5 Ethics and Security

5.1 Ethics

OVERALL ISSUES AND APPROACHES

We are very much aware that our proposal involves complex issues, and that new information may become available during the course of our studies that have ethical implications. Hence, we have included an independent Ethics Advisory Board. Professor [redacted], has kindly agreed to lead this aspect of our work. Through regular (quarterly) meetings, the Ethics Board will ensure that our ethical approaches are consistent across work packages and across countries wherever appropriate; provide expert advice to the rest of the consortium on any specific ethical issues arising in particular studies; engage key experts to provide additional advice where necessary (e.g. new EU legislation as it arises); and develop strategies to explore the ethical implications of novel results as they emerge from the consortium (see below). Professor [redacted] will ensure that the Ethics Board includes relevant independent expertise to monitor the ethics issues in this project and how they are handled. The Board will be consulted at least on the following points (inclusion of fetuses, infants at risk, imaging, animal models, genetic information, dedication, clinical trials and data protection). A report by the Ethics Board will be submitted as a deliverable at the end of each reporting period.

Any consideration of ethical issues has to be set in a context of the potential benefits and risks of the overall project. Undoubtedly, better options are needed for treating ASD. AIMS-2-TRIALS will address this issue using the latest techniques of animal and cellular models, genetics, proteomics, imaging and behavioural/cognitive phenotyping.

In pursuit of these objectives the ethical aspects to be addressed with respect to our proposal concern experiments using small laboratory animals (mice and rats, WP2) and investigations of human stem cells (WP2). To be clear our project does make stem cells derived from scalp hair (keratinocytes) or fibroblasts or existing human embryonic stem cell lines – but not new tissue from embryos. In the case of animal studies of AIMS-2-TRIALS we need to address the issues of necessity of animal experimentation and the choice of lowest species, the refinement of methods and the expertise of the individuals involved in handling, the reduction of animal use - insofar as possible with an eventual goal of replacement.

In this section we lay out a set of general principles which guide our overall approach in AIMS-2-TRIALS across both our human and 'basic' research, and we then address the individual issues is separate sections (e.g. in humans and animals).

Overall principles impacting on all our studies.

- The participating investigators of AIMS-2-TRIALS agree to adhere to all relevant international, IMI and national legislation and guidelines relating to the conduct of all our studies.
- All research activities within AIMS-2-TRIALS requiring approval on ethical and legal grounds through responsible local or national Ethics Committees and Regulatory Authorities will be conducted only after obtaining approval.
- AIMS-2-TRIALS will comply with the highest ethical standards, including those outlined in the Grant Agreement (Article 34 of the Model Grant Agreement) and the European Code of Conduct for Research integrity. The balance between the research objectives and the means used to achieve them will be given special attention.
- Most of the experimentation is being conducted in EU countries. However some work packages, e.g. WP2,3,4,and 6, which will also share some data from South African (SPS), Canadian (POND), US (ABC-CT), and Australian (CRC) sources). These will be subject to appropriate EU regulations and supplementary local regulations where appropriate. Also we will ensure that the research conducted outside the EU is legal in at least one member state.
- The experiments and studies are all being led by investigators who have established (usually two decades) of track record of excellent scientific work in their respective areas – thus assuring scientific expertise, established infrastructure and experience within the local regulatory authorities with the kind of work being proposed.
- While AIMS-2-TRIALS will use an array of procedures, genetic and other metabalomic analysis (and the specific ethical issues are addressed below), we are not introducing any radically new first-in-man procedures nor are we introducing any incrementally invasive techniques in animals.
- However where we do carry out animal experiments the anticipated benefits of the project justify their use due to the fact that alternative methods are not applicable. The 3 Rs rule (i.e. Replace, Reduce, Refine) will be strictly applied.
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Overall principles impacting mainly on human studies. In addition to the general principles described above, our foremost principles for the conduct of any research involving human participants within AIMS-2-TRIALS are:

- Respect for the rights, integrity and privacy of patients
- Protection of vulnerable patients
- Continuous monitoring of patients' safety
- Generation of meaningful, high-quality data

- Timely publication of results
- A comprehensive policy for incidental findings.
- Clarity with participants that they can leave the study at any time with no deleterious consequences

Moreover research in AIMS-2-TRIALS involving human participants will be conducted under the applicable international, IMI and national laws and regulations. This also applies when collecting, obtaining, or using any samples for research

In particular, the consortium is committed to

- The DECLARATION OF HELSINKI: Ethical Principles for Medical Research Involving Human Subjects (Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and last amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013)
- The standards of the International Conference on Harmonisation (ICH) / Good Clinical Practice (GCP)
- The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine, ETS No. 164, Oviedo, 4 April 1997; and the Additional Protocol on Biomedical Research (CETS No. 195), 2005. And the UNESCO: Universal Declaration on Bioethics and Human Rights (2005).
- Submitting copies of ethical approvals by the competent legal local/national Ethics Boards/Bodies to the IMI prior to the commencement of research.
- Being explicit in the Informed Consent Forms that data can be used in the future for research and commercial purposes.

Human issues. An overview

Our in vivo research with humans includes; 1) healthy volunteers (WP2), 2) phenotyping research with fetuses, infants, children, adolescents, and adults with and without ASD (WP2) that includes imaging studies (WP2).; 3) experiments (in adults only) that use pharmacological probes (WP3) to explore causes of brain dysfunction, and ionising radiation (PET) to explore specific neurochemical systems, 4) clinical trials using drugs (WPs 3-4), 5) the use of human biological samples and the collection of human data, and research involving processing and analysis of genetic information or phenotypic or personal data (WPs 2,3,4,6). We already have local and national ethics approval for our work with fetuses, infants, older children and adults (age > 6 years) and at KCL for children age 3-6 and for the pharmacological probe studies in those > 18 years). However, at other sites we will need to obtain ethics for our studies of 3-6 year olds (WP2), for studies of brain response to pharmacological probes (WP2) in adults, and for clinical trials (WP3 and 4) in children and adolescents/young adults. No clinical trials are proposed in any individuals who are less than 55 years of age, and no pharmaco-probe studies on brain function are planned in any individuals who are less than 18 years of age.

The human studies in AIMS-2-TRIALS raise a number of additional issues. These include the appropriateness of design of the studies, the expertise of the investigators carrying it out, the rigour of the local approval process and their ability to assess the balance risk and benefits, the validity of the informed consent procedure, the efforts to minimize any risks to volunteers/patients, protection of data and privacy and mechanisms for monitoring as well as sensitive mechanisms of response if things go wrong. For instance, major ethical siderationconsideration will be the collection of potentially sensitive data relating to health and specifically mental health, and the process of active and passive remote monitoring. We have described within section 5.1.4 steps taken to protect privacy, including deidentification of data, data security and training of all research workers in their duty to confidentiality. Any threats to privacy and all procedures for handling data will be explained to participants within the informed consent process.

Research with human participants will be conducted in the countries listed below in accordance with national and international regulations: UK, Ireland, Portugal, Spain, France, Italy, Netherlands, Belgium, Sweden, Germany, and South Africa.

Human issues. Shared themes impacting on recruitment/inclusion of subjects across all/most work packages

Our work packages involve the recruitment, assessment and follow up of patients with ASD and therefore demands the highest ethical standards. Key issues include competency to give written, informed consent, possible deficits in everyday concentration and attention, and fatigue and possible distress in the test situation. These studies will be conducted within 55 years across our clinical sites. In all cases the general (Helsinki and Madrid declarations) and the EU and local regulations regarding ethical conduct of human clinical trials will be followed. In specific, no patient legally detained under mental health legislation will be approached. Potential eligible subjects will be identified by advertisement and by clinical teams made aware of inclusion criteria. Such subjects, and/or their parents, will be approached by the research assistant with permission from the responsible medical officer, who will also be asked to confirm that the patient is competent to give informed consent, and that they are clinically stable. Some individuals we approach to take part in the study will have reduced capacity to give informed consent (e.g. those with intellectual disability). For details on our consent procedure please see below.

Human Issues. Consent

Subjects will be contacted by the research assistant and, in conjunction with a verbal explanation, provided with a written information sheet approved by national and local research ethics committees (the ethics review process in now a national Integrated Research Application System [IRAS] system in the UK). This will include the purpose of the study, why the person has been invited to take part, details of what they will be asked to do, how the data from the study will be kept secure and confidential in an appropriately pseudoanomised way, with identifiers removed, and assurance that participation or non-participation will not affect their clinical care. The information sheet will be left with the patient and/or their carer who will then be re-approached at their convenience to discuss any further questions and patients (and or their parent/carer in the case of children) who elect to participate will then provide written consent witnessed by a third party, usually a member of the clinical team.

Some recruited participants (i.e. fetuses, infants and children) will be under the age of consent, therefore informed consent will be obtained from the parents or guardians. Prior to obtaining consent, volunteers and their parents will have received an information sheet detailing all aspects of the study, including aims, methodology and benefits. Alternate information sheets using simplified language and images will be used for participants whose estimated developmental level is of an older child/younger adolescent, e.g. between 8 and 14 years. The study will be explained in detail to parents and guardians during telephone screening and on the screening visit. Assent will be sought from young people under the legal age for consent. At all points throughout recruitment and the study, participants and their parents will be encouraged to ask questions and air any concerns that may help clarify their role as a participant. For imaging studies of the fetus and newborn infants parents will be consented in the imaging centre, having been shown the MRI scanner and having had information, given to them, in some cases for some weeks prior. They will already have been in contact with the team to ask questions beforehand and on the day of scanning any remaining details of procedures involved are fully explained to them. All volunteers will be continually reminded that participation in the study is voluntary and that they are free to withdraw at any time. Participants and their families will be given a copy of the information sheet and signed consent form to keep.

The ethical approval from the relevant Local and/or National Research Ethics Service (NRES/HRA) that will be obtained before recruitment starts will contain explicit reference to the longitudinal follow up, and the possibility of the subjects being invited to participate in voluntary long-term follow up. There are a number of issues here:

- Follow up, as all research participation is voluntary and subjects can withdraw at any stage; this will be explicit in the patient information literature and the consent process. That is it will be made explicit that their consent is for the current contact/assessments and that they can then in future (when re-approached for follow up) again make an independent decision as to whether to continue to consent and/or withdraw (including withdrawal of retrospective data).
- The ethical permission granted before patient recruitment begins will include the taking of explicit consent for researchers to contact subjects or their families again in the long-term to invite their further participation.
- A related issue is the fact that even if subjects are not followed up long-term in the flesh, the images and DNA obtained will be used for many years, and there is a question about whether individuals should be asked for re-consent when they reach the age of majority for the use of their data and materials. We have taken advice on this from the Health Research Authority, which is the new national oversight body for all medical research. The Director, Professor [redacted], has given the opinion that in UK law consent for long-term follow up given by parents is adequate and appropriate for the long-term use of the materials, and that research could legally and ethically continue in the long term unless individuals asked for their materials to be destroyed. The right and process for this- which is ethically equivalent to voluntary withdrawal from the study- will be codified in the permissions granted by NRES before studies commence

Human issues. Determining capacity

Our study will use a similar protocol to assess capacity, and the same capacity assessment tool, as developed by KCL and approved by UK ethical review bodies (for use in an NIHR programme grant (ref. 09/H0807/72)). We are already using approach in EU AIMS.

