Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Regulatory Toxicology and Pharmacology 59 (2011) 19–27

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

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 Pomezia, RM, Italy

ARTICLE INFO

Article history:
Received 27 August 2010
Available online 13 December 2010

Keywords:
Callithrix jacchus
Marmoset
Non-human primates
Pharmaceuticals
Biotechnology-derived products
Nonclinical
Pharmacology
Pharmacokinetics
Toxicology
Regulatory

ABSTRACT

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.

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

* Corresponding author. Fax: +44 1767 681703.
E-mail address: aorsi@salupontconsulting.com (A. Orsi).

0273-2300 © 2010 Elsevier Inc. Open access under CC BY-NC-ND license.
doi:10.1016/j.yrtph.2010.12.003

Table 1
Growth characteristics of the marmoset.

Characteristics	Description
Weight (g)	
Birth	20–35
Weaning	60–150
Adult	200–600
Length (cm)	
Adult without tail	Up to 20
Adult tail length	Up to 35

material production is usually sub-optimal and therefore sufficient quantities can be rate limiting 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 (Badihi 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 m^2 for a breeding pair and 2.0 m^2 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-

Fig. 1. An example of a modern housing facility for marmosets. Each cage can be divided in length and contains wooden hand-made enrichments and entertainments. One side of each cage is removable creating larger cage for family groups.

ation for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

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 m^2 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). Continual 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

Fig. 2. Marmosets in a modern housing facility.

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 toxicology 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; (Commision 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							2
Education and training			1				
Other			18				13

* New World monkeys are predominantly marmosets, with a small number of tamarins included (SCHER, 2009; Commision 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 marmosets 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 (Hohjoh 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

Table 3
Examples of marmoset use in pharmacodynamic studies.

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 purpurea
Infectious disease	Hepatitis Eastern equine encephalitis Severe acute respiratory syndrome Acute oncogenesis Viral persistence HIV Measles pathogenesis Anthrax Smallpox virus infection Viral hemorrhagic fevers
Other	Reproductive biology and immuno-contraception Bone disease Human physiology Behavioral

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).

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 glucurondation 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 (Commision 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 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 (Inhibace®)	Oral: 2, 4 and 26 weeks; Intra-venous: 2 weeks	Angiotensin converting enzyme (ACE) inhibitor for the treatment of hypertension and congestive heart failure	Hoffmann-LaRoche (2008)
Anti-retroviral	Saquinavir mesylate (Invirase®)	Oral: 2, 4, 26 weeks Intra-venous: 2 and 4 weeks	Proteinase inhibitor for human, immunodeficiency virus (HIV)	Hoffmann-LaRoche (2009)
Anti-viral	Oseltamivir phosphate (Tamiflu®)	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 *Streptomyces caespitosus* which has antitumor activities	Matsumoto et al. (1987)
Lipid lowering agent	Fibrates (e.g. ciprofibrate 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	Eason et al. (1988), Spencer et al. (1989), Smith et al. (2001), Reddy et al. (1983), Makowska et al. (1992), Graham et al. (1994)
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 including schizophrenia	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)
Varies	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.

Class of compounds where marmosets may be a more suitable 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 pharmaceutical product development. These range from reduced material requirement due to their small size (up to15 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 maturation 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; Hillier et al., 1997)
To define the mechanisms of estradiol inactivation, i*n vitro* fertilization, embryo transfer and parthenogenesis (Husen et al., 2001; Summers et al., 1987; Marshall et al., 1997, 1998, 2003a; Luetjens and Wesselmann, 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. The manuscript was sponsored by Research Toxicology Center S.p.A., a contract research organization specialized in nonclinical safety studies for product registration.

References

Abbott, D., 2003. Aspects of common marmoset basic biology and life history important for biomedical research. Comp. Med. 53, 12.

Badihi, I., Morris, K., Buchanan-Smith, H.M., 2007. The effects of increased space, complexity, and choice, together with their loss, on the behavior of a family group of *Callithrix jacchus*: a case study. Lab. Prim. Newslett. 46, 1–5.

