Report by the Temporary Specialist 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.

1. Foreword

The "FAAH inhibitors" TSSC was set up by the Director General of the Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM), following the accident that occurred during the Phase 1, first-in-human clinical trial on the molecule BIA 10-2474, in Rennes on 10 January 2016.
The scientific missions of the TSSC, were, on the basis of the available data and expertise of its members:

- To analyse the mechanisms of action and potential toxicity of substances which, like BIA 10-2474, are presumed to have a direct or indirect effect *via* the endocannabinoid system.
- To put forward and, where possible, list hypotheses to be able to explain the toxicity observed in several volunteers in the trial conducted in Rennes by Biotrial.
- To enact, where appropriate, general recommendations aiming to tighten safety for volunteers, especially during first-in-human (Phase 1) trials.

The TSSC, from the time it was set up (25 January 2016) to issue of its report (18 April 2016), worked according to three methods:

- Individual expert appraisal of the documents provided to each member.
- Two one-day "open" meetings (15 February and 24 March 2016) during which the expert appraisals were read. Another open meeting between Bial and members of the TSSC also took place on 18 March 2016. The three meetings were held on the ANSM's premises, attended by two inspectors from the General Social Welfare Inspectorate (Christine d'Autume and Gilles Duhamel). A representative from the EMA (European Medicines Agency) also attended the meetings of 15 February (Hans-Georg Eichler) and 24 March (Jean Marc Vidal) as observer; two representatives from the Portuguese Medicines Agency (Ana Catarina Fonseca and Isabel Vieira) also participated as observers in the last meeting.
- Question and answer and group writing sessions for TSSC members only, which led to approval of the two versions (intermediate and final) of the present report.

This organisation made it possible to reserve discussion of the key points of the expert appraisal, conclusions and recommendations for TSSC members only, independently of the presence of the organiser (ANSM) and observers (IGAS general inspectors, European and Portuguese Agency representatives). This closed phase represented by far the largest part of the TSSC's work.

The mission of the TSSC's experts, although it represented a significant amount of expert appraisal work (estimated at more than 600 hours in total), did not serve to replace an inspection under any circumstances. Therefore, the conclusions of this report shall not prejudge those of the ongoing administrative and legal investigations.

Concerning the source documents used, there is a foreword to the BIA 10-2474 Investigator Brochure, written by Bial, which contains incorrect translations and transcription errors, especially in the tables and figures. This, in several places, gives rise to ambiguity and comprehension difficulties, including with respect to important information (see chapter 6). This deserves to be highlighted as the Investigator Brochure is a document used as reference during pre-approval phases of a health care product, as recalled by international rules and recommendations.
Finally, although the TSSC was set up by decision of the ANSM's Director General and received logistics support from the Agency, the Committee conducted its work and investigations fully independently during the two and half months of its existence, especially with regard to the ANSM, Bial, Biotrial, the volunteers that participated in the trial and their families and defence lawyers.

All TSSC experts worked on a voluntary basis for their entire mission.

Various draft versions of this report were submitted to the TSSC's experts alone, and the numerous discussions required to finalise it and to reach a consensus on the key points of the case took place among those experts alone at all times.

2. TSSC members

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3. Background

The molecule BIA 10-2474, by the Bial pharmaceutical company (Portela Ca, Portugal), belongs to the FAAH inhibitor family, an enzyme degrading anandamide, a biolipid acting as mediator in what is known as the endocannabinoid system.
More than ten inhibitors of this type are or have already been developed, none being marketed to date; for many due to efficacy considered to be disappointing. In terms of structural chemistry, these inhibitors mainly belong to two families: Molecules with a *urea* function and those with a *carbamate* function,.

Research in the field of FAAH inhibitors has been driven by strong hopes and the prospect of highly varied therapeutic indications: pain, vomiting, anxiety, mood disorders, Parkinson's disease, Huntington's chorea, various cardiovascular indications, to name but a few.
For Bial's product, the Investigator Brochure states that BIA 10-2474 was developed "*for the treatment of medical conditions in which there is advantage in enhance the levels of endogenous anandamide (AEA) and tonically increase the drive of the endocannabinoid system (Sic)*".
The indication which appeared to have been preferred, at least initially, was neuropathic pain; this was confirmed by Bial during its hearing on 18 March 2016.
First-in-human trials were entrusted to Biotrial Research in Rennes, a centre specialising in investigations and research of this type for almost twenty years. The accident which occurred mid-January 2016 led to the suspension of the clinical development of BIA 10-2474. Its severity and spectacular nature deeply affected the drugs industry, scientists and the public, as much in France as around the World. Understanding the circumstances, and if possible, the mechanisms of occurrence of this unprecedented accident is therefore a collective priority and reason behind the expert appraisal work conducted by the TSSC.

This expert report, after a reminder on the endocannabinoid system (prerequisite to introduction of the discussion on the mechanisms of action of the molecule and the hypotheses surrounding its toxicity) will analyse the molecule itself, its pharmacological properties, followed by animal toxicity studies, the protocol used by Biotrial, the symptoms observed in healthy trial volunteers, and pharmacodynamic and pharmacokinetic data. The second part will explore the hypotheses likely to explain the accident in Rennes. A conclusion will summarize the TSSC's opinions and positions as to the key points of the case. The report will conclude on recommendations affecting the conduct of first-in-human trials that the TSSC wishes to see implemented at European and international level.

4. Reminder on the endocannabinoid system

BIA 10-2474 is an FAAH inhibitor, serine hydrolase degrading anandamide, one of the main mediators of what is known as the endocannabinoid system. This equivocally-named system (it is in fact a lot broader and more complex than cannabis derivative targets) exists in a large number of species (vertebrates and invertebrates, except for

insects) and in mammals in particular. Knowledge is recent (the first receptor was identified by cloning in 1990) and as yet incomplete.
There are two types of receptors (CB1 and CB2), transmembrane and G protein-coupled (inhibiting adenyl cyclase).

- CB1 is a highly ubiquitous presynaptic receptor found at the surface of several cell types (neurons, astrocytes, pericytes, endothelial cells) and in a large number of cerebral sites (basal ganglions, cerebellum, hippocampus, cerebral cortex, olfactory bulb, etc.). CB1 is one of the G protein-coupled receptors expressed at the highest level in the central nervous system, with the noteworthy exception of the brain stem. CB1 is also found in peripheral organs (lungs, bowel, testicles, uterus, etc.). The exogenous agonist specific to this receptor is tetrahydrocannabinol (THC).
- CB2 is mainly found in immune system cells (immunomodulator effects).

Eight endocannabinoid agonists have been identified to date. They are bioactive lipids acting both as neurotransmitters and neuromodulators and produced and released "on demand", unlike conventional neurotransmitters which are released from storage vesicles.
The three main endocannabinoids are:

- anandamide (AEA); this was the first endocannabinoid to be characterised (1992),
- 2-arachidonylglycerol (2-AG), arachidonic acid ester,
- 2-AG ether (arachidonic acid ether).

Like THC, AEA has preferential affinity for the CB1 receptor and very low affinity for the CB2 receptor. Conversely, 2-AG has high affinity for both receptor types and it can therefore be seen as the main endocannabinoid system mediator, whereas AEA has almost no effect on CB2 and is able to interact with several other systems. Also, 2-AG is found at levels 200 to 800 times higher than anandamide in rodents.
Unlike 2-AG, anandamide is therefore little specific to the endocannabinoid system in the strict sense of the term and can also be considered to be an endovanilloid. It is able to activate TRPV1 (*transient receptor potential vanilloid 1*) which are non-selective cation channels from the TRP channels group.
AEA also acts on other systems:

- it is a good agonist for PPAR (*peroxisome proliferator-activated receptor*) alpha and gamma, nuclear receptors involved in the energy metabolism and inflammation,
- it interacts in NMDA (N-methyl D aspartate) glutamate receptors, both as stimulator by direct action and inhibitor acting indirectly *via* CB1,
- finally, like other endocannabinoids, it can lead to the activation of multiple transcription factors involved in neuroprotection phenomena by the MAP-kinase pathway and a chain reaction, which is a highly promising research approach.

The effects of endocannabinoid system stimulation are similar to those induced by cannabis derivatives. Low to moderate concentrations induce behavioural responses combining stimulant and depressant effects, whereas at high doses, the effects are always of the depressant type. We therefore mainly see the following in animals:

- antinociception,
- hypothermia,

- hypolocomotion.

Working memory is affected, without effect on reference memory. The effect on level of anxiety is biphasic: anxiolysis at low doses and anxiogenic at high doses.

