Minutes of the Temporary Specialist Scientific Committee (TSSC) meeting on "FAAH (*Fatty Acid Amide Hydrolase*) Inhibitors" of 15 February 2016.

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Foreword

This TSSC was set up by the French National Agency for Medicines and Health Products Safety (ANSM), following the accident that occurred during the Phase 1 first-in-man clinical trial on the molecule BIA 10-2474 in Rennes, in January 2016. The scientific missions of the Group, were, on the basis of the available data and expertise of its members:

- to better understand the mechanisms of action and potential toxicity of substances which, like BIA 10-2474, have a direct or indirect effect on the endocannabinoid system and, with that understanding,
- put forward and list hypotheses to be able to explain the accident which occurred at Rennes,
- to establish, where appropriate, general recommendations aiming to tighten safety during first-in-man Phase 1 trials.

The members of the TSSC, after having studied data from literature and the documents provided to them (BIA 10 2474 preclinical data, data on the trial conducted at Biotrial), met in a one-day plenary session on Monday 15 February 2016.

Reminder on the endocannabinoid system

BIA 10-2474, by the Bial pharmaceutical company (Portugal), is introduced as a "reversible" inhibitor of FAAH, an anandamide-degrading enzyme (hydrolase), 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 in several aspects.
There are **two types of receptors** (CB1 and CB2), transmembrane and G protein-coupled receptors (inhibiting adenylcyclase).
- 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, medulla oblongata, cortex, 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 endocannabinoids have been identified to date. They are bioactive lipids acting both as neurotransmitters and neuromodulators, produced "on demand", unlike conventional neurotransmitters which are released from storage vesicles. The three main lipids are:

- anandamide (AEA), isolated in 1992; its concentration in the brain is similar to that of dopamine or acetylcholine,
- 2-arachidonylglycerol (2-AG), arachidonic acid ester,
- 2-AG ether (arachidonic acid ether).

Like THC, anandamide has preferential affinity for the CB1 receptor and low affinity for the CB2 receptor. Conversely, 2-AG has high affinity for both receptor types and it can therefore be seen as the "real" endocannabinoid system mediator, whereas AEA, which has almost no effect on CB2, is able to interact with several other systems. Anandamide is therefore **little specific of the endocannabinoid system**. In effect:

- it is able to activate TRPV1 (*transient receptor potential vanilloid 1*) which are non-selective ion channels from the TRP channels group,
- it is a good agonist for PPAR (*peroxisome proliferator-activated receptor*) alpha and gamma, nuclear receptors involved in the energy metabolism and inflammation process,
- it interacts with 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 apoptosis and neuroprotection phenomena by the MAP-kinase pathway, 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,
- catalepsy.

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

After release 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, there are two FAAH isoforms in human; prevalence of the low activity form is believed to be around 38% in the general population.
Where there is inhibition of FAAH activity, AEA concentrations increase, however an additional degradation pathway takes over: that of the cyclo-oxygenases. 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, well documented.

Points emphasised by the Group:

- among the "endocannabinoids", anandamide is that which it is believed the TSSC should focus on as part of its investigations,
- this biolipid acts on several other systems, some of which could be relevant to the question raised: vanilloid system receptors (TRPV1), PPAR alpha and gamma, NMDA glutamate receptors,
- anandamide is usually degraded by a hydrolase (FAAH), the activity of which is inhibited by BIA 10-2474. In the brain however there is a large number of other hydrolases (around 200), with more or less similar structures, the roles of which are far from being fully elucidated. The ability of BIA 10-2474 to bind to some of them and inhibit their action cannot be ruled out,
- FAAH inhibition leads to an increase in anandamide concentrations which is then catabolised by an additional pathway (cyclo-oxygenases); this gives rise to several compounds, some of which could have a harmful effect, especially on brain circulation,
- even massive stimulation of the endocannabinoid system per se, is not known, alone, to lead to significantly serious toxic effects. This strongly suggests that an *off-target* effect could explain the accident in Rennes.

