The Journal of Clinical Pharmacology
2017, 57(6) 690–703

DOI: 10.1002/jcph.889

The Bial 10-2474 Phase 1 Study—A Drug Development Perspective and Recommendations for Future First-in-Human Trials

Philip Chaikin, PharmD, MD, FCP

Abstract

BIA 10-2474 (a fatty acid amide hydrolase inhibitor) was evaluated in a first-in-human phase 1 study in normal volunteers to assess safety/tolerability, pharmacokinetics, pharmacodynamics, and food effect. The dose-escalation process consisted of a single-ascending-dose phase (SAD) and multiple-ascending-dose phase (MAD). Prospective determination of the starting dose and maximal escalated dose was consistent with the usual clinical pharmacology principles for extrapolation of preclinical toxicology data to human equivalent doses. After only 5–6 days of multiple-dose administration of 50 mg daily in the MAD phase, several subjects became quite ill with central nervous system symptoms. One subject progressed to brain death within several days of symptom onset. Magnetic resonance imaging scans demonstrated signal abnormalities consistent with microbleeds affecting the hippocampus and pons, suggestive of possible cytotoxic or vasogenic edema compatible with a toxic/metabolic process. There were no findings at lower MAD doses or during the SAD phase. The toxicology program carried out in 4 preclinical species (mouse, rat, dog, and monkey) did not demonstrate significant neurotoxicity. The probable mechanism of neurologic toxicity demonstrated in humans at the 50-mg daily dose was inhibition of off-target cerebral receptors or through another mechanism. Additional recommendations have been proposed for future first-in-human studies to maximize subject safety. However, one must also accept the basic premise that, in general, first-in-human phase 1 studies are remarkably safe, and these rare events are not 100% avoidable during the drug development process.

Keywords

fatty acid amide hydrolase, serine hydrolase, anandamide, dose response, on- and off-target receptor binding

Healthy volunteers in phase 1 studies incur remarkably few adverse events (AEs) greater than moderate severity. In the 475 phase 1 studies published from 2008 to 2012 reviewed by Johnson, more than 27 000 participants had 284 serious adverse events (SAEs), only 15 were assessed as at least possibly related to study drug exposure, and 5 deaths occurred, none drug related.[1] In Emmanuel's review of safety experience in Pfizer's phase 1 units, of 11 028 participants exposed to active drug from 2004 to 2011, there were 255 severe AEs and a total of 34 SAEs, 11 of which were related to study drug and 7 to study procedures. No deaths or life-threatening events occurred.[2]

That is a remarkable track record, perhaps partly attributable to implemented lessons learned from the TeGenero first-in-human (FIH) disaster of 2006. Also remarkable is that retrospective investigations of the TeGenero near-deaths and of earlier FIH study deaths have indicated that these should have been preventable. So, although we might logically expect an irreducible minimum of FIH near-fatal or fatal events, they usually do not occur—that is, until January 2016.

It has been 10 years since the TeGenero incident involving TGN 1412 (a CD28 superagonist monoclonal antibody) and the serious adverse events observed in phase 1 volunteers treated at Northwick Hospital in London. However, as mentioned above, rare deaths have been reported in other phase 1 studies (recombinant adenoviral vector gene therapy product at the Hospital of the University of Pennsylvania, 1999) and at Johns Hopkins Hospital in 2001 following administration of hexamethonium bromide by inhalation and a methylcholine challenge.[3,4] Nevertheless, the nature of the serious neurologic adverse events and death reported in the Bial phase 1 first-in-human study of BIA 10-2474 in January 2016 was unprecedented for an orally administered small molecule and raised significant concern in the global clinical pharmacology circle as well as in regulatory agencies around the world.

President, Chaikin Associates LLC, Sarasota, FL, USA

Submitted for publication 4 January 2017; accepted 15 February 2017.

Corresponding Author:
Philip Chaikin, PharmD, MD, FCP, President, Chaikin Associates LLC, 8388 South Tamiami Trail, Sarasota, FL 34238
Email: philipchaikin@comcast.net

BIA 10-2474 was being developed by Bial-Portela & C, S.A. (Bial) for the potential treatment of various central nervous system indications (including neuropathic pain) based on its ability to increase the levels of endogenous anandamide (AEA) and increase the drive of the endocannabinoid system. BIA 10-2474 was designed as a long-acting and reversible inhibitor of fatty acid amide hydrolase (FAAH) that increases AEA levels in the central nervous system (CNS) and peripheral tissues. It was reported to be significantly less potent and less selective as an inhibitor of FAAH versus endocannabinoid agonists that were in drug development by other pharmaceutical companies.[5] The other FAAH inhibitors in development did not have significant safety concerns demonstrated in phase 1 studies (ie, Merck, Pfizer, Janssen, Vernalis), and several were discontinued because of lack of sufficient efficacy in phase 2 clinical trials.[5]

The Temporary Specialist Scientific Committee (TSSC) was commissioned in late January 2016 (several weeks following the serious adverse events in Rennes, France) by the director general of the French regulatory agency (Agence Nationale de Securite du Medicament [ANSM]) to analyze the BIA 10-2474 data and discuss some possible mechanisms that may have caused these serious adverse events in this first-in-human phase 1 study. The TSSC issued a comprehensive report in April 2016 detailing specific issues regarding the pharmacology of the drug (noting its relative low potency and nonselectivity) versus other FAAH inhibitors in development and put forth some hypotheses regarding what factors may have contributed to the neurologic toxicity in several volunteers and the one subject's death. The report also made some recommendations to minimize this type of occurrence in future phase 1 studies.[5]

The perspective offered in this article is the author's drug development and clinical pharmacology viewpoint. The assessment is based on the preclinical data presented in the protocol, review of the TSSC report, and that which is public knowledge in the lay press and previous scientific publications, as well as additional data provided to this author through personal communication with Biotrial, the contract research organization (CRO) that conducted the study. Some of these data have not been presented in previous articles. This allowed the author to draw conclusions, which in several respects, are somewhat different from what has been previously stated, and enabled the author to question some of the conclusions reported in the TSSC report. Further suggestions are made to improve the preclinical data base from which to make human risk assessments, and comments are provided on first-in-human study design and implementation to improve the safety of phase 1 participants for future studies.

Conduct of BIA 10-2474 FIH Trial at Biotrial Clinical Pharmacology Unit

To briefly summarize this FIH study of BIA 10-2474, the objective was to evaluate the safety, tolerability, food effect, pharmacokinetics, and pharmacodynamics of single and multiple ascending doses in healthy volunteers.[6] The single-ascending-dose (SAD) phase consisted of doses of 0.25 mg as a starting dose (1/400th of the maximal dose) and subsequent dose increases up to 100 mg (the human equivalent dose calculated from a 10 mg/kg no-observed-adverse-effect-level [NOAEL] dose in the rat, the most sensitive toxicology species). The multiple-ascending-dose part of the study (MAD) consisted of 5 cohorts receiving 2.5, 5.0, 10.0, 20.0, and 50.0 mg for 10 days (the 50-mg dose group was only dosed for 5 days before the first serious adverse event occurred, and there was a 100-mg cohort also planned). There were no severe adverse events reported in the SAD part of the study. The first 4 MAD dose cohorts completed doses of 2.5 up to 20 mg daily for 10 days without reported incident.[7]

