Drug Experimentation in Healthy Volunteers

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1. Introduction

Drug development is a complex process that includes drug discovery/product development, pre-clinical research (*in vitro/in vivo*) and clinical trials. The new chemical entities, which show promising pharmacodynamic activity in *in vitro* experiments on particular biological targets, thought to play critical pathophysiological roles in specific diseases, emerge from the process of drug discovery and are candidates to undergo safety and toxicity tests, as well as pharmacokinetic and metabolism evaluations in *in vivo* pre-clinical models. Moreover, pre-clinical investigations are focused on determining the dose and administration schedule to be used in the first human clinical trial (first-in-man or first human dosing).

Clinical drug development is currently arranged into four phases, with phase I traditionally representing the very early stage of drug development in humans. Phase I is conducted to establish safety and tolerability, to evaluate pharmacokinetics and to obtain preliminary data on pharmacodynamics. Phase I begins with the first administration of a new compound in humans (Pocock, 1983).

Based on differences in the experimental design, various types of phase I trials can be distinguished: (1) Single ascending dose studies, in which small groups of subjects receive a single dose of the test drug, afterward they are observed and examined for a given period of time. If subjects do not experience any remarkable adverse effect, and pharmacokinetic data are roughly consistent with pre-specified safety values, the dose is escalated up, and a new group of subjects is then given a higher dose (Buoen et al., 2005). This stepping-up dose is continued until the pre-calculated pharmacokinetic safety levels are achieved, or intolerable side effects occur, indicating the point at which the drug appears to have reached the maximum tolerated dose (Friedman et al., 1996). The first dose to be tested in phase I is estimated as a fraction of the so called "no adverse effect dose", which is the highest dose found not to harm animals under appropriate toxicity/safety testing. (2) Multiple ascending dose studies, characterized by an experimental design similar to single ascending dose studies, with the exception that, at each step, a small group of subjects undergoes repeated administration of the same dose of the test drug. Such studies can be conducted to better understand the pharmacokinetics and pharmacodynamics of the new drug at the steady state. (3) Short trials, designed to investigate variations in the absorption of the new drug following its oral administration in the presence of food.

The increasing cost of novel drug development, in conjunction with ethical considerations about the safety of first-in-man trials, has fostered the implementation of novel procedures with the purpose of optimizing, rationalizing, and enhancing the ability of eliminating redundancies in early phase clinical trials without compromising safety. Accordingly, a new procedure, designated as "phase 0", has been introduced into the very early stage of clinical drug development in order to gain insight into the clinical suitability of novel compounds before starting conventional phase I trials (EMEA, 2004; Pasqualetti et al., 2010).

Additional clinical studies, which are conducted in healthy volunteers, include bioequivalence pharmacokinetic tests. To assess bioequivalence between two medicinal products containing the same active ingredient, such as a commercially available brand product and a generic formulation under clinical development, cross-over pharmacokinetic studies in healthy volunteers are currently regarded as the most suitable experimental approach (Del Tacca et al., 2009). Furthermore, bioequivalence studies are performed in healthy volunteers during pre-registrative development phases, in order to assess different formulations of the new active ingredient.

The present chapter focuses on issues concerning the enrollment of healthy volunteers in the early clinical phases of drug development from different points of view, including regulatory, methodological, normative, ethical, and logistical perspectives.

2. Healthy volunteers in early clinical studies

Defining a healthy volunteer is not an easy task, since different criteria, underlying the concept of wellness, can be implied in this condition. The Royal College of Physicians has defined the healthy volunteer as an "individual who is not known to suffer of any significant illness relevant to the proposed study, who should be within the ordinary range of body measurements, such as weight, and whose mental state is such that he is able to understand and give valid consent to the study" (Royal College of Physicians, 1986). Moreover, in the Association of the British Pharmaceutical Industry guidelines for medical experiments in human volunteers, it is highlighted that the individual cannot be expected to receive therapeutic benefit from the proposed study (Association of British Pharmaceuticals Industries, 1988). The EMEA guideline proposes also a general definition of healthy volunteer for studies aimed at assessing pharmacokinetics: "healthy, adult volunteers, in well-defined and controlled conditions" (EMEA, 1998). On this basis, the selection of healthy volunteers is conducted by enrolling subjects without relevant pathologies and with organ functions, such as heart, liver and kidney, in the normal range. However, the general definitions of healthy volunteer, as those proposed by current guidelines, allow wide margins of discretion. For example, might an asymptomatic subject with allergic rhinitis or affected by knee swelling be considered eligible for a phase I clinical experimentation of a new antidepressant drug? Furthermore, might women under treatment with oestrogen derivatives be eligible for early phases of drug experimentation? These simple examples support the notion that we can use different definitions of healthy subjects and that a critical judgment is required (Pasqualetti et al., 2010).

3. Enrollment procedures

From the industry standpoint, recruitment problems translate into potential revenue losses resulting from delays in bringing a new drug into the market (Harris et al., 2005).

Accordingly, clinical research units must ensure a fast recruitment of healthy volunteers into phase I studies, since an inadequate enrollment may increase the study costs, delay the time to completion, and possibly invalidate the trial outcome due to insufficient study power. Recruiting volunteers is an unavoidable, often time-consuming, and difficult task in clinical research (Bramstedt, 2007). Clinical pharmacological units may overcome recruiting problems by facilitating the access of healthy volunteers to information about clinical experimentation. Internet advertising of clinical research studies can be accomplished in various ways, including websites dedicated to specific studies, clinical trial databases, which store basic information about studies, and direct e-mail solicitation to target populations. The U.S. Food and Drug Administration (FDA) requires that institutional review boards (IRB) examine and approve the advertising materials and methods used to recruit human subjects. Additionally, FDA has issued a guidance regarding internet advertising. In particular, FDA does not require IRB to review internet listings of clinical trials as long as the listings provide only "basic trial information" (e.g., study title, study summary, study location, contact information). Internet databases, which are designed with formal system limits (e.g., font size, font style, entry of only basic study information), satisfy this guidance, and thus, do not require IRB approval for each study listing. Bramstedt (2007) analyzed the incidence and nature of ethically inappropriate recruiting advertisements on internet, and provided a descriptive guidance to clinical investigators for responsible internet recruiting. The majority of advertisements satisfied the FDA guidance. However, 18% of them were ethically questionable with regard to font size, font style, and/or verbiage. This author concluded that inappropriate recruiting advertisements can be coercive and misleading.