Molecule BIA 10-2474

Bial would appear to have planned to develop BIA 10-2474 mainly as an analgesic. Examination of this molecule does not theoretically raise any specific questions. Its originality is relative as it could appear to be a "me-too" of several molecules previously developed as FAAH inhibitors such as PF-3845 by Pfizer and JNJ-42165279 by Janssen. They are heterocyclic compounds with pyridine, piperazine and pyridazine nuclei … and especially a urea function, which is the site of a nucleophilic attack from the oxygen in the enzyme's serine 241. Item of interest: clinical development of several of these compounds was abandoned after Phase 2 clinical trials due to insufficient effectiveness (analgesic especially) without any specific toxicity being noted in humans or animals.
From a structural viewpoint, BIA 10-2474 would effectively appear to be an irreversible inhibitor of FAAH (and not reversible as stated by the pharmaceutical company). To this effect, it is similar to the irreversible inhibitors already cited. The irreversible nature of the inhibition induced by the latter was clearly demonstrated in the covalent type bonding with the enzyme. Also, *reversible* FAAH inhibitors generally belong to other chemical compound classes.
An important difference with known inhibitors, in particular the compounds developed by Pfizer, concerns specificity for FAAH. It is, for example, extremely high for one of the Pfizer molecules, with a ratio of about 14,000 between the inhibitory concentrations (IC_{50}) for FAAH (7.2 nanomolar) compared to those inhibiting a panel of around twenty other hydrolases (100 micromolar). In the same way, Janssen & Janssen tested the selectivity of their JNJ-42165279 (for which, again, no toxicity has been seen in Phase 1) against 50 different enzymes.
The dossiers provided to date do not discuss the specificity of BIA 10-2474 for FAAH compared to other hydrolases. This has to be documented to be able to determine the plausibility of an off-target effect. Especially as the IC_{50} of BIA 10-2474 measured in rats (1.1 to 1.7 micromolar, equivalent to around 200 times the Pfizer molecule) is that of a compound with a **relatively poor specificicity for the endocannabinoid FAAH**.

Another shortcoming concerns the potential toxicity of identified BIA 10-2474 metabolites. The imidazole cycle is a "leaving group" that can produce an isocyanate to which many brain proteins are likely to bind. The potential "intracerebral" metabolism is undoubtedly a more promising avenue for the observed toxicity than the peripheral molecule metabolism. The latter appears to be low although in the dossier submitted by Bial, a high metabolism is mentioned ("*extensive metabolization*"). During single-administration trials in humans (see further on), four metabolites were identified in plasma, two undetectable and two measured but with much lower concentrations than those of the mother molecule. These small quantities (<3%) which do not legally have to be characterised, do not theoretically plead in favour of toxicity via this pathway, unless we accept that one of metabolites, is extremely reactive and toxic at very low concentration levels.

Points emphasised by the Group:

- BIA 10-2474 is structurally similar to other existing FAAH inhibitors; development of several of them was interrupted in Phase 2 due to insufficient effectiveness, without any specific toxicity being observed in humans,
- the structural relatedness and analysis of its chemical structure would more so bring us to consider BIA 10 2474 as an irreversible and not reversible FAAH inhibitor,
- BIA 10-2474 would appear to be a lot less specific to FAAH than its predecessors, making binding to other cerebral enzymes plausible. This possibility, and as it has been done for other compounds, must absolutely be documented by Bial.

Animal toxicology data

Opening remark: interpreting animal toxicology data is always complex. Studies are conducted at doses which can be very high, incommensurate with the highest doses tested in humans. Therefore, highly varied toxicity symptoms, often aspecific, clinical or only visible after sacrifice (in macroscopy or microscopy), are observed in most animals. There is therefore a strong probability that elements appearing to indicate toxicity that we would look for later on are found within the data. To avoid this conventional interpretation bias, the TSSC closely examined the particularly extensive dossier of animal studies conducted, which must be looked at as a whole and in its context.

Preclinical studies seem to have been conducted according to currently approved standards (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.
The studies covered, which is little common and therefore surprising (this point should be clarified), four different species (rats, mice, dogs and monkeys) in two centres accredited by the European Medicines Agency (EMA) of sound reputation (Harlan Laboratories SA in Spain and AnaPath GmbH in Switzerland).
The toxicology data for BIA 10-2474 appeared to be complex to analyse and the TSSC declared it essential to have more detailed information on several important points, before its meeting of 24 March (see further on).

