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Overview of the marmoset as a model in nonclinical development of pharmaceutical products

Antonia Orsi a,⇑, Daryl Rees a, Isabella Andreini b, Silvana Venturella b, Serena Cinelli b, Germano Oberto b

a Salupont Consulting Ltd., Kent Science Park, 940 Cornforth Drive, Sittingbourne, Kent, ME9 8PX, UK
b Research Toxicology Centre S.p.A, Via Tito Speri, 12/14 00040 Pomizia, RM, Italy

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A B S T R A C T

Callithrix jacchus (common marmoset) is one of the more primitive non-human primate species and is used widely in fundamental biology, pharmacology and toxicology studies. Marmosets breed well in captivity with good reproductive efficiencies and their sexual maturity is reached within 18 months of age allowing for rapid expansion of colonies and early availability of sexually mature animals permitting an earlier assessment of product candidates in the adult. Their relatively small size allows a reduction in material requirements leading to a reduction in development time and cost. Fewer animals are also required due to their ability to be used in both pharmacology and toxicology (nonclinical) studies. These factors, alongside a better understanding of their optimal nutrient and welfare requirements over recent years, facilitate the generation of a more cohesive and robust dataset. With the growth of biotechnology-derived pharmaceuticals, non-human primate use has, by necessity, also increased; nevertheless, there is also a growing public call for minimizing their use. Utilizing, the more primitive marmoset species may provide the optimal compromise and once the scientific rationale has been carefully considered and their use justified, there are several advantages to using the marmoset as a model in nonclinical development of pharmaceutical products.

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1. Introduction

Before evaluation in human volunteer or patient clinical studies, all investigational medicinal products (IMPs, e.g. pharmaceuticals including biotechnology derived products, gene therapies and vaccines) require evaluation in nonclinical toxicology and safety studies, generally in a rodent and a non-rodent ('second') species; (ICH-Topic-M3(R2), 2009). These nonclinical toxicology and safety studies allow a risk assessment to be performed to act as a safeguard for human subjects. The use of a non-rodent species in addition to a rodent species aims at limiting the uncertainty in the extrapolation process from animal toxicity and safety data to the human situation. Dogs, and in specific circumstances minipigs, are the more frequently used non-rodent species (ICH-Topic-M3(R2), 2009). However, when it is scientifically justified that these are inappropriate, regulatory authorities worldwide accept the use of non-human primates. Non-human primates are also used when they represent a well-established model of a compound class or when they are the relevant species (especially for biotechnology-derived products) for detecting known side effects or upon specific recommendations from regulatory authorities. “New World monkeys” such as Callithrix jacchus (the common marmoset) and the larger “Old World monkeys” such as Macaca Fascicularis (cynomolgus monkeys) and Macaca Mulatta (rhesus monkeys) are typically used (Weatherall, 2006; SCHER, 2009). This paper provides a general overview of the marmoset as model in nonclinical development of pharmaceutical products taking into account the recent regulatory changes in husbandry, new micro sampling technologies, the requirements for reducing primate use alongside the contrasting need for relevant species selection in biotechnology derived product development.

2. Marmoset characteristics

2.1. Regulatory acceptance

Regulatory authorities worldwide recognize the use of marmosets in nonclinical development and as they are one of the more primitive, non-human primate species and the most phylogenetically distant from humans, their use requires less ethical justification than the larger “Old World monkeys” (Home–Office, 2000).

2.2. Small body size

The marmoset is a relatively small animal, approximately the size of an adult rat (up to 500 g; Table 1). Their small size has several advantages during the early phases of IMP development, as
material production is usually sub-optimal and therefore sufficient quantities can be rate limited for studies. Since the amount of material required is roughly proportional to the size of the animals there are significant savings, both in cost and time, in the use of smaller species such as marmosets. The reduction in material requirement has been estimated to be 10–15-fold when comparing a 500 g marmoset to a ~5–10 kg macaque (Weatherall, 2006; Zuhlke and Weinbauer, 2003; Smith et al., 2001). Shorter synthesis time offers the potential for earlier administration to human subjects which in turn may lead to substantial cost savings (estimates range from 12 to 18 months’ reduction in development time). The reduction in the amount of material required for early development studies may also have a corresponding benefit in allowing more time for large scale synthesis optimization, with further opportunities for benefit in time and costs later in development (Smith et al., 2001). The marmoset also requires smaller cages than those for larger non-human primates and many methodologies and technologies used for the rat can also be used for the marmoset such as metabolic cages.