The fifth MAD cohort (50-mg once-daily dose) was added because the maximal tolerated dose (MTD) was not reached in the previous cohorts. Cohort 5 was scheduled to receive 50 mg daily for 10 days (6 subjects on active drug, 2 on placebo). One subject (a 49-year-old man) became ill after the fifth dose. This subject initially had moderate blurred vision and floating specks with a worsening of his clinical condition (gait disturbance and dysarthria), prompting transfer from the Biotrial Clinical Pharmacology Unit (CPU) to the emergency department at Rennes University Hospital.[8] A computed tomography (CT) scan of the head and a CT angiogram were interpreted as being normal. Personal communication with Biotrial revealed that although the emergency room physician proposed sending the subject back to the CPU, Biotrial medical personnel insisted that the subject remain at the hospital for a more extensive workup including magnetic resonance imaging (MRI) of the head the following morning. Apparently the MRI scanner was used on weekend evenings for emergencies only. The subject was expected to return to the CPU the next morning following the MRI scan; however, absent feedback from the hospital regarding his medical status and results from the MRI, a decision was made by the Biotrial physician to dose the remaining subjects in cohort 5, and they subsequently received the sixth dose of 50 mg at 8 AM. Two hours following dosing of the additional subjects, Biotrial was notified from the hospital that the subject had deterioration in his clinical condition, and results of the MRI scan showed a stroke with hyperintensities in the pons and hippocampus on fluid attenuated inversion recovery and diffusion-weighted sequences,

with multiple microhemorrhages in the pons.[8] This resulted in the code being broken for this 1 subject and the trial being discontinued. Four of the 5 additional participants who had received active therapy became ill over the following few days and were all hospitalized; 1 subject remained asymptomatic.[5,7,8] According to the TSSC report, the neurologic events reported were headaches, cerebellar syndrome, altered consciousness, and memory impairment. Other reported symptoms were diplopia, paresthesias, hemiparesis with tremor, spine pain, and stiffness.[5] Details of the hospital course for these subjects have been recently summarized.[8] The first subject hospitalized progressed to brain death 3 days after the onset of symptoms. The clinical picture in 2 of the other volunteers stabilized in several days. All subjects except the deceased received large doses of intravenous corticosteroids. According to the TSSC report, there was an absence of neurologic toxicity in the trial volunteers other than those in the 50-mg once-daily MAD cohort. MRI scans of 4 of the volunteers of cohort 5 demonstrated highly unusual signal abnormalities consistent with bilateral and symmetrical microbleeds affecting the hippocampus and pons. In the deceased volunteer, the scans also showed involvement of the thalamus and cerebral cortex.[5,8] The pattern of MRI signals was indicative of possible cytotoxic or vasogenic edema compatible with a toxic/metabolic process, although an inflammatory component could not be ruled out.[5,8] Personal communication with Biotrial investigators revealed that the deceased 49-year-old subject showed evidence of cerebral vascular angiopathy (possibly amyloidosis) that perhaps made him phenotypically more vulnerable to the effects of BIA 10-2474. Details from complete neuropathology reports are unavailable to the public, but critical to an understanding of this case. Subsequent MRI imaging of the subjects who received lower SAD and MAD doses showed no subclinical brain injury.[7] Recent data indicate that the distribution of the brain lesions did not exactly match the location of endocannabinoid receptors. Endocannabinoid receptors and FAAHs are apparently highly expressed in the hippocampus but not in the pons. In addition, severe toxic effects in the CNS as a result of an increased level of endocannabinoids have not been previously reported.[8]

After the shock of this tragedy, one can pose questions about how these events could have been prevented. Clearly Biotrial was an experienced CRO in the conduct of first-in-human phase 1 studies. It has been previously criticized for administering the sixth dose of 50 mg in cohort 5 to the remaining subjects prior to confirming the results of the index subject's medical status and the results of the MRI scan. It was also criticized for not asking the remaining volunteers to reconfirm their informed consent to participate in the study after one of the subjects was hospitalized. The sponsor, Bial, has also been criticized for not notifying the regulatory agency, ANSM, earlier of the multiple serious adverse events that occurred in this study. These issues and pertinent scientific issues pertaining to this study will be discussed in the article.

Review of Pertinent TSSC Findings

There are several issues noted by the TSSC report regarding the pharmacology profile of BIA 10-2474 that deserve repeating below[5]:

(a) The drug had relatively weak pharmacologic activity — inhibition of FAAH in vitro was in the μM range versus the nM range for similar drugs in development. The IC_{50} of BIA 10-2474 for inhibition of FAAH was reported to be 240-fold greater than for Pfizer's compound (PF-04457845), with some other compounds showing even lower IC_{50} data.

(b) The drug was relatively nonspecific for its binding to FAAH. Inhibition of various other enzymes occurred at 50–100 times those concentrations required for inhibition of FAAH, and the ratio was even lower for other cerebral hydrolases. These data are in contrast to Pfizer's compound (PF-04457845), which showed a ratio of approximately 14 000, making it much more specific for FAAH binding. In addition, the TSSC reported that BIA 10-2474 was tested against only 3 other serine hydrolases versus >20 hydrolases tested by other companies with their FAAH inhibitors.

(c) The dose–response curve was noted to be "steep" (50% inhibition of FAAH was achieved with a human dose of 0.25 mg and almost 100% at a dose of 5 mg), as was apparently demonstrated in this FIH study.

(d) Although stated to be a reversible inhibitor of FAAH, the duration of inhibition of BIA 10-2474 was prolonged and still almost complete after 8 hours, with residual effect observed in humans after 24 hours (despite nonquantifiable plasma concentrations). These data call into question whether the inhibition is truly reversible or irreversible in vivo.

From a research and development perspective, one might question the rationale to advance a new chemical entity further in clinical development given its significantly lower potency and less specificity versus other FAAH inhibitors in development. The lack of specificity could be viewed as a red flag, and it is noted that minimal off-target in vitro binding data are

mentioned in the TSSC report and no in vitro binding data are reported across toxicology species versus humans regarding inhibition of off-target receptors (especially in the brain), where their function is critical to signal transduction and metabolism.

Analysis of the Data in the TSSC Report Versus Author's Perspective

In Vitro Receptor-Binding Data

As part of the preclinical data package, it is imperative to include binding affinities (IC_{50} data) for each of the 4 toxicology species versus humans for the on-target effects (FAAH binding) and for off-target effects. In vitro IC_{50} binding data would allow an assessment of whether humans were more or less sensitive to on-target and off-target effects versus the 4 toxicology species. A comparison of the in vitro IC_{50} binding data to preclinical toxicokinetic (TK) systemic exposures and subsequently human pharmacokinetic (PK) data from the phase 1 study would allow for calculation of C_{max}/IC_{50} ratios. These data would provide insights regarding the magnitude of in vivo systemic exposures versus those necessary to inhibit both on- and off-target receptors and could potentially provide worthwhile information for the dose-escalation process.

Having access to the above data would allow an assessment of the relevance of the 4 animal toxicology species at the TK exposures achieved for demonstrating both on- and off-target toxicities. A very important question is whether humans were more sensitive to off-target enzyme inhibition at the systemic exposures achieved versus the preclinical toxicology species. Between-species differences in off-target receptor binding would have been important information to this assessment given that the human neurologic events observed in the fifth MAD cohort (50 mg once daily for 5 days) were completely unpredictable from the available preclinical toxicology data. It is conceivable that a threshold exposure was reached at the dose of 50 mg a day, at which excessive systemic and central nervous system (CNS) concentrations of unbound drug were present to inhibit other relevant brain serine hydrolases and/or additional off-target receptors in the brain. Could this have possibly been predicted based on early access to the systemic exposures obtained in this dose cohort compared with IC_{50} receptor binding data for the identified off-target receptors? Did the pharmacokinetics become markedly non linear because of saturable metabolism at this dose, as suggested in the TSSC report?

Furthermore, this author believes that the sponsor did not conduct a comprehensive evaluation to identify other major protein targets for BIA 10-2474 supported by in silico medicinal chemistry modeling. The TSSC authors discussed a specificity ratio of 100 for the various other enzymes against which BIA was tested, of which only 3 additional off-target serine hydrolase enzymes are mentioned in the TSSC report, that is, monoacylglycerol lipase, a carboxylesterase, and acetylcholinesterase (with a selectivity of 10 for inhibiting rat FAAH versus 50 for human FAAH).[5] Given that the family of serine hydrolases contains more than 300 enzymes, the in vitro receptor-binding data for other off-target proteins inhibited by BIA 10-2474 were very sparse.[5] For reference, Pfizer's FAAH inhibitor (PF-04457845) was reported to have a selectivity ratio of 14 000 and was tested on 68 different targets.[5] All the other FAAH inhibitors being developed apparently had greater potency for FAAH inhibition in the nM range and significantly greater specificity.[5]