Barrett, J.R., 2007. The testosterone test: phthalate inhibits leydig cell aggregation. Environ. Health Perspect. 115, A153.

Barton, R.W., Thrall, R.S., Neubauer, R.H., 1984. Binding of human lymphocyte-specific monoclonal antibodies to common marmoset lymphoid cells. Cell. Immunol. 84, 446–452.

Baskerville, M., 1999. Old World monkeys. In: Poole, T. (Ed.), UFAW Handbook on the Care and Management of Laboratory Animals, seventh ed. Blackwell Science, Oxford., pp. 611–635.

Boon, L., Brok, H.P., Bauer, J., Ortiz-Buijsse, A., Schellekens, M.M., Ramdien-Murli, S., Blezer, E., van Meurs, M., Ceuppens, J., de Boer, M., t Hart, B.A., Laman, J.D., 2001. Prevention of experimental autoimmune encephalomyelitis in the common marmoset (*Callithrix jacchus*) using a chimeric antagonist monoclonal antibody against human CD40 is associated with altered B cell responses. J. Immunol. 167, 2942–2949.

Boretius, S., Schmelting, B., Watanabe, T., Merkler, D., Tammer, R., Czeh, B., Michaelis, T., Frahm, J., Fuchs, E., 2006. Monitoring of EAE onset and progression in the common marmoset monkey by sequential high-resolution 3D MRI. NMR Biomed. 19, 41–49.

Broadmeadow, A., 2005. The common marmoset (*Callithrix jacchus*) in preclinical research and development of new pharmaceutical products. J. Toxicol. Sci. 30, S123.

Brysch, W., Brysch, I., Creutzfeldt, O.D., Schlingensiepen, R., Schlingensiepen, K.H., 1990. The topology of the thalamo-cortical projections in the marmoset monkey (*Callithrix jacchus*). Exp. Brain Res. 81, 1–17.

Bullock, P., Pearce, R., Draper, A., Podval, J., Bracken, W., Veltman, J., Thomas, P., Parkinson, A., 1995. Induction of liver microsomal cytochrome P450 in cynomolgus monkeys. Drug Metab. Dispos. 23, 736–748.

Butler, M.A., Guengerich, F.P., Kadlubar, F.F., 1989. Metabolic oxidation of the carcinogens 4-aminobiphenyl and 4,4′-methylene-bis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. Cancer Res. 49, 25–31.

Cilia, J., Piper, D.C., Upton, N., Hagan, J.J., 2001. Clozapine enhances breakpoint in common marmosets responding on a progressive ratio schedule. Psychopharmacology (Berl.) 155, 135–143.

Cline, J.M., 2007. Assessing the mammary gland of nonhuman primates: effects of endogenous hormones and exogenous hormonal agents and growth factors. Birth Defects Res. B Dev. Reprod. Toxicol. 80, 126–146.

Commision of the European Communities, E., 2007. Fifth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union. p. 277.

Cyranoski, D., 2009. Marmoset model takes centre stage. Nature 459, 492.

Datson, N.A., Morsink, M.C., Atanasova, S., Armstrong, V.W., Zischler, H., Schlumbohm, C., Dutilh, B.E., Huynen, M.A., Waegele, B., Ruepp, A., de Kloet, E.R., Fuchs, E., 2007. Development of the first marmoset-specific DNA microarray (EUMAMA): a new genetic tool for large-scale expression profiling in a non-human primate. BMC Genomics 8, 190.

Datson, N.A., Morsink, M.C., Steenbergen, P.J., Aubert, Y., Schlumbohm, C., Fuchs, E., de Kloet, E.R., 2009. A molecular blueprint of gene expression in hippocampal subregions CA1, CA3, and DG is conserved in the brain of the common marmoset. Hippocampus 19, 739–752.

Davy, C.W., Jackson, M.R., Walker, J., 1984. Reference intervals for some clinical chemical parameters in the marmoset (*Callithrix jacchus*): effect of age and sex. Lab. Anim. 18, 135–142.

