De-risking clinical trials: The BIAL phase I trial in foresight

COMMENTARY

De-risking Clinical Trials: The BIAL Phase I Trial in Foresight

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Disasters in early drug development are rare but, because they generally harm healthy young people, remembered for a long time. Few will forget the death of a volunteer after a new anti-arrhythmic drug (eproxindine) in 1985, the TGN1412 event in 2006 where several subjects just survived a cytokine storm after being exposed to a CD28 agonist, and the BIA-10-2474-101 trial in 2016 where several subjects had neurological damage and one died after receiving a FAAH-inhibitor.

In this issue of CPT,[1] investigators from the company BIAL describe the ill-fated experiment of 2016. The paper is co-authored by company staff and two toxicology consultants. The absence of clinical investigators as authors is concerning. The death and damage to a group of healthy people is the nightmare of each researcher involved in early drug trials fear. When it happens, as here, we would have anticipated the clinical team involved in the study to have underwritten this article.[2] Such a publication must nevertheless be applauded, because the only positive thing that emerges from such a tragedy is the opportunity to learn from it. For this learning to occur, the scientific community must have timely free access to all data. In 2016, the British Pharmacological Society published a call for immediate release of all data from the trial to the scientific community.[3] Unfortunately, this has yet to happen.

In this commentary, we wished to use as little hindsight as possible, generating learnings for future researchers who always face the problem of how to translate preclinical data into safe starting doses while simultaneously generating data with sufficient exposure to detect pharmacological effects and evaluate pharmacokinetics (PKs). The authors have supplied data in a supplementary file, but much of this was published with hindsight. We therefore decided to rely on the same data that the investigators must have had when planning the trial. We have thoroughly studied the Investigator's Brochure (IB; edition 2 of April 13, 2015) and the original study protocol (BIA-102474-101 of July 1, 2015[4]). We have requested release of the IB, but BIAL has exercised its right to deny this with a threat of legal action. This generates a problem, because we want to comment on the manuscript and educate the scientific community with a novel approach for dose-rationale based on information from the IB. Therefore, we only use data from the IB that has also been disclosed by BIAL. Despite this limitation, we demonstrate the importance of mechanistic and PK dose rationale, as a better alternative to tolerability studies in humans.

Prior to the execution of the clinical trials, the BIAL investigators only had in their possession the preclinical data from the IB. In general, such documents are extensive and do not make for easy reading, especially not when estimating an integrated view of the risks to subjects. We have applied a previously published approach to summarize data from an IB in a structured, tabular manner to the BIAL IB, using the publicly available IB-de-risk tool.[5] In other words, we have approached the BIAL phase I trial "in foresight," and discuss aspects of BIA 10-2474 that become apparent from the IB-de-risk approach that would otherwise be very hard—if not impossible—to conclude from a textual IB alone. Although we cannot disclose the full de-risk-table based on this IB, we supply the IB-de-risk table of published IB-data. An IB-de-risk table is provided in **Table 1**. This table combines all studies with PKs, pharmacodynamics (PDs), and toxicity data and gives a color code to the severity of findings. The table is sorted on observed or estimated maximum plasma concentration (C_{max}), but similar color-profiles are observed if data were sorted on any other PK-parameter (e.g., human equivalent dose). In general, one would *expect* that the results of a C_{max}-sorted table would lead to a rather consistent picture: first (at low C_{max} values) no clinical observations are expected (white color), followed by desirable, pharmacological effects (green color). At higher

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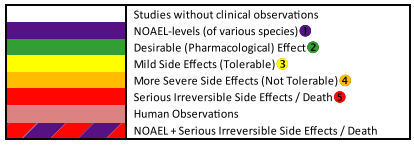
Table 1 IB-de-risk table of BIA 10-2474