4. Pharmacokinetic and pharmacodynamic investigations

Clinical pharmacokinetic studies are performed to examine absorption, distribution, metabolism, and excretion patterns of investigational or approved drugs in healthy volunteers and, where appropriate, in patients. Data obtained from early pharmacokinetic testing are useful for designing and conducting extensive clinical trials. Pharmacokinetic studies are also necessary in the post-marketing setting for bioequivalence assessments (Del Tacca et al., 2009). Moreover, clinical pharmacokinetic studies are also relevant for determining the appropriate use of medicines in particular populations, mainly in patients with impaired renal or liver function, for predicting the outcome of pharmacokinetic drug interactions and for assessing genetic variants in drug metabolism. Data on drug concentration-time profiles, obtained from clinical trials, can also provide information for therapeutic drug monitoring in clinical practice.

In a single-dose study, the concentrations of an investigational drug and its metabolites are measured in blood samples following a single administration to healthy volunteers and/or patients. Furthermore, the levels of the investigational drug and its metabolites in blood, urine and, when necessary, faeces are measured to evaluate the elimination pathways. Drug binding to plasma proteins, time-concentration profiles, and the effects of meals on its bioavailability should also be investigated in single-dose studies. In order to evaluate the relationship between the dose and the respective pharmacokinetic profiles, several doses should be tested. Some pharmacokinetic studies, both in healthy volunteers and patients, aiming at evaluating not only the relationship between dose and blood concentrations, but also that between pharmacological effects and blood levels, may provide valuable information for future clinical development.

As far as pharmacodynamic investigations during a clinical trial are concerned, drug effects can be studied both with hard endpoints, like the patient survival, or surrogate endpoints. A surrogate endpoint (or marker) is an effect measure of a certain treatment that may correlate with a real clinical outcome, but doesn't necessarily have a firm relationship with it. The US National Institutes of Health define a surrogate endpoint as "a biomarker intended to substitute a clinical endpoint" (Cohn, 2004).

Several authors have suggested the use of biomarkers as a method for obtaining early indications of drug effectiveness and safety, both for research and regulatory approval purposes, thereby reducing costs and development time (Frank & Hargreaves, 2003; FDA, 2004). Indeed, it has been estimated that small improvements in clinical trial outcomes and decision-making translate into hundreds of million dollars of development cost-savings and a faster time-to-market (DiMasi, 2002). Therefore, the use of biomarkers is encouraged to such an extent that they are routinely examined as a part of many new drug trials.

The traditional gold standard for evaluating drug safety and efficacy relies on prospective, randomized, well controlled, double-blind clinical trials (Merrill, 1996). Although this paradigm has served well the public health in ensuring that new drugs are thoroughly and scientifically evaluated before reaching the consumer, some investigators have argued that reliance on morbidity and mortality data – the so-called 'true endpoints' – could have had the unintended consequence of contributing to the high cost of pharmaceutical innovations. In an attempt of avoiding such cost increments, with the enactment of the FDA Modernization Act of 1997, the FDA was given explicit authority to approve drugs for the "treatment of a serious or life-threatening condition…upon a determination that a product has an effect on a clinical endpoint or on a surrogate endpoint that is reasonably likely to predict clinical benefit".

Traditional biomarkers, such as analytes assayed in serum, have been employed for decades in the clinical practice and drug development. However, a variety of new biomarkers have recently fostered great interest. In particular, the use of imaging biomarkers for the assessment of drug therapies – a field designated as "pharmaco-imaging" – has become very common in recent years. Not surprisingly, the rise in use of pharmaco-imaging methodologies has coincided with the impressive technical advances occurred in medical imaging, with particular regard for noninvasive *in vivo* imaging methodologies such as computerized tomography (CT), magnetic resonance imaging (MRI) (Rudin et al., 1999; Beckmann et al., 2004) and positron emission tomography (PET) (Fischman et al., 2002; Gambhir, 2002).

Several characteristics of imaging biomarkers differentiate them from traditional biomarkers. First, non-invasive imaging has been applied routinely to diagnosis and disease management for several decades, and the ability to identify a wide spectrum of pathophysiological conditions by means of imaging techniques is well established. Second, imaging biomarkers tend to be more closely associated with disease phenotypes, thus enabling a direct association between therapy and its effect. Third, imaging allows a marked versatility in providing continuous, structural and functional assessments of therapy, thus offering snapshots of drug bioactivity over time (Pien et al., 2005).

5. Efficacy-related surrogate endpoints

Several techniques can be used to measure indirectly the effect of drugs in healthy volunteers *(*Passchier et al., 2002*)*. Currently, drug dosing regimens for patients are based

mainly on the outcome of preclinical and phase I–II human studies, such as in vitro autoradiography, dose–effect relationships, plasma concentration, tolerability, electroencephalography (EEG), and functional MRI (fMRI) or PET. Although these measurements have their own merits, they cannot provide direct insight into the relationship between the amount of drug administered and the occupancy of its target, thus not allowing a correlation of the results with the effectiveness of the drug in treating the disease. For example, a drug that is assumed to exert its action on the central nervous system (CNS) may instead induce a systemic release of endogenous factors, such as cortisol or noradrenaline, thus leading to changes in EEG patterns or changes in cerebral blood flow, which can be misinterpreted as the drug having a direct effect on CNS. Likewise, measuring drug plasma levels following single or repeated administration, and assuming that target occupancy is linearly related to the dose or plasma concentration, may be incorrect.

Two cases can be easily identified in which plasma concentration does not reflect the actual target occupancy. First, if brain uptake is high and the drug off-rate from its target is slow, a significant fraction of the target receptor will remain occupied for prolonged periods, even if the clearance of the drug from plasma is rapid. Second, a drug can be effective on CNS, even if it shows a very little brain uptake and, consequently, a very low target occupancy. (Sawada et al., 1991; Gibson et al., 2000).

Two techniques are currently available to provide a direct measurement of drug-related receptor occupancy. PET and single-photon emission computed tomography (SPECT) both use radiolabelled ligands specifically designed to bind the desired target with high selectivity and specificity. Although SPECT has certain advantages, such as a relatively long half-life of radionuclides, it displays also major drawbacks, including low sensitivity, limited temporal resolution, impossibility of labelling the native drug since its conjugation with [99mTc], [131I], and [111In] may result in changes in its chemical properties (Sawada et al., 1991; Gibson et al., 2000).

Although various issues, concerning the study design and data interpretation, must be taken into account, PET can play a relevant role in the evaluation of novel drugs. Imaging studies may demonstrate whether the drug reaches its target, if there is a linear relationship between dose and target occupancy, if the occupancy reflects plasma drug levels, and how long the drug remains bound to its target. PET data from healthy volunteers can thus provide very useful information for the design of early clinical trials (Passchier et al., 2002).