In the past, in certain circumstances, their small size was considered a potential hurdle as larger volumes of blood were needed for pharmacokinetic and toxicokinetic investigations, resulting in an increased number of animals required with a consequent increase in material requirement and cost. Nowadays, the need for large blood volumes has diminished with the availability of more sensitive analytical techniques such as “dried spot detection”, newer clinical pathology techniques and enzyme linked immuno assay (ELISA) kits (Pastori et al., 2010; Dunbar, 2006; Hu and Wang, 2007; http://www.sfbr.org/SNPRC/detail.aspx?r=60, 2010).

2.3. Breeding and sexual development

The marmoset breeds well in captivity and nowadays nearly all marmosets used are captive bred and have been for sometime (F 4/5 generation), providing a more reproducible dataset (Scher, 2009). The marmoset has a high reproductive efficiency, typically producing twins twice a year with sexual maturity reached within 18 months of age (compared to approximately 4 years for “Old World monkeys”). This aspect is particularly important, as the issue of toxicity studies conducted in non-rodent species where the animals are not sexually mature has been of considerable concern. The high reproductive efficiency also allows for the rapid establishment of an in-house breeding colony, with easier availability of animals and more reliable results due to reduced stress from transportation and colony alterations. A secondary benefit is a reduction in overall cost. Furthermore, the life cycle of the marmoset is physiologically faster (7–20 years) than that of the “Old World monkeys” resulting in greater availability of aged animals, an important aspect for studies involving aging and bone disease.

2.4. Other characteristics

Marmosets are born as naturally occurring chimeras, so although the animals are genetically distinct, they share and are tolerant to each other cells (Niblack et al., 1977; Ross et al., 2007). This natural chimerism enables the study of cell transfer without initiating an alloresponse (Mansfield et al., 2004).

3. Marmoset management

The marmoset is an arboreal, diurnal species originating from the South American forests and as such a suitable environment and diet is required to reflect this natural habitat.

3.1. Husbandry

3.1.1. European guidelines

The natural behavior of marmosets indicates that the captive environment should have a degree of complexity and stimulation (OJ-L-197-(vol-50), 2007) and due to their arboreal nature the volume of available space and the vertical height of the enclosure appear to be more important than floor area (Badhi et al., 2007).

The European guidelines for their housing recommend the following:

- The marmosets should be housed in groups and during studies in pairs.
- Cages should be constructed with a height of at least 1.5 m (with the top of the cage at least 1.8 m from the floor) and a minimum floor area of 0.55 m² for a breeding pair and 2.0 m² for family groups (OJ-L-197-(vol-50), 2007).
- Rich and complex environments, including opportunities for climbing, foraging (e.g. via puzzle feeders high up in cages), gnawing, swinging, and social play, as well as rest places should be provided. The cages should be equipped with wooden bars and hiding provisions and should take into account that marmosets are used to an arboreal habitat. It is important to provide a certain degree of variability in orientation, diameter and firmness to allow the animals to perform appropriate locomotor and jumping behaviors (Weatherall, 2006; OJ-L-197-(vol-50), 2007) (Fig. 1 and Fig. 2).
- Marmosets frequently scent-mark their environment and the total removal of familiar scent may cause behavioral problems. Alternate cleaning and sanitation of the enclosure and the enrichment devices retains some of the territorial scent-marking. Regular handling and human contact are beneficial for improving the animals’ habituation to monitoring and experimental conditions and facilitate training to co-operate with some procedures.
- Since the marmosets originate from tropical rain forests, they should be kept at a relatively high temperature of 23–28 °C with a relative humidity of between 45 and 70%. A light and dark cycle of 12 h is recommended. The lighting source should illuminate uniformly the holding room. However, within the animal enclosures, a shaded area should always be provided.
- Special consideration should be given to minimize exposure to ultrasound and any sudden unexpected noise, within the hearing range of marmosets (OJ-L-197-(vol-50), 2007).