On the basis of the data that could be analysed to date, and generally, up to very high doses, we do not observe any specific toxicity, even if one of the most commonly observed toxic effects is that on spermatozoa.
The four BIA 10-2474 metabolites identified in plasma are likely to be those found in humans and apparently produced in very small quantities (around 1% of the parent product), and this in the four species. Therefore, specific toxicity studies for these metabolites were not legally compulsory and were not conducted.
We do not observe accumulation of the product or of its metabolites in repeated-dose studies (over 13 weeks).
The NOAEL (*No Observable Adverse Effect Level*) and NOEL (*No Observable Effect Level*) seem to have been correctly determined.
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, at this stage of analysis of the case, 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 some 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 (therefore, 650 and 195 times the highest dose, 50 mg, respectively, having been tested in repeated administration in the volunteers in Rennes). The damage, discussed by the Group given the context, appears to be common in rodents in such studies and does not in principle seem to be of the type to generate a signal. In the same way in primates and rats, cerebral damage and especially of the autonomic nervous system (Meissner's plexus in the bowel) was observed in some animals treated with a high dose.
In the group of dogs treated for 13 weeks, lung alterations clearly visible in macroscopy and confirmed in microscopy (bronchopneumonia/focal and multifocal acute alveolitis) were observed. These symptoms appear to be surprising due to their frequency. The toxicology report submitted by Bial links these lesions to bronchial inhalation of BIA 10-2474 powder. This hypothesis seemed little plausible to the TSSC experts. The relationship with the existence of high CB1 receptor density in the lungs, even if, without additional investigation, it cannot be ruled out, does not seem probable either; if only for the absence of similar symptoms in the other three species. Due to the symptoms, two dogs (one male and one female) from the high dose group had to be put down before the end of the study.
Various studies have been conducted in primates (*cynomolgus* or macaque). No mortality was observed in the long-term study (13 weeks at 75 mg/Kg after dose-escalation by level). However, in other groups, one female died after dose escalation over 12 days (10, 25 and 50 mg/Kg/24h) followed by 9 days administration at 75 mg/Kg/24h (the dossier does not say anything specific about this animal; this point requires more detailed information).
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 60mg/Kg/24h, the other animals having survived to the end of escalation at 110 mg/Kg/24h. These premature deaths among primates occurred

however for very high repeated doses, equivalent to 325, 162 and 78 times the highest dose tested in Rennes in repeated administration (50 mg) respectively.

The animal studies dossier, although robust and in theory not generating any specific signals contraindicating in-human administration, raises some comments which led to more detailed information being requested of Bial:

- use of four different species (of which 2 rodents), is, for a case of this type (studies prior to first-in-man trials), unusual. A study in rats and primates would have been expected for a product with potential effect on the central nervous system,
- in dogs, the doses administered were reduced during the study (*down titration*) from 100 to 50 then to 20 mg/Kg,
- whereas BIA 10-2474 appeared to be developed as an analgesic, the analgesic activity of this molecule was apparently only demonstrated in two animal pharmacology tests, without comparison to a benchmark analgesic (gabapentin not being considered as such). This seems too basic to justify continuing development, *a fortiori* in humans (see the TSSC's recommendations at the end of the minutes).

Points emphasised by the Group:

- BIA 10-2474 toxicology studies were carried out properly in accordance with current standards (those of the ICH especially),
- no toxicity, especially neurological (central or peripheral) comparable to that observed in the accident in Rennes, appears to have been demonstrated in animals, despite the use of 4 different species and high doses administered over long periods,
- Bial should however provide clarification to the TSSC as to:
    - the reasons for using four different species for the toxicology studies,
    - the circumstances of death by bronchopulmonary disease in dogs,
    - the circumstances of death during studies on primates at high doses,
    - the results of any microscopic examinations of the brains of deceased primates,
    - the reasons for down-titration in the 13-week study in dogs,
    - the reasons for the apparent lack of preclinical pharmacology studies for confirming, before transfer to humans, the analgesic effect of BIA 10-2474, especially compared to benchmark analgesics.

