.2016.07.004

20 Morgan P, Van Der Graaf PH, Arrowsmith J, et al. Can the flow of medicines be improved? Fundamental pharmacokinetic and pharmacological principles toward improving Phase II survival. *Drug Discov Today*. 2012;17(9-10):419-424. doi:10.1016/j.drudis.2011.12.020

21 Paul SM, Mytelka DS, Dunwiddie CT, et al. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat Rev Drug Discov*. 2010;9(3):203-214. doi:10.1038/nrd3078

22 Stanford SC. Some Reasons Why Preclinical Studies of Psychiatric Disorders Fail to Translate: What Can Be Rescued from the Misunderstanding and Misuse of Animal 'Models'? *Altern to Lab Anim*. 2020;48(3):106-115. doi:10.1177/0261192920939876

23 Belzung C. Innovative Drugs to Treat Depression: Did Animal Models Fail to Be Predictive or Did Clinical Trials Fail to Detect Effects? *Neuropsychopharmacology*. 2014;39(5):1041-1051. doi:10.1038/npp.2013.342

24 Trunnell ER, Baines J, Farghali S, et al. The need for guidance in antidepressant drug development: Revisiting the role of the forced swim test and tail suspension test. *Regul Toxicol Pharmacol*. 2024;151:105666. doi:https://doi.org/10.1016/j.yrtph.2024.105666

25 Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. *Nat Neurosci*. 2010;13(10):1161-1169. doi:10.1038/nn.2647

26 Leo A, Giovannini G, Russo E, Meletti S. The role of AMPA receptors and their antagonists in status epilepticus. *Epilepsia*. 2018;59(6):1098-1108. doi:10.1111/epi.14082

27 Guideline for good clinical practice E6 (R2). *Eur Med agency*. 2016;(EMA/CHMP/ICH/135/1995).

28 Gemkow MJ, Davenport AJ, Harich S, Ellenbroek BA, Cesura A, Hallett D. The histamine H3 receptor as a therapeutic drug target for CNS disorders. *Drug Discov Today*. 2009;14(9):509-515. doi:https://doi.org/10.1016/j.drudis.2009.02.011

29 Iasella CJ, Johnson HJ, Dunn MA. Adverse Drug Reactions: Type A (Intrinsic) or Type B (Idiosyncratic). *Clin Liver Dis*. 2017;21(1):73-87. doi:10.1016/j.cld.2016.08.005

30 Gribkoff VK, Kaczmarek LK. The Difficult Path to the Discovery of Novel Treatments in Psychiatric Disorders. *Adv Neurobiol*. 2023;30:255-285. doi:10.1007/978-3-031-21054-9_11

31 Neurocrine Biosciences Reports Positive Phase 2 Data for NBI-1065845 in Adults with Major Depressive Disorder. 04/23. Published 2024. Accessed August 8, 2024. https://www.prnewswire.com/news-releases/neurocrine-biosciences-reports-positive-phase-2-data-for-nbi-1065845-in-adults-with-major-depressive-disorder-302123889.html

32 Insel T, Cuthbert B, Garvey M, et al. Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry*. 2010;167(7):748-751. doi:10.1176/appi.ajp.2010.09091379

33 Gribkoff VK, Kaczmarek LK. The need for new approaches in CNS drug discovery: Why drugs have failed, and what can be done to improve outcomes. *Neuropharmacology*. 2017;120:11-19. doi:10.1016/j.neuropharm.2016.03.021

34 Tricklebank MD, Robbins TW, Simmons C, Wong EHF. Time to re-engage psychiatric drug discovery by strengthening confidence in preclinical psychopharmacology. *Psychopharmacology (Berl)*. 2021;238(6):1417-1436. doi:10.1007/s00213-021-05787-x

35 van der Doef TF, Zaragoza Domingo S, Jacobs GE, et al. New approaches in psychiatric drug development. *Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol*. 2018;28(9):983-993. doi:10.1016/j.euroneuro.2018.06.006

36 Zhu T. Challenges of Psychiatry Drug Development and the Role of Human Pharmacology Models in Early Development-A Drug Developer's Perspective. *Front psychiatry*. 2020;11:562660. doi:10.3389/fpsyt.2020.562660

37 Cuthbert BN, Insel TR. Toward the future of psychiatric diagnosis: the seven pillars of RDoC. BMC *Med*. 2013;11:126. doi:10.1186/1741-7015-11-126

38 Rutherford BR, Wall MM, Glass A, Stewart JW. The role of patient expectancy in placebo and nocebo effects in antidepressant trials. *J Clin Psychiatry*. 2014;75(10):1040-1046. doi:10.4088/JCP.13m08797

39 Reiter JE, Nickels S, Nelson BW, et al. Increasing psychopharmacology clinical trial success rates with digital measures and biomarkers: Future methods. NPP – *Digital Psychiatry Neurosci*. 2024;2(1):7. doi:10.1038/s44277-024-00008-7

40 Brietzke E, Hawken ER, Idzikowski M, Pong J, Kennedy SH, Soares CN. Integrating digital phenotyping in clinical characterization of individuals with mood disorders. *Neurosci Biobehav Rev*. 2019;104:223-230. doi:https://doi.org/10.1016/j.neubiorev.2019.07.009

41 ZhuParris A, de Goede AA, Yocarini IE, Kraaij W, Groeneveld GJ, Doll RJ. Machine Learning Techniques for Developing Remotely Monitored Central Nervous System Biomarkers Using Wearable Sensors: A Narrative Literature Review. *Sensors (Basel)*. 2023;23(11). doi:10.3390/s23115243

42 van der Graaf PH, Benson N. The Role of Quantitative Systems Pharmacology in the Design of First-in-Human Trials. *Clin Pharmacol Ther*. 2018;104(5):797. doi:10.1002/cpt.1145

43 Braakman S, Pathmanathan P, Moore H. Evaluation framework for systems models. CPT *pharmacometrics Syst Pharmacol*. 2022;11(3):264-289. doi:10.1002/psp4.12755