Study Design	Species	Dose (mg/kg)	HED (mg)(*75kg)	Effects		C_{max} (ng/mL)	Classification (see legend)
Single Dose Phase I - Cohort 1	*human*	*0.003*	***0.25***	*46% FAAH inhibition (predicted)*	Low C_{max}	*1*	
Single Dose Phase I - Cohort 2	*human*	*0.018*	***1.25***	*92% FAAH inhibition (predicted)*		*11*	
Single Dose Phase I - Cohort 3	*human*	*0.033*	***2.5***	*83% FAAH inhibition (predicted)*		*28*	
Formalin paw test	mouse	1	**6**	Anti-nociceptive effect only in combination with anandamide		35	
Dose dependent brain AEA from plasma BIA	mouse	1	**6**	85% of max anandamide		35	
Leukocyte FAAH inhibition	monkey	0.1	**2.4**	Max 72% inhibition, 88% FAAH inhibition peripherally, '~50% recovery after 168 h'		37	
Multidose study - Cohort 1	*human*	*0.033*	***2.5***	*108% FAAH inhibition (predicted)*		*43*	
Multidose study - Cohort 2	*human*	*0.067*	***5***	*110% FAAH inhibition (predicted)*		*76*	
Single Dose Phase I - Cohort 4	*human*	*0.067*	***5***	*100% FAAH inhibition (and no clinical effects observed)*		*81*	
Leukocyte FAAH inhibition	monkey	0.3	**7.2**	98% FAAH inhibition, '~50% recovery after 168 h'		117	
Single Dose Phase I - Cohort 5	*human*	*0.133*	***10***	*106% FAAH inhibition (predicted)*		*122*	
Multidose study - Cohort 3	*human*	*0.133*	***10***	*112% FAAH inhibition (predicted)*		*134*	
Single Dose Phase I - Cohort 6	*human*	*0.267*	***20***	*106% FAAH inhibition (predicted)*		*256*	
Dose dependent brain AEA from plasma BIA	mouse	3	**18**	81% of Max. anandamide, Analgesic/anti-inflammatory activity in the Formalin-Paw test		271	
Multidose study - Cohort 4	*human*	*0.267*	***20***	*113% FAAH inhibition (predicted)*		*278*	
Leukocyte FAAH inhibition	monkey	1	**24**	Max. (+100%) FAAH inhibition, '~50% recovery after 168 h'		503	
Single Dose Phase I - Cohort 7	*human*	*0.533*	***40***	*111% FAAH inhibition (predicted)*		*570*	
Multidose Study - Cohort 5	*human*	*0.666*	***50***	*5th Day: 5 severe toxicity, 1 brain dead*		*612*	
Dose dependent brain AEA from plasma BIA	mouse	10	**60**	100% of Max. anandamide, Analgesic activity in the Tail-Flick test		1127	
Three month toxicology	monkey	6.25	**150**	No severe effects and all reversible. Pasty or liquid feces, increased salivation		4256	
Repeat administration toxicology	dog	20	**810**	No effects		5235	
Toxicology 6 month.	rat	10	**120**	Rat NOAEL		5241	
Up-titration experiment in cynomolgus monkeys	monkey	10	**240**	(Side) Effects in single animals, Pasty or liquid feces, miosis		6169	
Repeated dose toxicology	dog	20	**810**	Dog NOAEL		6400	
Repeated dose toxicology	dog	20	**810**	Severe clinical signs leading to dose reduction (20 mg). Abnormal respiratory sounds		6640	
Toxicology 6 month.	rat	10	**120**	Rat NOAEL		7660	
Cardiovascular conscious animal study	dog	20	**810**	No effect on cardiovascular variables		8870	
Toxicology 6 month.	rat	30	**360**	Vacuolar degeneration in multiple organs, irreversible in CNS; hematological/biochemistry changes		9420	
Four week oral repeat administration	dog	50	**2025**	Dog NOAEL (2)		10600	
Safety pharmacology CNS/Respiratory	rat	30	**360**	Minor changes in body tone and piloerection, No respiratory/renal effects		11866	
Three month toxicology	mouse	25	**150**	NOAEL Mouse, slight body weight increase		12100	
Toxicology up-titration in cynomolgus monkeys	monkey	37.5	**900**	No serious (side) effects, slight tremors		14200	
Toxicology study 28d	rat	30	**360**	Rat NOAEL		17400	
Cardiovascular conscious animal study	dog	50	**2025**	Severe clinical signs leading to dose reduction after 4 weeks of treatment		17595	
Cardiovascular conscious animal study	dog	50	**2025**	No effect on cardiovascular variables		17595	
Repeat toxicology	dog	100	**4050**	Tremors, loss of balance, abnormal gait, decreased motor activity, weakness, vomits, salivation increase and miosis		19500	
Up-titration toxicology (four-week oral)	monkey	75	**1800**	Monkey NOAEL and 1 animal death (adverse effects (e.g. axonal dystrophy)		20600	
Up-titration study in cynomolgus monkeys	monkey	50	**1200**	Liquid feces, weakness, tremors, hunched back, miosis		21867	
Cardiovascular conscious animal study	dog	100	**4050**	Increase in arterial BP (considered non-significant)		22400	
PK Study	dog	30	**1215**	Not determined		27300	
Up-titration toxicology (four-week oral)	monkey	100	**2400**	Monkey NOAEL and 1 animal death (adverse effects (e.g. axonal dystrophy)		27625	
Up-titration toxicology (four-week oral)	monkey	100	**2400**	Liquid feces, weakness, tremors, (reversible) axonal dystrophy		27625	
Toxicology 6 month.	rat	90	**1080**	Hematological/biochemistry changes, vacuolar degenerationin multiple organs, irreversible in CNS		28433	
Toxicology 6 month.	rat	90	**1080**	Vacuolar degeneration in multiple organs, irreversible in CNS. Hematological/biochemistry changes		35650	
Three month toxicology	mouse	75	**450**	Slight body weight increase, hematological changes, hepatocellular hypertrophy		36400	
Toxicology study 28d	rat	90	**1080**	Vacuolar degeneration, Renal tubular damage; Hematological changes, decreased amount of CD3+/CD4+ (helper) T-cells		38900	
Dose ranging toxicology PK day 28 max	mouse	100	**600**	Mouse NOAEL and two deaths, cause/day unknown		49700	
Dose ranging rat	rat	150	**1800**	Stiff or abnormal gait		52900	
Toxicology study 28d	rat	150	**1800**	Vacuolar degeneration, Renal tubular damage; Hematological changes, decreased amount of CD3+/CD4+ (helper) T-cells		52900	
Dose ranging rat	rat	200	**2400**	Stiff and abnormal gait, Ataxia, killed in extremis due to severe clinical signs		65474	
Three month toxicology	mouse	150	**900**	Body weight increase, hematological changes, hepatocellular hypertrophy, Nephropathy, scaiatic single fiber degeneration		72300	
Dose ranging rat	rat	250	**3000**	Stiff and abnormal gait, Ataxia, killed in extremis due to severe clinical signs		81259	
Dose ranging toxicology PK day 28 max	mouse	300	**1800**	Study paused and dose reduced, Hematological changes, increased leukocytes. Increased organ/brain ratio		92100	
Safety pharmacology CNS/Respiratory	rat	300	**3600**	Minor changes in body tone and piloerection, No effects Respiratory/Renal		97044	
Toxicology study maximal PK values	mouse	500	**3000**	Serious toxicity and neuronopathy, death, decreased hematopiesis and splenomegaly. Proteinuria, Leucocyturia, et cetera	High Cmax	117000	
Dose ranging toxicology	mouse	1000	**6000**	Study paused and dose reduced, Death, ruffled fur, hunched posture and visible weight loss. Many symptoms prior to death		220400	