Participants who are aged 16 or over. Following the protocol set out by project ref. 09/H0807/72, the initial step in determining who requires a capacity assessment will be to approach the consultant psychiatrist or physician in charge of the patient's treatment. Where the view of this person is expressly that the person has capacity to consent, no further assessment is appropriate or necessary as is standard practice (as stated in the Mental Capacity Act, 2005: "a person must be assumed to have capacity unless it is established that he lacks capacity). Where capacity is in doubt, it will be further assessed by an appropriately trained member of the treating team, together with a member of the research team. We are aware the baseline position is that capacity to consent is assumed unless there is reason to suppose otherwise. However, some neurological, psychological and/or behavioural markers may suggest lack of capacity for some things at some times. We also recognise that capacity is fluid in time and with respect to subject.

Given the nature of the research, it is clear that some people who may be eligible to participate may lack capacity. In this situation, the views of senior medical / psychology staff with regard to possible absence of capacity will be sought. Indicators of potential lack of capacity include (but are not restricted to) the diagnosis of learning disability (i.e. mental retardation), significant deficits in adaptive functioning or other signs or symptoms of mental disorder that might compromise capacity to give informed consent.

Where it is considered necessary by the clinical team that capacity should be assessed, the capacity assessment tool will be used to address the following questions, which are based upon the principles outlined in section 3(1) of the UK Mental Capacity Act, 2005:

- Can the person understand the information relevant to the decision?
- Can the person retain that information?
- Can the person use or weigh that information as part of the process of making the decision?
- Can the person communicate his/her decision (whether by talking, using sign language or any other means)?

Where it is identified that the participant may lack capacity to consent, the study will be introduced using the Participant Information Sheet (Easy read version) (Appendix S**).** Participants will be asked if they have any questions about the information they have been given. They will then be asked the questions on the Capacity Assessment form as part of the test of capacity (Appendix T: Mental Capacity Assessment ASD).

In the case that their child may lack capacity to consent, the letter to the parent asks them to nominate another relative or close family friend (other than themselves) to act as their son/daughter's personal consultee. The nominated personal consultee will be given the Personal Consultee General Information Sheet (see Appendix U) explaining what it means to be personal consultee and the Personal Consultee Signature Form ASD (Appendix V). We will try to arrange for the consultee and informant to be independent (i.e. one parent acts as the consultee, the other as the informantIf there are any concerns about a conflict of interest then the parent will be asked to contact the research team, at which stage we will speak to them about the possibility of finding an alternative personal consultee.

Participants recruited from child clinics/charities. Where the participant has been identified by referral from child clinics or from charities, if in the researchers' initial telephone conversation with the family, or the clinic, they identify that the son/daughter is aged 16 or older and may lack mental capacity to consent to participate in the research due to a diagnosis of learning disability, or significant deficits in adaptive functioning or other signs or symptoms of mental disorder that might compromise capacity to give informed consent, after appropriate training the researchers will arrange to assess the participant's mental capacity using the amended assessment of capacity to give informed consent. As above we will introduce the study using the Participant Information Sheet (Easy read version) before assessing capacity using the Capacity Assessment form. The personal consultee will be appointed using the same procedures as outlined above.

All researchers in our network will receive training from KCL, under the guidance of our ethics committee, in assessing mental capacity to consent. In addition, given that they may have to assess capacity without a clinician being present they will also undertake additional training: Mental Health Act 2005 (or European equivalents) and DOLS Awareness Training and Training on the Protection of Vulnerable Adults/Children training (POVA/POCA). Where possible, the researchers will conduct assessments in collaboration with a member of the healthcare team, known to the potential participant, with experience in assessing mental capacity. Moreover, where possible decisions will be made in collaboration between the research team and mental health team.

Human issues. Distress and fatigue

For participants with ASD, much of the distress around research involvement relates to new surroundings and new tasks. With this in mind, a booklet using words and images describing the research environment and investigations will be given to participants at least several days before the research assessment. Testing will take place at the participant's convenience either at home or in a research institute. Each session can take up to four hours and, in the case of participant fatigue, can be split over two consecutive days. In the event of a study participant becoming fatigued or distressed the following course of action will be taken: The cause of fatigue or distress will be established and discussed if participant wishes. The researcher, in collaboration with the participant and their carer will decide whether to proceed with the interview, re-schedule or continue with inclusion in the study. The participant will be free to withdraw from the study at any time. Participants will be reimbursed for any financial expenses incurred and will be paid 10 Euros or equivalent for each hour of assessment time. Children receive vouchers for books/educational toys in EU countries in which that is allowed. **We have successfully used protocols of this type and length across many hundreds of mothers, infants, children and adults with excellent acceptance and satisfaction. Moreover we** frequently carry out post-visit questionnaires to inform future protocols.

Human issues. Pregnancy

Pregnant and breast-feeding women will be included in our non interventional studies – but will not be included in any studies involving exposure to drugs or ionizing radiation.

Human issues. Filing copies of ethics approval(s).

Copies of opinions/approvals by ethics committees and/or competent authorities for the research with humans will be kept on file (and will be specified in the grant agreement).

Human issues. Cells/tissues – documenting procedures.

In case of the use of human cells/tissues obtained commercially, within the project, from another project, or obtained from a biobank, details on the cell/tissue types and on the biobank and access to it will be it kept on file (and this will be specified in the grant agreement). Moreover, copies of relevant documents for using, producing or collecting human cells or tissues (e.g., ethics approval, import licence, accreditation/designation/authorisation/licensing) will be kept on file (and will be specified in the grant agreement). Last, an internal register will be established to keep track of necessary approvals of ethics committees and competent authorities and their status (applied, obtained, conditional, to be renewed etc.). A consortium member (currently [redacted]) will be responsible for establishing and up-dating this register. The register will be checked by the DMEB (Data Monitoring and Ethics Board) on a regular basis (e.g. every 6 months).

Human issues. Embryos/Fetuses

Our research does not use primary human fetal tissues/*cells* – but we do carry out *in vivo* MRI imaging studies of fetal brain and placental development. We already have ethics approval for this *in vivo* work (attached –please see appendix).

Human issues. The need for MRI and genetic and biomarker studies in fetuses, infants, and children.

We do not directly use embryonic or fetal tissues. However, as noted above, we will carry out prospective *in vivo* longitudinal studies of fetuses and infants at low and high familial risk for ASD, and recruit a new cohort of preschoolers with ASD, typical development or other conditions. This approach is critical to identifying stratification biomarkers that precede the onset of clinical symptoms; understanding the cascading effects of genetic and environmental risk factors on brain development that ultimately lead to autism and related conditions; and identifying proxy biomarkers for treatment outcome that could be used to determine whether early treatments are working before behavioral symptoms fully consolidate. It is essential that we start this work in fetal development because we know that the causative mechanisms of ASD begin from conception. However, that the longitudinal nature of our studies recognizes that informative results will likely come from the integration of data across timepoints, rather than relying upon solely any single timepoint.

The pathophysiology and aetiology of ASD are poorly understood. Given that brain abnormalities are likely to precede the expression of symptoms, and the early onset of ASD in the first years of life and the typical manifestations of ASD at childhood age, a detailed investigation of the neural, cognitive and biomarkers underpinnings of these disorders only carried out in adolescent or adult patients will not be able to fully address our research questions. Studies in adult patients are unable to address the very important issue of the development of cognitive functions and neural systems (e.g. the trajectories of the brain circuits, and the connectivity between different areas of the brain) and lack relevance for our understanding of the origins of these disorders. Thus, it is necessary to work with fetuses, infants, very young and school age children.

Our procedures have been risk assessed and ethics approval has already been obtained for all *in vivo* fetal and infant work. It is particularly important to note that all the fetuses we study are older than the legal limits for termination in all EU countries. This means that it is very unlikely that any fetal biomarker we discover in the third trimester could have future utility in guiding decisions about termination. However, it could help parents select appropriate treatment strategies that they could use in very early development, including parent-mediated strategies that have efficacy and were developed by our partner teams (BC).

Human issues. Care and protection of research participants and vulnerable individuals

All study teams are linked to hospitals with 24h urgency services per day, and the imaging centre for fetus and newborn studies is based within a neonatal intensive care unit with full intensive care facilities, and close to a large, well-staffed maternity unit. All study procedures will be designed to minimize burden of the patients. Study staff will have relevant experience – e.g. working with pregnant mothers and fetuses (e.g. neonatology), infants, child and adolescent psychiatry and neurology and paediatrics. Invasive examinations will be reduced to a minimum to minimize burden (e.g. frequency of blood-drawing). Local anaesthetics can be provided if requested in the case of drawing blood and instruments/tools will be used which are appropriate to the age of the patients (e.g. 22 G needle for collecting blood samples). No examination will be conducted against the will of the patient or the caregiver. Dosages of any medications used in adults during pharmaco fMRI experiments in WPs 2 and 3 will be selected on the basis of relevant previous studies and are in the range that has been shown effective and safe in previous studies. Safety assessments will guarantee detection of adverse effects and a high standard of patient's safety, which will be higher than in routine care at present. Patients in the placebo group of pharmaco fMRI experiments, or clinical trials, will not be devoid of a standard and effective treatment. Certain special populations will be excluded from participation in the trial. Pregnant and breast feeding women will not participate in pharmaco fMRI experiments or clinical trials. Concomitant medication which increases the risk for adverse effects by possible drug interactions will not permitted and therefore safety of participants will increase.

Human issues. Balancing benefits against risk and burden

Benefits.

Best standards of safety assessments which are rarely used in standard medical care will be provided for participants in the human studies. Last, a biomarker for later adverse outcomes could create potential ethical problems if the condition is untreatable, and we have considered this problem. First, this is not an uncommon problem in paediatric medicine, where the routine practice of universal early examination of infants to discover potential problems is of great value, but where the possibility of detecting untreatable conditions is always present. Second, early detection allows parents to be aware and symptomatic care plans to be developed. Third it allows infants to gain the advantage of inclusion in clinical trials of potential treatments at an early stage where success is more likely [122, 123]. Last, ASD is not an untreatable condition – and it can respond to early intervention [124-127].

Minimising risk and burden.

MRI. MRI scanning is carried out by professional teams with longstanding experience in using it with fetuses/infants/children/adults for both clinical and research purposes. Together we have many decades experience with research using human MRI and we include the premier centers for neurodevelopmental research using neuroimaging in Europe. The technique is non-invasive, and does not require administration of any contrast agent or ionizing radiation. It is generally accepted that non-invasive imaging by MRI carries minimal risk in general, no long-term harmful effects of MRI are known, and if requested we can provide data that shows this remains true in the specific population(s) under investigation [122, 123]. Provided appropriate precautions are taken regarding metallic objects, there are no known adverse effects. All research subjects (e.g. infants/children and parents) will be screened for metallic objects in clothes or inside their bodies and infants will be undressed to check for the presence of metallic clips etc.