44 Giordano D, Biancaniello C, Argenio MA, Facchiano A. Drug Design by Pharmacophore and Virtual Screening Approach. *Pharmaceuticals (Basel)*. 2022;15(5). doi:10.3390/ph15050646

APPENDICES

NEDERLANDSE SAMENVATTING

INTRODUCTIE

De ontwikkeling van geneesmiddelen voor psychiatrische aandoeningen blijft achter ten opzichte van andere therapeutische domeinen. Een aanzienlijk percentage van potentieel nieuwe geneesmiddelen voor psychiatrische aandoeningen wordt in een laat stadium van de ontwikkeling gestopt vanwege problemen met veiligheid of effectiviteit. Dit wordt vaak toegeschreven aan het ontbreken van diermodellen die de therapeutische effectiviteit van een nieuw middel kunnen voorspellen. Het verbeteren van deze diermodellen vereist echter een diepgaande heroverweging van psychiatrische ziekteconstructen, wat gezien de complexiteit hiervan niet eenvoudig te realiseren is.

Een eenvoudiger te behalen verbetering in de geneesmiddelenontwikkeling voor psychiatrische aandoeningen is het zo ontwerpen van studies dat fundamentele farmacokinetische (PK) en farmacodynamische (PD) kenmerken – zoals blootstelling op de plaats van werking en target-modulatie – al in de vroege ontwikkelingsfasen worden onderzocht. Een belangrijke voorwaarde voor therapeutische werkzaamheid is namelijk dat het onderzoeksmiddel de hersenen bereikt en daar zijn farmacologische effect heeft. Empirische analyses van geneesmiddelenontwikkelingsprogramma's laten zien dat in bijna de helft van de studies die faalden door gebrek aan werkzaamheid in fase II, zulke fundamentele eigenschappen niet werden onderzocht of aangetoond in de vroege fasen van klinische ontwikkeling. De 'IB-Derisk analyser' tool is ontwikkeld om rationele geneesmiddelenontwikkeling te ondersteunen door resultaten uit preklinische en klinische studies te integreren en daarmee inzicht te bieden in het farmacologisch profiel van een nieuw geneesmiddel. Het ontwikkelen van geneesmiddelen op deze rationele manier kan voorkomen dat de ontwikkeling van een nieuw middel in een laat stadium mislukt.

IB DERISK ANALYSER TOOL

De IB-Derisk analyser tool kan worden gebruikt om de vaak uitgebreide en complexe preklinische gegevens zoals beschreven in de 'Investigator's Brochure'(IB) samen te vatten in een gestructureerd overzicht van één pagina. Tijdens uitvoer van de klinische studies in mensen kan het overzicht worden bijgewerkt met nieuwe bevindingen uit deze studies. Zo kan getoetst worden of de daadwerkelijke uitkomsten overeenkomen met de voorspellingen. Door de resultaten van alle uitgevoerde preklinische en klinische experimenten in het overzicht op te nemen, ontstaat een volledig beeld van het onderzoeksmiddel. Dit helpt bij het identificeren van ontbrekende cruciale gegevens, het opsporen van veiligheidsproblemen, het duiden van bevindingen en het bevorderen van communicatie tussen onderzoekers.

Het IB-Derisk analyser overzicht wordt verkregen in vier stappen. In de eerste stap worden de doseringen van het onderzoeksmiddel en de bijhorende maximale concentratie (C_{max}) en totale blootstelling (oppervlakte onder de curve (AUC)) ingevoerd, afkomstig van farmacokinetiek (PK)-studies met een eenmalige dosering van het onderzoeksmiddel. Indien in een PK-studie verschillende doseringen zijn toegediend, wordt elke dosering als een afzonderlijk experiment beschouwd dat individueel in de IB-Derisk analyser tool wordt ingevoerd. In de tweede stap worden de resultaten van niet-PK-studies, zoals diermodellen voor ziektes of farmacologische veiligheidsstudies, ingevoerd. Voor dit soort studies worden PK-waarden vaak niet gerapporteerd, maar de ontbrekende C_{max}- en AUC-waarden kunnen redelijkerwijs worden afgeleid uit de PK-studies in dezelfde diersoort. De derde stap bestaat uit het invoeren van de resultaten van PK-studies waarin meervoudige doseringen van het onderzoeksmiddel zijn toegediend, en de resultaten van acute toxicologische studies. Voor deze studies kunnen ontbrekende PK-gegevens vaak worden geschat op basis van intra-en extrapolatie van de PK-studies. De laatste stap bestaat uit het toekennen van een kleurcodering aan de resultaten van de verschillende studies op basis van de waargenomen effecten. Wanneer de kleurcodering is toegevoegd, kunnen de experimenten worden gerangschikt op C_{max} of een andere PK-parameter, om een visuele indruk te krijgen van de dosis-responscurves. Het IB-Derisk analyser overzicht van een onderzoeksmiddel met een gunstig kleurpatroon begint met witte rijen voor doseringen zonder farmacologische effecten, gevolgd door groene rijen die gewenste effecten aanduiden. Ongewenste effecten verschijnen daarna, te beginnen met geel voor milde bijwerkingen, oplopend naar oranje en rood voor ernstigere bijwerkingen.

