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

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CHAPTER VII

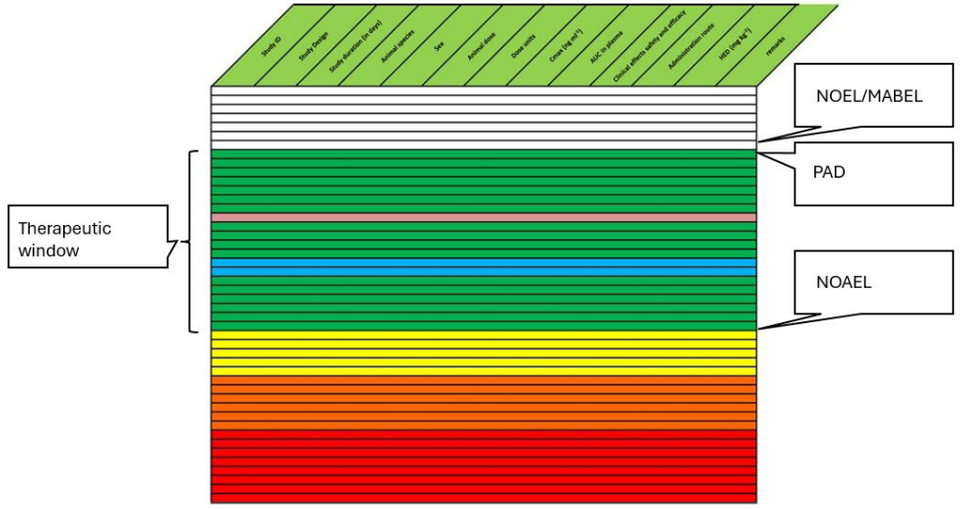
GENERAL DISCUSSION

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



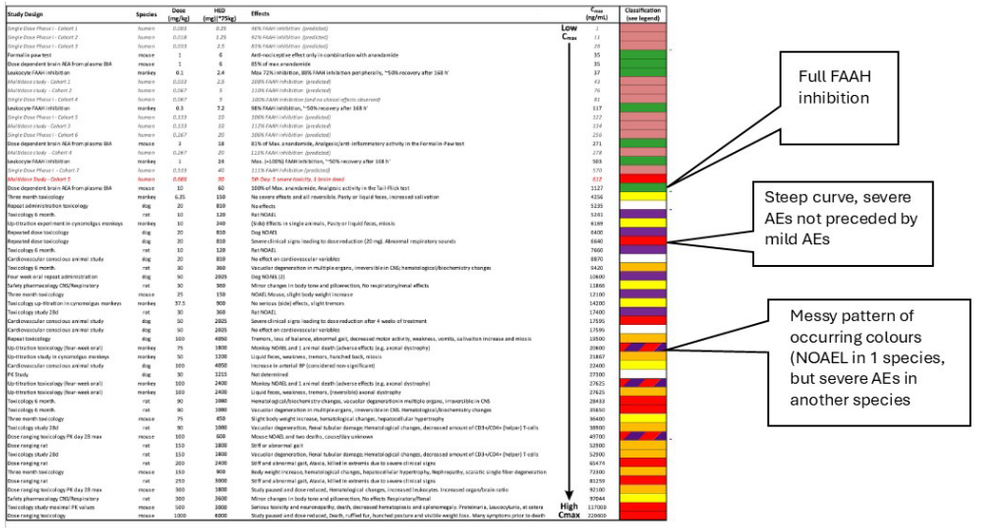
White: Studies without clinical observations / Blue: *In vitro* studies / Green: Safe or desirable effects (including doses that show pharmacological activity or beneficial effects on disease models) / Pink: Human observations inserted during first clinical studies / Yellow: Undesirable effects that 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

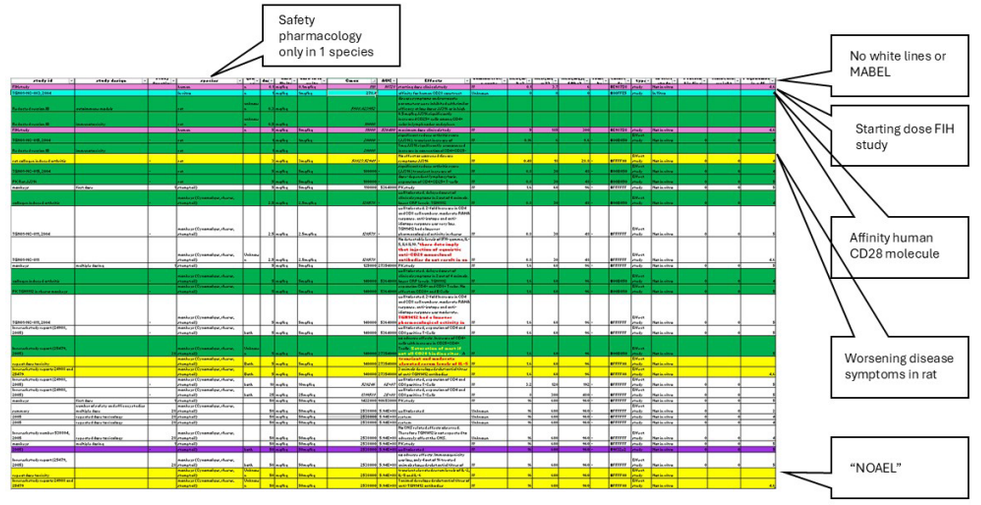
translatability – is dramatically illustrated by the case of BIA 10-2474.[1,8] In the first-in-human (FIH) trial with BIA-10-2472, a Fatty Acid Amide Hydrolase (FAAH) inhibitor, several healthy volunteers experienced neurological damage, and one volunteer died as a result of escalating the doses up to 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.

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