In terms of synaptic transmission, endocannabinoids act in a retrograde fashion (from the neuronal post-synaptic to pre-synaptic element) and generally reduce transmission in the short (few seconds) or long-term (several hours or days). They modulate both excitatory (glutamatergic) and inhibitory (GABAergic) transmission.
After being produced and released by the postsynaptic compartment, AEA is usually degraded by FAAH (membrane hydrolase) which also partly degrades the 2-AG but also a fairly large number of other bioactive lipids.
Unlike in animals, two FAAH isoforms (FAAH-1 and FAAH-2) can exist in the human species. The prevalence of carriers of the two isoforms is estimated at around 38% in the general population and that of carriers of the low activity isoform (FAAH2) 5%.
Where there is inhibition of FAAH activity, AEA concentrations increase, however an additional degradation pathway takes over: that of the cyclooxygenases. This leads to the formation of eicosanoids: leukotrienes and prostanoids (prostaglandins, thromboxanes, prostacyclins) with the ability to act on apoptosis and vasomotricity phenomena; the vasoconstrictor effect of 20-HETE (20-hydroxyeicosatetraeinoic acid) in the brain is, for example, confirmed.

5. Examination of molecule BIA 10-2474

Examination of the chemical structure of this molecule does not theoretically raise any specific questions, especially concerning potential toxicity. The functional groups and chemical nuclei it contains are commonly found in medicinal chemistry. For example, the *N-oxide* function is found in chlordiazepoxide (benzodiazepine sedative), minoxidil (potassium channel agonist developed as antihypertensive agent and used secondarily to develop hair growth), and in various antiretrovirals.
The originality of BIA 10-2474 is for the remainder, relative; it can be considered as a "variation" of molecules previously developed as FAAH inhibitors. For example, Pfizer's PF-3845 also contains a pyridine nucleus directly adjacent to the urea function. This compound, effective *in vivo* and selective, was proven to be a powerful FAAH inhibitor, well-tolerated in Phase I clinical trials, but without satisfactory efficacy in Phase 2 trials. In the same way, the *imidazole* nucleus, common in pharmaceutical chemistry, is contained in the compounds developed by Bristol-Myers Squibb (carbamate type inhibitors). However, in the case of BIA 10-2474, this nucleus is in the position adjacent to the molecule's electrophilic site which (see further on) potentially makes it a "leaving group".
All FAAH inhibitors developed are based on the formation of a covalent bond between hydrolase serine 241 and the carbamate or urea electrophilic carbon. FAAH inhibition can therefore be considered to be irreversible. According to Bial, BIA 10-2474 is effectively covalently bound to FAAH (therefore irreversibly) *in vitro* but partially reversibly *in vivo*. This has already been reported in the case of Janssen & Janssen's inhibitor (JNJ-42165279) with which partial enzyme activity is observed after 8 hours.

A significant difference between BIA 10-2474 and most known inhibitors concerns its **low specificity** for its target enzyme. Concentrations inhibiting FAAH activity at 50% (IC_{50}) range, on average, from 1.7 (1.5 – 1.9) micromolar (μM) in mice to 1.1 (0.9 – 1.3) μM in rats. They are believed to be 100 times higher at most for the various other enzymes against which BIA was tested, according to Bial. Bial therefore only tested its compound and one of its metabolites (BIA 10-2445) on three serine hydrolases: monoacylglycerol lipase (MAGL), a carboxyl-esterase and an acetylcholine-esterase (selectivity of 10 for rat FAAH, and 50 for human FAAH). The other enzymes tested were dopamine-beta-hydroxylase, glutamic acid decarboxylase, monoamine oxidases A and B and choline-acetyl transferase.
This contrasts with the results with other compounds such as Pfizer's PF-04457845 (tested against 68 receptors) which has an IC_{50} of 7.2 nanomolar (nM) for human FAAH (therefore 240 times lower than that of BIA 10-2474) and of over 100 μM for a panel of around twenty hydrolases. The specificity ratio of Pfizer's compound is therefore no longer 100, but around 14,000. The same applies for JNJ-42165279 by Janssen & Janssen tested on 50 enzymes. The low affinity/specificity of BIA for its target enzyme will further lead us to envisage "parasite" binding to other serine hydrolases during discussion of the toxicity mechanism observed in humans. It should be recalled that the serine hydrolase superfamily counts around 300 members and that it is therefore recommended developing inhibitors with the highest possible affinity for the enzyme targeted. Proteomic screening would probably have provided useful information in this respect.

Nine presumed BIA 10-2474 metabolites have been synthesised (compounds BIA 10-2639, 10-2583, 10-3258, 10-3827, 10-2445, 10-2631, 10-3844, 10-2580 and 10-3764). All have a structure that is very similar to that of the mother molecule. They correspond either to reduction of N-oxide, or to hydroxylation of the cyclohexane nucleus (which leads to the formation of more hydrophilic compounds), or to demethylation of the amine function, or to two concomitant changes. Theoretically, nothing in the chemical structure of these metabolites portends potential toxicity. Three of them have the potential to inhibit FAAH to a similar extent as that of the mother molecule. These metabolites are mainly found in very small quantities, even after 14 days' administration of BIA 10-2474 to animals. BIA 10-2631 (produced by N-oxide reduction and cyclohexane hydroxylation), is however found in larger quantities in primates.
During pharmacokinetic studies in humans (see further on), four of these metabolites were identified, 2 were undetectable and 2 measured at much lower plasma concentrations than those of the mother molecule (<3%).
Unless we presume a completely different metabolite exists, or that there is significant accumulation in cerebral tissues (very high tissue concentration/plasma concentration ratio, explaining the low circulating concentrations), involvement of these metabolites in the toxicity observed in the clinical trial in Rennes appears to be little likely.
In another respect, it should be recalled that the BIA 10-2474 molecule imidazole cycle, in adjacent position to the electrophilic carbon, site of binding to FAAH, can be considered to be a "leaving group» that may produce an isocyanate to which many brain proteins are likely to bind.

When in contact with hepatic microsomal enzymes, BIA 10-2474, at least up to the concentration of 30 μg/mL, only slightly inhibits cytochromes P450 2D6 and 3A4 and does not seem to inhibit cytochromes P450 1A2, 2A6, 2B6, 2C8, 2C9, 2C19 or 2E1. It

does not appear to act as an inducer, at least on cytochromes P450 from series 2B and 3A, with a doubt as to 1A2.

6. Preclinical pharmacodynamic data

With respect to FAAH inhibition (mechanism presented as central to its pharmacological activity), we can consider BIA 10-2474 as a compound:

- *with relatively weak activity*. 50% FAAH inhibition *in vitro* requires, for instance, concentrations in the mid-micromolar range whereas for most inhibitors developed to date, they are in the nanomolar range. As mentioned above, the IC_{50} of BIA 10-2474 for FAAH appears to be 240 times higher than that of Pfizer's PF-04457845, some inhibitors being characterised by even lower IC_{50}.
- *that is little specific*. Inhibition (again *in vitro*) of other enzymes occurs at concentrations 50 to 100 times those inhibiting FAAH. It is possible (this has not been tested) that the ratio is even lower with other cerebral hydrolases. As a comparison, this ratio is, as we have seen, of around 14,000 for PF-04457845.
- *of little progressive effect*. The dose-effect curves (here inhibition of FAAH activity) of BIA 10-2474 show this in particular when we enter an unusually narrow concentration range, going from absence of to almost complete inhibition. Even if it is fitting to take account of experimental variability, the dose-effect curve slope appears to be high and very steep, if we compare it to those of other enzyme inhibitors and more generally to other drugs.
- *that is long-acting*. Even if we consider that BIA 10-2474 is not typically an irreversible inhibitor, the FAAH inhibition it induces is extremely prolonged. It is still almost complete after 8 hours. In humans, inhibition persists well over 24 hours, whereas BIA plasma concentrations have fallen below the limit of quantification of the test method used (that is to say they are non-quantifiable).

  Concerning the dose from which BIA inhibits FAAH activity, there is apparently significant discordance between what could be extrapolated from animal studies and that which was observed in the volunteers in the trial in Rennes. Animal data shows that maximum effect is achieved from a dose of 0.3 mg/Kg in monkeys; a dose of 1 mg/Kg not making it possible to increase FAAH inhibition or anandamide concentrations. This (calculation not provided in this report but verified by the TSSC) made it possible to predict that complete FAAH inhibition would be achieved in humans for a dose of between 10 and 40 mg. However, in the volunteers of the Biotrial trial, we see that around 50% inhibition is achieved for a 0.25 mg dose and almost 100% for a 5 mg dose. This is equivalent to a ratio of at least 10 between the dose estimated on the basis of animal data and that measured in humans.
  As mentioned previously, the resulting inhibition is extremely prolonged as activity recovery is not complete 72 hours after administration, whereas the product has almost fully disappeared from the plasma.