Het verkregen IB-Derisk analyser overzicht moet vervolgens op de juiste manier worden gelezen om belangrijke eigenschappen van het onderzoeksmiddel te beoordelen. Allereerst moet de 'translatability' van het middel worden geëvalueerd. Hiervoor is het van belang na te gaan of vergelijkbare effecten optreden bij dezelfde blootstellingsniveaus in verschillende preklinische diersoorten. Een homogene verdeling over meerdere soorten vergroot de kans dat ook de mens in dit patroon zal passen. Vervolgens moet een inschatting worden gemaakt van de te verwachten farmacologisch actieve dosering waarbij gewenste effecten optreden. Dit is essentieel om een geschikte startdosering voor een klinische studie te kunnen bepalen. Daarnaast moet aan de hand van het overzicht worden beoordeeld of het optreden van effecten overeenkomt met het gewenste profiel. Dit houdt in dat bij de laagste doseringen geen effecten worden waargenomen (wit), gevolgd door het optreden van gewenste effecten (groen) bij oplopende doseringen. Ongewenste effecten treden pas op bij hogere doseringen (geel, oranje, rood). Wanneer deze ongewenste effecten het gewenste profiel volgen, nemen zij geleidelijk en voorspelbaar in ernst toe – zichtbaar in de kleurcodering van geel naar oranje en uiteindelijk rood. In dit beoordelingsproces speelt de kleurcodering dus een essentiële rol. Door het kleurpatroon te beoordelen, wordt in een oogopslag duidelijk of het middel een geordend patroon over meerdere prek-

linische soorten (goede translatability) vertoont en of gewenste effecten (groen) bij lagere blootstellingsniveaus optreden dan ongewenste effecten (geel, oranje, rood). Het kleurpatroon geeft tevens inzicht in de steilheid van de dosis-responscurve: ontstaan ernstige bijwerkingen geleidelijk, dan is dit zichtbaar als een opeenvolging van gele, vervolgens oranje en uiteindelijk rode effecten; treden ernstige ongewenste effecten daarentegen abrupt op, dan verschijnen direct rode effecten zonder voorafgaande gele of oranje effecten.

DEZE THESIS

In dit proefschrift is onderzocht hoe de IB-Derisk analyser tool toegepast kan worden in vroege fase geneesmiddelenstudies voor de ontwikkeling van psychoactieve stoffen. In **Hoofdstuk II** is een semi-kwantitatieve analyse uitgevoerd naar de nauwkeurigheid waarmee preklinische data, samengevat in een IB-Derisk analyser overzicht, farmacologische actieve en veilige doseerranges kunnen voorspellen. In **Hoofdstuk III**, **IV**, **V** en **VI** wordt beschreven hoe de IB-Derisk analyser tool werd gebruikt bij de ontwikkeling van drie individuele onderzoeksmiddelen. Daarnaast is onderzocht of het visualiseren van studieresultaten in het IB-Derisk analyser overzicht daadwerkelijk resulteerde in een beter inzicht in de farmacologie van het onderzoeksmiddel. Ten slotte is onderzocht of het verkregen IB-Derisk analyser overzicht nuttig was bij de besluitvorming rondom verdere ontwikkeling van de onderzoeksmiddelen.

TRANSLATABILITY

Het kleurpatroon in het IB-Derisk analyser overzicht van alle drie individuele onderzoeksmiddelen beschreven in **Hoofdstuk III**, **IV**, **V** en **VI** van dit proefschrift, wees op een goede translatability. De onderzoeksmiddelen veroorzaakten dus vergelijkbare effecten bij dezelfde blootstellingswaardes in verschillende preklinische soorten. Dit resultaat is enigszins vertekenend, aangezien middelen met een slechte transleerbaarheid niet zonder zorgvuldige overwegingen verder zouden zijn ontwikkeld. In de discussie van dit proefschrift wordt dieper ingegaan op twee middelen waarvan het IB-Derisk overzicht níet indicatief was voor een goede transleerbaarheid: BIA 10-2474, een FAAH-remmer, en TGN1412, een CD28-superagonistisch antilichaam.

In het geval van BIA 10-2474 maakte het IB-Derisk analyser overzicht duidelijk dat er aanzienlijke verschillen waren in de verdraagbaarheid van het middel tussen verschillende preklinische diersoorten. Bij het sorteren van het overzicht op blootstellingsniveau verscheen niet het verwachte kleurpatroon van witte lijnen (geen effect), via groen (gewenst effect), naar geel, oranje en rood (milde tot ernstige effecten). In plaats daarvan toonde het overzicht een rommelig kleurpatroon waarbij een bepaald blootstellingsniveau in de ene diersoort ernstige toxicologische effecten veroorzaakte, terwijl datzelfde niveau in een andere soort zonder problemen werd verdragen.

Ondanks deze duidelijke discrepantie werd hier geen nader onderzoek naar verricht. In de daaropvolgende klinische studie ontwikkelden gezonde vrijwilligers ernstige neurologische complicaties, waarvan één met dodelijke afloop. Analyse achteraf liet zien dat in de klinische studie met gezonde vrijwilligers doseringen veel verder waren opgehoogd dan nodig voor maximale FAAH-remming. Bij deze hoge doseringen beïnvloedde het middel waarschijnlijk ook andere receptoren die effecten veroorzaken waar mensen gevoelig voor zijn, wat heeft geleid tot de ernstige neurologische complicaties.

Het IB-Derisk overzicht van TGN1412 maakte onder andere inzichtelijk dat preklinische veiligheidsonderzoeken slechts in één diersoort waren uitgevoerd – een onvoldoende basis om de transleerbaarheid naar de mens betrouwbaar te kunnen inschatten. Dit werd echter niet herkend en bij de eerste toediening van dit middel aan gezonde vrijwilligers ontwikkelden zij allen een hevige cytokine storm waarvoor opname op de Intensive-Care nodig was. In beide gevallen had het IB-Derisk overzicht kunnen bijdragen aan het eerder signaleren van potentiële risico's, en zo mogelijk de ernstige gevolgen in de klinische studies kunnen voorkomen.