Although there are no data presented regarding comparative enzyme- and receptor-binding assays across species in the protocol or TSSC report, it has been reported from independent in silico medicinal chemistry modeling that BIA 10-2474 was predicted to bind to various other important targets as well as FAAH.[9] Independent research conducted by Dr. Sean Elkins demonstrated that in addition to FAAH as a primary target for BIA 10-2474, other proteins were highly inhibited that included histone deacetylases, which regulate gene transcription, and macrophage-stimulating protein receptor, which could trigger an immune reaction.[9] Unfortunately, we do not know at what exposures this off-target binding would have occurred, as no in vitro IC_{50} data are reported. This independent analysis also reaffirmed that BIA 10-2474 was much less specific for binding FAAH than other drugs tested (ie, Janssen and Pfizer compounds).[9]

A comprehensive preclinical evaluation of drug binding to the relevant on-target enzyme FAAH as well as other off-target cerebral protein receptors across preclinical species and in humans is critical to an understanding of species differences in potency for binding inhibition. In addition, these data are relevant for assessing whether the toxicology studies achieved sufficiently high exposures to adequately characterize the potential for pharmacologically related toxicities resulting from FAAH inhibition and whether sufficient safety margins were established to support the safety of the proposed dosing regimen in this FIH study for off-target toxicities. Systemic exposures, initially extrapolated to humans from preclinical data, and the exposures actually observed from a FIH dose-escalation study should be reviewed relative to the in vitro IC_{50} binding data to evaluate the potential for "on- and off-target" inhibition. These data were not commented on in the TSSC report and apparently were not part of the human risk assessment stated in the protocol.[5,6]

Finally, the authors of the TSSC report stated that humans were 10 times more sensitive to FAAH target inhibition than the representative animal toxicology species (page 27 of the TSSC report).[5] However, subsequent personal communication with Biotrial revealed the mean IC_{50} for FAAH inhibition in humans using a recombinant human enzyme to be 3.3 versus 1.1 μM (0.9–1.3) in the rat and 1.7 μM (1.5–1.9) in the mouse, making humans apparently 2–3 times less sensitive to on-target enzyme inhibition than rodents. There are no in vitro FAAH-binding data reported for dog or monkey.

Dose–Response Issues

With respect to dose–response issues, there are several important points that merit discussion. The TSSC report stated there was significant discordance between the dose observed in volunteers that produced complete inhibition of FAAH activity (5 mg) and that which could be extrapolated from animal studies. The report stated that "maximal inhibition of FAAH occurred at a dose of 0.3 mg in the monkey and that complete inhibition of FAAH was predicted to occur in man at a dose of 10–40mg" (page 7, TSSC report).[5] The report stated this would be "equivalent to a ratio of at least 10 between the human dose estimated on the basis of animal data and that actually measured in humans" to cause 100% inhibition of FAAH (5 mg). Note that it was not reported what method was used to predict human effective doses from preclinical animal data and whether PK/pharmacodynamic (PD) modeling was performed to estimate pharmacodynamic effect after multiple-dose administration. Use of standard allometric methods for scaling from animals to humans is accepted practice and included in the Food and Drug Adminisration guidance document for determination of a safe starting dose in FIH studies.[10]

It is unclear to this author how the TSSC calculated its predicted human dose range of 10–40 mg for complete inhibition of FAAH. A 0.3 mg/kg dose in the monkey is equivalent to a dose of 0.097 mg/kg in humans (0.3 mg/kg divided by the human equivalent dose [HED] conversion factor of 3.1), which equates to a human dose of 6 mg for a 60-kg person (which is very close to the 5-mg dose observed in the phase 1 study that apparently caused 100% FAAH inhibition).

It is worth making a few comments pertaining to the "steepness" of the dose–response curve, as mentioned in the TSSC report. The ED_{50} was reported to be 0.25 mg in humans, with almost complete inhibition of FAAH occurring at a single 5-mg human dose.[5] The dose–response curve in this case would be consistent with a Hill coefficient of 1, in that an ED_{90} dose of 2.5 mg is only 10-fold greater than the ED_{50} dose of 0.25 mg (Figure 1). However, what is more important is that acute inhibition of FAAH was not a reliable predictor of multiple-dose toxicity. It would have been useful to generate tissue distribution data regarding preclinical brain accumulation after multiple-dose administration.

Positron emission tomography (PET) imaging can be a useful tool in drug development to shed additional light on the magnitude of brain tissue concentrations achieved upon steady-state dose administration and the extent and duration of brain receptor occupancy. A recent publication evaluating PET in the rat actually demonstrated that BIA 10-2474 is a potent inhibitor of FAAH in rat brain, as measured by the radiotracer F18-DOPP with IC_{50} of 52–71 μg/kg, comparable in potency to other established FAAH inhibitors (URB 694 and PF-04457835). The authors concluded that it is quite likely that very high levels of FAAH inhibition were achieved in the CNS for the subjects in this FIH study for BIA 10-2474.[11] Although providing useful information regarding dose response and receptor occupancy and sometimes helpful for choosing human effective doses for CNS drugs, one has to appreciate that most sponsors do not routinely conduct preclinical PET studies, and certainly clinical PET studies are not conducted routinely prior to a first-in-human phase 1 trial. It is questionable how the availability of these data would have altered any decisions regarding dose strategy for this FIH study of BIA 10-2474, especially given that the goal was to determine MTD, not to curtail dosing at the minimal anticipated biologically effective dose (MABEL).

Regarding duration of inhibition of FAAH, it was previously stated in the TSSC report that the compound had a long duration of action, was covalently bound to FAAH in vitro, but was partially reversible in vivo, although no enzyme kinetics for T(off) were reported.[5] Personal communication with Biotrial revealed that anandamide (AEA) plasma concentrations continued to increase and persisted for more than 72 hours following single-dose administration of BIA 10-2474 to humans at doses up to 100 mg (keeping in mind that when FAAH is inhibited, AEA concentrations increase; Figure 2). These data not only underscore the long duration of action of the drug, but given that AEA concentrations continued to increase following single doses of BIA 10-2474 (from 1.25 to 100 mg), would be inconsistent with previous data that a 5-mg single dose produced maximal response for FAAH inhibition in humans.[5,7] Therefore, there are conflicting data concerning dose–response relationships for FAAH inhibition (Figure 1) and identification of the maximal effective dose from AEA plasma concentrations in humans (Figure 2). It is recommended that these data be further explored by the sponsor.

One would normally need to ensure adequacy of the assay methodology for ex vivo determination of FAAH

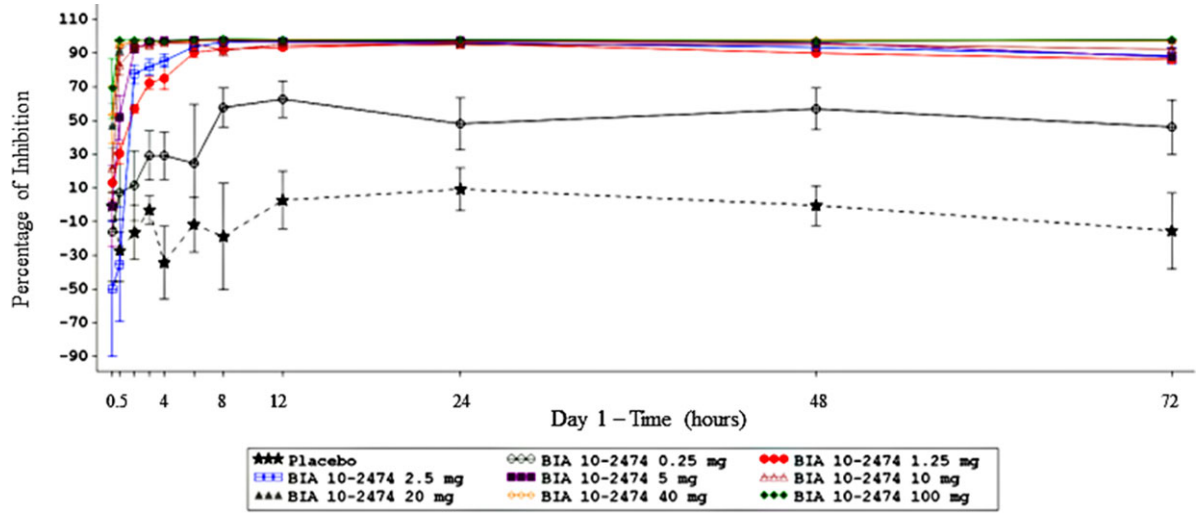

Figure 1. Percent inhibition of fatty acid amide hydrolase (FAAH) activity on day 1 following single doses of BIA 10-2474.