Dawson, T.M., Dawson, V.L., 2002. Neuroprotective and neurorestorative strategies for Parkinson's disease. Nat. Neurosci. 5 (Suppl), 1058–1061.

Diem, R., Demmer, I., Boretius, S., Merkler, D., Schmelting, B., Williams, S.K., Sattler, M.B., Bahr, M., Michaelis, T., Frahm, J., Bruck, W., Fuchs, E., 2008. Autoimmune optic neuritis in the common marmoset monkey: comparison of visual evoked potentials with MRI and histopathology. Invest. Ophthalmol. Vis. Sci. 49, 3707–3714.

Dunbar, S.A., 2006. Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection. Clin. Chim. Acta 363, 71–82.

Dutton, G.J., 1980. Glucuronidation of Drugs and Other Compounds. CRC Press, Boca Raton, Florida.

Eason, C.T., Spencer, A.J., Pattison, A., Howells, D.D., Henry, D.C., Bonner, F.W., 1988. Species variation in gastric toxicity following chronic administration of ciprofibrate to rat, mouse, and marmoset. Toxicol. Appl. Pharmacol. 95, 328–338.

Eden, R.J., Costall, B., Domeney, A.M., Gerrard, P.A., Harvey, C.A., Kelly, M.E., Naylor, R.J., Owen, D.A., Wright, A., 1991. Preclinical pharmacology of ropinirole (SK&F 101468-A) a novel dopamine D2 agonist. Pharmacol. Biochem. Behav. 38, 147–154.

Edwards, R.J., Murray, B.P., Murray, S., Schulz, T., Neubert, D., Gant, T.W., Thorgeirsson, S.S., Boobis, A.R., Davies, D.S., 1994. Contribution of CYP1A1 and CYP1A2 to the activation of heterocyclic amines in monkeys and human. Carcinogenesis 15, 829–836.

Eslamboli, A., 2005. Marmoset monkey models of Parkinson's disease: which model, when and why? Brain Res. Bull. 68, 140–149.

Eslamboli, A., Romero-Ramos, M., Burger, C., Bjorklund, T., Muzyczka, N., Mandel, R.J., Baker, H., Ridley, R.M., Kirik, D., 2007. Long-term consequences of human alpha-synuclein overexpression in the primate ventral midbrain. Brain 130, 799–815.

Gaudy, J.H., Sicard, J.F., Lhoste, F., Boitier, J.F., 1987. The effects of cremophor EL in the anaesthetized dog. Can. J. Anaesth. 34, 122–129.

Genain, C.P., Hauser, S.L., 1997. Creation of a model for multiple sclerosis in *Callithrix jacchus* marmosets. J. Mol. Med. 75, 187–197.

Graham, M.J., Wilson, S.A., Winham, M.A., Spencer, A.J., Rees, J.A., Old, S.L., Bonner, F.W., 1994. Lack of peroxisome proliferation in marmoset liver following treatment with ciprofibrate for 3 years. Fundam. Appl. Toxicol. 22, 58–64.

Guldin, W.O., Mirring, S., Grusser, O.J., 1993. Connections from the neocortex to the vestibular brain stem nuclei in the common marmoset. NeuroReport 5, 113–116.

Heger, W., Klug, S., Schmahl, H.J., Nau, H., Merker, H.J., Neubert, D., 1988. Embryotoxic effects of thalidomide derivatives on the non-human primate *Callithrix jacchus*; 3. Teratogenic potency of the EM 12 enantiomers. Arch. Toxicol. 62, 205–208.

Heger, W., Schmahl, H.J., Klug, S., Felies, A., Nau, H., Merker, H.J., Neubert, D., 1994. Embryotoxic effects of thalidomide derivatives in the non-human primate *Callithrix jacchus*. IV. Teratogenicity of micrograms/kg doses of the EM12 enantiomers. Teratog. Carcinog. Mutagen. 14, 115–122.

Hillier, S.G., Tetsuka, M., Fraser, H.M., 1997. Location and developmental regulation of androgen receptor in primate ovary. Hum. Reprod. 12, 107–111.