3.1.2. Other guidelines and recommendations

The United States National Research Council’s “Guide for the Care and Use of Laboratory Animals” integrates recently published data, scientific principles and expert opinion to recommend practices for the humane care and use of animals in research, testing, and teaching (National Research Council, 2010). The “Guide” is an internationally accepted primary reference on animal care for the scientific community and previous editions have served as the basis for accreditation of institutions worldwide by the Associ-
The “Guide” recommends:

- Minimum space based on the needs of pair or group housed marmosets; however the group composition should be critically considered.
- Cages with a minimum height of at least 76.2 cm and a minimum floor area of 0.20 m² for a breeding pair. However this recommendation highlights that in light of the marmosets arboreal nature, taller cages should be utilized, with species-specific environmental and psychological enrichments (National Research Council, 2010).

The marmosets’ climate tolerance, monogamy and preference for living in small family groups make it comparatively straightforward to provide housing and husbandry that meets the animals’ needs and promotes their well-being. This is a significant advantage over the larger non-human primates where it is considerably more difficult to provide laboratory housing, due not only to their larger physical size, but also their greater natural troop, home range sizes, and social characteristics, including complex dominance relationships and aggressive tendencies (Baskerville, 1999).

This, coupled with the physical and microbiological health hazards that the larger non-human primates pose to humans (as well as climate requirements of cynomolgus monkeys) suggests that husbandry of marmosets, is one of the major advantages in providing an acceptable quality of life for them as laboratory animals. From an ethical perspective there are several benefits in using marmosets, as their characteristics facilitate the provision of a more complex and enriched environments, being less destructive than larger non-human primates. Their greater wellbeing from this environment should also translate into more consistent and reliable results and over the last few decades extensive databases have also been generated within companies, toxicology service providers and breeding establishments providing essential background data to support this (Abbott, 2003; Smith et al., 2001).

3.2. Food

Marmosets eat fresh fruit, bread loaf, eggs, nuts high protein intake. A commercial supplement is also available (e.g. “New World monkey” prepared diet by Harlan Teklad). Biscuits and condensed milk are also typically used as remuneration food (RTC S.p.A.; internal procedures). The marmoset has a high requirement for protein and since they are unable to synthesize vitamin D3 without access to UV-B radiation, the diet must also be supplemented with adequate levels of vitamin D3 (OJ-L-197-(vol-50), 2007). Continuous improvements in diet as well as husbandry and environmental enrichments are helping to dramatically reduce the incidence of the marmoset ‘wasting syndrome’ (Tucker, 1984) resulting in the generation of more consistent and reproducible datasets.

3.3. Clinical pathology

All clinical examinations in vivo as laid out in the regulatory guidelines are feasible, established and reproducible in the marmoset. Once the marmoset is gently restrained, it is relatively easy for experienced staff to take blood samples from the animals or to give injections. However, the marmoset is too small to allow a large number of samples to be taken from the same individual for pharmacokinetic/toxicokinetic or clinical pathology studies. Blood samples are usually taken from the femoral vein, although the tail vein can be used for very small toxicokinetic samples. Typically only 15% of the circulating blood volume can be removed within one
month, requiring more sensitive analytical methods to be developed for a small sample size, similar to that for rodents (Smith et al., 2001; Zuhlke and Weinbauer, 2003), consequently the marmosets’ small size does not pose a problem if analytical methodologies are already in place for rodents. Furthermore, the volume of blood required for pharmacokinetic/toxicokinetic analysis is now significantly reduced with the development of “dried spot blood detection” (in some circumstances down to 30 µl) (Pastori et al., 2010).

The small size of marmosets allows for the collection of urine using metabolism cages similar to those used for rodents, eliminating additional difficulties associated with the use of larger non-human primates.

Examination of the eye may be performed with or without sedation and abnormalities are few with only the anticipated routine finding of hyaloid artery remnants in young animals. Screening compounds for ototoxicity can be performed in sedated marmosets using the Brainstem Auditory Evoked Response to monitor hearing loss (e.g. loop diuretics and aminoglycoside antibiotics; Kuzel et al., 1990). Screening potential effects on the cardiovascular system is an important component of both safety pharmacology and repeat dose toxicity studies (ICH-Topic-S7A, 2000; ICH-Topic-S7B, 2005; ICH-Topic-M3(R2), 2009). These can be determined in restrained or sedated marmosets, as in other species, but more informative data can be obtained by remote telemetry (Schnell and Wood, 1995) which can also monitor motor activity and body temperature.

The marmoset is a well-established laboratory species and many reviews of pathological changes have been reported as long ago as the early 1980s with database collection and marmoset pathology experience continuing within a number of establishments. The marmoset has its own range of background pathology and, as with any species, experience is needed in the interpretation of any lesions found during the course of a toxicology study (Broadmeadow, 2005; Zuhlke and Weinbauer, 2003; Smith et al., 2001).