As for the analgesic activity of BIA 10-2474 (therapeutic possibility theoretically favoured), two tests, usually used to test this possibility (not specially for neuropathic pain), have been carried out in mice:

- *Formalin paw test*. This test consists of injecting a 5% formalin solution into the tip of the paw of the hind leg. This causes persistent pain leading to repeated reflex licking by the rodent. The efficacy of the investigational molecule is assessed by the reduction in the number of times the areas is licked (*licking score*) over a given period (here: 15 to 50 minutes) after the injection. Three doses of BIA 10-2474 (0.3 mg, 1 mg and 3 mg/Kg) were compared, either administered alone or in combination with 5 mg/Kg AEA, also administered alone. Gabapentin, at the dose of 300 mg, was used as reference analgesic for this comparison; indeed, this gamma-aminobutyric acid derivative is used as anticonvulsant and to treat neuropathic pain.
  In this test, the effect of BIA 10-2474 used alone appeared to be prolonged but of moderate amplitude. In effect, for the three escalating doses, and compared to an inactive solvent, the score decreased by 29%, 28% and 41% respectively. The effect of AEA alone was of the same order of magnitude (35%). However, we see a marked dose-dependent effect for the BIA + AEA combination, the scores decreasing by 42% for the combination with 0.3 mg/Kg of BIA, 65% for the combination with 1 mg/Kg and 86% for the combination with 3 mg/Kg. In this test, gabapentin appeared to be clearly more effective than BIA since the variation is 76% compared to 41% for BIA alone at its highest dose.
  We note that in the Investigator Brochure the corresponding figure (4.6) is doubly false compared to the source document provided by the investigator (Porsolt & Partners, Report no. 09.770/2, 2010): the vertical axis shows seconds whereas they are score values and the column for the gabapentin score (nevertheless presented as reference for this comparative test) had been deleted from the bar chart.
- *Tail flick test* (reflex flick of a tail subject to heat stress). A significantly higher dose of BIA 10-2474 (10 mg/Kg) was used for this test. The antinociceptive effect was maximal in the eighth hour but, like in the previous test, of moderate amplitude (flick time increasing from around 4.8 seconds on average to almost 6, therefore a 1.2 second difference) but prolonged (a difference persisting in the 72nd hour). On the basis of the same test, BIA and URB 597 (FAAH inhibitor from the carbamates family), both administered at the dose of 1 mg/Kg, were compared with respect to potentiation of the analgesic effect of AEA. Potentiation was only observed with Bial's compound.

The doses used in these tests differ greatly (from 0.3 to 10 mg/Kg), without it being possible to trace a dose-effect curve or to estimate an effective dose 50 (which is a surprising shortcoming). We note that:

- The antinociceptive effect of BIA 10-2474 remains moderate when the molecule is administered alone (presumed conditions of future therapeutic use). BIA however strongly potentiates the antinociceptive effect of AEA.
- The antinociceptive effect of BIA 10-2474 increases little (score ranging from 29% to 41%) when we increase the dose from 0.3 to 3 mg/Kg, for the formalin test, that is to say we increase the dose by a factor of 10. This could mean that inhibition of the enzyme involved in this effect is almost complete from the 0.3 mg/Kg dose and therefore, that the range of doses tested, too narrow and/or badly chosen, does not make it possible to accurately determine an effective dose. This would constitute another problematic shortcoming with respect to the rational choice of doses in later development stages.

- The potentiating activity of AEA by BIA 10-2474 is, however, very clear and prolonged, regardless of the dose tested.

The relative lack of pharmacodynamic data compared to other preclinical studies would justify a recommendation from the TSSC concerning new drug development.

7. Animal toxicology data

7.1. Opening remark

Interpreting toxicology study data is always complex. These studies are conducted with doses which can be very high, incommensurate with those used in humans. Therefore, at the highest doses, various signs of toxicity, often little-specific or visible (in macroscopy or microscopy) only after sacrifice, are observed in most of the animals.
With an accident such as that in Rennes, there is therefore a strong probability of finding elements consistent with the type of toxicity identified, in animal data later on. This does not in any way mean that these elements constituted signals predictive of toxicity of this type. To avoid this conventional interpretation bias, the TSSC closely examined the particularly extensive dossier of animal studies conducted; this must be looked at as a whole and in its context.

7.2. Toxicology dossier

Studies conducted with BIA 10-2474 seem to have been conducted according to currently approved protocols (ICH recommendations especially) with a highly pure product (more than 99.9%), identical to that used for the manufacture of the capsules administered to the volunteers at the Biotrial centre.
These studies covered, which is little common (especially for a molecule that is not particularly innovative), four different species (rats, mice, dogs and monkeys) and were conducted in two centres of sound reputation: Harlan Laboratories SA in Spain (studies in non-rodents) and AnaPath GmbH in Switzerland (studies in rodents).
Another species (rabbits) was also used for studies on the potential effects of BIA 10-2474 on fertility and reproduction.
During its hearing by the TSSC on 18 March 2016, Bial explained that this particularly extensive toxicology programme was related to a delay in the start of clinical development, this meaning that additional toxicology studies were carried out or continued following first-in-human administration (i.e. carcinogenesis studies). Therefore, the TSSC did not find, in any of the data it analysed, anything to substantiate the hypothesis that this particularly comprehensive and costly toxicology programme was conducted due to doubts as to good tolerance to the molecule.

On the basis of the data that could be analysed to date, and generally, up to very high doses, we do not observe any toxicity from BIA 10-2474 specifically targeting a given organ and which could have been taken as a signal precluding clinical development. One of the toxic effects found the most often in treated animals, as for several other FAAH inhibitors, affects semen and more generally, gametes. This point, without a doubt the

clearest of the case, should be taken into account if BIA 10-2474 were to be brought to be used as a drug.

We note that Bial, unlike what has been done for several other FAAH inhibitors, did not define target organs in its toxicology programme.

The sensitivity of the assay methods used during toxicology studies only identified five peripheral (plasma compartment) metabolites among the nine which BIA 10-2474 is able to produce. These metabolites are theoretically identical to those found in humans and also produced in very small quantities (around 1% of the parent product), and this for the four species. Therefore, toxicity studies specifically for these metabolites were not legally compulsory and were not conducted.
We do not observe accumulation of the product or of its metabolites in multiple dose studies (over 13 weeks).

The NOAEL (*No Observable Adverse Effect Level*) and NOEL (*No Observable Effect Level*) doses seem to have been correctly determined. They varied according to species tested and interestingly to duration of administration, especially in mice. The NOAEL for the 4-week and 3-month studies are therefore respectively:

- 100 and 25 mg/Kg/24h in mice,
- 30 and 10 mg/Kg/24h in rats,
- 50 and 20 mg/Kg/24h in dogs,
- 100 and 75 mg/Kg/24h in monkeys.

On the bases of the calculated NOAEL, and by referring to Food and Drug Administration (FDA) procedures, it was in theory logical to test a dose of up to 100 mg in humans (96 mg according to the TSSC's calculation).

As in all toxicology protocols, the organs of the animals provided for in the protocol (40 organs) were systematically submitted for macroscopic and microscopic examination, without, according to the study centres' reports, noteworthy toxicity of a specific organ, *a fortiori* common to the four species studied, being observed. This also applies to both the central and peripheral nervous system, especially in primates.
However, in rats and mice, cerebral damage, especially in the hippocampus with gliosis and inflammatory cell infiltration were observed in three animals treated at very high doses. This concerned one male and one female in the study on mice at 500 mg/Kg/24h over 4 weeks and one rat in the study at 150 mg/Kg/24h over 4 weeks. This damage, discussed by the TSSC given the context, seems to be fairly commonly observed in rodents in studies of this type, and was not theoretically likely to generate a signal, although such damage does not appear to have been observed with other FAAH inhibitors. For the three animals concerned, as for their fellow creatures in the same group, the observation reports do not mention any neurological or behavioural disorders.
In the same way in primates and rats, cerebral damage and especially of the autonomic nervous system was observed in some animals treated with a high dose. In monkeys, during 4-week studies conducted with 10, 50 and 100 mg/Kg/24h doses respectively, *medulla oblongata* (spinal bulb) damage in the form of *axonal dystrophy* was noted in some animals from the 100 mg/Kg/24h group and not in those receiving lower doses. It is difficult to comment on the precise histological nature of the damage, due to the fact

that the two pathologists who interpreted the slice slides on behalf of Bial did not use the same terminology.
Again in monkeys, a clearly dose-dependent toxicity (only occurring in the 100 mg/Kg/24h group), and more significant than previously, concerned damage in the form of oedema of the Meissner plexus cells of the digestive tract.