5.1 Use of biomarkers in neuroscience

Treatment of neuro-degeneration or CNS diseases has been a daunting task, and therefore, substantial efforts have been made to identify and validate biomarkers to support the implementation of effective therapies (Bakhtiar, 2008). Notably, understanding the molecular bases of Alzheimer's and Parkinson's disease, and identifying novel brain molecular targets has been subject to intense research and high investment by industry, government, and academia. Examples of methodologies employed to identify biomarkers in CNS include brain imaging techniques such as PET, CT, MRI and SPECT. In addition, facial expression recognition task, Visual Analogue Mood Scale, anxiety tests, genetic markers, catecholamine concentrations, psycho-immunological markers, and neuroendocrine markers are among other complementary approaches (Bieck & Potter, 2005). Since drugs that directly target CNS receptors must show good permeability across the blood–brain barrier (BBB) and sufficient brain exposure, an approach is needed to determine the extent of receptor occupancy, which is defined as the percent of receptor population that is

occupied by the drug at a specific dose or concentration in plasma. The data obtained from such imaging biomarkers are then correlated with the pharmacokinetic data to generate a pharmacokinetic/pharmacodynamic (PK/PD) model, understand the mechanism of action, select the most promising compounds candidate to clinical development, guide dose selection, and/or develop predictive tools at early stages of clinical development (de Boer & Gaillard, 2007). There are two common approaches for obtaining information on BBB penetration: to apply an imaging technique (non-invasive); to sample cerebrospinal fluid directly from the central compartment (invasive). Recent advances in medical imaging have made it possible to employ PET in order to detect picomolar levels of radiolabelled drugs in both preclinical models and humans (Frank & Hargreaves, 2003; Lee & Farde, 2006).

5.1.1 Magnetic resonance imaging and pharmaco-MRI

In contrast with the use of ionizing radiations in CT, MRI employs radio-frequency pulses and magnetic fields to obtain signals from changes in nuclear magnetic moments (Pien et al., 2005). In particular, as the alignment and relaxation of protons occur in response to pulsed radio-frequencies, characteristic relaxation times can be measured, most notably T1 (longitudinal relaxation time) and T2 (transverse relaxation time) (McRobbie et al., 2002). While CT images result from a single parameter, namely the X-ray attenuation by the tissue along the propagation path, MRI is generated by multiple parameters, including proton density, T1, T2, flow, diffusion and susceptibility. MRI is useful for several applications, including central and peripheral nervous system function and visualization, both under basal conditions and after environmental stimuli.

Although MRI displays lower resolution and requires a longer time for data acquisition than CT, the former offers higher soft tissue contrast, thus making MRI the technique of choice in the brain, besides specific applications in musculoskeletal and gastrointestinal systems. MRI, with or without the aid of contrast agents, is also employed for a number of functional assessments, including tissue perfusion, tumour permeability, and blood oxygenation level-dependent (BOLD) studies (Le Bihan, 1995; Sorensen & Reamer, 2000).

Pharmaco-MRI allows in vivo visualization of human brain activity and enables non-invasive assessments of drug-related changes in this activity (Windischberger et al., 2010). Several studies have investigated the effects of antidepressant drugs, such as selective serotonin reuptake inhibitors (SSRIs) on neural activation (Anderson et al., 2008; Arce et al., 2008), indicating area-specific and dose-dependent effects on the BOLD response in both healthy subjects (Loubinoux et al., 2002; Del-Ben et al., 2005) and patients suffering from major depression and obsessive–compulsive disorder (Hoehn-Saric et al., 2004). These effects were particularly pronounced in the amygdala, the key brain region in processing and consolidating aversive emotional cues. For instance, a study on acute citalopram administration in twelve healthy male volunteers showed an increased BOLD signal in those brain areas typically involved in depression (McKie et al., 2005).

5.1.2 Positron emission tomography and single-photon emission computed tomography

The basis of radionuclide imaging is the use of bi-functional compounds containing a radiolabelled moiety, which confers detectability, and a chemical and/or pharmaceutical moiety, which determines uptake and distribution throughout the body. In the case of positron-emitting radioisotopes, the emitted positron passes through tissues and is

ultimately annihilated upon combination with an electron, resulting in two photons emitted in opposite directions. Detectors are arranged in a ring around the tissue of interest, and only triggering photonic stimuli arriving near simultaneously at diametrically opposite detectors can be recorded. Tomographic methods are then used to construct the resulting PET images. Several radioisotopes are used for nuclear imaging. These tracer isotopes can be substituted directly into drug compounds to mimic naturally occurring compounds, or can be conjugated with other molecules to form new compounds referred to radiopharmaceuticals. 2-^{18}Fluoro-2-deoxy-D-glucose (FDG), for example, is an analogue of glucose labelled with a positron-emitting form of fluorine, and it is used in PET imaging of metabolic activities that involve glucose uptake (Gambhir, 2002).

In the case of investigations concerning the dopaminergic system, other iodine radiolabelled compounds can be employed (e.g., [^{123}I] FP-CIT: N-ω-fluoropropyl-2β-carbomethoxy-3β(4-iodophenyl)tropane) (Figure 1).
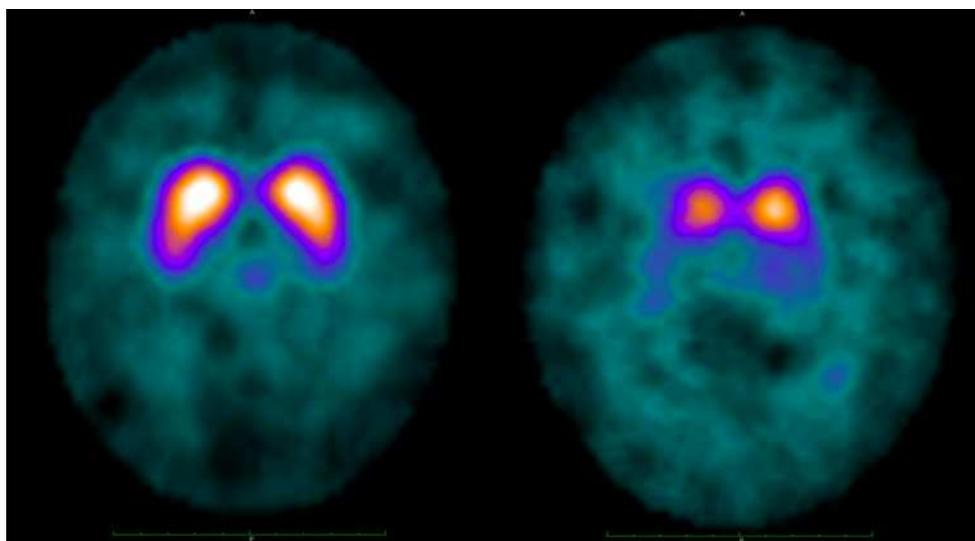

Fig. 1. Transversal sections of [^{123}I]FP-CIT SPECT from brain of a normal subject (left) and a patient affected by PParkinson's disease (right). Radiolabelled compound pattern shows a reduced uptake, both in putamen and caudatum, associated with the degeneration of nigrostriatal nerve fibers (by courtesy of Prof. G. Mariani, Nuclear Medicine Unit, University of Pisa)

It is this diversity in imaging capabilities that allows imaging biomarkers to address a spectrum of drug development issues (Pien, 2005).