### 3.4. Dosing

The proposed dosing route in humans determines the dosing route in the test species and oral, intravenous, intramuscular, subcutaneous, intranasal, intradermal and intraperitoneal are all feasible in the marmoset. Oral gavage is the most common method of dosing by way of a rubber catheter. Intravenous dosing is commonly performed using the saphenous vein (although some laboratories have successfully employed the cephalic vein). With experienced technicians, daily intravenous dosing can be performed relatively easily as well as continuous intravenous infusion (Ruiz de Elvira and Abbott, 1986) and repeated intravenous dosing, the latter using subcutaneously implanted vascular access ports (Smith et al., 2001; Zuhlke and Weinbauer, 2003). Intramuscular injections are usually given in the thigh muscle while subcutaneous injections are made into the upper back or abdomen in combination with lightweight backpack systems (Smith et al., 2001; Zuhlke and Weinbauer, 2003). Dietary administration of non-palatable products and inhalation are the only dosing methods that present challenges. For inhalation studies it is possible to provide masks for the marmoset in a similar fashion to those used for macaques; however, their small size makes this more difficult. A one month repeated inhalation study has been described using whole body exposure where the animals were exposed for 6 h per day to a vapor (Kurata et al., 1997).

### 4. The marmoset in nonclinical development

The marmoset has been used successfully by a number of pharmaceutical and biotechnology companies to support clinical trials and for product registration with the United States (US) Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (Zuhlke and Weinbauer, 2003; Smith et al., 2001).

The European Union distinguishes six nonclinical categories where experiments using marmosets may be conducted (Commission of the European Communities, 2007) as follows:

- (1) Biological studies of a fundamental nature
- (2) Research, development and quality control of products and devices for human medicine, dentistry and for veterinary medicine
- (3) Toxicological and other safety evaluations
- (4) Diagnosis of disease
- (5) Education and training
- (6) Other

The distribution of testing according to these categories is outlined in Table 2; (Commission of the European Communities, 2007).

The ability of the marmoset to be utilized in fundamental biology, pharmacology (pharmacodynamics and pharmacokinetics) and toxicology studies allows for a strong interconnected relationship between these disciplines, leading to a reduced number of animals used with a more complete data set at a lower cost (e.g. there is no need to repeat dose ranging studies, there are less pharmacokinetic/toxicokinetic animals required, less material is required and development time is reduced).

#### 4.1. Fundamental biology

Marmosets are used widely in the fields of neural and cognitive sciences, immunology, infectious disease, reproductive biology and stem cell research due to their close genetic, physiological and metabolic similarity to humans (Mansfield et al., 2004). These activities are centered on understanding fundamental elements of human biology as well as the pathogenesis of disease syndromes.

### Table 2

Marmoset use by category in each EU member state for 2005.

| Research area | New World monkeys (% of total number for each EU Member State) |
|--------------|-------------------------------------------------------------|
| EU member states | DE | ES | FR | IT | NL | SE | UK |
| Biological studies of a fundamental nature | 48 | 100 | 6 | 53 | 26 | 18 |
| Research, development and quality control of products and devices for human medicine, dentistry and veterinary medicine | 22 | 36 | 47 | 46 | 14 |
| Toxicological and other safety evaluations | 30 | 39 | 28 | 100 | 52 |
| Diagnosis of disease | 1 | 18 | 2 |
| Education and training | | | | |
| Other | | | |

* New World monkeys are predominantly marmosets, with a small number of tamarins included (SCHER, 2009; Commission of the European Communities, 2007).

DE = Deutschland; Germany; ES = Spain; FR = France; IT = Italy; NL = Netherlands; SE = Sweden; UK = United Kingdom.
of humans and providing relevant translational research for potential new medicines.

Recently, several international activities have been implemented to further support the use of the marmoset in nonclinical development of pharmaceuticals including biotechnology derived products, gene therapies and vaccines. These include the following: the significant progress in the marmoset genome sequencing (Mansfield et al., 2004; http://ucscbrowse, 2010), the successful creation of a marmoset specific DNA microarray (Datson et al., 2007, 2009), the public availability of a large collection (3215) of express sequence tags (ESTs;) of marmoset origin [GenBank: EF214838 –EF215447, EH380242 – EH382846] (http://www.biomedcentral.com/content/supplementary/1471-2164-8-190-S2.xls, 2007) and the creation of transgenic marmosets (Cyranoski, 2009; Sasaki et al., 2009).