Clinical trial conducted in Rennes by Biotrial

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

- *single ascending dose* (SAD) study,
- *multiple ascending dose* (MAD) study,
- an open-label food interaction 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-man trials. The ages of the six subjects hospitalised at Rennes University Hospital ranged

from 27 to 49 years. Furthermore, several volunteers considered to have a risk factor for certain drug-related adverse effects were included. Among others, the risk factors included history of severe head injury (loss of consciousness) in one volunteer; a PR interval measured at over 240 milliseconds on several pre-dose electrocardiograms in another and blood pressure of over 140/90 mm Hg over 4 pre-dose readings.
The choice of the first dose administered was careful, 0.25 mg, 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 8 cohorts of 8 volunteers (6 receiving the active treatment and 2 the placebo) for the 8 dose levels tested (0.25 mg to 100mg); 48 subjects were therefore exposed to the active treatment. For each level, 2 subjects (1 *active treatment* and 1 placebo) were tested before administration to the other 6.
The MAD part provided for 6 cohorts of 8 volunteers (6 *active treatment* and 2 placebo), therefore 48 subjects. The 6 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 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 toxic effects were observed in the volunteers from the previous level, following the monitoring committee's advice. As the MAD part was interrupted before cohort 6, 30 volunteers received the *active treatment* for this part of the trial.
The food interaction study covered 12 volunteers at the 40 mg dose.
90 subjects in total were therefore exposed to BIA 10-2474 during Phase 1, at highly variable doses.
The SAD part started on 9 July 2015 and ended (cohort 8: 100 mg) on 9 October.
The MAD part 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). On the evening of day five (10 January) and therefore of the 5th administration (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). The 5 volunteers receiving the active treatment, and not the 2 subjects receiving the placebo, were in turn hospitalised at Rennes University Hospital between 13 and 15 January, therefore between 2 and 4 days after the last administration. The trial appeared to have been effectively suspended on

[1] As a reminder, we recall the 2006 recommendations of the French Medicines Agency (AFSSaPS) for first-in-man trials (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".*

the 11th since the administrations, which were to continue until the 15th, were discontinued on that date.

Points emphasised by the Group:

- according to current standards, it is a conventional Phase 1, first-in-man clinical trial protocol, conducted in a specialized centre of sound reputation,
- the protocol did not in principle include any elements or provisions likely to contraindicate or delay authorisation of the trial,
- three points should however be highlighted:
  - the trial was not immediately suspended whereas one of the volunteers had been hospitalised for a sudden-onset event,
  - it is regrettable that, as it is a trial on a molecule theoretically targeting the central nervous system, volunteer selection did not apparently take neuropsychological assessment (clinical interview with cognitive assessments and tests) into account, whereas the "somatic" explorations appeared to be exhaustive,
  - especially, the increase in the doses administered, although sometimes common practice in Phase 1, appears to be problematic as too sudden at the end of escalation, as the opposite would have been expected. For example, dose skipping between the MAD cohorts 4 and 5 corresponds to a ratio of 2.5 (20 to 50 mg) whereas the ratio is only 2 between cohorts 1 and 2 (2.5 to 5 mg). This very important point should be included in recommendations (see end of minutes).

Symptoms observed in the hospitalised volunteers

All of the clinical, biological and radiological data available was analysed by the TSSC's doctors, and it was then summarized and made anonymous for presentation to the plenary group. For obvious reasons relating to subject protection and medical secrecy, this information will not be given in detail in this first report. Several important points deserve to be underlined however:

- the 6 volunteers (27 to 49 years) having received multiple doses of BIA 10-2474 50mg were hospitalised,
- the symptoms, of very rapid onset, **presented by 5 of the 6 volunteers**, although of varying severity, where of the same form, as much in clinical as in radiological terms, and only involved the central nervous system,
- brain imaging (MRI) showed damage of highly variable severity but also of the same form in terms of its characteristics, and essentially affecting the hippocampus and the pons,
- clinically, there was neither peripheral neurological symptoms, nor seizures, nor biological, metabolic or immunological anomalies,
- the entire picture, both clinical and radiological, was **therefore completely unusual**, with no relatedness to a known disease or toxicity.

Detection of signs of toxicity in the other volunteers

One of the most striking elements of the case is the absence of toxicity (adverse event of noteworthy intensity, *a fortiori* serious) in the other trial volunteers, some of which had received single-doses up to 100 mg or multiple doses of 10 times 20 mg, therefore a cumulative dose of 200 mg (NB: cumulative 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, there being no events of noteworthy seriousness or severity, and cardiovascular symptoms were predominant. It should be noted that two volunteers from the 10 mg MAD cohort however presented, *on two occasions*, blurred vision and diplopia (one episode on the 2nd and 6th day for one volunteer and on the 3rd and 7th day for the other). Apparently, the investigator and the monitoring committee did not consider this symptom to be relevant, and it was not observed in the volunteers in the 20 mg cohort.
Since suspension of the trial, the 84 volunteers having taken BIA 10-2474 (aside from the six from cohort 5) have been contacted for a full clinical examination and MRI exploration. Among the 62 volunteers (74%) seen at the TSSC meeting (15 February 2016), no clinical or MRI anomalies have been detected.