The utility of PET studies in healthy volunteers could be represented by drug effect evaluation in specific cerebral areas, in terms of receptor occupancy. In the study by Smith and colleagues (2007), the receptor occupancy produced by treatment with mirtazapine differed significantly among brain regions, showing that higher receptor occupancies were achieved by 7.5 and 15 mg of mirtazapine in high-binding regions (e.g., cortex, amydgala and hippocampus) than in regions with less binding properties (e.g., thalamus and putamen). Thus, PET studies of regional receptor occupancy using [^{11}C]mirtazapine may

provide a reliable mean for testing hypotheses concerning the role of central alpha2-adrenergic dysfunctions in psychiatric disorders (Smith et al., 2007).

5.1.3 Pharmaco-EEG studies of psychotropic drugs

EEG can detect with high sensitivity functional alterations of human brain. Specific EEG patterns related to various psychotropic drugs, such as antipsychotics, antidepressants, anxiolytics, psychostimulants and nootropics, have been reported (Mucci et al., 2006).

By a combination of computer-assisted quantitative analyses of EEG with statistical procedures (quantitative pharmaco-EEG) and mapping techniques (pharmaco-EEG mapping), Saletu and colleagues (2005) classified psychotropic substances and indirectly evaluated their bioavailability in the human brain. In particular, by means of pharmaco-EEG techniques it is possible to determine, at an early stage of drug development, whether a drug is effective on CNS as compared with placebo, what clinical efficacy will be likely achieved, at which dosage it acts, when it acts and equipotent dosages of different galenic formulations. Pharmaco-EEG patterns and maps of neuroleptics, antidepressants, anxiolytics, hypnotics, psychostimulants and nootropics/cognition-enhancing drugs differ, and these differences could reflect the influence of several factors, such as acute or chronic drug administration or differences between normal subjects and patients. Pharmaco-EEG evaluations of these drug classes could anticipate their effects on CNS, their PK/PD profiles and their therapeutic efficacy.

Saletu et al. (2002) investigated the relationship between alterations induced by mental disorders and psychotropic drugs by means of an EEG source analysis, designated as low-resolution brain electromagnetic tomography. Through this approach, they found that some neuroleptics, antidepressants, anxiolytics, hypnotics, psychostimulants and nootropics/cognition-enhancing drugs exerted EEG effects which appeared to counteract the EEG changes associated with mental disorders (i.e., schizophrenia and generalized anxiety disorder). Such a phenomenon has been termed "key–lock principle". In line with this principle, Yoshimura et al. (2007), who performed a placebo-controlled pharmaco-EEG study on two conventional antipsychotics (chlorpromazine and haloperidol) and four atypical antipsychotics (olanzapine, perospirone, quetiapine and risperidone) in healthy volunteers, observed that, under perospirone and haloperidol, the EEG pattern was opposite as compared with the pattern previously reported in schizophrenic patients, thus suggesting a key–lock mechanism.

A compatibility or incompatibility with the key–lock hypothesis does not necessarily have implications for the effectiveness of the drug, but rather for the potential mechanisms of action. Indeed, a key–lock pattern of drug response suggests that the drug acts upon bioelectrical processes that are present both in patients and healthy controls, although in patients such processes are quantitatively different, thus suggesting functional alterations (Yoshimura et al., 2007).

5.1.4 Facial expression recognition task

The area-specific and stimulation-dependent changes in human brain activation by SSRIs are important issues for improving our understanding of the mechanisms evoked by pharmacological treatments. For instance, dysfunctions of the emotion processing circuitry are associated with depression and anxiety disorders (Akimova et al., 2009), and SSRI-induced changes in reactivity within this circuitry can be taken as indicators of treatment response and efficacy (Anderson et al., 2008; Cipriani et al., 2009). In particular, it has been

suggested that, under SSRI treatment, the decrease in clinical symptoms can depend on changes in the processing of emotional stimuli (i.e., enhanced positive emotion processing in concomitance with an attenuated negative emotion processing) (Nathan et al., 2003).

Several studies have shown that changes in serotonin neurotransmission can modulate the identification of emotional faces, particularly fearful faces. Alves-Neto and colleagues (2010) performed their studies on healthy volunteers by using faces from the Pictures of Facial Affect Series, portraying six basic emotions (anger, disgust, fear, happiness, sadness, and surprise), which had been morphed to range from neutral (0%) to a standard emotion (100%), in 10% steps of emotion intensity. For each emotion, pictures of 2 males and 2 females were presented at each intensity level, thus comprising 40 stimuli for each emotion. The faces were displayed in the computer screen for 0.5 s, with an interval (blank screen) of 4.5 s among the various stimuli. Volunteers were requested to select the response that best described the emotion shown in the picture and to record their responses as soon as possible by pressing one of the labelled keys on the keyboard. A single oral dose of escitalopram (10 mg) or placebo was administered to healthy male volunteers 3 hours before testing the Facial Affect Series. Escitalopram facilitated the recognition of sadness and inhibited the recognition of happiness in male, but not female, faces. These results confirm that serotonin modulates the recognition of emotional faces, and suggest that the gender of the face subject can play a role in this modulation (Alves-Neto et al., 2010). Based on these findings, the facial expression recognition test represents an innovative model suitable for studying old and new antidepressant drugs in healthy volunteers.

5.1.5 Biomarkers for antipsychotic drugs

Studies of novel antipsychotics in healthy volunteers deal traditionally with pharmacokinetics and tolerability, but useful information can be also obtained from biomarkers of clinical endpoints. A reliable biomarker should meet the following requirements: consistent response across studies of different antipsychotics; clear response of the biomarker to a therapeutic dose; dose-response relationship; plausible relationship among the biomarkers, pharmacology and pathogenesis (Figure 2).