Hoffmann-LaRoche, 2008. Product monograph: Inhibace® (cilazapril).

Hoffmann-LaRoche, 2009. Product monograph: Tamiflu® (oseltamivir phosphate capsule).

Hoffmann-LaRoche, 2009 Product monograph: Invirase® (Saquinavir mesylate).

Hohjoh, H., Akari, H., Fujiwara, Y., Tamura, Y., Hirai, H., Wada, K., 2009. Molecular cloning and characterization of the common marmoset Huntington gene. Gene 432, 60–66.

Home–Office, 2000. Statistics of Scientific Procedures on Living Animals Great Britain.

Hornung, J.P., Fritschy, J.M., Tork, I., 1990. Distribution of two morphologically distinct subsets of serotoninergic axons in the cerebral cortex of the marmoset. J. Comp. Neurol. 297, 165–181.

http://ucscbrowser.genenetwork.org/goldenPath/newsarch.html#030610, 2010. (3 June 2010) – Updated Marmoset Genome Browser Available.

http://www.biomedcentral.com/content/supplementary/1471-2164-8-190-S2.xls, 2007. Overview of 3215 sequences submitted to GenBank and dbEST (Datson et al., 2007).

http://www.sfbr.org/SNPRC/detail.aspx?r=60, 2010. Immunology Core Laboratory.

Hu, R., Wang, J., 2007. A rapid, multiplexed new technology xMAP liquid chip for detection and identification of pathogens. Wei Sheng Yan Jiu 36, 759–762.

Husen, B., Adamski, J., Rune, G.M., Einspanier, A., 2001. Mechanisms of estradiol inactivation in primate endometrium. Mol. Cell. Endocrinol. 171, 179–185.

ICH-Topic-M3(R2), 2009. Guideline on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals.

ICH-Topic-S7A, 2000. Safety Pharmacology Studies for Human Pharmaceuticals.

ICH-Topic-S7B, 2005. The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals.

Kametani, Y., Suzuki, D., Kohu, K., Satake, M., Suemizu, H., Sasaki, E., Ito, T., Tamaoki, N., Mizushima, T., Ozawa, M., Tani, K., Kito, M., Arai, H., Koyanagi, A., Yagita, H., Habu, S., 2009. Development of monoclonal antibodies for analyzing immune and hematopoietic systems of common marmoset. Exp. Hematol. 37, 1318–1329.

Kendall, A.L., David, F., Rayment, G., Torres, E.M., Annett, L.E., Dunnett, S.B., 2000a. The influence of excitotoxic basal ganglia lesions on motor performance in the common marmoset. Brain 123 (Pt 7), 1442–1458.

Kendall, A.L., Hantraye, P., Palfi, S., 2000b. Striatal tissue transplantation in non-human primates. Prog. Brain Res. 127, 381–404.

Kendall, A.L., Rayment, F.D., Torres, E.M., Baker, H.F., Ridley, R.M., Dunnett, S.B., 1998. Functional integration of striatal allografts in a primate model of Huntington's disease. Nat. Med. 4, 727–729.

Kireta, S., Zola, H., Gilchrist, R.B., Coates, P.T., 2005. Cross-reactivity of anti-human chemokine receptor and anti-TNF family antibodies with common marmoset (*Callithrix jacchus*) leukocytes. Cell. Immunol. 236, 115–122.

Kirik, D., Annett, L.E., Burger, C., Muzyczka, N., Mandel, R.J., Bjorklund, A., 2003. Nigrostriatal alpha-synucleinopathy induced by viral vector-mediated overexpression of human alpha-synuclein: a new primate model of Parkinson's disease. Proc. Natl. Acad. Sci. USA 100, 2884–2889.

Klug, S., Felies, A., Sturje, H., Nogueira, A.C., Neubert, R., Frankus, E., 1994. Embryotoxic effects of thalidomide derivatives in the non-human primate *Callithrix jacchus* 5 Lack of teratogenic effects of phthalimidophthalmide. Arch. Toxicol. 68, 203–205.