Points emphasised by the Group:

- the symptoms seen in the volunteers other than those hospitalised, but exposed to BIA 10-2474 at highly variable doses, are non-specific and appear to be quantitatively and qualitatively similar to those seen in Phase 1 trials of this type, except for the fairly high frequency of cardiovascular symptoms (orthostatic hypotension and tachycardia),
- serious central nervous system symptoms exclusively, **only** appeared in the exposed volunteers from MAD cohort 5 (50 mg).

Pharmacokinetic data

Generally, pharmacokinetic studies conducted in animals do not give rise to any specific remarks, even if as is usually the case, pharmacokinetics appear to become non-linear with the highest doses, at least in dogs.
The choice of the doses administered in Phase 1 would however deserve to be discussed. Indeed, considering the inhibitory concentration 50 (IC_{50}) of BIA 10-2474 and its pharmacokinetic characteristics in humans, complete FAAH inhibition should be achieved at doses lower than 5 mg (probably from 1.25 mg). Even if the primary objective of a Phase 1 study is to ensure good acceptability of a molecule for doses significantly higher than those considered to be therapeutic, this raises the question of the need to test an escalation up to 20 to 80 times the dose inhibiting FAAH, if this molecule is supposed to have an effect *via* this mechanism.
The pharmacokinetic studies during the SAD cohorts show that the elimination half-life is extended when doses administered become high; the areas under the curve (AUC), reflecting exposure, increase more rapidly than the doses increase. 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, transporter induction

type, etc.), or, a lot more likely, by saturation of elimination at between 40 and 100 mg, without it being possible to more accurately identify the threshold dose at which non-linearity begins.
During MAD studies, the same non-linearity is observed, the AUC increasing more rapidly than the doses from 20 mg. We especially see that:

- dispersion in the pharmacokinetic parameters among the volunteers is higher at 50 mg than at 20 mg,
- again for 50 mg, and unlike what is observed for 20 mg, residual BIA 10-2474 plasma concentrations continue to increase up to the fifth administration. The plasma concentration steady state was not therefore reached in cohort 5, unlike what was predicted by the elimination half-life values calculated for lower doses.
- as in SAD, non-linearity is likely as of 50 mg multiple doses.

The four metabolites identified in animals are expected to be the same in humans, two of them (2639 and 2445) reached measurable plasma concentrations remaining however very low (<3% of those of the parent product). Without direct administration of the metabolites themselves, it is difficult to determine their individual characteristics. However, it seems that the variability in the pharmacokinetic parameters is higher for these two metabolites than that observed in animals, with, for example, elimination half-life estimated to vary from 4 to 23 hours.
Variability also affects, but to a lesser extent, the pharmacokinetics of the molecule itself. This is commonly observed with drugs due to interindividual variations in metabolism, among other things. In the case of a Phase 1, first-in-man trial, this variability can become problematic if the dose calculations for the multiple doses (MAD) are based, as is the case here, on the means of the parameters measured in other individuals previously. By definition, this approach does not take extreme values into account, distribution of which can vary from one group to another, and which can lead to fairly significant prediction errors (see recommendations by the Group at the end of the minutes).

Points emphasised by the Group:

- extrapolation of animal data to humans suggests that complete inhibition of FAAH activity is achieved for doses a lot lower (20 to 80 times) than the maximum doses the protocol planned to test in humans,
- BIA 10-2474 pharmacokinetics become non-linear somewhere between 40 and 100 mg administered. They are also subject to noteworthy interindividual variability. Also, the kinetics of the two main, non-specifically explored metabolites, are possibly non-linear during administration of multiple doses of parent product higher than 40 mg,
- this explains why in the 50 mg MAD cohort, residual plasma concentrations were not all stabilised on day five of administration, unlike that which was predicted by the calculations pertaining to mean elimination half-life.