In the group of dogs (beagles) treated for 13 weeks, the TSSC's attention was drawn to the presence of pulmonary changes clearly visible in macroscopy, confirmed in microscopy and termed as "bronchopneumonia/focal and multifocal acute alveolitis". These symptoms are surprising in their frequency. The toxicology report submitted by Bial links these lesions to bronchial inhalation of BIA 10-2474. This hypothesis seemed little plausible to the majority of the TSSC experts. In effect, the shell of the capsules administered was designed to resist gastric juices, making it little plausible that they were opened and caused powder inhalation. The same reason, and the method of administration of the capsules and absence of suggestive symptoms also make regurgitation following "choking" little plausible (i.e. explained by neurological toxicity of BIA 10-2474). No alternative hypothesis (infectious contamination, specific susceptibility of this group of dogs or other reason) was favoured by the TSSC for this symptom which is one of the highlights of the BIA 10-2474 toxicology dossier. The relationship with the existence of high CB1 receptor density in the lungs, in the absence of further investigations, cannot be considered as a possible explanation. Also, we note the absence of similar symptoms in the three other species (mice, rats and monkeys) and apparently similar signs in toxicology studies conducted with other FAAH inhibitors, including in dogs of the same origin. These clearly dose-dependent symptoms, notably interfered with the study plan scheduled in dogs. It planned to test (*versus* controls) doses of 20, 50 and 100 mg/Kg/24h over 4 weeks. Due to the significant symptoms (two dogs, one male and one female from the high dose group had to be sacrificed before the end of the study) and signs of motor incoordination, the doses had to be reduced in the 50 and 100 mg/Kg/24h groups to be able to complete the study. Therefore, the 20 mg/Kg/24h dose was considered to be the NOAEL. As it was impossible to test the highest doses this may explain why another species, the monkey, was used. It theoretically constituted a more appropriate model for the study of an FAAH inhibitor (better sensitivity to stimulation of the endocannabinoid system) even if it was seen not to be the case on the dose estimation plan. It would however seem that the studies in dogs and monkeys started at the same time

Various studies have therefore been conducted in primates (*cynomolgus* or macaque). Maybe due to that which was observed in dogs, these studies came after (unlike what was done for other FAAH inhibitors) an up-titration phase. According to Bial, this aimed to achieve *"stabilisation of the endocannabinoid system"* by monitoring the onset of suggestive signs such as hypothermia (examination of the dossier however shows that it never exceeded 1 degree Celsius), hypolocomotion or reduced dietary intake, etc.
No mortality was observed in the long-term study (13 weeks at 75 mg/Kg after up-titration by level). However, in another group, one female died after up-titration over 12 days (10, 25 and 50 mg/Kg/24h) followed by 9 days' administration of BIA at 75 mg/Kg/24h. The dossier does not say anything specific about this animal.
In the same way, several primates had to be put down *for ethical reasons* during ascending dose studies to test tolerance to the product at very high doses: the two animals from Group 1 on the fourth day of the final level at 250 mg/Kg, the two animals

from Group 2 (125 mg/Kg/24h) and one female from Group 3 after three administrations at 60 mg/Kg/24h, the other animals having survived to the end of escalation at 110 mg/Kg/24h. These premature deaths in primates nevertheless occurred for very high multiple doses, which in *Human Equivalent Doses* (HED), would be equivalent to 78 mg/Kg, 39 mg/Kg and 34 mg/Kg respectively. For purely information purposes, a HED of 78 mg/Kg would be equivalent to around 100 times the highest dose having been tested in humans in multiple doses (50 mg).
During the hearing on 18 March, Bial said it was planning a long-term toxicology study of BIA 10-2474 in monkeys (52 weeks).

The animal studies dossier on BIA 10-2474 generally appears to be of good quality and no aspects of the data that the TSSC has studied constituted a signal likely to contraindicate administration in humans. This is also true for neurological toxicity with central nervous system and autonomous nervous system damage having affected a small number of animals treated at the highest doses. The initially non-alarming nature of the neurological damage observed was confirmed by examination of the slices of tissue concerned, by the TSSC's experts (photographs provided by Bial following the TSSC's request on 18 March 2016).

Several comments deserve to be raised however:

- The reasons that may have led Bial to use four different species (including 2 rodents), which is, for a case of this type, unusual (no other FAAH inhibitor appears to have been studied on this basis) were discussed at length by the TSSC. It is however possible that this may be the result of a change to the trial plan during development: switch from dogs to monkeys due to the poor tolerance observed in the first species (dog and monkey studies appear however to have started on almost the same dates), postponement of the clinical development plan (official response from Bial). It is also plausible that the studies conducted in mice were in fact intended to determine the doses for the (long-term) carcinogenesis studies.
- Caution should be exercised as to the conclusions that could be drawn from relating the doses tested in animals to those administered to the Biotrial trial volunteers (single or multiple doses). The plasma concentration ratios and more specifically the areas under the curve should be used to compare the exposure densities between animals and humans. Regardless, the doses used in animals did not give rise any reservations as to the validity of the preclinical dossier. According to the data provided by the company on the TSSC's request, the reports on the animal/human areas under the curve, where the values are available, are close to 1, except in monkeys in which the margins are wider (ratio of around 6). This confirms that extrapolations from animals to humans in terms of exposure margins and doses to be tested, should consider all species used and not only that appearing to be the best pharmacological model.
- Even if it could not be used to highlight a specific toxicity signal, the safety of BIA 10-2474 during toxicology studies, generally and clearly appears to be under par compared to previously developed FAAH inhibitors. Besides the specific problems of lung toxicity in dogs, the other inhibitors (despite sometimes higher dose ratios than in the case of BIA) did not seem to lead to any toxic effects worth noting. In particular, damage to the Meissner plexus in monkeys was not observed and no early sacrifices were necessary. The better tolerance is for

instance confirmed by the fact that for one of the products, the absence of toxic effects observed led to the use of the highest dose administered in order to determine the NOAEL.

8. Clinical trial conducted in Rennes by Biotrial

The Phase 1, monocentric, *First-in-Human* (FIH) trial planned to include 128 healthy male and female volunteers in total, aged 18 to 55 years, and involved four parts:

- *single ascending dose* (SAD) study,
- *multiple ascending dose* (MAD) study,
- an food interaction open study, and
- a pharmacodynamics study (not done).

We see that dispersion of the ages of the volunteers recruited (18-55 years) is high, some being relatively elderly, compared to what is usually seen in Phase 1, first-in-human administration trials. The ages of the six subjects hospitalised at Rennes University Hospital ranged for instance from 27 to 49 years. Furthermore, several volunteers considered to have a potential risk factor for certain drug-related adverse effects were included. Among others, we note a PR interval measured at over 240 milliseconds on several pre-dose electrocardiograms and blood pressure of over 140/90 mm Hg over four pre-dose readings.

The choice of the first dose administered (0.25 mg) was careful for the SAD part, as it was equivalent to around 1/400th of the highest dose with no observable adverse effect level (NOAEL) in animals.

The SAD part[1] involved 64 volunteers in eight cohorts of 8 volunteers (six receiving the active treatment and two the placebo) for the 8 dose levels tested (0.25 mg to 100 mg); 48 subjects were therefore exposed to the active treatment. Two subjects (one active treatment and one placebo) were tested before administration to the other six.

The MAD part provided for six cohorts of 8 volunteers (six active treatment and two placebo), therefore 48 subjects. The six doses to be tested were: 2.5 mg; 5 mg; 10 mg; 20 mg; 50 mg and 100 mg. Each dose was to be administered for 10 consecutive days. The

[1] As a reminder, we recall the 2006 recommendations of the French Medicines Agency (AFSSaPS) for first-in-human administration (page 4):
"In the same group:
• *number of volunteers receiving the new active substance simultaneously. It is necessary, except otherwise justified with arguments, to limit the number of volunteers receiving the new active substance simultaneously, according to the risk factors identified.*
• *time between administration to one volunteer and administration to the next. A sufficiently long observation period should be provided for between administrations, especially depending on the product characteristics,*
the data available (pharmacokinetic, pharmacodynamic) and on the risk factors identified,
• *criteria for administration to the next volunteer,*
• *criteria for discontinuation of administration to volunteers not yet treated"*

subjects in each cohort were to stay at the Biotrial centre for 15 days (and 14 nights). From the 10 mg dose, administration was based on the pharmacokinetic data measured at n-2 (i.e. that for the 10 mg cohort to start administration of 50 mg). As is the rule in Phase 1, the next dose level was used only if no adverse effects were observed in the volunteers from the previous level, following the study monitoring committee's opinion. In these conditions, the molecule was administered at the same time to all volunteers in the cohort. According to the protocol, *severe* intolerance observed in 4 volunteers in the cohort (therefore 50% of total numbers) was to lead to discontinuation of the trial. Due to the accident that occurred among the volunteers of MAD cohort 5, only 30 volunteers (out of the 36 planned) received the active treatment for this part of the trial with multiple doses.

The food interaction study involved 12 volunteers at the 40 mg dose.

Ninety volunteers in total therefore were exposed to BIA 10-2474 during this trial, at very different doses (0.25 to 100 mg, therefore a ratio of 400), whether in single or multiple daily doses.
The SAD (single dose) part started on 9 July 2015 and ended (cohort 8: 100 mg) on 9 October 2015.
The MAD part (10 days' treatment) started on 6 October 2015. The penultimate cohort (cohort 5, 50 mg) began on 6 January 2016, therefore 19 days after the end of cohort 4 (20 mg).
We therefore note a three-day overlap between the SAD and MAD programmes. This was not at all problematic from a safety point of view due to the weakness (2.5 mg) of the first dose tested in MAD.