The MRI-procedure is painless and not uncomfortable, and considerable work has been done by the imaging team to define comfortable and safe positioning for mothers, and secure swaddling for infants. However it does require subjects to lie still with the head and part of the body in a tunnel-like device. Also, the MRI scanning procedure requires confinement in a small partially enclosed space. Some individuals find this to be uncomfortable and may exhibit symptoms of claustrophobia including nervousness, sweating or other minor discomfort. The sound of the MRI scanner can be quite loud. These side effects are minimized by appropriate screening and preventive procedures: children and adults (including mothers) are protected with noise-cancelling headphones, and infants with dental putty earplugs, proprietary ear defenders, and an acoustic shield. In our experience infants (when sleeping) and children/adults do not find the experience particularly problematic. Throughout the session subjects will be continuously monitored using visual and auditory channels as well as a pulse oximeter to ensure that they are comfortable, and contented. Should any infant/child/adult show any sign of distress the session will immediately be terminated. The parent or guardian will also be informed that they can terminate the session if they have any concerns. Where relevant a doctor with paediatric experience will be present during the scans. We aim to scan infants and young children in natural sleep, however in children older than 3 years some parents use sleep aids such as melatonin or Benadryl if their child is 'grizzly' and unable to settle. Where this is the case then we will follow this usual practice if requested by parents. This is has been agreed through our risk assessment and ethics procedures

We will also invite parents and their young children involved in MRI studies to attend for a "mock scan" visit if they wish. The mock scanner is a non-functioning MRI scanner which is used to practice scanning procedures and acclimatise research participants to the scanner. Although this involves the potential inconvenience of an additional visit, we would reimburse time and travel costs and we believe that the inconvenience is far outweighed by the additional benefit of allowing the parents to give fully informed consent by seeing first-hand exactly what is involved, and that this will increase the likelihood of a successful scanning session. We will reimburse reasonable travel costs and provide compensation in line with the approved protocols for research at each site. In our experience so far, parents are primarily motivated by the desire to engage in research and would consider this adequate compensation for the time and inconvenience involved.

We also argue that while the benefit for some participants will be limited, all scans are reported by a radiologist and there are some groups where the data may be of medical benefit: for instance children with ASD may benefit from early MR imaging in the short term if a primary condition is diagnosed (e.g. a tumour causing epilepsy), and retrospectively if biomarkers for subsets and treatment groups is defined by the project that can guide later treatment. Potential anxiety is addressed by preparing subjects for the MRI in a protocolised MR simulation (for more details see below). Before and after the simulation, the children, their parent and the researcher each rate anxiety on a Visual Analogue Scale (VAS). If a child does not successfully complete the simulation session, or indicates verbally that he or she no longer wishes to take part in the study, or if either the child, the parent or the experimenter assesses the child as too anxious, the MR scan is cancelled. We also are able to carry out our imaging during natural sleep in children if that is deemed helpful to an individual.

MRI sessions have breaks to accommodate the ability of mothers and children to be engaged in an MRI environment for a long time. Invasive examinations are minimized to lower the burden of the patient by study examinations. All study examinations are designed to the principle of minimizing the burden (e.g. collecting several blood samples for genetic tests, chemistry etc. by one puncture of the vein). Local anaesthetics are provided for minimizing pain by blood drawing.

Electrophysiology. For electrophysiogical recordings painless recording sensors are comfortably placed onto the infant's/child/adultsadults head using an adjustable cap. In babies this adjusts with Velcro and lace at the back and sides and has a ribbon underneath the chin so that the headgear can be resized for each baby. The fibres are wrapped in coban so that

they lead away from the infant and parent out of the infants reach. During the experiment, the infants sit on the parent's lap or on an infant chair (with the parent next to them) and watch a computer monitor or a stage, which presents them with various visual stimuli (abstract geometric forms, photographic or schematic faces, images of other objects, short videos of simple human actions, etc.) that may be accompanied by auditory stimuli (pure tones, environmental sounds, recorded or synthesized human speech) presented through loudspeakers. None of these stimuli are frightening or elicit distress, and all of them are presented well within the level of comfort and safety limits of human sensory organs. The infants' behaviour is monitored by an experimenter through a video camera, and recorded to a digital video tape for off-line coding. These studies usually last about 20 minutes, which is usually well-tolerated by infants in this range. The study is terminated if the infant become fussy or if the parent wishes to do so.

Eyetracking. Eye-tracking uses infrared lights to measure position of gaze. We can also use eye-tracking to learn about head motion and pupil dilation. Eye-tracking is very commonly used in research settings with infants as it is very baby-friendly. Our eye-tracking procedure is designed so that stimuli can be presented when adults/children/infants are attending to the screen. This allows us to present stimuli when (for example, infants) tell us they are ready to watch. While we record eye movements and other measures, we will study how babies respond to visual and auditory information in their environment. For example, we will show infants child-friendly pictures and videos containing social and/or non-social features (e.g. women telling nursery rhymes, toys moving, patterns). Eye-tracking will last around 20 to 45 minutes in total; we have found that this duration is well tolerated by infants in this age range.

Autonomic control. We are interested in how differences in autonomic control relate to early social, communication and attention problems. We will measure indices of autonomic control (heart rate and movement in foetuses; heart rate, breathing and perspiration in infants) using technologies that have been extensively used in fetal and infancy research. We will use these technologies at home (pregnancy) and during the participation in other methodologies (infancy e.g. during EEG, NIRS, eye-tracking, behavioural observations). This allows us to collect more information from infants without increasing testing time.

- We can record fetal heart rate using mobile sensors which can be provided for mothers to use at home.
- We have movement sensors that can be strapped to an infant's wrist. This measures activity levels. We will put a long-sleeved t-shirt on the infant so that they don't feel distracted by the sensor. This sensor is well tolerated by infants.
- A wearable system will be used to measure heart rate and perspiration. This involves the placement of four sensors on the child's back, stomach and/or foot. These sensors are well tolerated and designed for sensitive skin. This system has been extensively used in infancy research.

Near InfraRed Spectroscopy. NIRS will only be carried out in infants. NIRS uses weak light sources to measure haemodynamic changes in the brain. This can provide us with a measure of the naturally occurring brain activity in response to the presented stimuli. Our NIRS system uses a maximum of 16 pairs of light sources and 16 detectors. The laser sources conform to British standard 60825-1:1994 based on the maximum power that they can deliver and their output. The maximum emitted energy at each pair of light sources is 2 mW.

The NIRS technique is increasingly commonly used in research settings with infants, as it is such a baby-friendly method. This technique also provides an opportunity for scientific advancement, given the greater spatial resolution and accommodation of participant movement that it allows.

The NIRS headgear consists of optodes, each imbedded in a silicon and foam pad and sewn together within a soft headband (please see the photos below). This adjusts with Velcro at the back and has a ribbon underneath the chin so that the headgear can be resized for each baby. This headgear is known to be particularly good to use with young infants because it is comfortable and very quick to put in place.

We will use NIRS to help us study how infants respond to visual, auditory and/or tactile information in the environment. To study responses to visual information, we will show infants child-friendly videos containing social and/or non-social features (e.g. women telling nursery rhymes, toys moving). To study responses to auditory information, we will play social and/or non-social child-friendly sounds (e.g. laughing, toy rattling). All stimuli are designed to be safe and to be experienced as fun for infants in our age range. NIRS tasks will last around 20 minutes in total; we have found that this duration is well tolerated by infants in this age range.

Parent Questionnaires and Interviews. A number of parent-report measures will be used to assess behaviours in their child. For example in at risk infants we will ask about symptom levels in the older sibling, and family. The questionnaires and interviews provide complementary information. Questionnaires are posted to the parent before the visit or completed online. Alternatively, the researcher may schedule a time to call the parent at home and complete some of the questionnaires. The questionnaire packets are designed such that they should take 2 to 3 hours total time (across home and lab completion) per time point. Interviews will be done during lab visits or on a phone call and take 10 to 20 minutes in total for infant time-points and around 2 hours for toddler/child time-points. This length of questionnaire packet and interviews has been used successfully in our previous waves of data collection, and parents report in our feedback reports that they are satisfied with the general duration of time required.

Observational Assessments. We will also ask infants to participate in a number of behavioural observation tasks. Total behavioral testing time is around 1 to 2 hours at each time-point (depending on age and interest level). Games are all designed to be fun for infants. Parents report that their infants find our activities fun and engaging, and enjoy the testing sessions.

Blood sampling. Samples will not be taken from infants or very young children (age < 6 years); instead permission to obtain DNA from saliva/cheek swab will be requested. Local anaesthetic will be used to ensure comfort for children older than 6 years old. We will however, where possible, obtain samples of blood from the umbilical cord after a baby is born – this is painless.

Human issues. Feedback to participants of incidental findings

MRI. As noted above, all MRI scans are reviewed by a radiologist. If MRI, or other physical health abnormalities are found, a specialist (e.g. neuro-radiologist or neurologist) will be consulted. If findings are relevant to the subject's health, the family is contacted and advised to seek further medical assistance. This is indicated in the Informed Consent. A letter summarizing findings and recommendations is provided to the family doctor/care team for further appropriate action.

EEG. The data collected during our EEG study is not a comprehensive clinical EEG, however when an incidental anomaly is detected the following procedures are followed:

- Contact the responsible doctor to look at the images. In this stage the participant is not informed about the finding
- The doctor may contact a neurologist to examine the EEG.to determine the medical relevance of the finding.
- Where the finding represents a normal variant/has no clinical significance, the participant will not be informed.
- In all other cases the participant will be informed by the doctor in person and provided advice.
- A letter with findings and recommendations is provided to the family doctor/care team for further appropriate action.

Genetics. For genomic analyses, the strategy for dealing with incidental genetic findings is as follows. Genome sequencing harbours a small risk of incidental findings, i.e. potentially clinically relevant findings that are not related to the research question. To guard against such incidental findings, we will use a bioinformatic filter that will prevent analysis of genomic locations that are known to harbour highly penetrant mutations. The bioinformatic filter to be used in the study is derived from a recent paper by the American College of Medical Genetics (Green et al. 2013) containing recommendations on incidental findings. This filter includes the most frequent, highly penetrant genetic variants, which fall into the category of actionable findings. In the future, new actionable genetic variants might still be discovered, although these findings will become more rare and of lower frequency quickly, given the fast progress in gene finding for penetrant diseases throughout the last few years. In the unlikely instance of identifying an incidental finding despite the use of this bioinformatic filter at the RUMC, the research team will report the finding to a commission at the Human Genetics department of RUMC that will determine the clinical relevance. The commission will also determine whether or not the finding is actionable (i.e., the patient can be treated or the disease can be prevented). In case of an actionable finding, the research team will inform the patient's parents and they will be asked to seek genetic counselling.

Behavioural Observations, managing parental concerns and feedback to families. Families who have a child with ASD made aware of the increased risk to a younger sibling through their clinical services or public information channels. Despite awareness of the risk, parents may or may not have any immediate concerns about the younger sibling. For the younger ages, this is consistent with the emerging research findings in this area which indicate that early signs are likely to be subtle and not easily detected by parents or other observers. Services for this age group are limited and extremely variable in different parts of the EU. There are currently no validated diagnostic instruments nor interventions that can be used prior to 24 months of age. Moreover, current research on the topic is still limited and more research is required before it can be effectively translated into clinical application. In this study, every effort will be made to avoid inducing concerns in parents who were previously unconcerned about their child in accordance with the principle of avoiding inflicted insight. However, it is possible that some families enrolled in our projects will express concerns regarding their infant at some point during the study, for reasons unrelated to the study. The information sheet will emphasize that no individual feedback will be given to parents. If parents ask about signs of ASD in their child, we explain that providing feedback on specific cases lacks scientific grounds since there are currently no validated diagnostic instruments for the age of the child; in fact, developing such instruments is one of the long term goals of the study. In cases where the family does raise concerns, the research team will then respond in such a way to facilitate parents' concerns being addressed by the appropriate services or individuals. This would include encouraging them to speak to their GP, health visitor or paediatrician and offering to write a letter of support if this was felt to be helpful (which could be sent via the parents or directly, according to parental preference). Parental concerns are best addressed, monitored, and dealt in partnership between the parents and their clinically responsible health care providers, and not in the research setting.