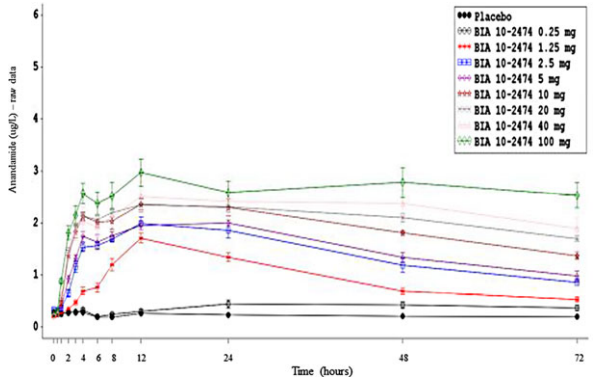

Figure 2. Anandamide (AEA) plasma concentrations (ug/L) following single doses of BIA 10-2474.

Table 1. No-Observed-Adverse-Effect-Level Doses and Associated Toxicokinetic Data in Toxicology Studies

	Duration of Study	NOAEL	AUC (ng·h/mL)	
Species	(Weeks)	(mg/kg/Day)	Male	Female
Mouse	4	100	160 000	149 000
	13	25	30 700	24 900
Rat	4	30	41 300	64 900
	13	10	18 900	23 100
	26	10	16 751	20 109
Dog	4	50	65 000	38 600
	13	20	24 100	66 200
Monkey	4	100	179 416	168 772
	13	75	138 325	130 702

AUC, area under the curve; NOAEL, no-observed-adverse-effect level.

inhibition during conduct of the phase 1 study to support any firm conclusions being drawn regarding dose response using this method. There is apparently some question regarding the reliability of this assay given the marked variability observed (personal communication with Biotrial). Subsequent PK/PD modeling of human anandamide (AEA) concentrations would be valuable information to generate for establishing dose–response relationships versus what has been previously reported in humans using the current ex vivo assay method for FAAH inhibition. This is a very important concept, given that much emphasis has been placed on the 5-mg dose producing 100% inhibition of FAAH and being on the plateau of the dose–response curve. If continued inhibition of FAAH were to occur at doses greater than 5 mg (as evidenced by continued increases in AEA concentrations up to 100-mg single doses), then this would provide additional support for the sponsor's choice of maximal dose (100 mg) for this FIH study. However, hindsight is 20-20, and none of this information was known in real time for dose-escalation decisions during the conduct of the trial. In fact, the analytical method for measuring FAAH inhibition was to be improved after the SAD phase, before the MAD phase; however, this was not possible, as all of the biological samples had been used (personal communication with Biotrial).

Toxicology Issues

During drug development, toxicology studies should be performed in the relevant animal species at sufficiently high doses and exposures to enable a proper human risk assessment based on demonstration of both "on-target" and "off-target" toxicities. According to the phase 1 protocol, animal toxicology studies were conducted up to 13 weeks in mice, dogs, and monkeys and up to 26 weeks in rats.[6] NOAEL doses and exposures are reported in the protocol (Table 1); however, there is no mention of any observed toxicities seen at higher doses. Unfortunately, there are no TK data (C_{max} and AUC) presented in the protocol nor in the TSSC report at doses higher than the NOAELs.[5,6]

The TSSC report (page 10) discusses cerebral damage in rodents (3 animals), especially in the hippocampus with the presence of gliosis and inflammatory cell reaction at high doses in rats (150 mg/kg) and in mice (500 mg/kg). The authors state that "this was not theoretically likely to generate a signal, although such damage does not appear to have been observed with other FAAH inhibitors." The authors also note that axonal dystrophy was observed in the medulla in some monkeys at 100 mg/kg, yet this was not observed at lower doses.[5] Perhaps these findings should have been further explored?

According to the TSSC report (page 11), 1 monkey died during the toxicology studies (after titration of doses up to 75 mg/kg/d), and several other monkeys had to be "put down for ethical reasons" during ascending-dose studies at very high doses: 250, 125, and 60 mg/kg. These doses translate to very high human equivalent doses of 80.6, 40.3, and 19.4 mg/kg, respectively (dividing by the HED conversion factor of 3.1), and translate to many-fold the doses administered to humans (100-mg single dose and multiple doses of 50 mg daily).[5] However, there are no monkey TK data presented in the TSSC report versus the PK data observed in humans during the SAD/MAD phases of the phase 1 study from which to draw more definitive conclusions and validate human safety margins. This begs the question regarding the magnitude of the systemic exposures in the monkeys that died or had to be euthanized versus what was achieved in humans at the highest single dose (100 mg) and at the exposures achieved in humans in the 50-mg once-daily dose cohort. There is no mention of premonitory signs and symptoms in the monkeys, and the deaths are unexplained. There is minimal discussion in the TSSC report regarding relevance to human risk. Also, no brain histopathology data from the deceased monkeys are presented, although the TSSC report states, "the non-alarming nature of the neurological damage observed was confirmed by examination of tissue slices by the TSSC experts."[5] An evaluation of the TK data and the IC_{50} data for binding inhibition to both target FAAH and other relevant off-target receptors across toxicology species and in humans would have been very instructive for a better understanding of this issue.

The TSSC report goes on to state that there was no toxicity from BIA 10-2474 targeting a specific organ that would have been a signal contraindicating administration to humans. Importantly, the report also mentions that the safety of BIA 10–2464 demonstrated during the toxicology studies was "under par" compared with previously developed FAAH inhibitors and that other FAAH inhibitors appeared to have a cleaner toxicology profile (absent dog lung toxicity and no early sacrifices needed in the primate toxicology studies).[5]

Toxicokinetics and Human Safety Margins

When initiating a FIH study, it is critical to evaluate the systemic exposures observed at the NOAEL and at doses that produced toxicities in the relevant preclinical species versus the systemic exposures observed during the conduct of the FIH study (safety margins).

The highest single dose administered in the SAD part of the study (100 mg) was chosen as it represented the human equivalent dose of 10 mg/kg NOAEL dose in the rat, which was the most sensitive toxicology species.[6] At this human dose of 100 mg, the mean AUC was approximately 23 000–24 000 ng·h/mL (personal communication with Biotrial). The mean AUC associated with the NOAEL in the rat at 10 mg/kg was 18 900 ng·h/mL (3-month toxicology study) and 16 751 ng·h/mL (6-month toxicology study); see Table 1. Therefore, the exposures achieved in humans after single-dose administration of 100 mg only modestly exceeded that obtained in the rat multiple-dose toxicology studies at the NOAEL. There is no need to correct systemic exposures and safety margins for unbound drug, as the plasma protein binding was similar across species (24.5% human, 24% rat, 21% dog; personal communication with Biotrial). However, we do not know the plasma protein binding in the monkey (personal communication with Biotrial). The TK exposures at the NOAEL in the other animal species (dog and monkey) were much higher than in the rat and would provide greater safety margins; however, safety margins using primate TK data cannot be corrected for unbound drug, as the plasma protein binding in the monkey is not reported (personal communication with Biotrial; Table 1).