BEPALEN VAN EEN GESCHIKTE STARTDOSERING

Om de startdosering te bepalen, is het van belang om goed te kijken naar het gedeelte van het IB-Derisk overzicht dat de lage blootstellingsniveaus toont waarbij nog geen waarneembare effecten optreden in preklinische modellen. Dit niveau wordt in het overzicht weergegeven door een reeks witte lijnen. Hierbij moet er rekening mee worden gehouden dat er verschillende redenen kunnen zijn waarom er geen effecten worden gerapporteerd in de IB. Ten eerste is het mogelijk dat er wel effecten zijn waargenomen, maar niet gerapporteerd omdat deze buiten het doel van de studie vielen – bijvoorbeeld milde gedragsveranderingen in een specifieke PK-studie. Ten tweede, en betrouwbaarder, kan expliciet worden bevestigd dat er geen effecten zijn waargenomen. Dit 'no-observed effect level' (NOEL) wordt in het IB-Derisk overzicht weergegeven als de hoogste dosering met een witte lijn, net onder het eerste doseringsniveau met een groene lijn. Als duidelijk is dat deze NOEL-lijn een dosering vertegenwoordigt waarbij wel enige targetactiviteit aanwezig is, maar nog geen van de effecten die bij de eerstvolgende (groene) dosering worden gezien, dan komt deze hoogste (witte) NOEL waarschijnlijk overeen met het 'Minimum Anticipated Biological Effect Level' (MABEL). Het laagste 'groene' niveau vertegenwoordigt dan de farmacologisch actieve dosering (PAD). Het vaststellen van deze niveaus wordt beschouwd als een degelijke basis voor het bepalen van de startdosering.

Het concept van MABEL werd in 2007 formeel opgenomen in de Europese richtlijnen voor 'first-in-human' (FIH) studies na het hierboven beschreven incident met TGN1412 in 2006, waarbij de startdosering ver boven de MABEL lag. In het IB-Derisk overzicht van TGN1412 kon geen NOEL of MABEL worden geïdentificeerd, wat blijkt

uit het ontbreken van witte lijnen bij de laagste doses/blootstellingen. Dit betekende dat uit de gepubliceerde dierstudies in de IB niet kon worden afgeleid bij welke blootstelling farmacologische effecten begonnen op te treden. Dit was een van de oorzaken van de foutieve inschatting van de laagste farmacologisch/biologisch actieve dosering van TGN1412, wat leidde tot de bovengenoemde ernstige gevolgen.

Hoewel MABEL sinds 2007 is opgenomen in de richtlijnen, bevatten veel IB's nog steeds geen witte lijnen die een niveau zonder observeerbare effecten aanduiden. De semi-kwantitatieve analyse van IB's voor centraal zenuwstelsel (CZS)-actieve verbindingen, zoals beschreven in **Hoofdstuk II**, toonde aan dat in 32% van de studies de laagst geteste preklinische dosering al farmacologisch actief was, wat betekent dat het volledige doseringsbereik niet is onderzocht. Ook bleek dat in 58% van de studies de startdosering werd gekozen zonder rekening te houden met MABEL of de farmacologisch actieve doseringsrange. Een belangrijke reden hiervoor is dat de startdosering voor FIH-studies nog vaak wordt berekend als een fractie (vaak 10%) van de hoogste 'veilige' dosering – de 'No Observable Adverse Effect Level' (NOAEL) – in de meest gevoelige diersoort.

Voor alle drie individuele onderzoeksmiddelen die in **Hoofdstuk III**, **IV**, **V**, en **VI** van dit proefschrift worden beschreven, werd het IB-Derisk analyser overzicht gebruikt om een geschikte startdosering te bepalen. In alle drie gevallen werd bij het bepalen van de startdosering rekening gehouden met de NOAEL en het blootstellingsniveau waarop farmacologische activiteit werd verwacht. De gekozen startdosering bevond zich in het IB-Derisk overzicht steeds op het grensvlak van de witte en groene lijnen. In lijn met de voorspellingen gebaseerd op het IB-Derisk overzicht werd de startdosering van alle drie onderzoeksmiddelen goed verdragen, en werd er geen farmacologische activiteit waargenomen.

Gezamenlijk illustreren de TGN1412-casus en de in dit proefschrift beschreven IB-Derisk analyser overzichten van de individuele onderzoeksmiddelen het belang van het in acht nemen van de te verwachten farmacologisch actieve doseerrange bij het bepalen van de startdosering voor een klinische studie.

VOORSPELLEN VAN DOSERINGEN WAARBIJ GEWENSTE EFFECTEN OPTREDEN

Een middel kan pas therapeutische effect hebben als deze de targetlocatie bereikt en daar de beoogde farmacologische werking uitoefent. Daarom is het opnemen van metingen van de beoogde farmacologische activiteit in FIH-studies van cruciaal belang. Metingen van farmacologische activiteit kunnen tijdens de uitvoer van de klinische studie worden gebruikt om dosis-escalatiestappen te sturen en om bevindingen farmacologisch te duiden. Het belang hiervan kan worden geïllustreerd aan de hand van het voorbeeld van de BIA 10-2472-studie zoals hierboven beschreven. In de FIH-studie met BIA 10-2474 werden beperkte metingen van farmacologische activiteit uitgevoerd. De resultaten hiervan waren niet beschikbaar tijdens het bepalen van de dosis-escalatiestappen, die uitsluitend gebaseerd waren op uitkomsten met betrekking tot veiligheid en verdraagbaarheid. Hierdoor werden de doseringen onbedoeld verhoogd tot niveaus die ongeveer 12 keer hoger waren dan nodig voor maximale remming van humane enzym 'fatty acid amide hydrolase' (FAAH). Bij deze doseringen ontwikkelden gezonde vrijwilligers de beschreven ernstige bijwerkingen, waarschijnlijk als gevolg van remming van niet-specifieke hydrolasen in het CZS. Als de FIH-studie met BIA 10-2474 metingen van farmacologische activiteit had opgenomen die samen met PK- en veiligheidsgegevens waren geëvalueerd ter ondersteuning van dosis-escalatiebeslissingen, was het toedienen van doseringen ruim boven de maximale FAAH-remming mogelijk niet gebeurd. Wanneer in dit geval de IB-Derisk analyser tool was ingezet, had mogelijk eerder herkend kunnen worden dat de dosis werd verhoogd tot een niveau dat ruim boven de benodigde dosering voor maximale FAAH-remming lag.