A review by de Visser and co-workers (2001) has identified 65 studies investigating the effects of 23 neuroleptics by means of 101 different neuropsychological tests, which could be clustered into seven neuropsychological domains. Subjective and objective measures of alertness, as well as those of visual-visuomotor-auditory and motor skills were most sensitive to antipsychotics, although over half of studies failed to show significant differences from placebo (de Visser et al., 2001). With regard for the subjective assessments, most individual analogue scales, in order to assess alertness, mood and calmness, have been proposed and applied to the evaluation of psychotropic drugs. Other scales can be used to examine anxiety, subjective psychotropic drug effects and extrapyramidal side effects (Norris, 1971; Bond et al., 1974).

In addition to EEG, as discussed above, other valid tools can be used for psychological and neurological assessment of novel drugs in healthy volunteers. Smooth pursuit and saccadic eye movements have been extensively validated to assess the side effects of psychotropic drugs. These effects are not specific for a class of drugs (van Steveninck, 1993), but they rather allow to quantify sleep/wake transitions. For instance, schizophrenic patients display abnormalities in stimulation-related potentials, which are postulated to reflect characteristic changes in the stimulus discriminability and decision making. Typically, these changes

consist of a reduction in the amplitude and a prolongation in the latency of the P300 component of evoked potentials.
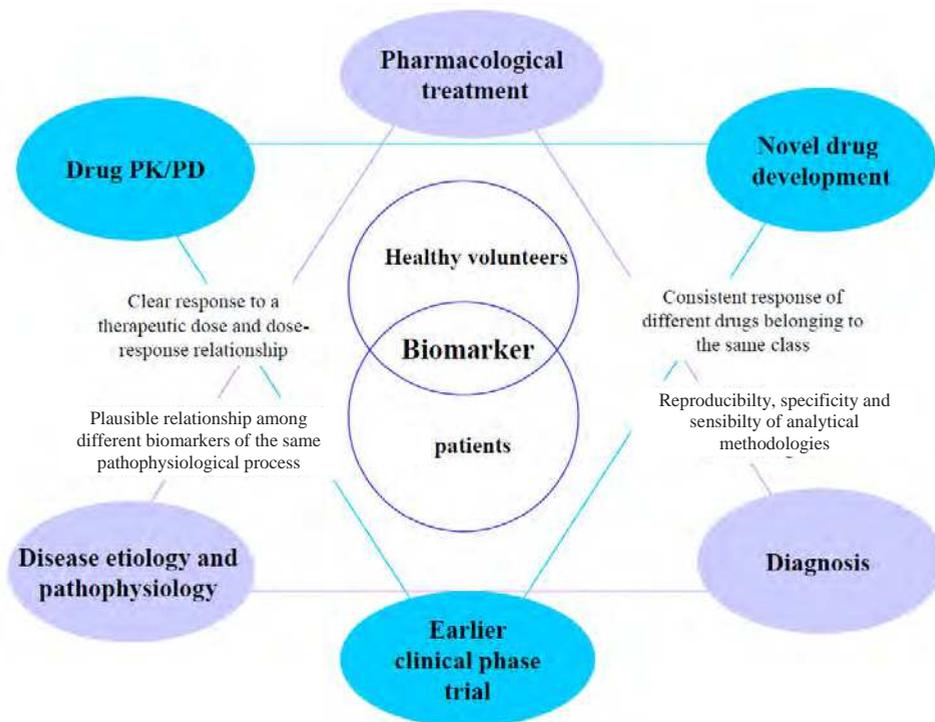

Fig. 2. Features and usefulness of reliable biomarkers and their investigational and clinical application targets. PK/PD: pharmacokinetic/pharmacodynamic relationship

Increments of serum prolactin response in healthy volunteers reflect the "therapeutic" antidopaminergic effect of antipsychotic drugs, while changes in growth hormone and cortisol secretion are studied as biomarkers for antipshycotic activity of drugs on the serotoninergic system. The most consistent effects have been associated with prolactin increase, where 96% of all studies reviewed by deVisser et al. (2001) showed statistically significant effects.

5.2 Biomarkers for anti-inflammatory drugs

Endotoxin or lipopolysaccharide (LPS) (Thorn, 2001; Kharitonov & Sjöbring, 2007) is a component of the outer cell wall of Gram-negative bacteria. LPS is a highly potent pro-inflammatory substance, which, upon inhalation, causes fever, chills and bronchoconstriction in a dose-dependent fashion. These symptoms are accompanied by a pro-inflammatory response, detectable in sputum and bronchoalveolar lavage fluid as elevation of neutrophils, macrophages and certain cytokines/chemokines. Such a response can be partly modified by specific drugs.

There is increasing evidence that diseases caused by organic dusts are mainly inflammatory in nature. Among the several agents found in organic dusts, LPS is a major candidate for the

pathogenesis of inflammatory reactions. Consistently with this view, the inhalation of LPS increases neutrophils, lymphocytes and fibronectin levels in the bronchoalveolar lavage fluid from healthy volunteers (Sandström et al., 1992). Changes in mediators of inflammation, such as eosinophilic cationic protein, myeloperoxidase, interleukin-8 (IL-8), IL-1beta, tumor necrosis factor alpha and C-reactive protein have also been found into the airways and/or blood. In particular, the inhalation of LPS elicits a neutrophilic inflammation with IL-8 elevation in both normal and asthmatic subjects (Kharitonov & Sjöbring, 2007).

Future studies with LPS inhalation need to be focused on relevant diagnostic tools for detecting the inflammatory reaction in subjects exposed to LPS-containing organic dusts, and evaluating whether the large inter-individual variations observed in the response to organic dusts or LPS could depend on differences in the molecular mechanisms underlying the toxicity of the injuring agent.

Models of provoked asthma are very valuable tools for understanding the pathobiology of asthma, aiding the diagnosis, helping to clarify the mechanisms of actions of effective drugs and supporting the development of new drugs. Some provoked models are useful in the clinical setting, particularly those that measure direct airway hyper-responsiveness (for instance, bronchoconstriction elicited by inhaled methacholine), while others, particularly those based on allergen challenge, can be used both in animal models and humans to study the mechanisms of allergen-induced airway inflammation and related pathophysiological changes, as well as in the development of new drugs for asthma (O'Byrne et al., 2009). In particular, investigations on novel bronchoactive drugs in healthy volunteers can be accomplished by testing their effects on basal airway calibre and induced bronchoconstriction. In this context, gaining further knowledge on the molecular and pathophysiological bases of the inflammatory response associated with LPS-induced bronchoconstriction is expected to foster the use of this model for investigating new antiasthmatic drugs.