Looking to the future – and reducing stigmatisation

We anticipate our work will lead to groundbreaking new knowledge about stratification biomarkers and treatment options for ASD. Thus, we need to not only consider the ethics of the direct experiments we are running, but also the potential implications of our discoveries. We have developed a set of strategies that we will employ as new results emerge from our project. Our over-arching strategy is to:

- *Take a proactive approach.* Work described within WP5 will involve engaging with the patient and client community to ensure that community voices are heard through every stage of the research process. We will begin discussing the

broader societal implications of the planned outcomes of our project from the outset, particularly for potentially sensitive areas like fetal biomarkers, genetic stratification or pre-emptive treatment for ASD. Our investigator teams have long histories of using similar processes to shape the research streams that feed into AIMS-2-TRIALS, and will continue to engage with the community at the site level. This will ensure that when new results emerge, we have already identified and begun to address the relevant community concerns.

- *Ensure dissemination is ethically-informed*. Sensitive public dissemination of the results of our work in AIMS-2-TRIALS will be critical. We will ensure the ethics of our dissemination in sensitive areas are fully considered by seeking the advice of our Internal Ethics Board on press releases and paper wording, who will consult our Ethics Advisor or engage other international experts where necessary. We will also continue to host webinars and public meetings around important topics and new findings as they arise, as we have successfully in EU-AIMS. This will ensure that we use the most appropriate framing of our research when we communicate it to the community and broader public. We will ensure that we publish results from our studies – and even if they are negative. Also the results from our RCTs will available through public repositories (e.g. clinicaltrials.gov and the EU clinical trials register).
- *Contribute to shaping new ethical frameworks*. Novel discoveries may require new ethical frameworks to be developed. This may be particularly applicable in the area of genetics, where the rapidity of progress will change our ability to make nuanced individual predictions. Another sensitive issue is the identification of fetal biomarkers; despite the acquisition of this data well beyond the legal limits for termination of pregnancy we recognize that there may be concern in the community. As the scientific progress within AIMS-2-TRIALS crystallises, we will contribute to shaping the new ethical frameworks required to govern use of our findings by working with relevant agencies to stimulate the development of relevant guidelines (e.g. World Medical Association; ICH; the EU commission). This will ensure that as AIMS-2-TRIALS produces the opportunity for new treatment approaches, the ethical frameworks necessary to govern their use are in place.
- *Reducing stigma.* Stigma in relation to people with neurodevelopmental disorders and/or mental illness can be understood as a combination of problems of knowledge (ignorance), attitudes (prejudice) and behaviour (discrimination). To reduce this a series of interventions have been identified which may be effective in reducing stigmatisation and discrimination at the following levels: individuals with neurodevelopmental disorders/mental illness and their family members; the workplace; and local, national and international. The strongest evidence for effective interventions at present is for (i) direct social contact with people with neurodevelopmental disorders/mental illness at the individual level, and (ii) social marketing at the population level. Hence our dissemination program will directly address each of these issues – for example our social marketing efforts include not only large scale use of social media but a progam of activities in art and science museums across the EU.

Specific WP issues

WP2. Task 2. Studying fetuses, neonates, and infants at high risk of autism; and young children with ASD.
This application involves the collection of data from pregnant women, newborn infants and young children, and samples containing DNA from infants. As noted above we already have ethical approval for all our studies in fetuses and infants (please see **Appendix W**). Nevertheless there are a series of ethical issues we will encounter including:

Recruitment, and safety of MRI, for fetuses. Mothers of 'at risk' and control fetuses will only be recruited in the UK. They are recruited by advertisement within antenatal services. Regulatory guidelines state that it is safe to use fetal MRI at 3T or less during the second and third trimester [130]. Moreover, in a recent study involving over 1.4 million pregnancies, exposure to MRI during the first trimester of pregnancy was not significantly associated with any adverse effects on the fetus or the developing child [131] . The fetal brain is most sensitive to heat during the embryonic and mid-gestation periods, i.e. prior to our scanning window [132]. In our Standard Operating Procedures, the fetal temperature is assumed to be 0.5°C greater than maternal temperature [133]. Hence, it is important to ensure that the maternal temperature does not rise by more than 0.5°C while in the MRI scanner, and that the fetal temperature is kept under 38°C. The temperature in the scanning room is therefore set to 18°C, with air-conditioning inside the bore of the magnet. The expectant mother's temperature is monitored before and after the scan. Sequences are run in an order that minimises long periods of the higher SAR sequences. The noise levels in the scanner are also kept to a minimum, and our mothers wear earplugs and headphones. There is at least a 30 dB sound attenuation as noise passes through the maternal abdomen and amniotic fluid to the fetus, and there is no evidence of hearing impairments in children who have undergone MRI examination during fetal life[134]. All our optimised sequences are acoustically tested to ensure we keep maximum noise levels at 100dB or below. Loud sequences are run for the shortest possible acquisition times.

Recruitment, and safety of MRI, for neonates. Mothers of 'at risk' and control neonates will only be recruited in the UK. They are recruited from the fetal cohort outlined above. MRI at 3T is safe for neonates [135]. Standard Operating Procedures are followed

to ensure highest safety – including monitoring of temperature, acoustic noise, and metallic implants – is in place. The infants are scanned at dedicated paediatric clinical imaging units. For example, a thorough metal check be complete to ensure that the individual is free of any ferrous metal. Exposure to acoustic noise is minimised by insulating the scanner bore with sound attenuating foam. The infant's ears are also protected with mouldable silicone-based dental putty and MiniMuff noise attenuators. In addition, an experienced clinician supervises the MR examination to monitor the infant's physiological parameters, including heart rate, oxygen saturation, and body temperature.

Sedation. We will not use sedation during any of our imaging paradigms for fetuses or infants. However in children older than 3 years some parents use sleep aids such as melatonin or Benadryl if their child is 'grizzly' and unable to settle. Where this is the case then we will follow this usual practice if requested by parents.

Informed Consent. We have undertaken MR imaging studies of fetuses, infants and children for two decades, and previously undertaken all of the procedures or practices required by this project. Each has been reviewed, most on multiple occasions by Regional Research Ethics Committees or latterly the National Research Ethics Service. In each case informed consent by the pregnant woman or parents of the infant was an essential part of the project, and in every case the review committee gave favourable opinions of the research proposal and allowed it to proceed. We are fully aware of the problems of gaining informed consent, and our processes have been independently reviewed and found to be better than those normally used in neonatal medicine [136]. For example we have prepared You Tube videos of our what families can expect during fetal [137] and neonatal scanning [138].

Fetuses and infants at−risk for autism, and children, cannot consent for themselves. Hence, informed consent will be obtained from the parent or guardian of the fetus/infant/child taking part in the study using an Information Sheet, which provides details on purpose, design and participation. The information sheet also includes descriptions of the assessment procedure where the infant's comfort and enjoyment are ensured and assessments are stopped at the parent's request or by the researchers in the unlikely case that the infant expresses discomfort.

Research in children which is not primarily for the benefit of the subject. Data collected in this study are not primarily of diagnostic intent. However, a small proportion of routine MR brain images of asymptomatic individuals show abnormalities. In some cases it will be of benefit to the individual to know about these findings (please see relevant section(s) above for more details).

Data management of parental/infant information. Data collected from parents include personal, full medical and family history, lifestyle, medication, genetic information as well as neurodevelopmental data of their children. This information will be stored and made available to the scientific community for a long period of time. As follow-up research is intended, there is a need to link data to identifiable individuals. Also through combination of data sets, individual participants may be identifiable. To address these issues fetal and infant data will be managed according to the regulations and guidance provided in each country. For example in the UK by the Health Research Authority, and in full compliance with the UK Data Protection Act 1988 which regulates the storage and use of personal data in the UK, and is in line with theEuropean General Data Protection Regulation , covering the potential issues noted above. The permission for research granted by NRES will require conformity with these legal instruments.

In particular this permission and the consent process for the study will explicitly take note that: personal data shall be obtained only for one or more specified and lawful purposes, and shall not be further processed in any manner incompatible with that purpose or those purposes; personal data shall be adequate, relevant and not excessive in relation to the purpose or purposes for which they are processed; that personal data shall be accurate and, where necessary, kept up to date; and that personal data processed for any purpose or purposes shall not be kept for longer than is necessary for that purpose or those purposes.

To this end we will explicitly obtain permissions for data storage and uses in a manner consistent with the act, including the provision of specific anonymous data linked to image data to the scientific community for defined scientific projects, and all data users will be required to commit to follow the tenets and spirit of the regulation, providing appropriate data control and safety, and committing to not transmitting data for third party usage.

Data will be fully anonymous at the point of use, and linkage of data will only be possible by the dHCP team; linkage keys will not be provided to other users, only the specific datasets requested for each project.

The permission granted by NRES to allow recruitment to begin will also cover two complex data issues; facial recognition by 3D reconstruction of cranial MR images; and personal identification by whole genome analysis. Safeguards, such as facial blurring or extraction, are possible and will be used as directed by NRES.

WP2. Longitudinal follow up of LEAP cohort (children > age 6, adolescents, and adults above the legal age for consent)

Ethical permission for this part of our proposal is already in place across all our centres; and approval letters have already been lodged with the IMI. The issues around use of sedation and determining capacity/obtaining informed consent have been discussed above. Blood samples be requested from young children at age >9: and permission to obtain DNA will be requested. We will also seek permission to take blood samples from adolescents and adults. If that is refused then permission to obtain DNA from saliva will be requested. We prefer to use blood for DNA where possible as it provides a better yield.

The issues pertaining to imaging and electrophysiological measures have been discussed above.

We anticipate that some families/individuals may not wish to return in future years. This issue has also been discussed above

WP2. Neurochemical Imaging (PET/autoradiography) methods for understanding epilepsy [WP2]

The objective of this part of WP2 is the use of PET imaging to examine endogenous transmitters and/or synaptic development in adult patients and healthy volunteers. **Infants and children beneath the age of consent will not be included in PET studies**. The PET centre at KCL has conducted approximately 5000 scans per year since opening in 1991 and over 30 drug development-related studies in the last 3 years using novel radiolabelled probes. The PET centres at the KI and UC are equally experienced. In general the experimental conditions have been tolerated well by the subjects. There are two major concerns with PET imaging – the direct response to the injected radioligand and the possible effects of radiation exposure. The injection of a tracer dose (typically less than 10 micrograms total per subject) of a radioligand has not had any pharmacological effects in nearly 10,000 studies worldwide and it is widely regarded as safe and tolerable – so long as it is done per the standard and well established procedures. Some subjects may experience transient pain and discomfort from blood vessel cannulation – this is clearly specified in the informed consent documents. The studies will meet all current EU requirements as described in the "Guideline to regulations for radiopharmaceuticals in early phase clinical trials in the EU" [139] and approved by the Radiation Safety Committee of the Karolinska University Hospital. Accordingly, the radiation dose is limited to below 10 mSieverts.