On the evening of day five (10 January 2016) and therefore of the fifth administration in the 50 mg MAD cohort (total dose of 250 mg), one of the 6 volunteers having received the active treatment was hospitalised in Rennes University Hospital in a serious condition. Biotrial did not initially consider the relationship between the acute symptoms presented by the subject and the molecule tested to be possible since the other 5 volunteers received their sixth dose the next morning, 11 January at 8 a.m. (total dose: 300 mg), without waiting for the results of ongoing tests (especially MRI scan) on the hospitalised volunteer. The 5 volunteers receiving the active treatment, and not the two subjects receiving the placebo, were in turn hospitalised at Rennes University Hospital between 13 and 15 January, therefore 2 to 4 days after the last administration. The trial appeared to have been effectively suspended on the 11th, since the administrations, which were to continue until the 15th, were discontinued on that date.

Several remarks on this crucial part deserve to be raised:

- The trial in Rennes was conducted in a specialized centre (Biotrial) of sound reputation. The reservation made above as to volunteer recruitment aside, the trial followed currently used protocols for first-in-human /Phase 1 trials. In particular, and unlike what has been wrongly cited by several information sources, there was no time overlap between the various cohorts, especially in MAD. On the contrary, an interval was provided for between the end of one cohort and the start of the next.
- Although not specific to this trial, we can only be astonished that, especially as it is a molecule acting *via* the central nervous system, volunteer selection, inclusion

and follow-up did not include a neuropsychological assessment with clinical interview and cognitive tests. Such assessments have been carried out using suitable scales among others for some other FAAH inhibitors. This point is one of the reasons behind the TSSC's recommendations.

- The dose escalation method (arithmetic, geometric and Fibonacci sequences, etc.) in Phase 1 trials is based neither on a consensus nor on clearly established international recommendations. In the case of the trial in Rennes, the dose escalation appears to be clearly problematic as too abrupt at the end of escalation whereas common sense would be in favour of the reverse. For example, the dose jump between MAD cohorts 4 and 5 (20 to 50 mg) is equivalent to a ratio of 2.5 whereas it is only 2 (2.5 to 5 mg) between cohorts 1 and 2, zone in which the risk can be considered to be low due to the minor difference in absolute value between the two doses. The sequence selected in Rennes is basically geometric (in essence less cautious than arithmetic and Fibonacci sequences) with a ratio (multiplying factor) of 2, also not respected between 20 and 50 mg. This type of sequence does not appear to have been used in trials on other FAAH inhibitors. It appeared to be important to the TSSC that the scientific and regulatory community address this aspect, a major element of volunteer safety (see recommendations at the end of the report).
- A difficult point to decide on in the case of the trial in Rennes is that of the choice of maximum dose to be tested in the volunteers. It was set at 100 mg, whether in single or multiple doses. We saw that this choice was theoretically logical if we extrapolate the data measured in animals to humans: extrapolation of the NOAEL results in a very close estimation (96 mg) and that of the concentration inhibiting FAAH leads to a dose of between 10 and 40 mg. As it is a product not considered to be at risk according to generally established criteria (especially by the European Medicines Agency), use of the NOAEL in the most sensitive species and not the MABEL (*Minimal Anticipated Biological Effect Level*) was legally justified. The problem comes from the apparently very different response in humans on this last point. In effect, we see that FAAH inhibition (alleged mechanism of the pharmacological activity of BIA 10-2474) is achieved in humans at 1.25 mg and is almost complete at 5 mg. In these conditions, the choice of 100 mg is tantamount to testing a dose 20 to 50 times higher than that presumed to be effective, which seems absolutely excessive, even if the rule, which stipulates we should not exceed a dose equivalent to the NOAEL in first-in-human trials, was observed in this case. This major safety issue could not be anticipated when the trial was approved or when it started (only data in animals was known). However, it would have been logical and expected that the dose escalation plan be reviewed in the light of the pharmacokinetic data collected from the volunteers, as it was collected for other FAAH inhibitors. This was not the case in this study.

9. Symptoms observed in the hospitalised volunteers

For obvious reasons relating to protection of privacy and medical secrecy, the information in this report only covers elements useful to the TSSC's mission and is presented in such a way to protect the identity of the volunteers'.

One of the records for the six volunteers in MAD cohort 5 having been exposed to BIA 10-2474 was not selected as the patient, during a routine examination, complained of no specific symptoms and their MRI scan only revealed an image interpreted as an "incidentaloma".

9.1. Clinical symptoms

The first volunteer was hospitalised in the evening of 10 January 2016, day of the fifth administration of the investigational product. Two other volunteers were hospitalised on 11 January (day of the sixth administration), two others on 12 January (day after the last administration) and the last volunteer on 13 January, therefore two days after the last administration.
The main clinical symptoms observed were:

- headaches, in all five volunteers, very severe in one but not occurring as a thunder clap headache,
- cerebellar syndrome in three volunteers,
- consciousness disorders (in three volunteers) ranging from sedation to coma (deceased volunteer),
- memory impairment in two volunteers.

Other symptoms were only noted once: diplopia, paraesthesia of the thighs, and hemiparesis with "tremor" of one side of the body, without pyramidal syndrome, spine pain and stiffness.

The initial clinical picture worsened in three volunteers. The first subject hospitalised progressed to brain death three days after onset of the symptoms. The clinical picture worsened in the other two over three to four days before stabilising (over two to three days), and improving. These two volunteers still had symptoms however (essentially cerebellar and mnestic) when they left Rennes Teaching Hospital.
The two volunteers in whom symptoms were mild, or due to this, hard to interpret, did not see any aggravation and they were able to leave the hospital without any apparent sequelae.
The four volunteers (except the one that died) were treated from 13 January with methylprednisolone (Solu-medrol°) at 1g/24h, without it being possible to determine whether this strong corticotherapy played a role in improvement of the clinical picture.

The clinical symptoms in the five subjects were remarkable in that they were purely neurological, suggesting that only the central nervous system was affected (but with no seizures), without any other symptoms to suggest other organ damage and without the least sign of infection. The bradycardia episodes and hemodynamic instability in the deceased subject aside, no cardiac or blood pressure anomalies were noted.
The progressive, isolated profile of the neurological symptoms goes against a vascular, tumoural or infectious process, and is hardly compatible with an inflammatory, metabolic or toxic process.

9.2. Blood and cerebrospinal fluid (CSF) tests

No metabolic anomalies or abnormal blood test results were observed in the five volunteers. The immunological assessment in the most severely affected volunteer was negative.
The CSF was tested in three subjects. It was normal in one of them, seat of isolated increased spinal fluid protein in another, and highly but non-specifically abnormal in the most severely affected volunteer, with increased spinal fluid protein and the presence of neutrophils, possibly suggesting tissue inflammation or necrosis.

9.3. MRI results

In one of the volunteers, the MRI-scan performed 24 h after administration of the investigational product, only revealed a minor punctiform hypersignal of the right hippocampal body, which was no longer seen on the MRI-scans two and four days later.
For the other four volunteers, the MRI-scan showed anomalies of highly variable intensity, affecting the hippocampus and the pons (protuberance) predominant in the anterior part (extending at times to the bulb or to the mesencephalon)**, bilaterally and symmetrically**. In the deceased volunteer, and only in this person, the MRI-scan two days after the initial examination, showed involvement of the thalamus and of the cerebral cortex.
The anomalies observed in the four volunteers were of the same type but of variable severity, with:

- 1. Diffusion hypersignal and drop in the apparent diffusion coefficient (ADC) seen once, and indicating water diffusion restriction (possibly suggesting cytotoxic oedema, regardless of the cause, but also inflammatory cell infiltration for instance). In the most severely affected volunteer, the diffusion hypersignal came with an increase in ADC in the posterior part of the brain stem, which could suggest vasogenic oedema.
- 2. Hyposignal in SWI (*Susceptibility Weighted Imaging*), interpreted in this context as suggesting the presence of blood (haemoglobin), in the form of multiple, small, rounded hyposignals reflecting microbleeds.
- 3. Non-specific, FLAIR (*Fluid Attenuated Inversion Recovery*) hypersignal, possibly related to an increase in water content, demyelination, gliosis or necrosis.

These signal anomalies are identical in the hippocampus and pons in the four volunteers, and in the cerebral cortex in the most severely affected of them. This suggests that the cortical damage was caused by the same mechanism and not by anoxia from the bradycardia episodes.