4.2. Pharmacodynamics

Marmosets are key pharmacodynamic models in the fields of neuroscience, immunology, and infectious disease and also serve as useful models in other disease areas (Table 3).

4.2.1. Neuroscience

Marmosets are widely used models for a number of neurological disorders including multiple sclerosis, Parkinson’s disease and Huntington’s disease (Mansfield et al., 2004) as well as for studies on normal neurophysiology (Brysch et al., 1990; Hornung et al., 1990; Guldin et al., 1993; Peretta, 2009).

Multiple sclerosis is a chronic progressive autoimmune disease of the brain and spinal cord (Rumrill, 2009). The experimental autoimmune encephalomyelitis (EAE) model in marmosets represents a chronic relapsing multiple sclerosis (Genain and Hauser, 1997; t Hart et al., 2000; Merkler et al., 2006) whereas the EAE model, in rodents and in rhesus monkeys, is more reminiscent of acute disseminated encephalomyelitis. Several nonclinical studies have been successfully conducted in the EAE marmoset model using in vivo magnetic resonance imaging (MRI), visual evoke potentials and neuropathology as measures of efficacy (Boretius et al., 2006; Diem et al., 2008; t Hart and Massacesi, 2009). New potential therapeutic antibodies against human cluster of differentiation (CD) 40 or the shared interleukin-12p40 (IL-12p40) subunit of interleukins 12 and 23 have been successfully tested in the EAE marmoset model (Boon et al., 2001; t Hart et al., 2005).

Parkinson’s disease (PD) is a neurodegenerative disease characterized by insufficient production of dopamine from the substantia nigra area of the brain which leads to significant impairment of movement (Dawson and Dawson, 2002). The marmoset is a recognized model of Parkinson’s disease (Owen et al., 1997) using selective neurotoxins to destroy the dopamine neurons. The toxins most commonly used are 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA). More recently, transgenic models of the disorder have been developed in marmosets leading to over-expression of proteins such as α-synuclein using viral vectors (Kirik et al., 2003; Eslamboli et al., 2007). The experimental use of marmosats is an important intermediary step between rodent studies and controlled clinical trials when the phylogenetic similarity to humans is pivotal for assessing and optimizing the efficacy of candidate therapies. However, the marmoset is not the most suitable non-human primate species to use when specific behavioral tests are required (e.g. cognitive testing, tremor rating) (Eslamboli, 2005).

Huntington’s disease (HD) is a fatal genetic neurodegenerative disorder with most animal models falling into two broad categories, non-genetic (e.g. quinolinic acid; QA) and transgenic. Historically, the former have dominated the field of HD research (Ramaswamy et al., 2007). The unilateral QA putamen lesion in the marmoset provides a useful and recognized tool for the investigation of potential treatments (Kendall et al., 1998), however this model does not replicate the pathology or the symptoms of HD but rather develops a consistently reproducible lesion with motor impairments (Kendall et al., 1998, 2000a,b). Recently the marmoset huntingtin (Htt) gene has been isolated and identified, resulting in the potential to prepare a transgenic HD marmoset model, that could provide further information on the underlying pathology and the ability to test therapeutic candidates and gene therapies (Hohljoh et al., 2009; Ramaswamy et al., 2007).

4.2.2. Immunology

For the development of IMPs it is of critical importance that potential effects on the immune system (either intended pharmacodynamic or resultant toxicological effects) are investigated in appropriate models. Non-human primates are typically considered superior to other models to investigate immunological, pharmacodynamic and potential immune toxic effects on IMPs (Weatherall, 2006).

The marmoset is a key species for several reasons.

- The marmoset represents a unique species for the study of T and B cell-mediated diseases, because they are born as naturally occurring bone marrow chimeras (Niblack et al., 1977).
- A large variety of surface receptors on lymphocytes and other blood cells have been found to cross-react with monoclonal antibodies against the corresponding human epitopes, indicating a high degree of similarity between these species (Barton et al., 1984; Neubert et al., 1996; Riecke et al., 2000; Kireta et al., 2005; Kametani et al., 2009).