The work will be approved by the Ethics Committees of Stockholm and Coimbra and the national Integrated Research Application System [IRAS] system in the UK, and conducted in full compliance with the Declaration of Helsinki (and Declaration of Madrid when psychiatric patients are included). Subjects will be included after written informed consent. They are covered by the general insurance of the Swedish, Portugese, and UK health care systems and free to withdraw their consent without any explanation or further consequences.

Incidental findings such as unexpected brain anomalies will be followed up by appropriate clinical functions at Karolinska University Hospital for Swedish volunteers, University of Coimbra for Portugese participants, or Kings College Hospital for those in the UK. The studies are prospective and will not include collection of previously collected data. All image files and subsequently generated data in the quantitative analyses will be assigned a code. The key will be stored in a locked safe accessible to one person only. The studies will be conducted in compliance with the relevant Swedish, Portugese, and UK Biobank Laws to ensure adequate protection of data and privacy.

We will also validate our findings using autoradiography on brain tissue resected as part of normal operative procedures for treating epilepsy at KCL. To be clear - we will not remove any additional tissue for this study, we will only use tissue that is normally resected for clinical purposes. This will require specific consent from the individual. Also autoradiography will be performed on resected epileptic tissue after clinical neuropathological examination. Subject to ethical approval and informed consent, tissue will be stored and analysed in compliance with the UK Human Tissue Act (2004). Paper copies of consent will need to be held for **7** years, and electronic for **30** years. Some tissue may also be exported and shred with other non UK collaborators (e.g. UCB). Patients will need to give specific consent for this to happen. Also we will follow Code of Practice 8 of the UK Human Tissue Authority which states 'Exported material should be procured, used, handled, stored, transported and disposed, in accordance with the consent which has been given, with due regard for safety considerations and with the dignity and respect accorded to human bodies, body parts and tissue in codes in England, Wales and Northern Ireland. This includes providing donors with adequate information upon taking consent, that their samples may be transported as exported samples for use abroad. [140]

WP2. Functional imaging/EEG models for drug testing/discovery.

One objective of human experimentation in these WPs is to establish cross-species homology and to identify specific brain activations which may serve as targets for drug development. The main focus of this work includes MR Imaging and EEG, though personal and genetic data will also be collected.

The issues pertaining to imaging and electrophysiological measures have been discussed above. Personal (epidemiologic) and genetic data are being collected as part of this study. Since linking genetic information to multimodal imaging and outcomes data is at the core of our work, the collection of this data is necessary. Specific consent for this is obtained. Data are pseudo-anonymized. The amount of sample donation will not exceed national and local regulations, neither the amount specified in the informed consent leaflets.

WP2. Metabalomic and other biomarkers for autism.

This work package requires the analysis of blood samples (serum and plasma) from both existing studies and our new studies. This will include samples from patients with psychiatric symptoms - and in WP 3 and 4 those before, during and after drug treatment with a view to identifying and validating biomarkers with potential utility to aid the diagnosis and treatment of diseases such as autism. Written informed consent has been obtained in all the current available samples being considered; and will be obtained from new patients by both academic partners and **EFPIA** member companies carrying out any future clinical trials in

accordance with EU ethical standards and local institution/country review board requirements. For data from other studies that may seek to collaborate with us in future the academic and **EFPIA** applicants will review the informed consent to make sure that the subjects have consented to have their sample used for the scope of the present research. No patient's samples will be included in the biomarker study without informed consent. For anyone in our LEAP study who reaches their age of majority (>16) and who asks for their data to be removed, this will be complied with.

Only fully trained clinical staff will take and process blood samples. Standard blood doning and sample handling protocols will be applied in all centres. Samples will aliquoted and stored at -80°C, and will be blinded and anonymized by providing a specific code for each patient before shipment to the Leboyer labs, for biomarker profiling. This code is subject to data protection in accordance with EU laws. No data enabling the identification of patients or volunteers will be provided to sample users. As no intervention will be decided on the basis of these studies, and as it is highly unlikely any biomarker data would be available in a timescale useful for the acute treatment of the patients, there are no plans to provide any follow up information to the patients.

Since a key feature of this application is to translate clinically relevant features of psychiatric disease into better and more useful preclinical models to enable more efficient and targeted drug discovery – the WP will also include the testing of biomarkers in samples from animal models that are generated in the course of WP 2 and/or are already available with **EFPIA partners**. The animals will be housed, cared and treated as laid out in the sections dealing with other WPs above – only their terminal samples will be sent to this part of the WP for analysis. This element of validating animal models by examining them for biomarkers will in time lead to the further refinement of models and could also lead to the reduction of the total numbers of animals used in development.

WP3. 'Shiftability' and Fast Fail Trials.

The issues of consent, compensation for time, and procedures to be used (e.g. MRI and EEG) have already been addressed in prior sections.

A particular risk of these components are those of safety and tolerability of the pharmacological compounds we may use.

Part of WP3 will entail the administration, to adults only, of a pharmacologic challenge – (e.g. one dose of an SSRE) to assess the effect of these compounds on brain function. All of the compounds which we investigate will be safe for the intended purpose, and approved for use in adults. In addition, these interventions will be restricted to Partners of our network which are fully equipped for clinical research and where a research nurse and a doctor will monitor the patients. Patients will be informed verbally as well as in writing (via the patient information sheet) about the potential side-effects of any pharmacological probes. In the interest of patients' safety, any patient suffering from moderate to severe hypertension, hypo/hyperthyroidism, history of drug or alcohol abuse and epileptic seizures will not be included in pharmaco-challenge studies. Pregnant and breast-feeding women will not be included because of potential embryotoxicity. Thus, a combination of an experimental design informed by previous experiments, careful patient selection, appropriate consent procedures, dedicated facilities and monitoring will allow us to conduct this study in a manner that minimizes risk.

The doses of medication we have initially chosen were selected to minimize possible side-effects with maximium likelihood of target engagement. We have previously adopted this approach for single dose studies of a range of compounds and found excellent tolerability in all our studies so far. With regard to the specific compounds.

A) Tianeptine. The medication tested in this study (tianeptine) is manufactured by pharmaceutical company Servier. We will use the manufacture's recommended dosage of tianeptine - 12.5 mg/tid. We already have ethics approval in place for the 'shiftability' study at KCL for cases vs controls, and we will now seek an amendment of this to allow intra-ASD comparisons. We will need to also obtain ethics approval at UC; and for the 'fast fail' study in both centres. Tianeptine is generally well tolerated and has been described as having a safety profiles [141] similar to other antidepressants and anxiolytics. The side effects profile is much like SSRIs, and includes nausea, constipation, abdominal pain, headache and dizziness. Clinical trials in healthy controls of 12.5 mg/tid have reported no adverse effects on cognitive, cardiovascular or psychomotor performance. When compared to placebo, only headache appeared more often with tianeptine. In a placebo-control trial in [142] patients with major depression the most commonly reported side effect(s) included nausea (8%), constipation (14%), headache (16%), and dry mouth (8%). A comparison of tianeptine to the SSRI paroxetine, in patients with depression and anxiety, reported the following side effects for tianeptine: nausea (7%), headache (6%), insomnia (1%) and dizziness (1%). Overall tolerability for tianeptine was significantly better than paroxetine. There has been one published report of a suicide death due to an overdose of Tianeptine in combination with alcohol [143]. At the single doses we plan to use, we do not expect to see significant adverse effects. Nevertheless, a clinician will review each participant before and after drug administration. Also, the participants will be asked to stay at the research unit until at least 4 hours after drug administration. Before leaving, each participant will complete an 'adverse effect checklist' with the clinician who will act on any concerns as appropriate. The randomization code can be broken at any time during the session. In the 'fast fail trial' all participants will be prescribed a 4 weeks' worth of tianeptine at a time by a registered doctor, which they will receive from the pharmacy at the outpatient department of the Maudsley Hospital and/or UC. Standard clinical guidelines will be followed. We will write a letter to the participants General Practitioner (GP) informing them of their patient's choice to take part in the study. We will include a product summary of the medication the patient is taking. Safety will be monitored by a medical doctor.

B) AZD7325. The company have furnished the PI with the Investigator Brochure for AZD7325 (This will be shared with the IMI if requested). The compound has been used in five Phase I and two Phase II studies in the US and EU. Single dose acute studies have used up to 100mg and fixed dosing of up to 15mg BID have been investigated. Across these studies AZD7325 has been well tolerated. Adverse effects are dose dependent and not prominent at the doses selected for this study. The most common adverse effects reported include dizziness, somnolence, fatigue and headache. Also reported, but less common, were; feeling hot, euphoria, feeling "drunk", and nausea. At the single doses we plan to use, we do not expect to see significant adverse effects. Nevertheless, a clinician will review each participant before and after drug administration. The participants will be asked to stay at the research unit until at least 4 hours after drug administration. Before leaving, each participant will complete an 'adverse effect checklist' with the clinician who will act on any concerns as appropriate. The randomization code can be broken at any time during the session.

WP4. Clinical trials.

WP4 will conduct 2 RCTs. RCT1 will be a randomized, double-blind, placebo-controlled study of the efficacy, safety, and tolerability of arbaclofen in children and adolescents (ages 5-18) with autism spectrum disorders. RCT2 to be conducted in part 2 will be determined based on results from other WPs and availability of a novel compound by industry or academic partner. These studies will be conducted according to the principles of the World Medical Association Declaration of Helsinki and its most recent amendment (Fortaleza, Brasil, October 2013). The Investigator will conduct all aspects of this study in accordance with all national, state and local laws of the pertinent regulatory authorities. Privacy laws and regulations will be adhered to during all procedures related to studies in WP4. The clinical trials will be conducted in accordance to the Regulation EU No 536/2014 (https://ec.europa.eu/health/human-use/clinical-trials/regulation_en). This Clinical Trial will also follow the guidance in 'Good Clinical Practice: Consolidated Guideline' (ICH E6) adopted by the ICH, 1 May 1996 and the protocol developed has followed the Statistical principles for clinical trials [144].

The issues of consent, compensation for time, procedures to be used (e.g. blood draw and EEG) and ethical aspects regarding sample use have already been addressed in prior sections.

Regarding the compound to be used in RCT1, arbaclofen, there has no been significant safety or tolerability concerns from previous studies with the drug in children and adolescents. The dose used have been previously used for the age range to be included in the study. The most common adverse events reported have been agitation (22%), irritability (22%), fatigue (16%), psychomotor hyperactivity (16%), diarrhorea (13%) and insomnia (13%). The majority of AEs were mild and resolved spontaneously or with dose adjustment. More recent studies published in a) animal models given R-Baclofen show that the compound had produced minimal side effects and b) human studies with children and adolescents arbaclofen was well tolerated with affect lability (11%) and sedation (9%) being the most common adverse events. Racemic baclofen and arbaclofen are excreted renally. Any participants with renal disease will be excluded from the study. Pregnant or breast feeding women will also be excluded. In addition, females of childbearing potential who are sexually active will have to agree to use an accepted form of contraception.

Pharmacokinetics with arbaclofen has been assessed in paeadiatric population (2-17 years, n=61) with cerebral palsy [145]. Forty-nine subjects had at least one post-dose PK blood sample and were included in the population pharmacokinetics (PopPK) analysis. R- and S-baclofen showed identical concentration-time profiles. Both baclofen enantiomers exhibited linear and dose/kg-proportional PK, and no sex differences were observed. Average baclofen terminal half-life was 4.5 hours. A 2-compartment PK model with linear elimination and transit absorption steps adequately described concentration-time profiles of both baclofen enantiomers.