More important, one should focus on the systemic exposures obtained in the MAD cohorts, especially in the 50-mg dose group (cohort 5, MAD) compared with the TK data in the preclinical toxicology species. Based on personal communication with Biotrial, although the PK data were not obtained at steady state for the 50-mg dose cohort, the steady-state AUC was estimated based on PK data obtained on day 1 using an accumulation ratio of 1.6 (which is what was observed for the 10- and 20-mg MAD cohorts). The estimated mean AUC at steady state for the 50-mg once-daily dose cohort was 12 200 ng·h/mL (day 1 mean observed AUC, 7642 ng·h/mL). This is lower than the NOAEL exposure in the rat of 16,751 (6-month study) and 18 900 (3-month study). Therefore, this would provide a human safety margin of only 1.4–1.5 for the rat NOAEL. Using the monkey TK data from a NOAEL dose of 100 mg/kg in the 4-week study (179 416 ng·h/mL) and the NOAEL dose of 75 mg in the 3-month study (138 325 ng·h/mL), the safety margins are roughly 11–15 (which is acceptable); see Table 1. It is noted by this author that the reported NOAEL dose of

100 mg/kg in the 4-week monkey titration study did produce some neurologic signs (weakness, incordination, tremor; personal communication with Biotrial). In addition, histopathology findings demonstrated some axonal dystrophy in the medulla. So it is not certain to this author how the sponsor arrived at 100 mg/kg as the NOAEL dose. Nevertheless, the human safety margin for the 50-mg once-daily dose cohort was 11–15 using the monkey NOAEL. However, it still remains unanswered why humans demonstrated significant neurotoxicity at much lower doses and systemic exposures (50-mg once-daily cohort) and for a much shorter duration (5 days) versus the monkeys (4-week and 3-month toxicology studies) and the other toxicology species. Without review of between-species IC_{50} data for both on- and off-target receptor binding, it remains somewhat difficult to assess the adequacy of the toxicology studies for achieving sufficiently high exposures to characterize the potential for pharmacologically related on-target and off-target toxicities.

Pharmacokinetic Issues

Much has been stated previously regarding the role that pharmacokinetics (PK) may have played in contributing to the outcome of the Bial 10-2474 study.[5] The TSSC report mentions nonlinear PK occurring in humans after single ascending doses in that "the elimination half -life was gradually extended when doses administered became high and the AUC also increased more rapidly than the doses increased." The report further goes on to state that this was likely because of saturable elimination and decreased clearance, yet it was not possible to accurately identify the threshold dose at which nonproportionality began.[5] The report also states that nonlinearity was observed in the MAD phase of the study, in which the AUC increased more rapidly than did dose at a dose of 20 mg and greater. This implies that a more cautious dose-escalation process and enhanced scrutiny of PK from each MAD cohort prior to further dose escalation would have somehow minimized the clinical outcome of this case. However, this author believes that PK contributed very little to the outcome of this study. Personal communication with Biotrial disclosed the mean elimination half-life ($T_{1/2}$) observed on day 1 of cohort 4 (20 mg once daily) to be 7.7 hours (range, 6.9–9.5 hours) and the mean elimination $T_{1/2}$ (day 1) at 50 mg once daily to be 8.5 hours (range, 6.4–12.3 hours). Accumulation ratios calculated for the 10- and 20-mg once-daily cohorts at steady state were 1.57–1.59, consistent with or a little higher than would be expected for a mean elimination half-life of 12 hours or less (personal communication with Biotrial). In contrast to what was stated in the TSSC report, mean C_{max} and AUC for the single-dose cohorts were relatively dose proportional, with only a slight non–dose proportionality at the higher doses for AUC (40 and 100 mg; Figures 3 and 4).

In this author's opinion, a review of real-time PK data from each MAD cohort prior to further dose escalation would generally be warranted for a drug with a narrow therapeutic index, steep dose response curve, and significant nonlinear PK. This did not appear to be the case for BIA 10-2474. As stated earlier, the dose-response relationship for FAAH inhibition in humans was not that "steep," as was described in the TSSC report. However, comparison of the PK data with the TK data should always be evaluated after each MAD cohort to calculate safety margins. Also, in most MAD studies, it is customary practice for a review of the PK data from the preceding dose cohort (N-1) before further dose-escalation decisions are made, yet the N-2 cohort PK data were used to make the dose-escalation decision (PK data from 2 lower-dose cohorts). Therefore, the decision to escalate from 20 to 50 mg once daily was made on the basis of PK data from the 10-mg once-daily cohort. This is somewhat unusual and not consistent with general clinical pharmacology practice given that there was a 2.5-fold dose increase based on PK data from a significantly lower dose. One could easily argue that the use of N-1 PK data would have made better sense, but this author believes that this decision had very little impact on the negative outcome of this study. As reported by Biotrial, the PK were linear and only slightly non–dose proportional at the higher single doses. In addition, we now know that the mean human systemic exposure (AUC) for the 100-mg single dose was only slightly greater than the AUC at the rat NOAEL (10 mg/kg dose), and the human exposures estimated for the 50-mg once-daily cohort at steady state were less than the AUC at the rat NOAEL dose (rat being the most sensitive toxicology species). The TSSC report states that departure from linear PK and saturable elimination might have occurred somewhere between the 20- and 50-mg MAD dose cohorts, making it unwise to escalate 2.5-fold (from 20 to 50 mg once daily) when using PK data from the 10-mg cohort.[5] However, we know that the PK were dose proportional in the 10- and 20-mg dose cohorts at steady state and that there was dose proportionality at least for day 1 between the 20- and 50-mg cohorts (personal communication with Biotrial). In addition, using the accumulation factor observed for the lower-dose cohorts (1.6), the 50-mg once daily cohort was predicted to have a steady-state AUC equivalent to a single dose of 80 mg, making the dose selection reasonable. In this case, the AUC exposure at the 100-mg dose from the SAD phase would have covered the AUC at the highest dose studied in the MAD phase (50 mg). The bottom line is that abnormal PK behavior in humans did not appear to be a contributing parameter to the

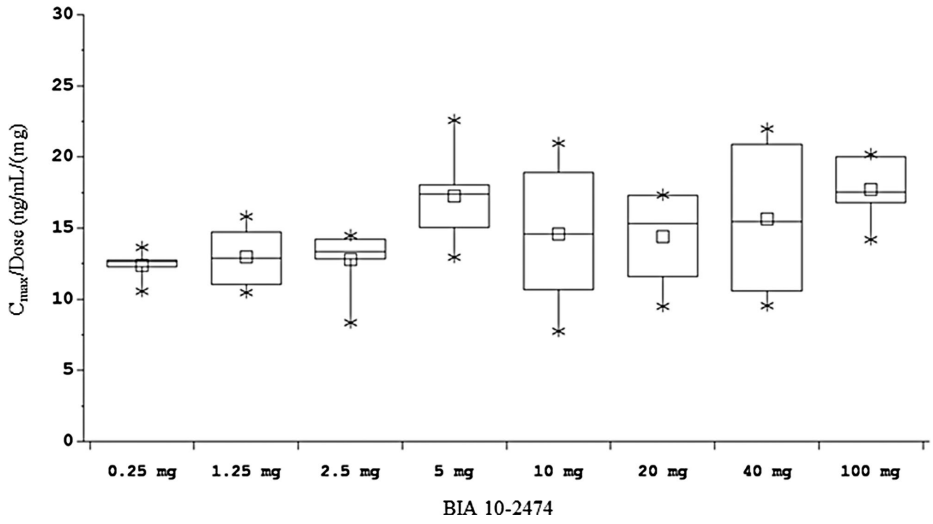

Figure 3. Dose of BIA 10-2474 versus dose-normalized maximum plasma concentrations (C_{max}) following single doses.

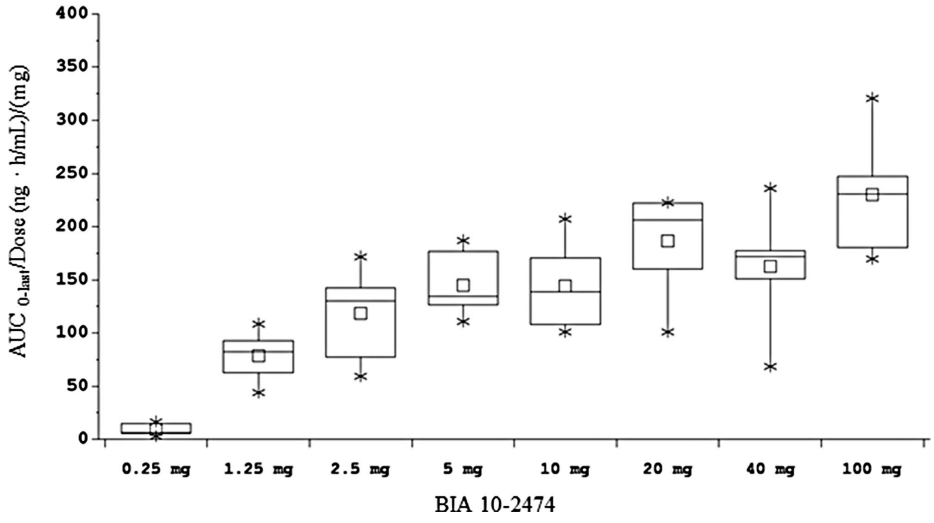

Figure 4. Dose of BIA 10-2474 versus dose-normalized area under the curve (AUC) following single doses.

negative outcome of this study. However, one might argue that in general for first-in-human phase 1 studies, as one continues to dose-escalate to MTD, the latter dose-escalation steps should be smaller as the doses become larger on the approach to MTD. In addition, it is also important to note that the subject who died was not a PK outlier and was not distinguishable from the other volunteers in the fifth MAD cohort, none of whom demonstrated excessive plasma concentrations (personal communication with Biotrial).