Hypotheses to look into in an attempt to explain the accident in Rennes

The TSSC's first conclusion concerns the astonishing and unprecedented nature of the accident in Rennes, as much in terms of:

- its seriousness (6 volunteers hospitalised, 1 death),
- the fact that the toxicology studies, although conducted on four animal species with doses up to 650 times the dose absorbed by the hospitalised volunteers, do not apparently show any lesions or picture likely to predict such toxicity,
- the very unusual nature of the clinical and radiological pictures which are like nothing potentially ever seen before,
- the fact that to date, 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 abandoned due to their insufficient effectiveness and for which no neurological or other toxicity had been observed.

Toxicity occurring in only one of the 14 cohorts of volunteers having received BIA-2474, can only in theory 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 total BIA 10-2474 dose that the subjects received.

Exploration of the first hypothesis is not within the scope of the TSSC's missions but it seems that this explanation is little likely. For example, the product used for the toxicology studies was the same as that used in the capsules administered to all volunteer groups, and was later tested and revealed to be of the highest purity.
The Group therefore mainly discussed the other two hypotheses.

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

Several possibilities were discussed:

<u>1.1. Hypothesis of an interaction with other products</u>
Cited regularly by the media, 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, and especially as the 6 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). The same theoretically applies for an interaction with food or consumption of chocolate by the volunteers. Chocolate only contains very small quantities of anandamide and hyperstimulation of the endocannabinoid system is not known to be able to produce symptoms of the type seen in Rennes (see further on).
To date, there are no credible arguments in favour of narcotic consumption immediately before or during the stay at Biotrial. Besides the serious breach of Phase 1 good practices that it would represent, and due to the fact that once again all cohort 5 volunteers would have to have taken the same substance, this hypothesis comes up against two observations:

- blood narcotic (including cannabis) and alcohol tests and tests for other substances, whether medicinal products or not, are negative to date,

- the same argument applies to cannabis, namely that it seems to be accepted in neuroscience, that direct or indirect, even massive stimulation of endocannabinoid receptors, CB1 in particular, would not induce toxicity of the type seen in Rennes. Even if in certain subjects it can induce severe psychiatric effects (i.e. psychotic episode), neither cannabis, nor its main component tetrahydrocannabinol lead to acute toxic brain damage, even experimentally and at very high doses.

1.2. Hypothesis of a specific genetic or metabolic characteristic or common pharmacological response among the subjects in the 5th MAD cohort

There are several genetic factors, among others, likely to modulate individual response to administration of an FAAH inhibitor. For example, this enzyme has two isoforms with different activity; in the same way, the cytochrome P450 system is found at several levels, the activity of which can vary widely (by induction or inhibition) from one individual to another. As appealing as it may seem, this hypothesis clashes with statistics laws. For the FAAH example, if low activity isoform prevalence is 38% in the general population, the probability of finding it in 5 out of the 6 exposed cohort subjects is 0.0295 (a less than 3 in 100 chance) and 0.003 (3 in 1000 chance) in the six subjects exposed. 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 cohort 5.

2. Hypotheses of a threshold effect relating to cumulative BIA 10-2474 dose

Even if this second set of hypotheses appears a lot more likely, the potential mechanisms are especially numerous and some purely hypothetical or very little known. They may involve the molecule itself or a mediator such as anandamide.
Let's not forget, first of all:

- the highly unusual nature of this dose-dependent toxicity, which was not observed in animals even at very high doses, theoretically with no portent signs in the volunteers having been exposed to lower doses of a compound similar to molecules having previously been seen to be little effective, without specific toxicity. It happened "*as if something gave way or swung suddenly at a specific dose or concentration threshold which is typical of an on-off effect*". This threshold effect could be encouraged by the fact that BIA 10-2474 pharmacokinetics become non-linear above 40 mg,
- that it is almost certainly an *off-target* effect due to (i) the fact that complete and prolonged (8 hours) FAAH inhibition is achieved for BIA 10-2474 doses of 1.25 to 5 mg, (ii) that this molecule appears to be little specific for FAAH, and, especially, (iii) that stimulation of endocannabinoid receptors by anandamide cannot theoretically induce toxicity of this type.