The derived dose and C_{max} values are based on the manuscript by Rocha *et al*. and its supplement that contains the various publications with (references to) preclinical data on BIA 10-2474.[1] More information on the IB-de-risk process, as well as examples of compounds with acceptable IB-profiles, can be found elsewhere.[4] We also included data from the human phase I clinical trials that were performed. The table is sorted on observed or estimated C_{max}.
C_{max}, maximum drug plasma concentration; CNS, central nervous system; FAAH, fatty acid amide hydrolase; HED, human equivalent dose; NOAEL, no observed adverse effect level; PK, pharmacokinetics.

concentrations, tolerable side effects would be observed and no observed adverse effect level (NOAEL) established (yellow and purple, respectively). At even higher concentrations, orange and red colors indicate irreversible effects in animals that were either not tolerable or fatal. Such a table illustrates the potential margin of safety, especially when the progression of effects through adverse effects is orderly and based on exposure. Even at higher C_{max} values, it is common that there are study results for which no clinical observations were reported (e.g., because clinical observations were not part of the primary or secondary aims of the study). This will result in white rows in between other color classifications.

Strikingly, BIA 10-2474 does not show this orderly white→green→yellow→orange→red color-coded pattern. Instead, yellow, orange, and red color labels alternate. This pattern is of concern, as it indicates that lower plasma concentrations do not necessarily mean that fewer (severe) side effects are to be expected in humans. Looking more carefully, one can observe that the sensitivity also differs among the four species. Dogs show serious irreversible side effects (leading to dose reduction) at concentrations in which rats and monkeys show only minor side effects. Besides, C_{max} values for the NOAEL sometimes overlapped with findings labeled as red (mortality).