Several studies have been performed resulting in the generation of a data bank from more than 400 animals, the largest data bank available for surface receptors (Neubert et al., 1993). However, in both marmosets and humans, some qualitative and quantitative differences exist with respect to surface markers on lymphocytes relevant for studies on immunotoxicity: the marmoset lacks natural

| Field                | Disease/model                                      |
|----------------------|---------------------------------------------------|
| Neuroscience         | Multiple sclerosis                                |
|                      | Parkinson’s                                        |
|                      | Huntington’s                                        |
|                      | Stroke                                            |
|                      | Alzheimer’s                                        |
|                      | Absence epilepsy                                   |
|                      | Aging                                              |
|                      | Spinal injury                                      |
| Immunology           | Thymic epithelium                                  |
|                      | Interleukin 2 and 4                                 |
|                      | Amyloid A (AA) amyloidosis                         |
|                      | Induced thrombocytopenia purpura                   |
| Infectious disease   | Hepatitis                                          |
|                      | Eastern equine encephalitis                        |
|                      | Severe acute respiratory syndrome                  |
|                      | Acute oncopnesis                                   |
|                      | Viral persistence                                  |
|                      | HIV                                                |
|                      | Measles pathogenesis                               |
|                      | Anthrax                                            |
|                      | Smallpox virus infection                           |
|                      | Viral hemorrhagic fevers                           |
| Other                | Reproductive biology and immuno-contraception      |
|                      | Bone disease                                       |
|                      | Human physiology                                  |
|                      | Behavioral                                         |
killer cells with typical markers found in humans (CD2-CD56+), conversely cytotoxic killer effector CD4+ cells (CD4+CD56+) found in marmosets are not found in humans (Neubert et al., 1993).

4.2.3. Infectious disease

Marmosets have been used extensively in infectious disease research due, in large part, to their unique sensitivity to a number of viruses, bacteria and parasites responsible for human infectious disease (Mansfield et al., 2004). Furthermore, in an era where bioterrorism has become a reality, there is an increased demand for non-human primate models and regulatory authorities have established new guidelines for testing vaccines and therapeutics for pathogens that governments have determined are potential biological weapons.

4.3. Pharmacokinetics

The marmoset is also used to investigate the absorption, distribution, metabolism and excretion of new IMPs. In addition, techniques that are not practical in larger non-human primates such as whole-body autoradiography can be performed in the marmoset (Broadmeadow, 2005). Furthermore, the marmoset represents a key model for IMPs that are substrates of cytochrome P450 (CYP450) such as CYP1A2, CYP3A4 and for investigating glucuronidation in vitro.

4.3.1. CYP1A2

CYP1A2 is constitutively expressed at a high level in the human liver and plays an important role in the mutagenic activation of heterocyclic amines (Butler et al., 1989; Shimada et al., 1994). CYP1A2 is also constitutively expressed in the liver of marmosets (Edwards et al., 1994; Bullock et al., 1995) but not in cynomolgus monkeys. Furthermore, the marmoset and human CYP1A2 respond to similar inducers, and appear to be under the same or similar control mechanisms (Sakuma et al., 1997).

4.3.2. CYP3A4

CYP3A4 constitutes the largest fraction of human hepatic CYP450 and is involved in the metabolism of 45–60% of medicines currently in use (Koehler et al., 2006). This broad substrate specificity represents the basis for many clinically relevant “medicine-medicine” interactions and the marmoset is a key model for studying these metabolic interactions as there is a strong similarity between marmoset and human genes, proteins and their functions (McArthur et al., 2003; Nelson et al., 2004; Williams et al., 2004; Koehler et al., 2006) (Table 4).

4.3.3. Uridine diphosphate glucuronosyltransferases

Uridine diphosphate glucuronosyltransferases (UGT) are a large family of membrane bound enzymes responsible for the detoxification of a wide range of xenobiotics and endogenous compounds. The large number of products glucuronidated, whether directly or following the Phase I step, necessitates a thorough understanding of glucuronidation for efficient pharmaceutical development (Dutton, 1980). Typically, rodents are used as the primary animal model and the dog commonly used as the second animal model for pharmacokinetic studies. However a wide range of products are more extensively glucuronidated by dog liver microsomes when compared with human hepatic microsomes (Soars et al., 2001). Furthermore, qualitative differences in glucuronidation are observed between dogs and humans with extrahepatic glucuronidation being markedly reduced in dogs (Soars et al., 2001). By contrast, there are quantitative and qualitative similarities between human and marmoset glucuronidation in vitro and as such the marmoset is a useful model especially when the rate and pathway of glucuronidation of a medicine are known to differ significantly between rodents, dogs and humans (Soars et al., 2001). The significant extrahepatic (e.g. kidney) glucuronidation observed in marmosets in comparison with other animals merits its use for preclinical pharmacokinetic and safety evaluation studies. Nevertheless, further work should also be performed to confirm that observations in different species in vitro accurately represent the situation in vivo (Soars et al., 2001).