In de semi-kwantitatieve analyse, beschreven in **Hoofdstuk II**, werd onderzocht of doseringen waarbij farmacologische effecten optreden bij mensen voorspeld kunnen worden op basis van preklinische studies. Dit werd onderzocht door de overlap te berekenen tussen waargenomen farmacologisch actieve doseringen in preklinische en klinische studies. Farmacologisch actieve doseringen werden gedefinieerd als doseringen waarbij zowel primaire (gewenste) als secundaire (ongewenste) farmacologische effecten optraden, mits deze gerelateerd waren aan het werkingsmechanisme van het middel. Uit de analyse bleek dat er een overlap van 84% was tussen de humane equivalente dosis (HED) van preklinisch en klinisch waargenomen farmacologisch actieve doseringen. Dit wijst erop dat preklinische modellen de dosering nodig voor farmacologische activiteit in klinische studies goed kunnen voorspellen. Deze overlap van 84% is waarschijnlijk een overschatting omdat het zowel gewenste als ongewenste farmacologische effecten omvat. In de semi-kwantitatieve analyse werd de overlap tussen doseringen specifiek gekoppeld aan gewenste farmacologische effecten in preklinische en klinische studies niet onderzocht. Echter, in de overgrote meerderheid van de geïncludeerde klinische studies werd het onderzochte middel goed verdragen tot aan de hoogste toegediende dosering. Dit suggereert dat het klinisch farmacologisch actieve dosisbereik voornamelijk bestond uit beoogde farmacologische effecten die verband hielden met het werkingsmechanisme van de stof. De waargenomen overlap van 84% laat daarom zien dat gegevens uit preklinische modellen over het algemeen goed voorspellen bij welke doseringen farmacologische activiteit optreedt en gewenste effecten bij mensen kunnen worden verwacht.

In **Hoofdstuk IV** van deze thesis worden de resultaten van een FIH-studie met ALKS 7119 beschreven. Dit middel was ontworpen als een NMDA-receptor antagonist, maar had ook affiniteit voor andere receptoren. Het IB-Derisk analyser overzicht gaf een indicatie van de affiniteit van ALKS 7119 voor de verschillende receptoren

ten opzichte van elkaar. Op basis hiervan kon een dosis-concentratiebereik worden voorspeld, waarvan werd verwacht dat het voornamelijk invloed zou hebben op de NMDA-receptor. Uit het IB-Derisk analyser overzicht bleek dat ALKS 7119 de hoogste affiniteit had voor de 'Serotonin Transporter' (SERT)-receptor. Zoals voorspeld op basis van het IB-Derisk-analyser overzicht, bleek uit de PD studie (NeuroCart en neuro-endocriene effecten) met ALKS 7119 dat ALKS 7119 inderdaad vergelijkbare farmacologische effecten veroorzaakte als SERT-inhibitoren. Het werd ook duidelijk dat doseringen nooit zouden kunnen worden opgehoogd tot doseringen nodig voor NMDA-antagonisme vanwege het optreden van ongewenste effecten bij deze doseringen. Er is daarom besloten ALKS 7119 niet verder te ontwikkelen.

Voor TAK-653, beschreven in **Hoofdstuk V** en **Hoofdstuk VI** van deze thesis, konden de blootstellingsniveaus waarbij gewenste farmacologische effecten optraden rechtstreeks worden vergeleken tussen preklinische diersoorten en mensen. Dit was mogelijk doordat in zowel ratten als mensen vrijwel identieke biomarkers voor farmacologische activiteit werden gebruikt. Op basis van het IB-Derisk analyser overzicht werd een doseringsniveau zonder voorspelde farmacologische activiteit als lage dosis aangemerkt en een doseringsniveau met voorspelde farmacologische activiteit als hoge dosis. In overeenstemming met de voorspellingen, leidde de hoge dosis TAK-653 tot een toename van de door transcraniële magnetische stimulatie (TMS) opgewekte motorische respons bij mensen, terwijl de lage dosis dit effect niet liet zien. Daarnaast was het NeuroCart-profiel van TAK-653 consistent met het werkingsmechanisme en indicatief voor stimulerende effecten op het CZS. In dit geval hielp het IB-Derisk analyser overzicht om vergelijkbare effecten bij gelijke blootstellingsniveaus in zowel preklinische als klinische studies te identificeren.

Samenvattend toont de semi-kwantitatieve analyse beschreven in **Hoofdstuk II** van deze thesis aan dat gegevens uit preklinische modellen doorgaans goed voorspellen bij welke doseringen farmacologische effecten bij mensen kunnen worden verwacht. Daarnaast laten de studies met individuele geneesmiddelen beschreven in **Hoofdstuk IV**, **V** en **VI** van deze thesis zien hoe het IB-Derisk analyser overzicht gebruikt kan worden om de aard van farmacologische effecten van nieuwe middelen bij mensen te voorspellen. De individuele studies beschreven in **Hoofdstuk IV**, **V** en **VI** van deze thesis illustreren hoe de IB-Derisk analyser tool ingezet kan worden om klinische bevindingen in context te plaatsen, en zo beter farmacologisch begrip van nieuwe middelen te verkrijgen.

VOORSPELLEN VAN DOSERINGEN WAARBIJ ONGEWENSTE EFFECTEN OPTREDEN

Om te onderzoeken of de IB-Derisk analyser tool goed verdragen doseringen in mensen kan voorspellen, werd in **Hoofdstuk II** van deze thesis een semi-kwantitatieve analyse beschreven. Daarin werd de verhouding berekend tussen de hoogste goed

verdragen doseringen in uitgevoerde klinische studies en de NOAELs die in preklinische studies waren vastgesteld. De analyse toonde aan dat in een minderheid van de studies (4 van de 25 [16%]) dosisbeperkende effecten bij mensen optraden bij doseringen of blootstellingsniveaus die lager waren dan de preklinisch vastgestelde NOAELs. De dosisbeperkende effecten in deze studies – met een $GABA_A$-modulator, twee histaminerge verbindingen en een partiële agonist van de 'Trace Amine-Associated Receptor' (TAAR) – kwamen overeen met de werkingsmechanismen van de onderzochte middelen en bestonden uit ataxie, hypotensie, slaperigheid, slapeloosheid en misselijkheid. Opvallend was dat beide histaminerge middelen bij mensen niet werden verdragen op doseerniveaus die in preklinische diersoorten goed verdragen werden. Eén van deze stoffen betrof oxathridine, beschreven in **Hoofdstuk III** van deze thesis, die pseudo-hallucinaties veroorzaakte bij gezonde vrijwilligers. Retrospectief waren er mogelijk al aanwijzingen voor psychiatrische bijwerkingen van dit middel, aangezien apen in preklinische studies opvallend gedrag vertoonden – zoals onverwacht menselijk contact zoeken en accepteren – al werd dit pas geobserveerd bij blootstellingsniveaus 300 keer hoger dan de niveaus waarbij pseudo-hallucinaties bij mensen optraden.