Safety will be assessed by evaluating reported AEs, suicidality assessment, clinical laboratory test results, ECG findings, physical examination findings and concomitant medication usage.

To assure the continuance of strict pharmacovigilance, an independent Data Safety Monitoring Board (DSMB) will be constituted to review the safety data collected in the study (including AEs, physical exams, vital signs, clinical laboratory tests, suicidality assessments, ECGs, and/or other information necessary to determine subject safety). For more information on the role and procedures of the DSMB see the RCT1 study protocol.

The Principal Investigator at each site will have extensive clinical experience caring for patients with ASD. The Principal Investigator or a designated substitute will be available 24 hours a day, 7 days a week. We will set up a 24/7 emergency unblinding system with each centre pharmacy. Results of the physical examination and laboratory values will be evaluated by the staff at each center for evidence of immediate safety concerns or for changes that indicate safety concerns. At each clinic visit and phone call during the study period, investigators will question parent/caregiver/legal representative about changes in health, inter-current

illnesses and concomitant medications. Any AE (adverse event) will be documented regarding the time it occurred, duration, severity, and relationship to the study drug. The Investigator will have to report promptly all SAEs to the sponsor in accordance with the procedures detailed in the RCT1 protocol. The Investigator, or responsible person according to local requirements, will comply with requirements related to the reporting of SAEs (serious adverse events) to their IRB or Independent Ethics Committee (IEC). For more information on reporting adverse events see the RCT1 study protocol.

WP6. Analysis Methods

This part of the WP mainly entails the analysis of cognitive, behavioural, electrophysiological, genetic, and scan data already collected in prior projects by our partners and as part of these WPs, though some specific and targeted MR image acquisition will be required over the five years. The acquisition and use of this data is governed by specific ethics applications for each clinical project. A broad ethics application has previously been granted to all our clinical recruitment sites involved in WP2 who will feed data to WP6. That allows data to be stored pseudoanonymously and securely on computer systems within all our clinical recruitment sites for possible future research ("Establishment of an MRI library of normal and diseased brains of adults and children", 033/03). We will use this stored data (and others for which appropriate informed consent was obtained by the academic and/or industry partners) to train, test and evaluate new software being developed by our network. To make full use of data collected in clinical research studies they must be analysed with computer software. To be able to develop, debug, test and evaluate such software requires access to larger amounts of data from a wider variety of subjects than are usually collected for individual projects. These data are needed to train and test the software and in particular to ensure that it can cope with age and sex differences and the subtle variations in (for example) the appearance of the brain caused by the range of neurological and neuropsychiatric conditions that are the basis of this IMI project. We also propose to pool data (e.g. electrophysiological recordings and brain scans) acquired and stored during previous research studies by our network and partner companies to test and evaluate future analysis techniques.

Informed consent issues. We will be analysing data from both new and existing studies. Research participants will have passed the inclusion criteria associated with those specific studies. We will include data from all subjects unless (a) they have not consented for their scans to be used in future work (b) the analysis technique under consideration is not appropriate for their clinical status or data−type (c) there are technical problems with the data (e.g. loss of quality caused by movement during a scan) (d) there is a risk of breaking anonymisation by inclusion.

Data protection issues. Some limited demographic information (age, sex, handedness, clinical status only) may be requested from the PI of the original study for certain kinds of testing. If supplied this data will be linked to the data using an existing pseudo−anonymisation code, for which only the study PI has an unblinding list. In this case researchers working under this ethics proposal will have access to these pseudo−anonymised demographics only − unblinding is not required at any stage of technical evaluation. These pseudo−anonymised demographics will be stored electronically and securely on central computers.

WP6. Identifying risk pathways via Genetics

The major objective of this WP is to collect genetic, transcriptomics and clinical data and link them to understand pathways of illness and their relevance to drug development. The major ethical issue in this effort are those related to consent, data protection and privacy of subjects.

AIMS-2-TRIALS has already genotyped ~ 1000 subjects, and will acquire genetic samples from ~ 10,000 more (including follow up studies of 1500 infants (now children) from the EU infant connectome project and the 7000 infants (now children) from the Safe Passage Study (South Africa). Phenotyping will be done by researchers (e.g. psychiatrists and psychologists) blind to genotype status. Standardized interviews and questionnaires used worldwide for diagnosing psychiatric patients will be employed.

In view of the continued expansion of data related to human genetics, concern for protection of the personal privacy of individuals who participate in such studies is paramount. While some have advocated the use of complete anonymity as a way out of this dilemma, others argue that the future cannot be predicted, and that future benefits may be lost when the link between genetics data and the individual subject is totally lost. Therefore, some ethical bodies have suggested a reversible third-party encryption system.

EFPIA members and Charity partners (e.g. SFARI) may also contribute data and data analyses from clinical trials (or other work) that were generated before the start or during this grant. The data analyses shared within this WP will be generated in line with the scope of the respective study protocols and the corresponding informed consent forms. Also, only samples from subjects enrolled in clinical trials that have been collected according to GCP standards, and include informed consent for the type of analyses performed in WP6 will be contributed to this WP. In addition, all samples will be handled according to Standard Operating Procedures (SOPs) within the contributing industry partners. These SOPs ensure that the privacy of individuals participating in clinical trials is protected, e.g. by (double) coding samples obtained from participants and restricted access to the keys holding the codes.

Based on the above, there may be a small set of samples/data where the participant has provided the consent for the use of data, but, not for the sharing of samples; and in some cases for the sharing of the results of analysis rather than the raw data itself. In such cases, biological analyses will be performed through the respective owner of the clinical trial samples (i.e. the respective **EFPIA partner**) and only the resulting and consented data or results of analysis (as allowed per initial consent) will be shared. All efforts will be undertaken by the participants of this WP to ensure that only those biological samples will be contributed for which the respective informed consent covers the planned analyses, e.g. CNV status and transcriptomics and association of these molecular markers with the treatment response. Samples for which the informed consent does not cover the scope of the analyses outlined in this WP, will not be used, unless a new and revised informed consent is obtained. In view of the multi-centre nature of most clinical efficacy trials, a re-contact of subjects from previous clinical trials would be a major logistical effort and is currently not planned by us. This possibility will only be pursed if there is an acute need for more samples – in which case the allowance in the original consent form will be strictly adhered to.

WP6. Identifying biomarkers of response and personalized medicine – a focus on the predictors of outcome and pharmacogenomics of autism. Data Protection issues.

The issues around consent, and 'deep phenotyping' (e.g. EEG, imaging, cognitive and behavioural assessments and genetic material) have already been discussed above.

In this part of the work package, however, we may also combine our data with samples previously collected in a number of studies for the research on the clinical and genetic determinants of treatment response in autism. Such re-use of combined existing data presents an opportunity to derive large benefits with no or very little additional burden on participants. However, as this project includes genetic samples and sensitive data including information on participants' mental health, the project raises issues with regard to informed consent, data protection and handling of biological genetic samples.

It is paramount that any research performed with previously collected data conforms to what the participants consented to be done with their data. The academic and EFPIA applicants will have reviewed the informed consent to make sure that the subjects have consented to have their sample used for the scope of the present research. Specifically, all participants will have consented that the data and samples they provide would be used for the research on autism treatment and its genetic determinants. Before any sample would be shared between EPFIA member and IMI collaborators, availability of informed consent for each of the research subjects would be checked. Furthermore all Academic and EPFIA partners would make sure that all samples to be investigated as part of this project have obtained appropriate approval by local Ethic Review Board committees to be used for the same research aims as the present project.

As the proposed research includes use of genetic and mental-health related information, adequate data protection procedures have to be in place to prevent inadequate use of such sensitive data. We take several precautions to comply with the highest standards of data protection. First, we limit data collection and sharing to data immediately relevant to the research questions. The data will be fully anonymised and no personally identifiable information, such as name, initials, date of birth, or address will be included. There is no need to re-contact research participants and therefore, data is not only anonymised but also unlinked so there is no possibility for the researchers to reverse the anonymisation process. The anonymised and unlinked data will be stored exclusively on password protected servers and will be only accessible to approved individuals who are researchers on this project.

Research with human participants will be conducted in the countries listed below in accordance with national and international regulations.

EU countries	
Country	*Applicable National Regulation(s)*
France	French Public Health Code, Legislative section, First part: general protection of health, First book: personal protection, Second Title: biomedical research, ch. 1 to 3 (this law also lists the relevant national authorities for regulatory and ethical approval). Ibid., Third Title: genetic examination. Ibid, Fourth Title: Tissues, cells, human body products and their derivatives. In the French Public Health Code, related regulatory parts on biomedical research. Loi n° 78-17 du 6 janvier 1978 on Data Protection and Liberties, art. 2, 53-61.
Germany	Arzneimittelgesetz (AMG) 24.08.1976, last amended 07.07.2017 (this law also lists the relevant national authorities for regulatory and ethical approval); GCP-Verordnung (GCP-V) 09.08.2004, last amended 19.10.2012, Arzneimittel- und Wirkstoffherstellungsverordnung (AMWHV) 03.11.2006, last amended 28.10.2014; Berufsordnung für Ärzte (Medical Association's professional code of conduct);
Ireland	The European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations, 2004. The Control of Clinical Trials Acts 1987 to 2006 (for any other clinical trials on medicinal products not covered by the above Regulations).

	No other legislation is involved for other clinical research projects. Good Clinical Practice (GCP) regulations and the requirements of the Research Ethics Committee of St. James's and Tallaght Hospitals.
Italy	Ministerial Decree of 15 July 1997: Transposition of guidelines of the European Union in good clinical practice for the conduction of clinical trials with medicines" Legislative Decree no.211 of June 24, 2003: Transposition of Directive 2001/20/EC relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for clinical use" Ministerial Decree of May 12, 2006: Minimum requirements for the institution, organization and functioning of Ethical Committee for clinical trials with medicines Ministerial Decree of 14 July 2009: Minimum requirements for insurance policies which safeguard participants to clinical trials of medicinal products Determine n. 451 of 2016 "Requirements for Phase I Centers" Ministerial Decree of 21 December 2007 "For the Request for Authorization to the Competent Authority and the Ethics Committees" Regulation n. 536/2014 of 16 April 2014 "New European Regulation on Clinical Trials of Medicinal Products in Europe" Ministerial Decree of 8 February 2013 "Criteria for the composition of the Ethics Committees that integrate the DM of 2006" Ministerial Decree 17 December 2004 "For non-profit studies"
Netherlands	Local Ethics approval from an accredited Medical Ethics Committee (METC) and positive judgment by the local authority according to the Medical research involving human subjects act (Wet Medisch-Wetenschappelijk Onderzoek met Mensen- WMO) (see www.ccmo.nl) Declaration of Helsinki De Nederlandse Gedragscode Wetenschapsbeoefening (Rules of Conduct for Scientific Studies of Dutch Universities) (see www.vsnu.nl) Wet Bescherming Persoonsgegevens (see www.cbpweb.nl), in accordance with the European Data Protection Reform (see www.ec.europa.eu) Good Clinical Practice Directive EMA: European Clinical Trials Directive Local: Approval by the Data Protection Representative of each partner
Spain	Biomedical Research Act 4/2007; Royal Decree on Biobanks 1716/2011 Royal Decree 1090/2015 (RD 1090/2015), of 24 December, governing the conduct of clinical trials with medicinal products.
Portugal	Directive 2005/28/EC or Good Clinical Practice Directive, of 8 April 2005 of the European Parliament and of the Council, lays down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products. Directive 2001/20/EC or Clinical Trials Directive of 4 April 2001, of the European Parliament and of the Council on the approximation of the laws, Regulations and administrative provisions of the Member States relating to implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. European General Data Protection Regulation on the protection of individuals with regard to the processing of personal data and on the free movement of such data. The EU Charter of Fundamental Rights; Helsinki Declaration (adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964; amended bythe 29th World Medical Assembly, Tokyo, Japan, October 1975; the 35th World Medical Assembly, Venice, Italy,October 1983; the 41st World Medical Assembly Hong Kong, September 1989, the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000); Universal Declaration on the human genome and human rights adopted by UNESCO; Applicable National Regulations: monitored by Entidade Reguladora da Saúde, CEIC and INFARMED. The conducting of clinical trials on medicines for human use is governed by Law n.º 46/2004 of 19 August that implements Directive 2001/20/EC of the European Parliament and of the

	Council of 4 April in Portuguese legislation. Approval by local review boards will be obtained, and all trials will be registered in the PNEC portal.
United Kingdom	The Medicines for Human Use (Clinical Trials) Regulations 2004 SI 2004/1031 (providing standardisation of procedures for ethical and competent authority consideration and authorization in the UK), last amended 2008 (SI 2008/941). The Human Tissue Authority Code of Practice 8. The UK Human Tissue Act 2004 (c30).