Koehler, S.C., Von Ahsen, N., Schlumbohm, C., Asif, A.R., Goedtel-Armbrust, U., Oellerich, M., Wojnowski, L., Armstrong, V.W., 2006. Marmoset CYP3A21, a model for human CYP3A4: protein expression and functional characterization of the promoter. Xenobiotica 36, 1210–1226.

Kurata, Y., Takechi, M., Toyota, N., Tsuchitani, M., Katoh, M., Rusch, G.M., Trochimowicz, H.J., Shin-Ya, S., 1997. Four-week repeated inhalation study of HCFC 225ca and HCFC 225cb in the common marmoset. Toxicol. Lett. 92, 209–219.

Kuzel, R.A., Smith, J.M., Trennery, P.N., 1990. Screening procedure for assessment of ototoxicity in the common marmoset. J. Pharmacol. Methods 24, 9–18.

Luetjens, C.M., Weinbauer, G.F., Wistuba, J., 2005. Primate spermatogenesis: new insights into comparative testicular organisation, spermatogenic efficiency and endocrine control. Biol. Rev. Camb. Philos. Soc. 80, 475–488.

Luetjens, C.M., Wesselmann, R., 2008. The fate of paternal mitochondria in marmoset pre-implantation embryos. J. Med. Primatol. 37, 128–140.

Makowska, J.M., Gibson, G.G., Bonner, F.W., 1992. Species differences in ciprofibrate induction of hepatic cytochrome P450 4A1 and peroxisome proliferation. J. Biochem. Toxicol. 7, 183–191.

Mansfield, K., Tardif, S., Eichler, E.E., 2004. White Paper for Complete sequencing of the common marmoset (*Callithrix jacchus*) genome. http://www.genome.gov/Pages/Research/Sequencing/SeqProposals/MarmosetSeq.pdf.

Marshall, J.W., Cummings, R.M., Bowes, L.J., Ridley, R.M., Green, A.R., 2003a. Functional and histological evidence for the protective effect of NXY-059 in a primate model of stroke when given 4 h after occlusion. Stroke 34, 2228–2233.

Marshall, V.S., Browne, M.A., Knowles, L., Golos, T.G., Thomson, J.A., 2003b. Ovarian stimulation of marmoset monkeys (*Callithrix jacchus*) using recombinant human follicle stimulating hormone. J. Med. Primatol. 32, 57–66.

Marshall, V.S., Kalishman, J., Thomson, J.A., 1997. Nonsurgical embryo transfer in the common marmoset monkey. J. Med. Primatol. 26, 241–247.

Marshall, V.S., Wilton, L.J., Moore, H.D., 1998. Parthenogenetic activation of marmoset (*Callithrix jacchus*) oocytes and the development of marmoset parthenogenones in vitro and in vivo. Biol. Reprod. 59, 1491–1497.

Matsumoto, K., Ochiai, T., Sekita, K., Kawasaki, Y., Yasuhara, K., Nakaji, Y., Furuya, T., Kurokawa, Y., Tobe, M., 1987. Six-month toxicity study with mitomycin C in marmosets. Jpn. Soc. Toxicol., Tokyo.

McArthur, A.G., Hegelund, T., Cox, R.L., Stegeman, J.J., Liljenberg, M., Olsson, U., Sundberg, P., Celander, M.C., 2003. Phylogenetic analysis of the cytochrome P450 3 (CYP3) gene family. J. Mol. Evol. 57, 200–211.

Merkler, D., Schmelting, B., Czeh, B., Fuchs, E., Stadelmann, C., Bruck, W., 2006. Myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis in the common marmoset reflects the immunopathology of pattern II multiple sclerosis lesions. Mult. Scler. 12, 369–374.

Millar, M.R., Sharpe, R.M., Weinbauer, G.F., Fraser, H.M., Saunders, P.T., 2000. Marmoset spermatogenesis: organizational similarities to the human. Int. J. Androl. 23, 266–277.

Committee for the Update of the Guide for the Care and Use of Laboratory Animals; National Research Council, 2010. Guide for the Care and Use of Laboratory Animals: Eighth Edition. The National Academies Press.