The signal anomalies observed reflect therefore the presence of microstructural changes with a vascular component (microbleeds) which are not specific to a given mechanism. However, their bilateral and symmetrical topography and the presence of hyposignals very early on in SWI, theoretically mean we can rule out an inflammatory process. The highly unusual topography makes a primary microvascular mechanism unlikely and is more so compatible with a toxic/metabolic process.

9.4. Comments

The circumstances of occurrence, the stereotypical nature and progressive profile of the neurological symptoms strongly suggest that the product tested caused them. The anomalies seen on the MRI-scan, which are consistent with the clinical symptoms, also strongly suggest a toxic or metabolic mechanism, given their signal characteristics and their bilateral and symmetrical topography. Imputability of the investigational product to the neurological damage therefore appears to be clear.

The highly unusual topography (and perhaps thus far unique) of the lesions (hippocampus, pons, thalamus, cortex) is a key element in identifying the mechanism by which the substance tested could have caused such effects.

10. Detection of signs of toxicity in the other volunteers

One of the most striking elements of the BIA 10-2474 case is the absence of toxicity (adverse event of noteworthy intensity, *a fortiori* serious), in particular neurological, observed in the trial volunteers other than those in MAD cohort 5. This, despite administration of single doses of up to 100 mg or 10 multiple administrations of up to 20 mg/24h, for a total dose of 200 mg (note: the total doses in the hospitalised volunteers ranged from 250 to 300 mg).
Among the 76 volunteers (except MAD cohorts) having received the active treatment, 18 adverse events were observed, 11 of which (frequency: 14.5%) were cardiovascular (orthostatic hypotension, reflex tachycardia, PR or QT interval prolongation on the electrocardiogram, etc.), and there were cases of mild dizziness or headaches.
The observations were of the same type for the volunteers in the MAD cohorts, no events of noteworthy seriousness or severity, and cardiovascular symptoms being predominant. It should be noted however that two volunteers from the 10 mg MAD cohort presented with *blurred vision* on two occasions. These episodes, which lasted between ten and thirty minutes each time, cannot, as is, be qualified as diplopia, a symptom which corresponds to a specific definition in neurology. Therefore, the trial investigator and the monitoring committee did not consider this symptom to be relevant, and also it was not observed in the volunteers in the cohorts exposed to higher doses.

Since suspension of the trial, all volunteers to whom BIA 10-2474 was administered, have been contacted for a full clinical assessment and brain MRI-scan. To date, no clinical or biological or MRI anomalies likely to be related to administration of the molecule or the trial conditions have been detected.

The safety data from first-in-human and Phase 1 trials of other FAAH inhibitors seems to have the same qualitative profile (sedation, digestive disorders, orthostatic hypotension, dizziness, etc.) with one difference, however, in the frequency of the adverse effects, which although they vary greatly from one product to another, generally appears to be lower than in the case of BIA 10-2474.

11. Pharmacokinetic data

Generally, pharmacokinetic studies conducted in animals do not give rise to any specific remarks, even if as is fairly often the case, pharmacokinetics appear to become non-proportional with the highest doses, at least in dogs and monkeys. For example, the ratios of the areas under the curve (AUC) and of the doses administered, which are supposed to remain constant, were 960 at 0.1 mg/Kg and 1,886 at 1 mg/Kg.
A study of (oral and intravenous) administration of the radiolabelled product showed the very good tissue diffusion of BIA 10-2474 and its very large volume of distribution.
The pharmacokinetic studies conducted on the volunteers from the SAD cohorts showed that the elimination half-life of BIA 10-2474 is gradually extended when doses administered become high; the areas under the curve (AUC), reflecting exposure, also increased more rapidly than the doses increased. This, from a purely theoretical standpoint, could be explained by the acceleration in absorption beyond a certain threshold (of the barrier breach, facilitation of passage, carrier induction type, etc.), or, a lot more likely, by saturation of elimination processes for a dose of between 40 and 100 mg, without it being possible to more accurately identify the threshold dose at which non-proportionality begins.
During MAD studies, the same phenomenon was observed, the AUC increasing more rapidly than the doses from 20 mg. We especially see that:

- Dispersion in the pharmacokinetic parameters among the volunteers had a strong influence on individual AUC values, which were larger at 50 mg than at 20 mg.
- Again for 50 mg, and like what was less clearly observed for 20 mg, residual BIA 10-2474 plasma concentrations continued to increase slightly up to the fifth and sixth administration. The plasma concentration steady state did not appear to be reached for some subjects in MAD cohort 5. This strongly suggests that the elimination half-life of BIA 10-2474 was longer in these subjects than estimates made from single doses or lower multiple doses (10 mg). For instance, the elimination half-life calculated in the 100 mg SAD group was around 10 hours on average, a value theoretically incompatible with what was observed in the 20 and 50 mg MAD cohorts.
- As in SAD, non-proportionality is therefore likely as of 50 mg multiple doses.

The four metabolites identified in animals are expected to be the same in humans, two of them (BIA 10-2639 and BIA 10-2445) reached measurable plasma concentrations but remained however very low (<3% of those of the parent product). Without directly administering the metabolites themselves, it is difficult to determine their individual characteristics. However, it seems that the variability in the pharmacokinetic parameters was higher for these two metabolites than that observed in animals, with, for example, an elimination half-life estimated (highly approximately given the very low concentrations) to vary from 4 to 23 hours.
Variability also affected, but to a lesser extent, the pharmacokinetics of the molecule itself. This is commonly observed with medicinal products due to interindividual variations in metabolism, among others; some (qualified as *outliers*) can have very different parameters from the other members of the group. In the case of a Phase 1 trial, variability can become problematic if the dose calculations are based, as is the case here, on the means of the key parameters measured in a small number of volunteers from lower doses levels. By definition, such an approach does not take account of the extreme

values in subjects expressing a specific response, distribution of which can vary from one group to another. This is likely to induce fairly significant prediction errors (see recommendations at the end of the report).

12. Hypotheses studied in an attempt to explain the accident in Rennes

The accident that occurred during the trial on BIA 10-2474 at the Biotrial centre in Rennes is unquestionably astonishing and unprecedented in:

- its seriousness (several volunteers from the same cohort having to be hospitalised, one of the being deceased in the days following admission),
- the fact that apparently, the toxicology studies, although conducted on four animal species up to very high doses, did not reveal any lesions or picture that could predict such a specific neurological toxicity,
- the highly unusual nature of the clinical and radiological picture of the brain damage observed in several volunteers in MAD cohort 5, unlike anything seen to date,
- the fact that no patent neurological or radiological signs of this type have been found in the other volunteers (some having absorbed up to 100 mg in a single dose or total dose of 200 mg over 10 days),
- finally, the fact that the accident occurred with a molecule similar to other compounds (around ten) the development of several of which was abandoned due to insufficient efficacy, without any neurological or other toxicity being observed.

In terms of formal logic, the fact that toxicity only occurred in one of the 14 cohorts of volunteers having received BIA-2474, can only be explained by:

- an administration error or procedure specific to this cohort,
- a common feature among the six subjects having presented with signs of toxicity,
- an effect relating to the cumulative BIA 10-2474 dose that the subjects received.

Exploration of the first hypothesis did not fall within the scope of the TSSC's missions but this hypothesis seems very little likely. Procedures for controlling the dose administered are very tight in Phase 1 trials. Also, the product contained in the capsules administered to all volunteer groups was the same as that used for the toxicology studies and was later tested and revealed to be of very high purity.
The TSSC therefore mainly focussed on the other two hypotheses.

12.1. Hypothesis of a common feature among the volunteers in the fifth MAD cohort

Several possibilities were discussed:

12.1.1. Hypothesis of infectious contamination

The close living conditions of the volunteers in the same cohort and consumption of the same foods several times a day could support this hypothesis, especially as certain infections, possible in this context, can be expressed by the central nervous system. For example, there is a rhombencephalic form of listeriosis with lesions located in the same areas as those observed in Rennes. This hypothesis is however very little plausible.

Firstly, this clinical form is very rare in humans (the most common neurological form of listeriosis being meningoencephalitis). It would have then had to only affect volunteers exposed to the active treatment. The two conditions make up a highly unlikely scenario in statistics terms. Finally, and above all, analysis of the symptoms presented by the volunteers and of the MRI images does not fit with this hypothesis.

12.1.2. Hypothesis of an interaction with other products

Mentioned several times in the days following the announcement of the accident in Rennes, an interaction with medicinal products, foods (such as chocolate) or recreational substances (alcohol, narcotics including cannabis, etc.) could have occurred. The "medicinal products" hypothesis appears to be resolutely unlikely given Phase 1 good practices. Especially as the six subjects hospitalised would have to have taken one or several of the same medicinal products even though they were of different ages (27 to 49 years) and had very different profiles.