Several hypotheses possibly explaining such a human-specific ***on-off/off-target effect***, were discussed during the TSSC meeting:

2.1. Inhibition of other cerebral enzymes by BIA 10-2474

This is one of the TSSC's preferred avenues. Bial's molecule is in fact significantly less FAAH-specific than molecules developed to date, and its binding to other, i.e. off-target, cerebral hydrolases or enzymes is therefore plausible, especially when concentrations of

BIA 10-2474 or its metabolites increase. Let's not forget that BIA 10-2474 was administered to the volunteers in MAD cohort 5 at a dose (50 mg) 10 to 40 times higher than that supposedly inducing complete FAAH inhibition. The TSSC therefore asked to receive additional information on:

- the affinity of BIA 10-2474 for other cerebral enzymes,
- the geographic distribution of these enzymes in the brain, and
- the consequences, if they are known, of inhibition of their activity.

2.2. Toxicity from a BIA 10-2474 metabolite

Bial's molecule (which has a leaving imidazole group) could produce a toxic isocyanate that is able to bind to many brain proteins and induce widespread lesions. This hypothesis is very interesting to look into, even if, like the previous hypothesis, it comes up against the absence of central nervous system toxicity observed in animals.
Toxicity from one of the 4 peripherally-circulating metabolites (plasma) in humans and animals could also be envisaged. Their specific activity and toxicity have 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 is higher in humans. It is also possible that these peripherally-produced metabolites are of the hydrophilic type and therefore have difficulty crossing the blood-brain barrier, unless we assume there is a specific carrier and/or efflux pump inhibition during the rise in circulating concentrations from repeated doses.

2.3. Suspected anandamide-related toxic effects

FAAH activity blockade leads, at least temporarily, to an increase in intracerebral anandamide concentrations, which has several possible consequences:

- 2.3.1. *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. Some of these mechanisms involve ion channels which may help explain the sudden threshold effect. This avenue is currently being explored by the TSSC's experts.

- 2.3.2. *Toxicity from anandamide degradation products*
  In the event of FAAH inhibition, anandamide can be degraded by the cyclo-oxygenases 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 cohort 5 volunteers. This avenue is also being explored by the TSSC.

In its meeting on 24 March, additional data will be studied by the TSSC, mainly concerning points 2.1, 2.2 and 2.3.
The plausibility of hypotheses 2.3.1 and 2.3.2 is however challenged by the fact that (i) this effect has not been observed with other apparently more specific inhibitors of FAAH and (ii) complete and lasting FAAH inhibition, and therefore, theoretically, the rise in intracerebral anandamide concentrations, is achieved from the lowest BIA 10-2474 doses tested by MAD, for which no toxic effect has been observed.

Recommendations that the TSSC would like to see put to French and international authorities

In the TSSC's opinion, the seriousness of the accident in Rennes warrants that legislation and good practices governing first-in-man trials move forward to become clearer and tighter on a certain number of points. More comprehensive recommendations, that the TSSC would like to see applied at international level, will be put forward at the TSSC meeting on 24 March 2016. Several of them can already be listed:

- Firstly, demonstration of pharmacological activity, comparative whenever possible, should be a requirement in the future before in-human administration or even before continuing toxicology studies can be envisaged. Preclinical pharmacology studies should be conducted as early as possible, on an adequate dose range (dose-effect curves) and should be designed so as to be reasonably predictive of real-life, future therapeutic efficacy.
- A neuropsychological assessment with clinical interview and cognitive tests should be a compulsory part of assessment during volunteer screening and inclusion in a Phase 1 trial for drugs with "central nervous system" tropism.
- Detailed and well-supported arguments for the choice of maximum dose to be tested in volunteers with respect to the presumed effective dose should be provided. For example, in this case, it appears unjustified to plan to test a dose (100 mg) 80 times higher than that presumed to induce complete and prolonged FAAH inhibition (claimed mechanism of action of the drug tested).
- A large-scale consensus process should cover Phase 1 dose-escalation strategies to establish recommendations for more reasonable and careful practices than those applied, for example, in the case of BIA 10-2474. Dose skipping, which is highly paradoxical, was observed between cohorts 4 and 5, and therefore in the risk area, and was more substantial than for the first apparently risk-free levels (i.e.: a ratio of 2.5 between 20 and 50 mg compared to 2 between 1.25 and 2.5 mg).
- Pharmacokinetic parameter variability and extremes, and not only the mean, should be taken into account for setting the next dose level.