5.1.1 Human Cells/Tissues

All tissue samples will be held and handled in compliance with the UK Human Tissue Act 2004 (c30).

Most of our work involves the use of established cell lines of human origin. They raise few ethical issues since as established lines, anonymised in accord with current European regulations, they fall outside of the legislation relevant to primary human tissue and require no contact with patients or primary human material. We will, however, also generate new human lines, and this raises ethical issues that we will address.

The novel lines will be generated from hair biopsies taken from ASD patients and control individuals. The main issue here is informed consent, anonymisation, and procedures for the appropriate storage and access to material. This consent procedure must accommodate the fact that many of the patients are children, and others who might not be able to give informed consent. At **KCL**, we have established a protocol for this informed consent, and routinely collecting scalp hair biopsies for the generation of iPS lines. We have generated information datasheets for children and parents/carers, including an appropriate level outline of the biopsy procedure and the intended use of the sample. We have specified procedures for the collection, anonymisation, and storage of this material, and the lines derived from it. The protocol has been reviewed by our local ethics committee, and received approval. We intend to use this established protocol, appropriately amended to meet local needs, as the prototype in extending our project to other centres as required.

5.1.2 Management and Protection of Personal Data

Data Management (WP6).

Within AIMS2-TRIALS we will (through WP6, lead by RUNMC, IP, Roche) institute a data management structure based on the Pasteur Data Warehouse solution (PaDaWan). This structure will integrate all data sets collected in WPs 1-4 and meets digital security and data management standards on the basis of FAIR principles.

RUMC and IP will establish an informatics platform for safe, secure data storage and deliver end-to-end privacy protection for sensitive data while enabling full data access functionality and implementing the principles of ‘data security by design’, meeting the legal requirements of the EU General Data Protection Directive (GDP Regulation (EU) 2016/679).

The security and access rights procedures will take into account national, and international legislation so as to ensure - with our US and other partners the legal, ethical, and efficient reuse of data by the scientific community.

Participation in the consortium as data will require any partner organisation to adapt the EU-AIMS2-Trials consortium Data Management Plan and include any applicable national regulation (see appendix for the example template). Access to budget and data from the consortium will require the partner to complete the consortium DMP along with any local institutional DMPs and submit copies to IP along with written confirmation by the local institutional Data Protection Officer (DPO) that allm data data handling and storage activities follow local institutional, national as well as international laws and proceeds according the requirements of the local ethical institutional review board.

Open Access Data will become available to those who register an account with the AIMS2TRIALS consortium and agree to the Open Access Data Use Terms. This includes agreement to comply with institutional rules and regulations.

Investigators are expected to meet one of the following criteria to be qualified to receive access to AIMS2TRIALS Data:

1. You are a Principal Investigator (PI) of scientific research at a university, a research organization (including commercial entities) or a government agency who is the leader of a laboratory or research team or who is working independently; or
2. You provide the name of the PI who is overseeing your research and is approved for access under #1.

3. If you do not meet either of the above criteria you may be considered qualified based on a track record of scientific publications or on the basis of a written reference from someone who meets qualification #1, verifying that the data will be used only for the purpose of legitimate scientific research.

The Open Access Data Use Terms will require a legally binding commitment to that investigators

1. Will not attempt to establish the identity of or attempt to contact any of the included human subjects.
2. Investigators will under no circumstances link these data to Protected Health Information provided to the investigator.
3. Investigators will comply with all relevant rules and regulations imposed by the institution, all national and international laws as well as the legal requirements of the EU General Data Protection Directive (GDP Regulation (EU) 2016/679). This may mean that Investigators need their research to be approved or declared exempt by a committee that oversees research on human subjects, e.g. a local IRB or Ethics Committee. Registration as a Data User requires relevant copies of local approvals being produced.
4. Investigators will acknowledge the use of primary AIMS2TRIALS data and data derived from AIMS-2-TRIALS data, incl. funding through the IMI mechanism, when publicly presenting any results or algorithms that benefitted from their use.
5. Papers, book chapters, books, posters, oral presentations, and all other printed and digital presentations of results derived from AIMS2TRIAL data will contain specific wording in the acknowledgments section that will be agreed with the IMI.
6. Failure to abide by these guidelines will result in termination of my privileges to access AIMS-2-TRIALS data.

Personal Data Privacy Regulation (WP2-6).

Electronic data capture and data integration for research purposes is at the core of the AIMS-2-TRIALS project. Personal privacy legislation is the cornerstone of EU personal data protection, based on the "Data Protection Directive" (DPD) which has been translated into various national privacy legislations in EU. The DPD is also used as a reference for handling sensitive data by non-EU countries.

The main tenets of the DPD for consideration in the AIMS-2-TRIALS project are:

- Data is collected for specified, explicit and legitimate purposes and not to be further processed in a way incompatible with those purposes (art. 6b);
- The collection is adequate, relevant and not excessive in relation to the purposes for which data is collected (art. 6c);
- The collected personal data shall be kept in a form which permits identification of data subjects for no longer than is necessary for the purposes for which the data were collected or for which they are further processed (art. 6e).
- Furthermore, according to art.8a, health data is considered a special (sensitive) data category that can only be disclosed in a very restricted number of situations that include:
- the data subject has given his explicit consent to the processing of those data, except where the laws of the Member State provide that the prohibition may not be lifted by the data subject's giving his consent(art 8.2a);
- data processing is necessary to protect the vital interests of the data subject, or of another person where the data subject is physically or legally incapable of giving his consent (art 8.2c).

Art. 8.3 allows the processing of health data when the data is required for the purposes of preventive medicine, medical diagnosis, the provision of care or treatment or the management of healthcare services, and where those data are processed by a health professional subject under national law or rules established by national competent bodies to the obligation of professional secrecy, or by another person also subject to an equivalent obligation of secrecy.

Privacy legislation clearly distinguishes primary and secondary use of data. In most cases, the primary collection of EHR will be done according to art. 8.3 in a diagnostic or therapeutic context. Subsequent use for clinical research will be considered secondary use, and will therefore require informed consent from the data subject and/or de-identification of the data.

In cases where the patient consents to be enrolled in a clinical research study prior to data being collected, the data collection is then considered to be primary and requires the informed consent of the patient.

Regardless of the primary or secondary nature of the collection or processing, researchers should be aware that privacy legislation is not the only reason why the informed consent from the data subject needs to be obtained. Patients may have other ethical objections for participating in a clinical research study, which may be motivated by reasons entirely different than privacy-related.

Hence, the main principles that will be followed for data management in AIMS-2-TRIALS will be de-identification and anonymisation (used in combination with access control of the data, codes of conduct and other procedural regulations for entities handling personal and de-identified data).

The ethical task force stresses that effective electronic data protection is always the result of combining ICT-security (authorised access only to systems and repositories), de-identification and other privacy enhancing technology, and plain organisational

preventive countermeasures (such as codes of conduct for personnel and non-disclosure obligations). Therefore, the EU-AIMS project will establish a clear privacy policy.

All biological samples will be stored and handled in accordance with UK and EU regulations. Most samples will be collected within the European Union. For samples collected in the US, genotyping will be performed in the US (this is ethically approved separately, and we are not seeking approval for this related work that is going to be carried out in the USA) and only electronic data will be shared to comply with the US regulations.

RUMC and IP will establish an informatics platform for safe, secure data storage [113] and deliver end-to-end privacy protection for sensitive data while enabling full data access functionality and implementing the principles of 'data security by design', meeting the legal requirements of the EU General Data Protection Directive (GDP Regulation (EU) 2016/679). The security and access rights procedures will take into account national, and international legislation so as to ensure - with our US and other partners (cf WP6 Task2) - the legal, ethical, and efficient reuse of data by the scientific community. All consortium partners will be required to check against relevant national regulations and ensure compliance, and we will store all relevant declarations centrally. Moreover, we will keep on file (also stored centrally); 1) detailed information on the procedures for data collection, storage, protection, retention, and destruction, and confirmation that they comply with national and EU legislation; 2) evidence that transfer of personal data from/to a non-EU country or international organisation, is authorised and complies with national and EU legislation; and 3) relevant authorisations for further processing of previously collected personal data. All these issues will be specified as part of the grant agreement.

Protection of research participants' confidentiality

The European Guidance for Healthcare Professionals on Confidentiality and Privacy in Healthcare will be respected and all study procedures will follow this guidance. All documents and data will be handled with strict confidentiality. Names and person-related data will be subject to the conditions of the national Protection Acts and European Directives and rules. In the documentation and data analysis phase, the patient-related data will be recorded pseudonymously and will be identifiable only by randomisation number, initials and birth date. The investigating physician will provide the monitor access to the patient file according to the monitoring process (quality control of data recording, archiving of source documents, ensuring that queries are processed immediately and completely, and review of SAE documentation).

The proposed trials will handle data of patients fairly. Data will processed for limited purposes of the studies. Therefore only relevant data will be collected. Data will be processed accurately (see above according to protection acts and monitored according to GCP). Data will be used for the analysis of the results of the proposed trials and will be not kept longer than is needed, but as long as to protect safety of the patients. No data will be transferred to countries without adequate protection.

5.1.3 Animals

We will not carry out any work in non human primates. Moreover, we will keep on file copies of ; 1) relevant authorisations for animal experiments (covering also the work with genetically modified animals); and 2) training certificates/personal licenses of the staff involved in animal experiments. This will be specified in the grant agreement.

This work will be undertaken in laboratories of five academic partners (**UNIBAS, KCL, RG, UEDIN** and **UULM**); and we address the specific issues herein. This research inevitably requires the use of laboratory animals as the key objective of this part of the project which is the study of the genetic and environmental influences on behaviour, the living brain, and the study of brain circuits involved. Since the expected relevant results are caused by complex interactions between cells, tissues and brain areas, the use of alternative methods would not be advisable to achieve the experimental goals. The anticipated benefits of the project justify the use of animals due to the fact that alternative methods are not applicable. We will use genetically modified rodents – to allow specific genetic questions to be addressed and to relate our work in humans (e.g. people with SHANK3 or TSB2 mutations) to rodent models of the disorder. The 3 Rs rule will be strictly applied:

Refinement: minimise suffering and distress. All reasonable steps will be taken to ensure the humane treatment of animals, so as to minimise discomfort, distress and pain. All surgical procedures will be performed under anaesthesia, with good post-operative care. Euthanasia will not be performed in presence of other animals. Animals will be humanely killed at defined end points according to national ethical rules through cervical dislocation or CO2 exposure.