Nelson, D.R., Zeldin, D.C., Hoffman, S.M., Maltais, L.J., Wain, H.M., Nebert, D.W., 2004. Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. Pharmacogenetics 14, 1–18.

Neubert, R., Foerster, M., Nogueira, A.C., Helge, H., 1996a. Cross-reactivity of antihuman monoclonal antibodies with cell surface receptors in the common marmoset. Life Sci. 58, 317–324.

Neubert, R., Hinz, N., Thiel, R., Neubert, D., 1996b. Down-regulation of adhesion receptors on cells of primate embryos as a probable mechanism of the teratogenic action of thalidomide. Life Sci. 58, 295–316.

Neubert, R., Stahlmann, R., Korte, M., van Loveren, H., Vos, J.G., Golor, G., Webb, J.R., Helge, H., Neubert, D., 1993. Effects of small doses of dioxins on the immune system of marmosets and rats. Ann. N. Y. Acad. Sci. 685, 662–686.

Niblack, G.D., Kateley, J.R., Gengozian, N., 1977. T-and B-lymphocyte chimerism in the marmoset. Immunology 32, 257–263.

Oberto, G., Conz, A., Giachetti, C., Galli, C.L., 1990. Evaluation of the toxicity of indomethacin in a 4-week study, by oral route, in the marmoset (*Callithrix jacchus*). Fundam. Appl. Toxicol. 15, 800–813.

OJ-L-197-(vol-50), 2007. Commission Recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (notified under document number C(2007) 2525).

Owen, S., Thomas, C., West, P., Wolfensohn, S., Wood, M., 1997. Report on primate supply for biomedical scientific work in the UK. EUPREN UK working party. Lab. Anim. 31, 289–297.

Pastori, F., Salerno, D., Oberto, G., Villa, S., Ricci, R., Calfapierta, F.R., 2010. Comparison and validation of Dried Blood Spot (DBS) and Solid Phase Extraction (SPE) techniques. In: XII International Congress of Toxicology. Toxicol. Lett. 196S, Barcelona, Spain.

Peretta, G., 2009. Non-human primate models in neuroscience research. Scand. J. Lab. Anim. Sci. 36, 77–85.

Ramaswamy, S., McBride, J.L., Kordower, J.H., 2007. Animal models of Huntington's disease. ILAR J. 48, 356–373.

Reddy, J.K., Scarpelli, D.G., Subbarao, V., Lalwani, N.D., 1983. Chemical carcinogens without mutagenic activity: peroxisome proliferators as a prototype. Toxicol. Pathol. 11, 172–180.

Riecke, K., Nogueira, A.C., Alexi-Meskishvili, V., Stahlmann, R., 2000. Cross-reactivity of antibodies on thymic epithelial cells from humans and marmosets by flow-cytometry. J. Med. Primatol. 29, 343–349.

Ross, C.N., French, J.A., Ortí, G., 2007. Germ-line chimerism and paternal care in marmosets (*Callithrix kuhlii*). Proc. Natl Acad. Sci. USA 104, 6278–6282.

Ruiz de Elvira, M.C., Abbott, D.H., 1986. A backpack system for long-term osmotic minipump infusions into unrestrained marmoset monkeys. Lab. Anim. 20, 329–334.

Rumrill, P.D.J., 2009. Multiple sclerosis: medical and psychosocial aspects, etiology, incidence, and prevalence. J. Voc. Rehabil. 31, 75–82.

Sakuma, T., Igarashi, T., Hieda, M., Ohgiya, S., Isogai, M., Ninomiya, S., Nagata, R., Nemoto, N., Kamataki, T., 1997. Marmoset CYP1A2: primary structure and constitutive expression in livers. Carcinogenesis 18, 1985–1991.