De semi-kwantitatieve analyse toonde ook aan dat in vijf van de 25 studies (20%) de dosering in de klinische studies werd opgehoogd tot blootstellingsniveaus boven de NOAEL. In deze studies konden verwachte bijwerkingen goed worden gemonitord met behulp van intensieve cardiovasculaire monitoring of NeuroCart-metingen, wat verklaart waarom dosisverhoging boven de NOAEL mogelijk was. In twee van deze studies, beide met een cannabinoïd-agonist, werd verdere dosisverhoging echter beperkt door reversibele psychiatrische effecten, waaronder derealisatie, auditieve en visuele hallucinaties en angst – effecten die in preklinische studies niet waren waargenomen. Voor beide middelen gold echter dat de NOAEL was gebaseerd op cardiovasculaire effecten, die bij mensen voldoende nauwkeurig gemonitord konden worden om het verhogen van de dosering op tijd te stoppen.

Het IB-Derisk analyser overzicht van de individuele middelen beschreven in **Hoofdstuk III**, **IV**, **V** en **VI** van deze thesis toonde een gunstig kleurpatroon waarbij gewenste effecten optraden bij lagere blootstellingsniveaus dan ongewenste effecten. Voor ALKS 7119 en TAK-653, beschreven in respectievelijk **Hoofdstuk IV** en **V** en **Hoofdstuk VI**, was dit patroon ook zichtbaar bij gezonde vrijwilligers. Voor oxathridine, beschreven in **Hoofdstuk III**, gold dit echter niet: in de studie met gezonde vrijwilligers konden de doseringen niet worden opgehoogd tot blootstellingsniveaus die in preklinische studies met gewenste farmacologische effecten waren geassocieerd, vanwege het optreden van pseudo-hallucinaties.

Concluderend tonen de semi-kwantitatieve analyse en de individuele studies beschreven in deze thesis aan dat preklinische data over het algemeen betrouwbaar goed verdragen doseringsniveaus in mensen kunnen voorspellen. De studies met

oxathridine en cannabinoïd-agonisten benadrukken echter dat onderzoekers alert moeten blijven op mogelijke psychiatrische bijwerkingen bij het onderzoeken van nieuwe centraal werkende geneesmiddelen, aangezien psychiatrische of psychomimetische effecten niet eenvoudig uit preklinische data zijn af te leiden. In de meeste gevallen kunnen dergelijke effecten worden verwacht bij activatie van specifieke farmacologische mechanismen, zoals 5HT2-, DA2-, en CB1-receptoren, zoals beschreven in **Hoofdstuk III**. Aan de andere kant zijn sommige effecten die de NOAEL in dieren bepalen het gevolg van reversibele farmacologische mechanismen die goed begrepen zijn en nauwkeurig in mensen kunnen worden gemeten. In dergelijke gevallen kan voorzichtige dosisverhoging boven de NOAEL in klinische studies verantwoord zijn, mits intensieve monitoring wordt toegepast.

ONTWIKKELINGSSTATUS VAN ONDERZOEKSMIDDELEN BESCHREVEN IN DIT PROEFSCHRIFT

In dit proefschrift worden studies beschreven waarin in de vroege klinische ontwikkelingsfase fundamentele farmacologische eigenschappen van onderzoeksmiddelen zijn onderzocht, zoals blootstelling op de doelwitlocatie en targetmodulatie. Het is daarom interessant om te bekijken hoe de verkregen inzichten zijn toegepast in de verdere ontwikkeling van deze middelen en of deze aanpak daadwerkelijk leidt tot een lagere uitval in latere fasen van klinische ontwikkeling.

Van de 25 middelen die zijn opgenomen in de semi-kwantitatieve analyse beschreven in **Hoofdstuk II**, bevinden zich er nog elf in klinische ontwikkeling – wat voor vroege fase I-studies een relatief groot aandeel lijkt. Van één verbinding kon de ontwikkelingsstatus niet worden achterhaald, en de ontwikkeling van de overige dertien verbindingen is stopgezet. Bij het vergelijken van de IB-Derisk-analyser overzichten van de inmiddels gestaakte studies en de studies die zijn doorgegaan naar een volgende ontwikkelingsfase, bleek dat de mate van overlap in farmacologische activiteit tussen preklinische en klinische studies vergelijkbaar was voor beide groepen. Wel was het percentage IB-Derisk-analyser overzichten met het gewenste kleurpatroon hoger bij de momenteel nog lopende studies (64%) dan bij de stopgezette studies (38%). Hoewel het aantal middelen in de semi-kwantitatieve analyse te klein is om definitieve conclusies te trekken, suggereren deze bevindingen dat het IB-Derisk-overzicht waardevolle inzichten biedt in de kans op succesvolle ontwikkeling van nieuwe geneesmiddelen.