5.3 Biomarkers for immune-allergology drugs

Allergic rhinitis is a chronic inflammatory disorder of upper airways which evokes characteristic signs and symptoms in sensitized individuals exposed to relevant allergens. Allergic airway inflammation is characterized by immunoglobulin E (Ig E)-triggered mast cells and activated eosinophils, which release pro-inflammatory mediators, such as histamine and leukotrienes. Furthermore, allergic rhinitis is associated with elevated serum IgE levels and positive skin prick test (SPT) to corresponding allergens. In addition to their diagnostic value, serum specific IgE levels and SPT tests may serve as biomarkers to monitor the disease activity in response to anti-allergic therapy (Boot et al., 2008).

Nasal challenge with allergens is a validated, reproducible clinical model for investigating the pathophysiology of allergic rhinitis, which also allows the evaluation of kinetics of nasal inflammatory responses (Doyle et al., 1995). As such, it may serve as a tool to study the effects of anti-allergic interventions, targeting specific inflammatory mechanisms related to the upper airway response (de Graaf-in't Veld et al., 1995). Although nasal biopsies are the golden standard for investigating the cellular inflammatory response, there are some limitations to this invasive technique (Godthelp et al., 1996): a) it can only be performed by an experienced ear-nose-throat physician; b) it provides information just on a limited part of upper airways; c) it does not allow repeated sampling within short time intervals (Jacobson et al., 1999). By contrast, nasal brushing is a less invasive method, which has emerged as a

possible viable alternative for interventional trials requiring repeated sampling. In patients with allergic rhinitis, Jacobson et al. (1999) showed that seasonal changes in the number of mast cells and eosinophils in nasal brush samples correlated well with those found in nasal biopsies. Likewise, intranasal fluticasone produced a similar degree of reduction in these inflammatory cells in both brush and biopsy samples. Nasal lavage is another relatively non-invasive sampling technique which allows serial assessments of the effects of anti-inflammatory drugs on soluble factors released by upper airway inflammation (de Graaf-in't Veld et al., 1997).

In the study performed by Boot and colleagues (2008), SPT, serum specific IgE levels and inflammatory biomarkers in nasal lavage and material obtained by nasal brushing were analyzed in 20 subjects with mild allergic rhinitis, randomly assigned to undergo an intranasal challenge with a relevant allergen (n=10) or diluent (n=10), in order to simultaneously assess the kinetics of several biomarkers of allergic airway inflammation. The authors concluded that serum specific IgE assay and SPT displayed good reproducibility in patients with clinically stable allergic rhinitis, and that nasal allergen challenge, when used in combination with nasal lavage and brush sampling, is a suitable research tool for early drug development.

5.4 Biomarkers for anticancer drugs

In oncology, besides toxicity, alternative endpoints have been proposed for early phase trials evaluating novel drugs targeted against relevant molecular factors, including the assessment of target inhibition in tumours or surrogate tissues, and/or the evaluation of biomarker pharmacokinetics (Kelloff et al., 2004; Goulart et al., 2007; Arrondeau et al., 2010). In this respect, phase 0 trials can provide critical human PK/PD data to support the design of future studies (Kummar et al., 2009). Healthy volunteers, as indicated by international guidelines (EMEA, 2004), can be enrolled to perform phase 0 and early phase I trials to study new anticancer drugs, evaluating specific surrogate biomarkers of antitumor activity and preliminary pharmacokinetics.

For instance, sunitinib, a thyrosin kinase inhibitor, endowed with antiproliferative and antiangiogenic effects, was tested in 12 healthy volunteers at the oral dose of 50 mg for 3–5 consecutive days in order to perform a preliminary evaluation of its PK/PD profile. The parameters assessed in this trial were blood pressure, plasma concentration-time-course of sunitinib, its major metabolite SU12662, vascular endothelial growth factors VEGF-A and VEGF-C, as well as soluble VEGF receptor-2 (sVEGFR-2). The authors found that the time-course of blood pressure under treatment with sunitinib was highly consistent with published data in patients, while changes in circulating biomarkers were greater in patients, as compared with simulations suggested for healthy subjects. Overall, the tumour-independent pharmacological response to sunitinib in healthy volunteers can be described by PK/PD models, thereby facilitating model-based investigations of novel antitumor antiangiogenic drugs, using blood pressure and circulating proteins as biomarkers (Lindauer et al., 2010).

Another interesting example of early phase trial on healthy volunteers in oncology is represented by the study carried out by Reid et al. (2011). In order to accelerate the clinical development of SR13668, an orally active Akt pathway inhibitor, which has demonstrated cancer chemopreventive potential in preclinical studies, these authors designed and conducted a phase 0 trial for evaluating and comparing the effects of food and pharmaceutical formulation on bioavailability of the novel drug. Healthy adult volunteers

were randomly assigned to receive a single 38-mg oral dose of SR13668 in one of five different formulations, with or without food. Blood samples were obtained pre- and post-drug administration for pharmacokinetic analyses and the area under the plasma concentration-time curve (AUC) was defined as the primary endpoint. Overall, the authors found that the AUC values of SR13668 were higher in the fed state and different across the formulations. Moreover, they identified a lead formulation of SR13668 for further clinical research, supporting the use of phase 0 trials to accelerate the development of new anticancer drugs (Reid et al., 2011).

6. Safety of experimental drugs

One of the main goals of phase I trials is the gathering of preliminary data on the safety profile of new compounds in humans, and also to ensure the safety of enrolled subjects. Participation in phase I trials can involve significant risks, and there is no expectance of medical benefits, particularly for healthy subjects. Preclinical data must support an acceptable level of risk for a new drug given to humans for the first time. On this basis, to optimize safety in first-in-man trials, a comprehensive understanding of the new molecule, its target, and its expected pharmacokinetics in both normal and pathological tissues is required. This concept is particularly important for biopharmaceuticals with slow elimination, such as monoclonal antibodies, where the potential for persistent target modulation and alteration of downstream cellular processes requires careful assessment (Tibbitts et al., 2010). However preclinical toxicology, as testified by several toxic reactions observed in early phase trials (Kenter & Cohen, 2006), may not provide suitable information on drug safety to predict which potential adverse effects might occur in humans. For this reason, adverse reactions must be intensively monitored throughout all early phase studies, and first-in-man administration must be conducted in an appropriate pharmacological unit, which can rapidly provide intensive cares. Guidelines issued by both American and European agencies (EMEA 2007a, 2007b, FDA, 2005, 2009) have been implemented to improve the safety of first-in-man trials. In particular, they provide important information to investigators and organizations involved in the design and interpretation of preclinical programs supporting first-in-man trials. Selecting the first dose to be administered in man represents a crucial step for preserving the safety of participants to phase I studies and it requires the integration of data provided by multidisciplinary approaches (Tibbitts et al., 2010). Once sufficient preclinical data are available, the first dose in humans is selected on the basis of both relevant toxicological and toxikinetic endpoints, such as the dose not eliciting any adverse effect in animals (no observed adverse effect level, NOAEL) and the evidence obtained in PK/PD animal models. Moreover, the first dose is calculated by application of a safety factor, which takes into consideration the overall robustness and quality of preclinical data, as well as the potential for adverse effects in the target population (Tibbitts et al., 2010) (Figure 3).