Possible Mechanisms for Toxicity in Humans

The authors of the TSSC report offer several possible reasons for this highly unusual clinical and radiologic picture presenting in MAD cohort 5 (50 mg once daily) and conclude that it was most likely because of the cumulative daily dose administered and that the mechanism accounting for this toxicity should "be looked for outside the endocannabinoid system."[5] The TSSC authors also underscore the nonspecificity of BIA 10-2474 and its ability to inhibit other cerebral hydrolases (yet as stated earlier in this article, there are no data reported for IC_{50} binding to off-target cerebral serine hydrolases). They underscore that the dose given to cohort 5 (50 mg) was 10 times higher than that which completely inhibits FAAH for a long period (5 mg), and the 100-mg maximal dose in the SAD phase was 20 times higher than the maximally effective dose of 5 mg. However, this author has previously raised the discrepancies between maximal effective dose based on FAAH binding and that noted by increasing AEA plasma concentrations at doses up to 100 mg. In addition, the ex vivo FAAH human binding data were batch-analyzed at the completion of the SAD phase before the MAD phase began, and therefore, the dose that produced maximal FAAH inhibition was unknown

at the time the SAD phase was actually conducted (personal communication with Biotrial).

The TSSC authors go on to propose several mechanisms for the reported human toxicity: (1) inhibition of other cerebral hydrolases by BIA 10-2474, (2) BIA 10-2474 toxicity by another mechanism, (3) toxicity from a BIA 10-2474 metabolite, and (4) suspected anandamide-related toxic effects.[5]

This author agrees with the TSSC report that the biologic plausibility of the first 2 mechanisms are most likely to explain the neurologic outcomes in this clinical study. As stated previously, perhaps the IC_{50} for off-target receptor binding was lower in humans and the systemic exposures achieved at the 50-mg daily dose cohort were in excess of that necessary to bind the off-target receptors. Could there have been more off-target binding at lower systemic exposures achieved in humans in the 50-mg once-daily cohort than was necessary to produce off-target toxicities in the 4 animal species at the higher concentrations observed in the toxicology program? Were other cerebral serine hydrolases or protein targets inhibited to a greater extent in humans in cohort 5 (50 mg once daily) versus what was observed in the animal toxicology species, or did we achieve sufficient inhibition of these other "off-target" protein receptors in the preclinical toxicology species yet for some reason not elicit the neurologic toxicity because of lack of human homology?

Another hypothesis is whether BIA 10-2474 could covalently bind another molecule, forming an adduct.[9] Dr. Elkins noted the chemical structure of BIA 10-2474 to contain a pyridine oxide, a reactive group infrequently found in other drugs. Adducts are often recognized as foreign substances immunologically that could provoke massive inflammation, consistent with the clinical scenario described in the hospitalized subject who died.[9] The TSSC report also mentions that the imidazole leaving group can produce isocyanate that could bind other proteins in the CNS. This could yet be another possible mechanism for off-target neurotoxicity.[5]

A recent proteome wide-screening approach was used to evaluate in silico binding of BIA 10-2474 to 80 923 protein structures.[12] Target disease association analyses identified 11 proteins as potential targets inhibited by BIA 10-2474, with the 2 highest-scoring proteins being factor VII and thrombin. Both these clotting factors are serine proteases and members of the same superfamily as BIA 10-2474, sharing many structural features. These data suggest that BIA 10-2474 may have anticoagulant properties mediated through inhibition of factor VII and thrombin that could facilitate intracranial hemorrhage.[12] Clearly, knowledge of the IC_{50} data for BIA 10-2474 binding to these clotting factors relative to the systemic concentrations achieved in humans during this FIH study would have been critical for an evaluation of potential human toxicity and mitigation of risk. This author is not aware of any reported data regarding coagulation abnormalities for the volunteers in this study.

For the sake of completeness, this author agrees with the TSSC report that there is little likelihood that human toxicity was attributed to any of the 4 circulating metabolites of BIA 10-2474 identified in animals, 2 of which reached measurable plasma concentrations but remained very low (<3% of parent drug), with chemical structures being fairly similar to the parent drug.[5] Although several metabolites have the potential to inhibit FAAH with intensity similar to the parent drug, the likelihood of metabolites accumulating in human brain with very high concentrations versus what was the case in preclinical toxicity studies is small. In addition, there was no mention of any unique human metabolites that were not observed in the toxicology species.[5]

Regarding the contribution of anandamide to this neurologic toxicity, in theory, it may interact with several types of receptors (TRPV1, PPAR, NMDA, and MAP-kinase) and may degrade to eicosanoids (leukotrienes and prostanoids).[5] However this type of toxicity has not been observed in animals or humans with other more specific FAAH inhibitors during administration of high multiple doses.[5] Therefore, as stated above, the most likely hypotheses to explain this mechanism of toxicity are: (1) inhibition of other cerebral hydrolases and (2) toxicity by an unknown mechanism.

Interestingly, during the legal proceedings of this case, additional investigation from the Paris prosecutor's office stated that the deceased subject was "a carrier of an occult intracranial vascular pathology, which could explain his fatal outcome, unlike the other volunteers within the cohort" (personal communication with Biotrial). From this author's perspective, this might have implications on the upper age limit for normal volunteers to participate in first-in-human studies (given that this subject was 49 years old).

Discussion

It is the opinion of this author that the French regulatory agency (ANSM), the ethics committee, and the Biotrial investigators may have missed an opportunity to ensure that Bial clarify its rationale and preclinical basis for translating BIA 10-2474 risk to human volunteers. Along these lines, this author suggests the investigator brochure (IB), preclinical reports, and relevant sections of the investigational medicinal product dossier (IMPD) be made available for scrutiny by clinical pharmacology experts for full and complete

transparency so that lessons can be learned to help to prevent such disasters in the future. In the absence of these data, lessons to be learned may be missed and doomed to be repeated. Where were mistakes made? Was it a lack of generation of relevant preclinical data or a possible misinterpretation of the available data for an adequate human risk assessment, or both?

In future FIH studies, it is imperative that a comprehensive preclinical data package be generated to properly profile a new chemical entity for clinical development to allow for a multidisciplinary assessment of human risk. This includes the relevant pharmacodynamic models from which to make an assessment of the pharmacologic profile of the drug for the proposed indication, construction of dose/exposure response curves suitable for modeling human effective doses, and a thorough assessment of on-target and off-target pharmacology. The IB should contain an integrated assessment of the pharmacology/toxicology data, translation to humans including anticipated efficacious dose range, anticipated human toxic dose/exposure range, and the intended therapeutic dose range in humans. Also, the IB should contain an assessment of brain tissue exposures, safety margins versus projected human exposures, and species differences for on- and off-target inhibition. The comprehensive data package should always contain in vitro data regarding receptor binding across species to assess interspecies differences in potency and a preclinical toxicology program to allow for sufficiently high systemic exposures for evaluating both on-target and off-target human risk assessment (with an early assessment of safety margins in humans). In addition, it is important to generate a thorough understanding of the absorption, distribution, metabolism, and excretion processes across species as well as profiling of major metabolites, and so forth. This would also include multiple-dose tissue distribution kinetics in critical organs and possibly assessment of brain receptor occupancy kinetics using PET imaging for CNS drugs.