Reduction: minimise number of animals used. For assessing preclinical and translational validity, live mice and rats will have to be used. All partners have developed in depth expertise in animal experimentation and use validated and optimised protocols that will reduce the number of animals needed to obtain significant results. The experimental design has been specifically powered to achieving statistically significant differences using the minimum possible number of animals. Thus, for example, the groups for comparison between treatments and conditions are generally powered to 10 animals to allow paired data comparison using the same animal for controls and tests. Alternative designs based on independent group comparison would require a much larger number of animals, and only be used when paired data comparison are not possible (for example, effects of a given condition in different model mice strains). Web sites as: Dr. [redacted] **http://www.3rs-reduction.co.uk**, or

http://www.biomath.info/ will be used for the experimental design and to estimate the sample size. Also, to implement our standards we will use factorial experimental design to maximize the data collected from each animal. In addition, we will develop a database to register every piece of tissue obtained from these animals, which will be efficiently stored for any refinement of our experiments and for future studies

Replacement: avoid the use of living animals. Replacement: avoid the use of living animals. Mice share 95% of the genes with humans and genes linked to disabilities in cognitive function in humans they also have been demonstrated to cause similar deficits in mice. They are easy to maintain, reproduces quickly, and are very amenable to genetic manipulations and analysis. For all the above reasons, mice are the most appropriate animals for our experiments, as complex CNS behaviour (e.g. EEG, neurophysiological signals related to information processing, social behaviour, learning and memory) can be studied only in intact animals. It is important to mention that, our understanding on the neural circuits function and the consequence of disrupting this circuitry is still very limited. Therefore, it is difficult to built computer models based on what is still unknown. Mice are the most commonly used species to investigate genetic risk factors through gene targeting and drug action on CNS neuronal activity, particularly at neurocircuitry level. This is particularly facilitated by the availability of mouse lines available from repositories for mutations in genetic disease risk factors and cre-recombinase expressing lines that allow for conditional, cell type-specific gene ablation. Nevertheless, where it is possible, we will use in vitro techniques (cell cultures, brain slices) for electrophysiology, because this can reduce the number of living animals used in our experiments. Over the first 18 months we anticipate using 500 mice, and over the five years a total of 2000 mice. Animals will be maintained on a 12-h light/dark cycle (lights on at 07:00 hours) and housed three-four per cage. Food and water will be always freely available. All experimental procedures will be carried out in strict accordance with European Communities Council Directive on “Protection of Animals Used in Experimental and Other Scientific Purposes” and national regulations (Home Office personal and project licenses under UK Animals, Scientific Procedures) 1986 Act. The experimental procedures in use have been already approved by the Institutional Animal Care and Use Committees. Our work is performed according to the European Union regulations (O.J. of E.C. L358/1 18/12/1986) for the use of laboratory animals and the consortium will follow the Council Directive 90/219/EEC on the “Contained use of genetically modified micro-organisms” and is aware of the guidelines given to the European Commission by the “European Group on Ethics in Science and New Technologies” on “Ethical aspects of genetic modification of animals” (Number 7, 21st of May, 1996).

Animal models of cognitive dysfunction that relate to clinical endpoints [WP2]. One of our aims is to improve the predictive validity of animal assays of cognition used for the discovery of drugs capable of addressing the major unmet clinical need of cognitive dysfunction associated with autism. By employing sensitive, translatable tests of cognition it is possible to measure such functions in rodents that appear to be controlled by homologous regions of the human brain. Animal studies are particularly necessary for establishing the possibility of adverse side-effects and the brain location and the causal mechanisms of action of the medication. The behavioural measures cannot be provided by computational modelling, especially as too little is known for accurate computer simulations of the neural control of behaviour. Tissue culture studies hardly reproduce the complex networks in vivo. For example, many of the inputs and therefore, modulation to the cerebral cortex come from subcortical regions or subtypes of neurons (chandelier cells), which will be absent in vitro. More important, tissue cultures cannot capture the complexity of the systems underlying behaviour and brain imaging in humans is limited by its correlational nature and its lack of spatial and temporal resolution.

Current assays will be **refined** by careful dissection of experimental design and statistical analysis, including power analysis, together with assessment of inter-and-intra laboratory reliability and reproducibility across pharmacological standards and experimental manipulations. Animal numbers will be ultimately **reduced** by allowing the industry to focus on those tests thought most likely to; (i) translate to man; (ii) give adequate signal:to:noise to allow drug effects to be seen; (iii) be reproducible across a wide variety of environmental conditions; and (iv) engage the circuitry relevant to the clinical disorder. There will also be focus on obtaining the maximum behavioural information from each animal by further development of software for apparatus control and data capture. By careful integration across work plans, assays, models and drugs will be consistently applied to achieve converging validity through the use of complementary techniques, for example in vivo electrophysiology in WP2. Over the first 18 months we anticipate using 500 mice, and over the five years a total of 1500 mice. For the present we cannot predict a time when the replacement of animals in research of this nature will be feasible. However, we plan to use in most cases rodents as these infra-primate mammals bear some evolutionary relationship to humans in terms of their basic learning and cognitive capacities, as well as in the structure and function of their brains. Thus, the vast majority of studies will be in rodents: mice are necessary for transgenic studies whereas rats are better suited to the study of brain systems involving manipulations of the central nervous system because of their greater size and because much is already known about this species from neuropharmacological and neuropsychological studies.

Research on animal models will be conducted in the countries listed below in accordance with national and international regulations.

EU countries	
Country	*Applicable National Regulation(s)*
Germany	Tierschutzgesetz (TierSchG) (Animal Protection Act) of 18 May 2006 (BGBl. I S. 1206, 1313), last modified 28.07.2014. Contains federal law on the protection of laboratory animals. Gentechnikgesetz (GenTG) (Act on the Regulation of Genetic Engineering) of 16 December 1993 (BGBl. I S. 2066), last modified 07.08.2013. Contains federal law on the handling of genetically modified organisms, including laboratory animals. Inflammation experiments proposed were already approved by the Committee for Animal Experimentation of the University of Ulm and the regional administrative authority in Tübingen (TVA No. 1293).
Netherlands	The GELIFES institute is a research institute of the Faculty of Mathematics and Natural Sciences at the University of Groningen, the Netherlands which is licensed by the Ministry of Economic Affairs to perform animal experiments (license number: 10500). The laboratory animal facility of the Faculty of Mathematics and Natural Sciences meets all legal requirements for housing of and performing experiments with laboratory animals as set out in Annex III of European Directive 2010/63/EU. According to the Dutch Experiments on Animals Act and conform the EU standards (European Directive 2010/63/EU), project proposals (animal experiments) are evaluated by the Animal Ethics Committee (DEC), the Animal Welfare Body (IvD), and the Central Authority for Scientific Procedures on Animals (CCD).
Switzerland	The project involves the use of small laboratory animals (transgenic and non-transgenic). All experimental procedures have been reviewed by the cantonal veterinary office Basel-Stadt, Switzerland and will be performed in compliance with relevant EU and national legislation (Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes).
UK	All experiments described in this proposal have been reviewed by the corresponding local and national committees and approved under Home Office (HO) licenses PPL 70/8331 (Oscar Marín) and PPL 70/8322 (Beatriz Rico), in compliance with relevant UK and EU legislation (Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes).

5.1.4 Non-EU countries

Our research involves South Africa and Canada and they will study local people with and without ASD. Personal data from those individuals will be imported to the EU (including cognitive/behavioural measures, blood and genetic samples, and EEG/MRI data). In turn the researchers in South Africa and Canada will have access to our (anonymised) data. The same ethical standards will be followed in both South Africa, Canada, and Europe. There are no differences between South Africa, Canada and Europe in the risks to the individuals taking part in our research.

We will ensure that; 1) the research conducted outside the EU is legal in at least one EU Member State; 2) details on the materials which will be imported to/exported from the EU will be kept on file; and 3) copies of import/export authorisations, as required by national/EU legislation will be kept on file. We have given detailed information on the measures to minimise the risks to research participants and staff involved in this project that also impact in non EU countries (at pages 143-145).

Extracted DNA will be exported to Canada – and will be subject to all relevant EU data safeguarding legislation.

5.1.5 Environment Protection

We will use of PET imaging to examine the endogenous transmitters in adult patients and normal volunteers. **Infants and children beneath the age of consent will not be included in PET studies.** There are two major concerns with PET imaging – the direct response to the injected radioligand and the possible effects of radiation exposure. The injection of a tracer dose (typically less than 10 micrograms total per subject) of a radioligand has not had any pharmacological effects in nearly 10,000 studies worldwide and it is widely regarded as safe and tolerable – so long as it is done per the standard and well established procedures. Some subjects may experience transient pain and discomfort from blood vessel cannulation – this is clearly specified in the informed consent documents. The studies will meet all current EU requirements as described in the "Guideline to regulations for

radiopharmaceuticals in early phase clinical trials in the EU" [139] and approved by the Radiation Safety Committee of the Karolinska University Hospital. Accordingly, the radiation dose is limited to below 10 mSieverts. For new radioligands dosimetry will be estimated from whole-body PET-measurements in non-human primates and/or rodents (see above).

The work will be approved by the Ethics Committees of UC (Coimbra), KI (Stockholm) and for KCL the national Integrated Research Application System [IRAS] system in the UK. All work will be conducted in full compliance with the Declaration of Helsinki (and Declaration of Madrid when psychiatric patients are included). Subjects will be included after written informed consent. They are covered by the general insurance of the Portugese, Swedish and UK health care systems and free to withdraw their consent without any explanation or further consequences.

Incidental findings such as unexpected brain anomalies will be followed up by appropriate clinical functions at **Karolinska University Hospital** for Swedish volunteers, **Coimbra University Hospital** for those in Portugal, **or Kings College Hospital** for those in the UK. The studies are prospective and will not include collection of previously collected data. All image files and subsequently generated data in the quantitative analyses will be assigned a code. The key will be stored in a locked safe accessible to one person only. The studies will be conducted in compliance with the relevant Portugese, Swedish and UK Biobank Laws to ensure adequate protection of data and privacy.

The PET-center at the KI has over the years conducted about 9000 single PET-measurements on brain neurotransmission markers in human subjects. The PET centre at the **IOP**/**KCL** has conducted approximately 5000 scans per year since opening in 1991 and the MTIC (Hammersmith Hospital) over 30 drug development-related studies in the last 3 years using novel radiolabelled probes. In general the experimental conditions have been tolerated well by the subjects. UC has carried out similar numbers of PET scans.

5.1.6 Dual Use

The research conducted in AIMS-2-TRIALS does not have any potential for military applications.

5.1.7 Misuse

The research conducted in AIMS-2-TRIALS does not have the potential for malevolent/criminal/terrorist abuse.

5.1.8 Other Ethics Issues

There are no other ethics issues currently identified beyond those discussed above.

5.2 Security

• Activities or results raising security issues: (NO)

• 'EU-classified information' as background or results: (NO)