In March 2006, TGN 1412, a new monoclonal antibody directed against a human lymphocytic antigen, which was studied in a first-in-man clinical trial at the Northwick Park Hospital of London, caused a catastrophic systemic organ failure in six healthy subjects exposed to the new drug (leading to hospitalization of all six volunteers in intensive care units), despite being administered at a supposed sub-clinical dose of 0.1 mg per kg, which was about 500 times lower than that estimated as safe in animals (Kenter & Cohen, 2006).

After the London tragedy, in an attempt of mitigating the risk associated with phase I clinical trials, the regulatory agencies, including EMEA, issued new guidelines to aid sponsors in the transition from preclinical to early clinical development, including the "Guideline on requirements for first-in-man clinical trials for potential high-risk medicinal products" (EMEA, 2007a) and "Strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products" (EMEA, 2007b). The first guideline provides criteria to classify new investigational drugs as potential high-risk medicinal products. The second one is intended to assist sponsors in the transition from preclinical to early clinical development. These efforts have been collectively gathered under the slogan "New Safe Medicines Faster" in Europe and "The Critical Path" in the U.S. (Buoen et al., 2005). For these reasons, a new procedure, designated as "phase 0," has been introduced into the very early stage of clinical drug development in order to gain early insights into the clinical suitability of novel compounds to shorten the duration of phase I studies.
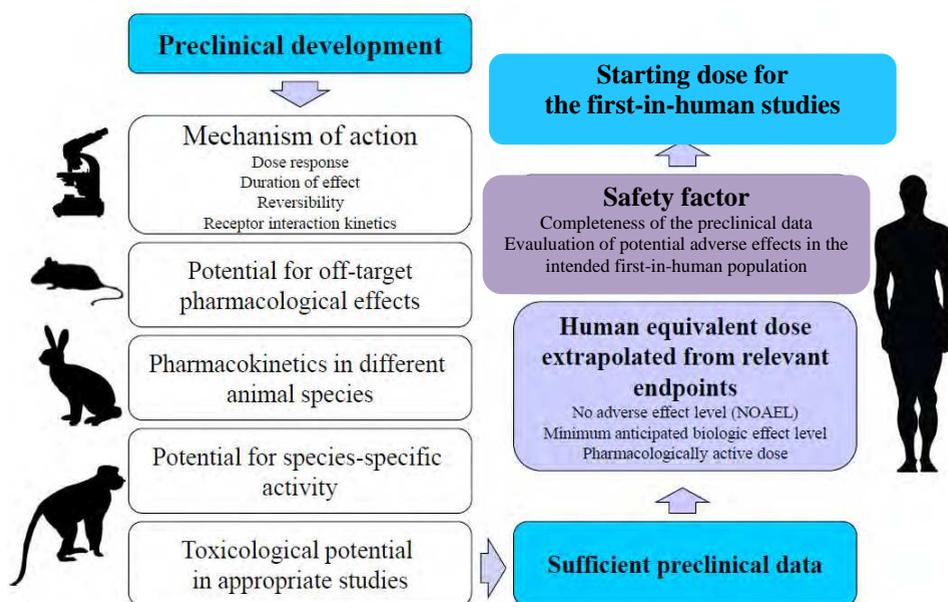

Fig. 3. Flow chart for determining starting dose in the first-in-human studies. The acquisition of sufficient preclinical data and a suitable safety analysis can allow to obtain the human equivalent dose and selecting the starting dose for the first-in-human study (Modified from Tibbitts et al., 2010)

As anticipated above, phase I clinical experimentation encompasses an array of studies, which can be performed on healthy volunteers or patients and deal with the determination of experimental drug tolerability and its PK/PD profile. In the setting of patients affected by a particular disease, these studies can include the evaluation of pharmacodynamic activity, mainly for those drugs with a balance between expected therapeutic effect and toxicological risk not justifying their administration to healthy subjects. In particular, studies evaluating high-risk drugs should be performed in patients when the potential therapeutic effects of

the test drug are expected to overcome its known toxicity (e.g., antiblastic, anti-HIV drugs) or when the expected risks are not acceptable for healthy volunteers. These concepts have been widely accepted until recently, when the new "phase 0" or "early phase I" studies were introduced, according to which the so-called high risk drugs (e.g., antiblastics, antipsychotics, antiepileptics, antiarrhythmics) can be administered to a small number of healthy subjects in subtherapeutic micro-dosing studies, with a consequent reduction of toxicity risk. Accordingly, phase 0 trials are not intended to replace the traditional dose escalation, safety, and tolerability studies, and they cannot indicate whether a candidate drug will have a positive impact on the target disease (Schellens, 2009). The scientific rationale underlying phase 0 trials includes determining as early as possible whether a new drug is capable of modulating the therapeutic target in humans and/or generating pharmacokinetic data, such as the biodistribution and metabolism. This early knowledge is critical in the process of drug development and it may avoid larger phase I and II trials for drugs shown to have unfavorable pharmacologic properties in phase 0 trials (Hill, 2007).

7. Reimbursement procedures

The main goal of early phase trials is to gain knowledge about the clinical suitability of novel compounds, without pursuing specific therapeutic or diagnostic purposes. As anticipated in the previous section, innovative drugs, investigated in these early phases, could be harmful to the health of volunteers. Therefore, it is reasonable to assume that altruism and idiosyncratic interests alone are unlikely to motivate a sufficient number of healthy subjects to act as volunteers in phase I trials. Without payments, recruitment can be slow, resulting in a thwarted and unfitting phase I. For these reasons, there is no doubt that an adequate remuneration must be offered to subjects recruited in early phase investigations. At the same time, coercion and excessive psychological influence should be avoided when obtaining consent (Dickert et al., 2002).

For many years, there has been an ongoing ethical-scientific debate on how to take decisions about fair payment of research subjects, viewed as an attempt of compensating them for their "lost wages" and "discomfort" due to their participation in clinical trials. As in the U.S. and other European countries, in Italy a detailed guideline or a specific law about research volunteer reimbursement is still lacking (Pasqualetti et al., 2010).