Aside from the scientific issues pertinent to the preclinical data base for BIA 10-2474 as discussed in this article, it is also important to make some comments regarding study conduct and execution that played a role in the unfolding of this tragic event. However, it must be acknowledged that only the investigator at Biotrial conducting the study had the relevant information to make an informed decision regarding continuation of the trial. It is quite appropriate to ask what data the medical personnel had available at the time a decision was made to continue dosing additional subjects in cohort 5. It is true that the study was not stopped despite the index subject being sent to the hospital for evaluation (with subsequent admission, fulfilling the criteria for a serious adverse event). As discussed previously, the subject was expected to be returned to the CPU after his MRI scan the following morning. Personal communication with Biotrial confirmed that attempts to reach the sponsor's study medical monitor to discuss the situation were unsuccessful that Sunday evening. There had been no additional feedback from the hospital to Biotrial the following morning, and the investigator made the decision to dose the additional subjects in cohort 5. One can propose that a higher index of suspicion for causality should have been made and increased vigilance applied such that no further dosing of subjects would take place until the results of the MRI scan and the clinical status of the volunteer were known to Biotrial medical personnel. It would follow that the informed consent would be modified to reflect the current condition of the index subject, with additional subjects reconsented prior to further dose administration. However, the medical personnel at Biotrial did not have the requisite information regarding the index subject's status at the time the additional subjects were dosed. Clearly waiting a couple of additional hours for feedback from the hospital would have minimized further exposure of additional volunteers to the investigational drug and the extent of this tragedy. Yet this was an experienced CRO with extensive experience conducting phase 1 studies. These are important lessons to be learned for future first-in-human studies.

In November 2016, the European Medicines Agency (EMA) published a draft revision for the "Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products."[13] There are several recommendations mentioned in this draft guideline and additional perspective provided from this author that merit mentioning below and are relevant to the Bial phase 1 study and future FIH studies[13]:

Recommendation 1: Special attention should be given to the estimation of the initial dose to be used in humans and to the subsequent dose escalations to a predefined maximum dose. This author believes that both the starting dose and maximal escalated dose of BIA 10-2474 were appropriate based on accepted clinical pharmacology convention and allometric scaling from toxicology data.

Recommendation 2: Consideration should be given to the novelty and the extent of knowledge of the supposed mode of action, as well as the characteristics of the target. When analyzing risk factors, previous exposure of humans to compounds that have similar or related modes of action needs to be taken into consideration. This author notes that BIA 10-2474 was the third or fourth in the series of FAAH inhibitors under development, so the pharmacologic concept was not that novel or unique.

Recommendation 3: It is important to conduct repeat-dose preclinical pharmacology studies and to include pharmacodynamic end points in repeat-dose toxicology studies when possible. PK/PD modeling should be used to characterize repeat-dose pharmacodynamic effects and to estimate multiple-dose pharmacodynamic effects in humans for both on- and off-target effects. This author agrees with this statement, especially given that acute inhibition of FAAH was not a reliable predictor of multiple-dose toxicity.

Recommendation 4: Sponsors should discuss the relevance of the preclinical models to humans, taking into account the target, structural homology, distribution, and nature of the pharmacologic effects. Relevance of the nonclinical models should be discussed in the IB. This is critical to the BIA 10-2474 situation, as the preclinical toxicology species did not demonstrate significant neurotoxicity. This author believes that additional investigational toxicology studies can be important to supplement a standard toxicology program. This is especially true once an unusual toxicity is observed in humans. Further guidelines are needed for new drug targets whose human homologues are not well modeled by animal species.

Recommendation 5: Systemic exposures at pharmacologically active doses in relevant animal models should be determined and PK/PD modeling used to predict the human effective dose range. In the author's opinion, this is routinely employed as standard practice during drug development. However, it must also be appreciated that there are several limitations because of species differences in PK/PD relationships versus humans. It is also questionable as to how robust the preclinical PK/PD modeling was for BIA 10-2474 regarding estimation of the human effective dose range.

Recommendation 6: Exposures achieved at the NOAEL dose in the most relevant and sensitive animal species should be used for estimation of an equivalent exposure in humans using appropriate PK/PD, physiologic PK, or allometric scaling. This author believes that in fact, this was done for dose selection for BIA 10-2474, as the estimated top doses in the SAD phase (100 mg) and subsequently in the MAD phase (50 mg) were selected based on the human equivalent dose of the NOAEL in the rat (the most sensitive toxicology species).

Recommendation 7: PD effects should be quantified in preclinical pharmacology studies including the use of ex vivo and in vitro studies using human tissues if feasible. These data should be used to determine the MABEL in humans and an estimation of the pharmacologically active dose and/or anticipated therapeutic dose range in humans. Differences in sensitivity between humans and animals for the mode of action of a new chemical entity should be taken into consideration. These data and methods used should be clearly summarized in the IB for risk assessment. Whenever possible, determination of these doses should consider target binding and receptor occupancy studies in vitro in target cells from humans and the relevant animal species and at exposures at the pharmacologic doses in the relevant animal species. All these data should be integrated into a suitable modeling approach for the determination of the MABEL, pharmacologic active dose, and anticipated therapeutic dose range. This author in general supports this recommendation, but recognizes that it may be somewhat overreaching and difficult to implement for drug development programs of small molecules.

Recommendation 8: Dose-escalation steps should be guided by the dose exposure toxicity or the dose exposure effect relationship, as determined in the preclinical studies and by emerging clinical data from a FIH study. This author believes that in certain situations, real-time collection of PK and PD data should be implemented, especially for drugs with a narrow therapeutic index and if there is evidence of nonlinear PK. However, it must be emphasized that access to these data would not have made a difference for the BIA 10-2474 study, given that dose escalation was to achieve maximal tolerated dose (MTD) and the PK exposures for cohort 5 were expected to be equal to or only slightly less than the TK exposures at the NOAEL in the most sensitive animal species (rat). The estimated PK exposures at the 50-mg once-daily dose were even further below the TK exposures in the other animal species at the respective NOAEL doses. This author also believes that smaller dose-escalation steps should be implemented, especially in the latter steps of a SAD/MAD study as MTD is approached.

Recommendation 9: A maximum predefined dose or exposure margin should be justified and stated up front in the protocol. This maximal dose or exposure should generally not be exceeded in the FIH study without requisite ethics committee approval of a protocol amendment. This author agrees with this recommendation, although the systemic exposures associated with multiple doses of 50 mg daily of BIA 10-2474 were estimated to be less than or equal to the NOAEL in the most sensitive toxicology species (rat).

Recommendation 10: A new recommendation by EMA is that the exposure at the expected human therapeutic dose range should not be exceeded in FIH studies in normal volunteers, unless scientifically justified. Target inhibition and saturation should be taken into account. If the intended therapeutic effect is linked to enzyme inhibition, then the maximum dose should be considered when complete inhibition is achieved and no further therapeutic effect is to be expected by increasing the dose. This author recognizes the

implication that pharmacodynamic data would need to be collected real time for subsequent dose escalation decisions, which may be impractical in many cases. In addition, one must keep in mind that target enzyme inhibition and saturation in the plasma compartment or in a circulating blood based tissue compartment (ie, peripheral blood mononuclear cells) may not be fully indicative of a similar extent of target inhibition in the central nervous system or for example in a solid tumor, and that MTD studies will still play an important role for subsequent dose decisions in the clinical development program. The new EMA guidance states that it is unethical to conduct a phase 1 study to identify the MTD in normal volunteers.[13] This concept will be discussed in more detail below.

Recommendation 11: In the MAD phase of a FIH study, the exposures (C_{max} and AUC) should have been covered during the preceding SAD phase of the trial. Note that in the Bial case, there was sufficient PK coverage for the highest dose from the SAD phase (100 mg) versus the 50-mg multiple-dose cohort.