One could argue that BIAL could not have foreseen that humans acted much more sensitively than other species studied. However, there are many more data in the IB that indicate the steep dose response curve and above all the large variability in the NOAEL values. Both the sudden death in dogs as well as the NOAEL levels in monkeys and mice that coincide with

severe central nervous system effects (axonal dystrophy or death) illustrate the steepness of the dose response curve (indicated by red/purple striped rows). Thus, severe side effects in animals were not preceded with tolerable, monitorable side effects, and there were no indications that humans would behave differently. This is very difficult to obtain from a textual format of the IB, but becomes apparent from the IB-de-risk table. Hence, we believe that use of such a tool might have alerted investigators, and regulators, to the potential risks. All this taken together is a clear warning sign to proceed carefully and, in our view, not beyond the maximal pharmacological effect.

Besides the tabular color coding used with the IB-de-risk tool, the investigators would have recognized from the data that this was a compound that fully inhibited brain and liver fatty acid amide hydrolase (FAAH) in primates at a C_{max} of around 100 ng/mL and had analgesic effects in mice at ~ 300 ng/mL. Notably, there was a certain amount of noncompetitive binding, as inhibition outlasted plasma concentrations. Toxicology was done in dogs, mice, rats, and cynomolgus monkeys, and we have no information about why more species were studied than usual. In these species, NOAEL levels (as a traditional measure for toxicity) varied from around 10 mg/kg in the rat to around 100 mg/kg in the mouse. C_{max} values varied accordingly between 5,000 and 50,000 ng/mL. The investigators took a traditional approach in their protocol, following the guidelines about an appropriate margin of safety between the first dose and the lowest NOAEL. They then performed a series of ascending dose experiments, first in single doses, and then with multiple doses. The aims of these experiments were to study the PKs and tolerability of the compound and evaluate FAAH-inhibition and anandamide concentrations to get an indication of the PDs of the compound. In later phases, more extensive functional PDs were planned, including nociceptive tests and psychometrics.

Altogether, the protocol impresses as a competent and professional approach to the study of a new compound. The study started, progressed to a dose level of about 0.5 mg/kg and C_{max} levels of 600 ng/mL in the first multidose study, when serious neurological damage was caused by the drug and one subject died. The paper by Rocha *et al*. describes this course of events, and state that "serious toxicity observed after repeat administration of 50 mg BIA 10-2474 could not have been anticipated from the previous dose cohorts."

Several points are worthy of reflection. Importantly, the compound itself was presented with surprisingly little work on its pharmacology beyond some studies of its inhibitory activity and anti-nociceptive activity. There was a lack of any data on other receptor systems, which may be of relevance in view of later findings.[6] The toxicity of the compound was studied, extensively, in "clinical" toxicology studies using traditional methods, including studies of histopathological damage. Dosing was then decided on the absence of evidence of such problems, through the NOAEL approach. Importantly, the TGN1412 disaster was based on the same principles. The compound was considered "safe" in animals, because the experimental animals did not have the required mechanisms to establish the toxicity, although there were clear indications of pro-inflammatory effects.[7] A calculation of TGN1412 receptor binding would have revealed a large overdose. Because of that, the purely NOAEL-based approach was discouraged and calculations of a pharmacologically active dose were recommended. The issue remains that there were indications of off-target toxicity in the BIAL toxicology, comparable, severe, and irreversible side effects, as seen in animals (e.g., axonal dystrophy), and a potential mechanism of these off-target toxicity has been published.[6] In most species, axonal degeneration (swelling and dystrophy) occurred in the brain and this was found at quite varying dose levels. These effects were in some instances described as reversible, sometimes as irreversible. Regardless of the reversibility, neurotoxicity and the unpredictability of the dose at which this happened, should raise major concerns when starting ascending dose studies in humans.