Pharmacodynamic or pharmacokinetic interaction between BIA 10-2474 or one of its metabolites and a food also appears little plausible. Analysis of literature did not reveal any examples of central nervous system toxicity, with symptoms suggesting that observed in Rennes, caused by drug/food interaction of any type. The same applies to consumption of chocolate by the volunteers (as the accident occurred in the two weeks after Christmas). Chocolate only contains small quantities of anandamide. Overall, hyperstimulation of the endocannabinoid system is not known to generate a picture of the type observed in Rennes (see further on).

Modification of the BIA metabolism by food or beverages (i.e. acting as inhibitor or inducer of the cytochromes P450 pathway) cannot, per se, be ruled out, but seems little plausible.

To date, there is no evidence to support the hypothesis of consumption of narcotics by the volunteers, immediately before or during the stay at the Biotrial centre. The results of ongoing inspections and investigations will from this standpoint be able to invalidate or confirm such a hypothesis. However, besides the serious breach of Phase 1 good practices (in terms of volunteer monitoring especially), that such, theoretically collective, consumption would represent (all MAD cohort 5 volunteers hospitalised would have to have taken the same substance and none from previous dose levels would have to have taken it), this hypothesis comes up against the previous argument. It effectively seems to be accepted by neuroscientists, that direct or indirect, even massive stimulation of endocannabinoid receptors, CB1 in particular, would not induce toxicity such as that seen in Rennes. Even if in certain subjects they can induce severe psychiatric effects (i.e. psychotic episode), neither cannabis, nor its main active substance, tetrahydrocannabinol lead to acute toxic brain damage, even experimentally and at very high doses. Clearly, such consumption could at best be considered a cofactor, but certainly not a triggering factor and even less so the cause of the accident.

12.1.3. Hypothesis of a specific genetic or metabolic characteristic or common pharmacological response among the subjects in the fifth MAD cohort

There are several genetic factors, among others, likely to modulate individual susceptibility to administration of an FAAH inhibitor. For example, as we have seen, this hydrolase exists as two isoforms (FAAH-1 and FAAH-2) with different activity. In the same way, the cytochrome P450 system is found at several levels, the activity of which can vary significantly from one individual to another. As appealing as it may seem, this

hypothesis clashes with statistics laws. For the FAAH example, if we consider that prevalence of carriers of both isoforms is 38% in the general population, the probability of finding it in 5 out of the 6 exposed cohort subjects was a less than 3 in 100 chance and 3 in 1000 chance in the six subjects exposed. If we recalculate, which would be more relevant, not with 38% but 5% (prevalence of carriers of the low activity isoform only), the results thus completely rule out such a possibility. The same applies to the probability of having included by accident a majority of rapid metaboliser subjects in a previous cohort, which could have biased the pharmacokinetic predictions for calculation of the dose to be administered to volunteers in MAD cohort 5.

It is therefore evident that the symptoms presented by the volunteers in cohort 5 can only be related to the cumulative dose of BIA 10-2474 administered to them in multiple daily doses.

12.2. Hypotheses of a threshold effect relating to total BIA 10-2474 dose

Even if this second set of hypotheses appears a lot more likely, the potential mechanisms are especially numerous, some remaining purely hypothetical. They may involve the molecule itself and/or a mediator such as anandamide.
Let's not first of all forget the highly unusual nature of this dose-dependent toxicity, apparently with no portent signs reported in volunteers having been exposed to lower doses. It happened "*as if something gave way or swung suddenly at a specific dose or concentration threshold*". Expression of this type may be compatible with the fact that BIA 10-2474 is characterised by a very steep dose-effect curve (ranging, within a fairly narrow concentration range, from absence of FAAH inhibition to complete and highly extended inhibition) and by the fact that the pharmacokinetics of BIA become non-proportional in humans from a dose of between 40 and 100 mg.
Let's also not forget that BIA has the characteristics of a molecule with the ability to bind (and, therefore, potentially inhibit) cerebral hydrolases other than that which is its pharmacological target. The specificity of BIA 10-2474 for FAAH is clearly lower than that of other inhibitors developed previously. It was also administered to the volunteers in MAD cohort 5 at doses around 10 times higher than that which appears to completely inhibit FAAH in humans for a very long period.
It is therefore highly likely, not to say almost certain, that the mechanism causing the accident in Rennes should be looked for outside the endocannabinoid system, especially as stimulation of endocannabinoid receptors by anandamide cannot theoretically cause this type of toxicity.

Several mechanisms can be discussed:

<u>12.2.1. Inhibition of other cerebral hydrolases by BIA 10-2474</u>
This avenue and the next (see 12.2.2) should be favoured in the initial analysis due to their biological plausibility. Let's not forget that BIA 10-2474 was administered to the volunteers in MAD cohort 5 at a dose (50 mg) probably more than 10 times higher than that fully inhibiting FAAH activity. Increasing tissue concentrations beyond those already completely inhibiting the enzyme cannot, in any circumstances, increase the pharmacological effect, but has every chance of promoting (especially with such a little-specific product) binding to other serine hydrolases. This could, via a direct or indirect mechanism (unknown to date), be the cause of the brain damage observed in Rennes.

It is interesting to note that such damage was not apparently observed in animals, despite the use of very high doses, or in the six volunteers having received a single 100 mg dose of BIA. It occurred from day five of daily administration of 50 mg. This finding is by no means incompatible with the hypothesis:

- the mechanism involved could be attributed to, as is fairly common in toxicology and pharmacovigilance (i.e. cumulative toxicity), to late expression,
- the specific pharmacokinetic features of the molecule (non-proportional pharmacokinetics, large volume of distribution) described above, make gradual accumulation of the molecule in the brain tissue plausible. Concentrations could have reached a trigger point on day five or six of administration.

The difference in animal/human susceptibility seems more difficult to explain. Such features, one way or the other, have already been observed however. It is not a moot point either to note that the doses required to achieve FAAH inhibition were around 10 times lower in humans than those reached during the studies in animals.

12.2.2. BIA 10-2474 toxicity via another mechanism

The starting point, as in the previous scenario, is believed to be an excess of free BIA 10-2474 in brain tissue related (1) to a too high dose administered for endocannabinoid FAAH blockade, and, undoubtedly, (2) to the molecule's pharmacokinetic characteristics (kinetics becoming non-proportional, possible accumulation in tissue from multiple doses, etc.). The difference here is that the pathogenic mechanism is not believed to occur by inhibition of other serine hydrolases but by *in situ* toxicity of the molecule on the cell structures or proteins involved, for instance, in exchanges in the blood-brain barrier. A credible hypothesis targets the imidazole-pyridine "leaving" group of BIA 10-2474 which could either bind to brain proteins or inhibit the cytochromes P450-epoxide system by leading to vasospasm. In effect, in the case of BIA 10-2474, and unlike other FAAH inhibitors, the imidazole nucleus is in adjacent position to the electrophilic carbon, the site of binding with FAAH. As before, this hypothesis comes up against the fact that no toxicity of this type was observed in animals, despite administration of high doses. The responses to this objection are the same as above.

12.2.3. Toxicity from a BIA 10-2474 metabolite

Toxicity from one of the four peripherally-circulating metabolites (plasma) in humans and animals could also be envisaged. Their specific toxicity has not been tested by Bial, however these metabolites are produced in very small quantities (<3% of BIA 10-2474 circulating concentration) even if pharmacokinetic variability seems to be higher in humans than in animals. All of the known BIA metabolites have a structure similar to that of the mother molecule, and there is no evidence in the dossier to support specific toxicity from any one of them. It is also possible (as is often the case during metabolisation processes) that these derivatives are more hydrophilic than the parent product. This would tend to make crossing of the blood-brain barrier more difficult, unless it is presumed that there is a specific carrier and/or efflux pump inhibition during the rise in circulating concentrations from multiple doses. This assertion should however be weighed against the fact that Bial said it planned to develop several of these metabolites as FAAH inhibitors, which theoretically suggests that they cross the blood-brain barrier in significant amounts.

The purely theoretical hypothesis of a metabolite with strong tissue tropism, non-quantified in humans due to the very high tissue/plasma ratio and very low resulting plasma concentrations remains to be envisaged.
Finally, the effect of genetic polymorphism, leading for example to the production of a metabolite in larger quantities in some individuals, would have to be ruled out it seems for statistics reasons (if this exists, it would have to have been present in all hospitalised volunteers in MAD cohort 5).

12.2.4. Suspected anandamide-related toxic effects

FAAH activity blockade leads, as we have seen, to an increase in intracerebral anandamide concentrations, which makes it possible to envisage several possibilities:

- *Anandamide binding to other receptors*
  Anandamide is a mediator, the ubiquity of which largely exceeds the endocannabinoid system. It is able, especially when its concentrations increase, to interact with several types of receptors (at least TRPV1, PPAR and NMDA) and with the MAP-kinase pathway, having possible consequences on apoptosis and neuroprotection.

- *Toxicity from anandamide degradation products*
  In the event of complete and prolonged FAAH inhibition, anandamide can be degraded by the cyclooxygenases pathway, giving rise to various compounds (leukotrienes and prostanoids) some of which have known effects on cerebral vasomotricity, which may be compatible with some of the lesions observed in the MAD cohort 5 volunteers.