De individuele middelen beschreven in **Hoofdstuk III**, **IV**, **V** en **VI** van dit proefschrift, bevinden zich in verschillende fasen van ontwikkeling. De ontwikkeling van oxathridine, beschreven in **Hoofdstuk III**, werd gestaakt na de FIH-studie vanwege onacceptabele bijwerkingen in de vorm van pseudo-hallucinaties. De ontwikkeling van ALKS 7119, beschreven in **Hoofdstuk IV**, werd eveneens stopgezet, aangezien uit de FIH-studie bleek dat verdere dosisverhoging niet zou leiden tot de plasmaspiegels benodigd voor relevante modulatie van de NMDA-receptor. Het in **Hoofdstuk V** en **VI** beschreven TAK-653 is zonder twijfel het meest succesvolle voorbeeld dat in dit proefschrift wordt beschreven. Voor dit middel gaven de bevindingen uit de PD studie bij mensen aanwijzingen voor targetmodulatie en droegen bij aan een goed farmacologisch begrip van het middel. Op basis hiervan werd besloten de ontwikkeling voort te zetten naar een fase II-studie, waarin voorlopige therapeutische werkzaamheid werd aangetoond.

CONCLUSIE

In dit proefschrift is onderzocht hoe de IB-Derisk analyser tool ingezet kan worden in vroege fase geneesmiddelenonderzoek naar psychoactieve stoffen, en of het visualiseren van studieresultaten in het IB-Derisk overzicht daadwerkelijk leidt tot beter inzicht in de farmacologie van het onderzochte middel. Tevens is beoordeeld of het IB-Derisk overzicht behulpzaam was bij besluitvorming over de verdere ontwikkeling van de middelen.

De studies in dit proefschrift, aangevuld met de casussen van BIA 10-2474 en TGN1412, laten zien op welke manieren de IB-Derisk analyser gebruikt kan worden om de translatability van een nieuw middel te beoordelen en farmacologisch actieve doseringen in mensen te voorspellen. Ook wordt duidelijk hoe de beoordeling van het kleurprofiel van (ongewenste) effecten in het IB-Derisk overzicht kan bijdragen aan het opzetten van een veilige en informatieve dosis-escalatie en monitoring tijdens klinische studies.

Bij alle drie afzonderlijke middelen die in dit proefschrift zijn beschreven, leverde het gebruik van het IB-Derisk overzicht waardevolle inzichten op in hun farmacologische profiel. Dit stelde onderzoekers in staat om in alle gevallen een onderbouwde beslissing te nemen over het al dan niet voortzetten van de ontwikkeling van het middel.

Uit de semi-kwantitatieve analyse in **Hoofdstuk II** en de studie met oxathridine in **Hoofdstuk III** bleek dat psychiatrische bijwerkingen, zoals pseudo-hallucinaties, nauwelijks voorspelbaar zijn op basis van dierstudies. Daarnaast werd aangetoond dat psychoactieve middelen bij mensen vaak al effect hebben bij lagere doseringen dan bij proefdieren. Mogelijke verklaringen hiervoor zijn een hogere gevoeligheid van mensen voor psycho-actieve middelen of het gebruik van sensitievere meetmethoden in klinisch onderzoek.

Samenvattend laat dit proefschrift zien dat de IB-Derisk analyser tool, ondanks de blijvende onzekerheden bij het vertalen van preklinische data naar humane effecten, waardevol kan bijdragen aan een rationele en zo veilig mogelijke ontwikkeling van nieuwe geneesmiddelen.

CURRICULUM VITAE

Francis Marijke Dijkstra was born on the 27th of December 1988 in Valkenswaard, the Netherlands. In 2007 she graduated from secondary school (Gymnasium, Maaswaal college, Wijchen). Motivated by her interest in the human brain and behaviour, she went to study Psychobiology at the University of Amsterdam, followed by a year of Psychology at the Vrije Universiteit Amsterdam. In 2009 she was admitted to medical school at Vrije Universiteit Amsterdam, the field of her true interest. As part of her master's, she spent six months at the Institute of Psychiatry, King's College London (UK), where she contributed to studies on comorbidities in children with autism spectrum disorder. She obtained her medical degree in 2015 and began her professional career as a PhD student and research physician within the Psychiatry group at the Centre for Human Drug Research and Leiden University Medical Centre (LUMC). Under the supervision of dr. G. Jacobs, dr. R. Zuiker, and Prof. J. van Gerven, she worked on her thesis. As part of her research training, she was educated as clinical pharmacologist. In 2021 she started working as a resident at the department of child- and adolescent psychiatry at the LUMC, Curium. She subsequently completed residencies in consultation-liaison psychiatry at Haaglanden Medical Centre and in acute psychiatry at Parnassia Group, The Hague. In 2024, she discovered her passion for Intellectual Disability Medicine, and in 2025 she began her specialist training in this field at Erasmus Medical Centre in Rotterdam. Francis currently lives in Leiden with her fiancé Allard Aukema and their two children Jaap (2021) and Jet (2023).

LIST OF PUBLICATIONS

Dijkstra, **F. M.**, Zuiker, R. G. J. A., Heuberger, J. A. A. C., Kanhai, K. M. S., de Kam, M., Duvauchelle, T., Lecomte, J. M., Schwartz, J. C., & van Gerven, J. M. A. *Administration of oxathridine, a first-in-class histamine-3 receptor partial agonist in healthy male volunteers: Central nervous system depression and pseudo-hallucinations*. British Journal of Clinical Pharmacology (2023), **90**(1), 321–335. https://doi.org/10.1111/bcp.15910

van Kraaij, S.J.W., Gal, P., Borghans, L.G.J.M., Klaassen, E.S., **Dijkstra**, **F.M.**, Winrow, C., Glasser, C., & Groeneveld, G.J. *First-in-human trial to assess safety, tolerability, pharmacokinetics, and pharmacodynamics of zagociguat (CY6463), a CNS-penetrant soluble guanylyl cyclase stimulator*. Clinical and Translational Science (2023), **16**(8), 1381–1395. https://doi.org/10.1111/cts.13537

Dijkstra, **F.M.**, Ferreira, G.S., Veening-Griffioen, D.H., Boon, W.P.C., Schellekens, H., Moors, E.H.M., van Meer, P.J.K., Stuurman, F.E., & van Gerven, J.M.A**.** *Translatability of preclinical to early clinical tolerable and pharmacologically active dose ranges for central nervous system active drugs* (G. S. Ferreira & F. M. Dijkstra contributed equally). Translational Psychiatry, **13**(1) (2023), Article 74. https://doi.org/10.1038/s41398-023-02353-1