To summarize the position before the trial, BIA 10-2474 could be described as a compound with pronounced and partially irreversible pharmacological action, causing limited and only summarily evaluated functional effects on nociception without clear safety pharmacological concerns. The toxicological effects demonstrated adequate safety margins but with high variability between species and some concerning neurotoxicological effects. No additional pharmacological measures were applied in the human trials to counter this risk, for example, including additional biomarkers related to PDs, like FAAH inhibition, in addition to performing interim analyses of the data. The pharmacological effect of BIA 10-2427 could have been studied by FAAH-inhibition assays and anandamide concentrations. This measure also provided an ideal bridge between animal and human experiments. Yet, despite the possibility to study pharmacological effects and to let these, rather than tolerability, determine the dose escalation, the study progressed to its tragic conclusion. Dose selection based on pharmacological effects is now part of regulatory guidance.[8] The compound could have been safely dosed to maximal FAAH-inhibition (rather than to intolerability) which would have required C_{max} levels of ~ 50 ng/mL. This conclusion is based upon measurements derived from data presented by BIAL at the British Pharmacological Society meeting in 2016. At these dose levels, it is unlikely that neurological damage (which share similarities with the axonal degeneration seen in animals) would have occurred and the study would then have progressed to its PD phase and, if no effects were seen on pain, the compound would likely have been discontinued (Temporary Specialist Scientific Committee report,[9] and the minutes of the Temporary Specialist Scientific Committee meeting[10]). Thus, despite that there was no consequence on the choice of the starting dose in the BIA 10-2474 study, we emphasize that strict monitoring of clinical adverse events and PK data are cardinal during ascending dose studies to decide on rational stopping dose.

Rocha and colleagues indicate in their paper that measurement of FAAH-inhibition in humans failed quality standards and apparently this was the reason that this study was not accompanied by PDs with the time course of leukocyte FAAH activity. This was convenient for proceeding rapidly with the study, but with hindsight, a disaster for the study and its subjects. Moreover, it was at variance with

the protocol which stipulates good laboratory practice-validated measurements, and, in any case, these measurements were already done in primates. A more prudent approach would have been that the study was not continued until the analytical quality control problem was rectified. The reason that the study could progress anyway is because objectives beyond tolerability are generally termed secondary or exploratory. This approach has been criticized before[8,9] and appears to be a practice that is difficult to change despite clear existing guidance, but is an important problem that led to the BIAL tragedy.

The average IB is often so obtuse in its supplied interpretation of the data that a structured, tabular summary—as we propose with the publicly available IB-de-risk tool (https://www.ib-derisk.org)—should be introduced by regulatory authorities in order to maximize understanding of what can otherwise seem an uninterpretable mass of data. A recent study has also demonstrated that preclinical information is often insufficient and that a structured approach could also serve to highlight these deficiencies.[11]

When studying the evidence, we believe that most clinical pharmacologists would have started this study, with questions about selectivity and potential usefulness in the clinic (given that other, more potent, and selective compounds had already been abandoned, but could have progressed in a different and more rational manner). In view of potential and unpredictable toxicity seen in animals, prudence would have dictated a dosing strategy to the maximal level of inhibition, and an estimate of the noncompetitive binding could have been modeled to obtain a safe dose for multiple dose experiments, which would have been about 10–20-fold lower than used. The BIAL case demonstrates that one of the best preventative measures is still in mitigation of unnecessary high doses.

To prevent disasters like the BIAL case,[1] a more structured approach to IB interpretation is crucial to minimize risks. There is a clear need for such a risk-based approach, and Leach *et al*. recently published recommendations for such an approach for dose selection in first-in-human clinical trials. They propose to address key risk factors to provide a consistent, data driven-based, and risk-based approach for selecting first-in-human starting doses.[12] In addition, preclinical dossiers of medicines should always be made public immediately in cases with serious outcomes. The learning that results from this will assist in the prevention of future disasters. Legal considerations should not play a role in providing transparency. We recommend a standardized summary of the preclinical data that helps scientists and regulators interpret IBs to develop rational and safe dosing regimens. Clearly, the tolerability of a new medicine is important, but when tolerability is the occurrence of serious injury or death one must be assured that this is prevented by all possible other means and the integration of as much preclinical information as possible in the decision process. In general, all early human trials should be based upon a mechanistic and PK dose rationale, with inclusion of PD-related biomarkers whenever possible. This unfortunate trial shows again that studies primarily focused on tolerability and NOAEL-based dosing in humans should be a thing of the past. Such classical phase I tolerability studies are not pharmacologically or clinically justifiable and should no longer be permitted by regulatory authorities and ethics committees.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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