Dickert et al. (2002) reported that only 37.5% of clinical research organizations included in their analyses (academic research centers, pharmaceutical companies, contract research organizations, and independent institutional review boards) had written guidelines about the payment of healthy subjects. These organizations disclosed that investigators and IRBs make decisions about payments and that in some studies both healthy and ill subjects are paid for reimbursement of their time, inconvenience, and travel, as an incentive, or for incurring risk. Dickert et al. (2002) underlined also some methods adopted by different organizations to establish "how to pay" by specific formulas: payment for time by the hour, payment per inpatient day or outpatient visit, payment by a flat rate per day or visit, supplemental payment for the "inconvenience" associated with certain procedures. Overall, most organizations require that the estimated payment must be delivered to subjects as prorated (i.e., fractional payment based on the amount of time or procedures actually accomplished) rather than as a contingent payment at the completion of the study (Iltis, 2009).

Several authors support the opportunity of a reimbursement to healthy volunteers and propose different hypotheses to resolve this issue (Pasqualetti et al, 2010). Among the

proposed ways, those indicated by Iltis (2009) and Resnick (2008) appear to be quite interesting. Iltis (2009) underlines the conflict existing between the practice of providing appropriate payments, thus avoiding undue influence, and the requirements of justice when recruiting normal healthy volunteers for phase I clinical trials. By keeping payments low intentionally, in line with recommendations by IRBs, investigators might target or systematically recruit healthy subjects from lower socio-economic levels, thus not fulfilling criteria of social justice. On the other hand, higher reimbursements to volunteers might prompt more persons to enter clinical studies, while not discouraging the less well-off from enrollment. Although investigators would likely prefer to achieve the goal of having a sufficient number of subjects within a reasonable time-frame, the latter method might appear as a wrong influencing procedure for the recruitment of research volunteers.

An alternative way to decrease or increase payments homogeneously is to offer reimbursements based on various and personalized contributions, which may differ according to the specific procedures required by trial protocols (Iltis, 2009). Resnick (2008) describes five distinct models of payments to research subjects: (1) free market model: subjects are paid for providing services (such as completing surveys and undergoing tests and procedures), goods (such as blood, tissue or other biological samples), and to cover potential risks; (2) wage payment model: participants earn a wage equivalent to that of a typical unskilled labourer; (3) reimbursement model: in this setting, participation in clinical research is regarded as a public service (altruism) and volunteers can be paid for their travel expenses, lost wages, baby sitting expenses, etc.; (4) appreciation model: subjects are not compensated for the costs of participation, but they receive money or gifts, such as t-shirts, mugs, gift certificates, as a sign of the investigators' appreciation; (5) fair benefits model: subjects are neither paid labourers nor paid volunteers, but partners in research, sharing the benefits of research, including the economic ones.

The first two models represent a form of "compensated labour" and could motivate individuals to take part in a clinical trial merely to obtain consistent gains. By contrast, the remaining models reflect a "free and voluntary contribution" to community, but they can't embrace the actual motivations of healthy volunteers, who might be seeking free clinical tests or monetary gain (Resnick, 2008).

The lack of international and local guidelines about some crucial aspects related to the recruitment of healthy volunteers in early phase clinical trials, such as the definition of healthy status, payments, advertisement, and participation of the same subject in different experimentations, stimulated a proposal by the Centre for Clinical Drug Experimentation of Pisa University Hospital to implement specific operative procedures (Pasqualetti et al., 2010), attempting to properly address the following issues: (1) advertising to healthy volunteers for recruitment in early phase clinical trials after approval by the local ethics Committee; (2) evaluation of the clinical and psychological status of a potential healthy volunteer; (3) creation of a database containing information on selected healthy subjects to be contacted for possible enrollment in a clinical trial, and allowing a proper monitoring of their participation in different experiments; (4) calculation of adequate reimbursements to healthy volunteers participating to clinical trials. The latter operative procedure is based on criteria which, although not yet fully comprehensive, provide a useful frame to estimate the amount of fair payment to healthy volunteers. In particular, it encompasses a number of items for which the amount of specific reimbursement is indicated, including the number of blood samples, peripheral venous catheter or needle placement, number of clinical laboratory analyses performed during the screening and follow-up (blood and urine

sampling, ECG, psychopathological test administration); other biological fluid sampling; restrictions imposed by a specific study protocol (for example, smoking and alcohol ban, physical exercise, etc.); investigational drug administration route (invasive: intramuscular, intravenous, by naso-gastric tube; not invasive: oral); filling in questionnaires and diaries; time spent at the clinical pharmacology centre (per hour); discomfort or distress caused by study procedures (classified as minor, moderate and major).

8. Conclusion

Drug experimentation in healthy volunteers is aimed at investigating pharmacokinetics and pharmacodynamics as well as documenting safety and tolerability of new compounds. Therefore, strategies to evaluate risks versus benefits during phase I and phase II clinical studies need to be refined continuously to improve efficiency and quality. At the same time, there is a clear need to better predict inter-subject variability, off-target toxicity, clinical outcomes and disease pathophysiology (Cohen, 2006).

Since drug development is a complex, costly and risky process, evidence has now been accumulated concerning the promising role of imaging in highlighting risks and costs associated with clinical drug experimentation by validating targets, confirming mechanisms of action, obtaining early markers of pharmacological activity, assessing pharmacokinetic profiles and providing prognostic indicators. Despite these interesting promises, consideration must be given to the possibility of confounding factors, which could mislead surrogate endpoints, and the extent to which biomarkers are validated for their intended purposes.

The issue of validation of new imaging biomarkers needs to be addressed in a more systematic and rigorous way, including the search for correlations among imaging and molecular biomarkers, elucidating the relationships between particular imaging biomarkers and the purported pathophysiological pathways. With recent advances in genomic, proteomic imaging and computational sciences, pharmaco-imaging is gaining an important role in drug development (Bakthiar, 2007).

Other critical aspects of drug experimentation in healthy volunteers include both legislative and ethical issues, pending the lack of international guidelines suggesting specific procedures for the recruitment and reimbursement of healthy subjects enrolled in clinical studies.

In order to clarify the main unresolved questions, the international scientific community needs to address and resolve urgently the following points: a) international definition of healthy status based on standard physical, psychological and clinical parameters; b) appropriate advertisement addressed to potential participants to first-in-man clinical trials; c) international standard criteria for offering fair payments to healthy volunteers enrolled in phase I trials; d) need of a national register, following the examples of some Countries (Resnik & Koski, 2011), in order to monitor the participation of healthy subjects and avoid their simultaneous enrollment in early clinical trials at the same or other centre for drug experimentation.

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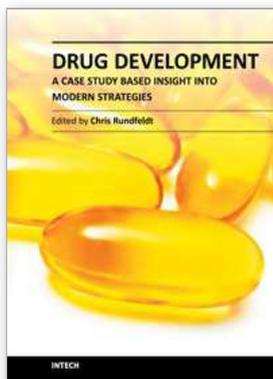
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