4.4. Toxicology

Toxicological and other safety evaluations, represent the largest use of marmosets in nonclinical development with typical use being for acute, sub-acute, chronic and toxicokinetic studies (Commission of the European Communities, 2007). Interestingly, long term studies of up to 6.5–7 years duration have also been reported (Smith et al., 2001) (Table 5).

The marmoset can be used for pharmacology as well as for toxicology studies. In addition, there are well-established classes of compounds where the marmoset may well offer an alternative species to the dog that shows hypersensitivity or idiosyncrasy (Smith et al., 2001; Gaudy et al., 1987) (Table 6).

4.4.1. Teratology, reproductive and related studies

Marmosets represent a useful model to investigate the teratologic potential of pharmaceuticals and to investigate aspects of reproductive physiology and immune-contraception due to their early sexual maturity, high reproduction efficiency and similarities in placentation to humans (Mansfield et al., 2004) (Table 7).

By contrast, there are several aspects of the reproductive process in marmosets that exhibit discrepancies to those described for humans (Cline, 2007; Luetjens et al., 2005; Zuhlke and Weinbauer, 2003). The differences for females include multi-ovulation, postpartum conception, the lack of external signs of menstruation and for males include alterations or even absence of genes known to be essential for reproductive development. The duration of the menstrual cycle in marmosets varies, on average, from 16 to 28 days and unlike women, the luteal phase is comparatively long at the expense of the follicular phase with 1 and 4 eggs ovulated. A mid-phase follicle stimulating hormone (FSH) peak and the presence of multiple dominant follicles in marmosets represents another difference to humans (Zuhlke and Weinbauer, 2003). For juvenile toxicology studies, useful background data has been generated in the marmoset over the years examining differences in clinical pathology endpoints and organ weights between young and adult marmosets (Wadsworth et al., 1981; Davy et al., 1984).

### Table 4

Summary of marmoset CYP3A characteristics.

| CYP3A characteristics |
|-----------------------|
| CYP3A21 is the dominant hepatic CYP3A protein in marmosets |
| The sequence similarity between human CYP3A4 and CYP3A21 in marmoset across the first 7.5 kb of the cloned CYP3A21 promoter is 88%. |
| The marmoset CYP3A21 gene is clustered with the human CYP3A genes, while rodent CYP3A genes form several separate clusters (McArthur et al., 2003; Nelson et al., 2004; Williams et al., 2004). |
| The marmoset CYP3A21 cDNA exhibits high similarity to human CYP3A genes (Williams et al., 2004). |
| The enzymatic activities of CYP3A21 are similar to those of CYP3A4 (Koehler et al., 2006). |
Table 5
Examples of marketed products where marmosets were used in their development.