At this point, it is appropriate to make a few comments regarding the concept of MTD in phase 1 dose-escalation studies, given that the new EMA guidance document states it is unethical to push for MTD in normal volunteers. In the Bial study, the dose was escalated to 100 mg in the SAD phase of the study, which was reported to be 20-fold the single 5-mg dose that produced 100% inhibition of FAAH and 10-fold higher for the 50-mg multiple-dose cohort. As stated earlier, the 100-mg single dose and the 50-mg once-daily multiple dose were chosen based on the human equivalent dose for the NOAEL (10 mg/kg) observed in the rat (the most sensitive toxicology species), which is consistent with normal clinical pharmacology principles. It has been customary practice to elucidate dose-limiting symptoms as an implicit goal of a first-in-human dose-escalation study.[14] In fact, early phase 1 FIH studies are the only place to actually determine dose-limiting toxicity (DLT) and a maximal tolerated dose. Identification of the MTD is important for optimal choice of doses in phase 2 clinical efficacy trials as well as the need to understand DLT at high doses in the event of an overdose situation and as a benchmark for conducting clinical pharmacology studies in subjects with impaired excretory function (renal/hepatic) and for cardiac repolarization studies (thorough QT), which require evaluation of a supratherapeutic dose. However, the decision to stop dosing in phase 1 when a particular target receptor or enzyme has been fully occupied or whether to continue to push the dose to demonstrate DLT depends on the drug and whether we are dealing with normal volunteers or patients, consistent with the new EMA guidance document. In some cases depending on whether the drug has a narrow therapeutic index or binds a specific enzyme, when the desired PD effect can be measured and one achieves maximal effect, one can possibly make an argument not to push the dose further to DLT in normal volunteers.[14]

One also needs to properly integrate pharmacokinetics into the assessment of the highest dose tested in phase 1 studies. For example, curtailing dose escalation when a target systemic exposure has been reached based on relevant animal models to avoid systemic exposures that could place subjects at risk may be appropriate. This would apply to those drugs with delayed toxicities or a narrow therapeutic index.[14] Systemic exposures associated with on- and off-target preclinical toxicities can be used as a guide regarding further dose escalation. Defining the MTD will ultimately take into account a combination of dose-limiting toxicity and pharmacokinetic-pharmacodynamic factors. Perhaps in the future we will see more phase 1 studies (for nononcology drugs) determine the MTD in the actual patient population and the studies conducted in normal volunteers geared more toward the pharmacologically active dose. This might consist of a SAD/MAD study in normal volunteers to cover the anticipated pharmacologic dose range followed by SAD/MAD study in patients, escalating the dose to DLT to find the MTD. This is actually how phase 1 MTD studies are conducted for many CNS drugs (especially in Alzheimer's disease and for antipsychotic drugs beause of the increased tolerance to adverse events shown by patients versus normal volunteers). Use of normal volunteers in FIH phase 1 studies for these types of drugs would inevitably lead to a much lower MTD, which could impact the choice of doses for subsequent clinical efficacy trials.

One must also appreciate that the EMA recommendation to conduct all MTD studies in patients versus normal volunteers would add an additional level of complexity given the presence of concomitant diseases and the potential for drug–drug interactions. It is this author's opinion that there is still a role for conducting MTD studies in normal volunteers unless the drug is deemed too toxic, which was not the case for BIA 10-2474 based on the available preclinical data.

Conclusions

It must be acknowledged that, in general, first-in-human studies are remarkably safe.[1,2] In this author's opinion, although the sponsor seems to have chosen the doses correctly for this FIH study based on available preclinical data, it now seems probable that the systemic exposures achieved in humans at the 50-mg multiple-dose cohort exceeded the IC_{50} data for off-target receptor inhibition, more so than what was achieved in the 4 preclinical toxicology species at higher systemic exposures. In addition, this mechanism

for human toxicity was unable to be fully expressed in the toxicology species, possibly because of lack of human homology for off-target binding. Investigative "predictive" toxicology studies using toxicogenomic and proteomic methodology and a systems biology approach can provide important information for a drug development program to support the traditional toxicology package mandated by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use.[12,15] These studies can provide further insights into potential human toxicities that may not be observed in a standard preclinical toxicology package. Clearly, additional work is needed to understand the nuances of this case and is especially important given the recent in silico proteomic screening approach suggesting that BIA 10-2474 may have anticoagulant properties through inhibition of factor VII and thrombin.[12]

The TSSC report comments on many of the deficiencies in the BIA 10-2474 preclinical data package. This article has raised further issues relevant to conducting a thorough risk assessment and describes some additional preclinical data that may have been helpful to potentially mitigate risk.

Finally, we are left with several unanswered questions: (1) Why were the 4 preclinical toxicology species inadequate for demonstrating this unique human toxicity? (2) Were early warning signals missed on review of the preclinical data base? (3) Were there critical gaps in the preclinical data base that did not allow for a thorough risk assessment? Could this disaster have been prevented? Or was this a chance encounter? Only a complete review of the preclinical study reports included in the regulatory submission for this protocol will help to clarify these issues. In the meantime, additional investigational toxicology studies are encouraged for a more complete understanding of this phenomenon. At the end of the day, one must answer the question of whether these serious neurologic events were completely unpredictable in humans based on the preclinical data package generated by the sponsor or whether there were gaps in the preclinical knowledge base and/or signals that were missed that could have potentially decreased the disastrous outcome of this first-in-human study.

It should be understood that not all relevant issues have been discussed in this article. This article should be seen as a starting point and a call for further discussion and evaluation by clinical pharmacologists and other drug development scientists to generate additional ideas for further understanding of this case to maximize subject safety in future first-in-human studies.

Acknowledgments

The author acknowledges Biotrial for providing the figures and additional data and for providing helpful discussions.

Declaration of Conflicting Interests

The author is a consultant to Upsher-Smith Laboratories and Akros Pharma.

Funding

There are no financial disclosures relevant to this article.

References

1. Johnson RA, Rid A, Emanuel E, Wendler D. Risks of phase I research with healthy participants: a systematic review. *Clin Trials*. 2016;13(2):149–160.
2. Emanuel EJ, Bedarida G, Macci K, Gabler NB, Rid A, Wendler D. Quantifying the risks of non-oncology phase I research in healthy volunteers: meta-analysis of phase I studies. *BMJ*. 2015;350:3271.
3. Sibbald B. Death but one unintended consequence of gene therapy trial. *Can Med Assoc J*. 2001;164(11):1612.
4. Olgive RI. The death of a volunteer research subject: lessons to be learned. *Can Med Assoc J*. 2001;165(10): 1335–1336.
5. Report by the Temporary Scientific Committee (TSSC). "FAAH (fatty acid amide hydrolase)," on the causes of the accident during a phase 1 clinical trial in Rennes in January 2016. Saint-Denis, France: AgenceNationale de Securite du Medicament et des Produits de Sante. http://ansm.sante.fr/var/ansm_site/storage/original/application/744c7c6daf96b141bc9509e2f85c227e.pdf.
6. Study protocol BIA 102474-101. Version 1.2, July 2015-A double blind, randomized, placebo controlled, combined single and multiple ascending dose study including food interaction, to investigate the safety, tolerability, pharmacokinetic and pharmacodynamic profile of BIA 10-2474 in healthy volunteers.
7. Eddleston M, Cohen AF, Webb DJ. Editorial. *Br J Clin Pharmacol.* 2016;81:582–586.
8. Kerbrat A, Ferre JC, Fillatre P, et al. Acute neurologic disorder from an inhibitor of fatty acid amide hydrolase. *N Engl J Med.* 2016;375(18):1717–1725.
9. Alzforum-networking for a cure. Will death in French drug trial lead to tighter phase 1 rules? January 2016. www.alzforum.org/news/community-news/will-death-french-drug-trial-lead-tighter-phase-1-rules.
10. Guidance for industry-estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), July 2005.
11. Tong J, Mizrahi R, Houle S, et al. Inhibition of fatty acid amide hydrolase by BIA 10-2474 in rat brain [published online ahead of print 2016]. *J Cereb Blood Flow Metab*.
12. Molinski SV, Shahani VM, MacKinnon SS, Morayniss LD, Laforet M, Woollard G, et al. Computational proteome wide screening predicts neurotoxic drug protein interactome for the investigational analgesic BIA 10-2474. *Biochem Biophys Res Commun*. 2017;483:502–508.
13. Guidelines on strategies to identify and mitigate risks for first in human and early clinical trials with investigational medicinal products, Nov 10, 2016, EMA/CHMP/SWP 28367/07 Rev 1.
14. Chaikin P. Industry perspectives on the MTD: growing acceptance in clinical development programs. *J Clin Pharmacol.* 2000;40:1191–1197.
15. Bai JPF, Abernathy DR. Systems Pharmacology to predict drug toxicity: integration across levels of biological organization. *Annu Rev Pharmacol Toxicol*. 2013;53:451–473.