Sasaki, E., Suemizu, H., Shimada, A., Hanazawa, K., Oiwa, R., Kamioka, M., Tomioka, I., Sotomaru, Y., Hirakawa, R., Eto, T., Shiozawa, S., Maeda, T., Ito, M., Ito, R., Kito, C., Yagihashi, C., Kawai, K., Miyoshi, H., Tanioka, Y., Tamaoki, N., Habu, S., Okano, H., Nomura, T., 2009. Generation of transgenic non-human primates with germline transmission. Nature 459, 523–527.

SCHER, 2009. The need for non human primates for biomedical research, production and testing of products and devices. European Commission. pp. 1–36.

Schnell, C.R., Wood, J.M., 1995. Measurement of blood pressure and heart rate by telemetry in conscious unrestrained marmosets. Lab. Anim. 29, 258–261.

Sharpe, R.M., Martin, B., Morris, K., Greig, I., McKinnell, C., McNeilly, A.S., Walker, M., 2002. Infant feeding with soy formula milk: effects on the testis and on blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity. Hum. Reprod. 17, 1692–1703.

Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., Guengerich, F.P., 1994. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J. Pharmacol. Exp. Ther. 270, 414–423.

Smith, D., Trennery, P., Farningham, D., Klapwijk, J., 2001. The selection of marmoset monkeys (*Callithrix jacchus*) in pharmaceutical toxicology. Lab. Anim. 35, 117–130.

Soars, M.G., Riley, R.J., Burchell, B., 2001. Evaluation of the marmoset as a model species for drug glucuronidation. Xenobiotica 31, 849–860.

Spencer, A.J., Barbolt, T.A., Henry, D.C., Eason, C.T., Sauerschell, R.J., Bonner, F.W., 1989. Gastric morphological changes including carcinoid tumors in animals treated with a potent hypolipidemic agent, ciprofibrate. Toxicol. Pathol. 17, 7–15.

Summers, P.M., Shephard, A.M., Taylor, C.T., Hearn, J.P., 1987. The effects of cryopreservation and transfer on embryonic development in the common marmoset monkey, *Callithrix jacchus*. J. Reprod. Fertil. 79, 241–250.

t Hart, B.A., Brok, H.P., Remarque, E., Benson, J., Treacy, G., Amor, S., Hintzen, R.Q., Laman, J.D., Bauer, J., Blezer, E.L., 2005. Suppression of ongoing disease in a nonhuman primate model of multiple sclerosis by a human-anti-human IL-12p40 antibody. J. Immunol. 175, 4761–4768.

t Hart, B.A., Massacesi, L., 2009. Clinical, pathological, and immunologic aspects of the multiple sclerosis model in common marmosets (*Callithrix jacchus*). J. Neuropathol. Exp. Neurol. 68, 341–355.

t Hart, B.A., van Meurs, M., Brok, H.P., Massacesi, L., Bauer, J., Boon, L., Bontrop, R.E., Laman, J.D., 2000. A new primate model for multiple sclerosis in the common marmoset. Immunol. Today 21, 290–297.

Tan, K.A., Walker, M., Morris, K., Greig, I., Mason, J.I., Sharpe, R.M., 2006. Infant feeding with soy formula milk: effects on puberty progression, reproductive function and testicular cell numbers in marmoset monkeys in adulthood. Hum. Reprod. 21, 896–904.

Tucker, M.J., 1984. A survey of the pathology of marmosets (*Callithrix jacchus*) under experiment. Lab. Anim. 18, 351–358.

Wadsworth, P.F., Budgett, D.A., Forster, M.L., 1981. Organ weight data in juvenile and adult marmosets (*Callithrix jacchus*). Lab. Anim. 15, 385–388.

Weatherall, D., 2006. The Use of Non-Human Primates in Research. Academy of Medical Sciences, London. p. 147.

Williams, E.T., Rodin, A.S., Strobel, H.W., 2004. Defining relationships between the known members of the cytochrome P450 3A subfamily, including five putative chimpanzee members. Mol. Phylogen. Evol. 33, 300–308.

Zuhlke, U., Weinbauer, G., 2003. The common marmoset (*Callithrix jacchus*) as a model in toxicology. Toxicol. Pathol. 31 (Suppl), 123–127.