Dijkstra, **F.M.**, Zuiker, R.G.J.A., O'Donnell, P., Klaassen, E., Quanhong, L., Buhl, D.L., Ruijs, T.Q., Asgharnejad, M., Murthy, V., Rosen, L., & Jacobs, G.E. *Central nervous system effects of TAK-653, an investigational a-amino-3-hydroxy-5-methyl-4-isoxazole receptor (AMPAR) positive allosteric modulator in healthy volunteers*. Translational Psychiatry (2022), **11**(1), Article 325. https://doi.org/10.1038/s41398-021-01451-2

Dijkstra, **F.M.**, Zuiker, R.G.J.A., Siebenga, P.S., Leigh-Pemberton, R.A., Sun, L., Manthis, J.D., von Moltke, L.L., Rezendes, D., & van Gerven, J.M.A. *Pharmacological profile of ALKS 7119, an investigational compound evaluated for the treatment of neuropsychiatric disorders, in healthy volunteers*. British Journal of Clinical Pharmacology (2022), **88**(6). https://doi.org/10.1111/bcp.15229

Dijkstra, **F.M.**, van de Loo, A.J.A.E., Abdulahad, S., Bosma, E.R., Hartog, M., Huls, H., Kuijper, D.C., de Vries, E., Singh, J.B., Aluisio, L., Zannikos, P., Solanki, B., Stuurman, F.E., Jacobs, G.E., & Verster, J.C. *The effects of intranasal esketamine on on-road driving performance in patients with major depressive disorder or persistent depressive disorder* (F. M. Dijkstra & A.J.A.E. van de Loo contributed equally). Journal of Psychopharmacology (2022), **36**(5), Article 02698811221078764. https://doi.org/10.1177/02698811221078764

Dijkstra, **F.M.**, O'Donnell, P., Damar, U., Quanhong, L., de Goede, A., Lin, X., Buhl, D.L., Zuiker, R.G.J.A., Ruijs, T.Q., Asgharnejad, M., Murthy, V., Rotenberg, A., & Jacobs, G.E. *Transcranial Magnetic Stimulation as a Translational Biomarker for Modulation of AMPA Receptor Function* (P. O'Donnell, U. Damar & F. M. Dijkstra contributed equally). Translational Psychiatry (2021), **11**(1), Article 325. https://doi.org/10.1038/s41398-021-01451-2

Dijkstra, F.M., Jacobs, G.E., & Cohen, A.F. *Question-based Drug Development for psilocybin*. The Lancet Psychiatry (2016), **3**(9), 806–807. https://doi.org/10.1016/S2215-0366(16)30214-0

Lommerse, K.M., **Dijkstra**, **F.M.**, Boeke, A.J.P., Eekhoff, E.M.W., & Jacobs, G.E. *Psychiatrische beelden bij afwijkende cortisolproductie, een vaak laat gestelde diagnose*. Nederlands Tijdschrift voor Geneeskunde (2016), **160**(31)

DANKWOORD

Het schrijven van dit proefschrift was me niet gelukt zonder de steun en het vertrouwen van de mensen om mij heen. Allereerst wil ik mijn promotor, professor Joop van Gerven, bedanken voor het delen van zijn passie voor en kennis van de (psycho) farmacologie. De gesprekken over neurotransmitters en geschiedenis tijdens een borrel of etentje op een congres hebben me altijd enorm geïnspireerd. Daarnaast wil ik mijn co-promotoren, dr. Gabriël Jacobs en dr. Rob Zuiker, bedanken. Gabriël, jouw gedrevenheid en humor hebben mij steeds gemotiveerd om mij volledig in te zetten als projectleider voor een zorgvuldige uitvoering van de studies, en om de manuscripten beter te schrijven dan ik voor mogelijk hield. Rob, als mijn mentor bij het CHDR heb jij met jouw vertrouwen en kalmte een cruciale rol gespeeld in mijn professionele ontwikkeling en in het versterken van mijn zelfvertrouwen. Het uitvoeren van de studies voor mijn proefschrift was niet mogelijk geweest zonder het werk van alle verschillende afdelingen van het CHDR, dus dank aan recruitment, adminCRU, de keuringsunit, datamanagement, data programming, lab, stats, iedereen op de CRU, secretariaat, receptie, de CTAs, education, R&D, quality assurance, finance, facilitair en IT. Natuurlijk wil ik ook de deelnemers aan de studies bedanken, zonder jullie deelname zou het ontwikkelen van nieuwe methodes of geneesmiddelen niet mogelijk zijn. Daarnaast wil ik mijn collega-projectleiders, en in het bijzonder de 'eilanders 2.0', bedanken voor het delen van praktische tips, inhoudelijke discussies, steun bij tegenslag en de dagelijkse gezelligheid. Ook dr. Karen Broekhuizen, dank voor jouw waardevolle hulp bij het schrijven van de laatste onderdelen van dit proefschrift. Gelukkig had ik ook buiten het CHDR mensen die mij tijdens de periode van het uitvoeren van mijn PhD hebben gesteund. Jasmijn, dank je dat je altijd naar me wilt luisteren en voor je nuchtere, soms ongevraagde adviezen. Juul en Marthe, mijn paranimfen, dank voor de onvergetelijke feestmomenten en voor jullie trouwe vriendschap door de jaren heen, in zowel goede als moeilijke tijden. Aan mijn familie: pap, mam, Esther en Inge, dank voor jullie onvoorwaardelijke steun en jullie oprechte interesse in wat mij bezighoudt. Allard, mijn grote liefde en veilige haven, dank dat je mij accepteert zoals ik ben en mij altijd hebt gesteund bij het schrijven van dit proefschrift. Tot slot, Jaap en Jet, dank dat jullie er zijn en mij iedere dag laten zien wat echt belangrijk is in het leven.