| Therapeutic class       | Product name                  | Toxicity study: route/duration | Notes                                                                 | References                     |
|------------------------|-------------------------------|--------------------------------|----------------------------------------------------------------------|--------------------------------|
| Anti-hypertensive       | Cilazapril (Inhibe®)          | Oral: 2, 4 and 26 weeks; Intravenous: 2 weeks | Angiotensin converting enzyme (ACE) inhibitor for the treatment of hypertension and congestive heart failure | Hoffmann-LaRoche (2008) |
| Anti-retroviral         | Saquinavir mesylate           | Oral: 2, 4, 26 weeks; Intravenous: 2 and 4 weeks | Proteinase inhibitor for human, immunodeficiency virus (HIV)         | Hoffmann-LaRoche (2009) |
| Anti-viral              | Oseltamivir phosphate         | Oral: 1, 4 and 39 weeks        | Neuraminidase inhibitor for seasonal flu and more recently swine flu | Hoffmann-LaRoche (2009) |
| Chemotherapeutic        | Mitomycin C (Mutamycin®)      | Oral: 39 weeks                | Antibiotic isolated from the broth of Streptomycetes coepitidis which has antitumor activity | Matsumoto et al. (1987) |
| Lipid lowering agent    | Fibrates (e.g. clofibrate and clofibrate) | Oral: several studies up to 7 years | Fibrates are hypolipidaemic agents which, in chronic studies in rats or mice, have produced tumors or pre-neoplastic changes not predictable for humans. Clofibrate and ciprofibrate toxicity studies up to 7 years duration conducted by two laboratories have shown that the marmoset is predictive for humans and in the marmoset studies, unlike rodents, there is no induction of liver tumors | |
| Non steroidal anti-inflammatory | Indomethacin                  | Oral: 4 weeks                 | Non-steroidal anti-inflammatory to reduce fever, pain stiffness and swelling. It inhibits prostaglandin release | Oberto et al. (1990) |
| Parkinson's disease     | Ropinirole                    | Oral-safety studies           | Ropinirole at microgram/kg doses produced significant decreases in the number of postures and significant increases in the time spent at the cage front in the “human threat” test. In rodents the effects were seen at mg/kg | Eden et al. (1991) |
| Psychotic disorders     | Clozapine                     | Oral, intramuscular-safety studies | Clozapine over a wide dose range, unlike other antipsychotics, increased the motivational state of the marmoset suggesting a unique clinical profile | Cilia et al. (2001) |
| including schizophrenia | Varieties                     | Thalidomide                   | The marmoset has been used to comprehensively investigate the mechanism of thalidomide teratogenesis | Heger et al. (1994); Neubert et al. (1996) |

Table 6
Compound classes where marmosets may be more suitable as a model than dogs.

- Vomiting with morphine compounds
- Atrio-ventricular block with calcium antagonists
- Reflex tachycardia with anti-hypertensives
- Allergic reactions with vehicles such as cremophor

5. Conclusion

Once the scientific rationale has been carefully considered and its use as the non-rodent species justified, there are considerable advantages in using the marmoset as a model in pharmacological product development. These range from reduced material requirement due to their small size (up to 15 fold less), a predicted acceleration of development (up to 18 months faster), an earlier assessment of product candidates in the adult due to their early sexual maturity and reduced numbers of animals required due to their ability to be used in both pharmacology and toxicology studies. These factors, alongside a better understanding of their optimal nutrient and welfare requirements over recent years, facilitate the generation of a more cohesive and robust dataset. With the growth of biotechnology-derived products and gene therapies, the use of non-human primates has, by necessity, also increased since these are often the only relevant species that provide an adequate safety assessment. Nevertheless, there is a growing public call for minimizing their use now and in the future. Utilizing the more primitive marmoset species may provide the optimal compromise.

Table 7
Examples of marmoset use in teratology and reproductive studies.

- Marmoset use in teratology and reproductive studies
- Higher non-human primates, such as the cynomolgus and rhesus monkeys, have generation times of about 5 years, making multigenerational studies impractical.
- However, multigenerational studies are feasible in the marmoset, which has a comparatively short generation time (14–18 months) (Marshall et al., 2003b)
- To investigate the mechanism of thalidomide teratogenesis: metabolites of thalidomide may cause the down-regulation of surface adhesion receptors thereby altering cell to cell and cell to extracellular matrix interactions within the developing limb bud (Heger et al., 1988, 1994; Klug et al., 1994; Neubert et al., 1996)
- To examine the effect of luteinizing hormone and follicular stimulating hormone on granulosa cell development and steroidogenesis (Barrett, 2007; Millar et al., 2000; Heger et al., 1994; Cilia et al., 2001)
- To define the mechanisms of estradiol inactivation, in vitro fertilization, embryo transfer and parthenogenesis (Husen et al., 2001; Summers et al., 1987; Marshall et al., 1997, 1998, 2003a; Luetjens and Wesseln, 2008)
- To determine whether infant feeding with soya formula milk, which contains high levels of plant estrogens, poses any immediate or longer-term health risk to the developing testis and reproductive system of the male. An initial study found that testosterone levels were suppressed in animals fed with soya formula milk. A longer term follow-up analysis of these animals indicated that infant feeding with soya formula milk had no gross reproductive effects in male marmosets, but that it does alter testis size and cell composition. The authors concluded that similar changes are likely to occur in adult men fed soya formula milk as infants (Sharpe et al., 2002; Tan et al., 2006)
Conflict of interest

The authors declare that there are no conflicts of interest.

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