The plausibility of the last two hypotheses is however strongly challenged by a serious of counter arguments:

- Administration, including at high doses, of anandamide or its non-metabolisable analogues (i.e.: methanandamide) to animals, is not known to induce neurologic toxicity, at least of the type examined in this report.
- This type of toxicity has not been observed (in humans or in animals) with other theoretically more specific FAAH inhibitors, including with those described as irreversible, even during administration of high multiple doses.
- Complete and lasting FAAH inhibition, and apparently therefore the intracerebral anandamide concentration plateau, would appear to be reached in humans from BIA 10-2474 doses of around 5 mg, whereas no neurological toxicity was observed in the volunteers having received multiple doses (10 days), of up to four times that dose (20 mg).

13. TSSC conclusions

The accident affecting several volunteers in the trial conducted by Biotrial would clearly appear to be related to the molecule tested.
It is very little likely that the toxicity be related to stimulation of the endocannabinoid system *via* FAAH (pharmacological target of BIA 10-2474) inhibition. A toxic mechanism relating to the increase in intracerebral anandamide concentrations can also apparently be ruled out.

In the current state of knowledge, imputability to one of the known BIA 10-2474 metabolites does not appear to be favoured either. We should note however the time to onset of the toxic effects observed in MAD cohort 5 which could be compatible with the production of a metabolite which, due to the longer elimination half-life than that of the parent compound, could accumulate in the tissue compartment gradually during administration until the concentration reaches a trigger point.
Notwithstanding, the most likely hypothesis to date is that of toxicity specific to the molecule *via* its binding to other brain cell structures, facilitated by:

- its low specificity for its target enzyme,
- use of multiple doses a lot higher than those leading (at least in humans) to complete and lasting FAAH inhibition, and
- its probable gradual accumulation in the brain, undoubtedly related to the specific pharmacokinetic features of BIA 10-2474. This is likely to explain why the accident in Rennes only occurred on day five of administration of a 50 mg dose, and not in the volunteers having received a single dose that was twice as high.

At this stage, it is difficult to favour one toxicity mechanism out of the two most likely: inhibition of other serine hydrolases, or harmful effect from the imidazole-pyridine "leaving" group.

The fact that this type of toxicity was not observed in animals despite administration of very high doses, remains unexplained so far. We should note however that BIA 10-2474 is around 10 times more active in humans than in animals in terms of FAAH inhibition.
The sudden onset of the toxic symptoms could be related to BIA 10-2474 being characterised, with hindsight, as "little manageable" due to relative low efficacy (inhibitory concentrations in the micromolar range), low specificity and a particularly steep concentration-effect curve. In these conditions, the little comprehensible acceleration in dose escalation in the 20 and 50 mg MAD cohorts probably significantly contributed to the accident. With this in mind, such an increase in dose was all the more risky given that due to cohort sequence timing and the time required for tests to be carried out, the latest pharmacokinetic data available was that of the 10 mg cohort subjects. Such a sequence made it almost impossible to adjust the dose to be administered in the light of the emerging non-proportionality. This would have been even more problematic for the last dose initially planned, that of 100 mg (dose for which the pharmacokinetics are probably clearly non-proportional) since adjustment for safety reasons would have been based on the 20 mg cohort data.

It was not within the TSSC's scope (unlike the two ongoing inspections) to comment on the basis to the trial authorisation issued by the ANSM following the opinion from the Brest ethics committee. In scientific terms, the TSSC considers however that:

- BIA 10-2474 could not, theoretically, be considered as an at-risk product, according to the terms of applicable recommendations especially the *Guideline on strategies to identify and mitigate risks in first in human clinical trials with investigational medicinal products (Committee for Medicinal Products for Human use, CHMP, EMA*, 2007).
- The data provided, especially the Investigator Brochure, did not contain information, especially data on toxicology, suggesting a specific risk during first-in-human use. We should recall however that the brochure contains many

mistakes, inaccuracies, figure inversions or incorrect translation of source documents, making understanding difficult in several aspects. This is highly surprising given the regulatory importance of this document.

14. TSSC's recommendations for the conduct of first-in-human trials

The seriousness of the accident in Rennes calls for international legislation and good practices on first-in-human trials to be amended in several areas. In effect, even if the BIA 10-2474 dossier and protocol of the trial conducted by Biotrial are in keeping with applicable provisions and recommendations, it's more in the rules than in the mind. Meeting regulatory requirements should forego neither the bases to pharmacology and clinical practice, nor the ultimate therapeutic aim of drug development. The supremacy of rules over common sense and scientific logic suggest a potentially dangerous change of direction and calls for collective awareness to be raised. The accident in Rennes illustrates this in a tragic manner.
The TSSC therefore puts forward six recommendations that it would like to see examined by European and international regulatory bodies, and by other relevant associations and bodies.

- 1. First of all, a medicinal product is developed in the ultimate aim of demonstrating its therapeutic effectiveness and its utility to public health. Therefore, justification and demonstration of pharmacological activity predictive of efficacy in humans cannot be considered to be secondary. In the case of BIA for example, out of the 63 pages of the Investigator Brochure summarizing the preclinical data, fewer than two discuss demonstration of pharmacological activity for the apparently planned indication. The tests did not make it possible to determine an effective dose either before lengthy, costly and never risk-free preclinical and clinical development took place. The tests revealed BIA 10-2474 to be a product with, at best, potential moderate efficacy in the planned indication, and in any case clearly lower efficacy than the comparator product (data deleted from the Investigator Brochure).
  A prerequisite essential to any form of clinical development, would be to conduct sufficiently comprehensive preclinical pharmacology studies, comparative whenever possible, on a sufficiently broad dose range (to establish a dose-effect curve where appropriate) so as to be reasonably predictive of real-life, future therapeutic efficacy. Prior justification should be clearly emphasised by the sponsor and studied as a priority during the course of initial opinion requests (ethics committees) and authorisation applications.

- 2. A neuropsychological assessment with clinical interview and cognitive tests should be a compulsory part of assessment during volunteer screening, inclusion and clinical monitoring in a Phase 1 trial for drugs with "central nervous system" tropism. This (not provided for in the Rennes protocol) could identify potentially at-risk subjects and to detect behavioural changes or neuropsychological disorders early on during exposure to the investigational product.

- 3. All first-in-human and Phase 1 protocols should, unless unnecessary, provide for the doses to be tested in volunteers to be adjusted according to the data collected in volunteers already having been exposed during the trial. This obviously concerns (as is usually the case, especially in the protocol in Rennes) dose adjustment according to the pharmacokinetic parameters from the previous dose level (in fact, from level n-2 in the case in Rennes). It should also concern the pharmacodynamic data. In the case of BIA 10-2474, if it was confirmed that FAAH inhibitory concentration was 10 times lower in humans than in animals, the choice of the 100 mg highest dose (20 times the dose inducing complete inhibition) was no longer justified and could carry a risk. Concerning dose adjustment according to the pharmacokinetic parameters measured, variability and its extremes, and not only the mean of these parameters, should be taken into account in the calculations in order to provide for a worst case scenario.

- 4. During first-in-human and Phase 1 trials, volunteer safety should take precedence over any practical, economic or regulatory considerations. To this effect, pluridisciplinary work at international level is required to redefine methodology options that provide for both an acceptable study duration and optimal level of safety. For example, as for single-dose study practices, the dose administration sequence could be transferred to MAD so as not to expose all subjects from the same cohort at the same time. In the same way, the timing of the various cohorts should make it possible to have the pharmacokinetic parameters from subjects from the dose level immediately below (n-1); a larger jump may be problematic in the event of non-proportionality of the pharmacokinetics with the dose administered.

- 5. Dose escalation strategies in first-in-human and Phase 1 trials should take account of considerations based on common clinical and pharmacological sense. For example, in the text by the European agency cited above, it only says the following: "*Dose increase should proceed with caution taking into account identified risk factors from non-clinical studies*". As we have seen, the triggering factor in the accident in Rennes could be the choice of dose to be tested which was too high, given the new data collected in humans and more rapid dose increase within the potential risk area. With this in mind, the geometric sequencing (especially of 2 or more) maintained until the end of dose escalation did not appear to be reasonable. The TSSC therefore recommends that geometric sequencing be avoided where possible, or at least the ratio be reduced at the end of escalation.

- 6. Finally, the TSSC would like, notwithstanding industrial property considerations, to see a debate opened at European and international level, on access to data from ongoing or previous first-in-human and Phase 1 trials. This would unquestionably be an advance in terms of the protection of subjects participating in biomedical research. For example, comparison with the protocols of studies on products developed previously, or easier access to toxicology and clinical safety data would enable highly useful comparative analysis, especially when analysing protocols in view of issuing opinions or authorisations.

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