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ARTICLE

Using an animal model to predict the effective human dose for oral multiple sclerosis drugs

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Abstract

The objective of this study was to determine the potential usefulness of an animal model to predict the appropriate dose of newly developed drugs for treating relapsing remitting multiple sclerosis (RRMS). Conversion of the lowest effective dose (LEffD) for mice and rats in the experimental autoimmune encephalomyelitis (EAE) model was used to predict the human effective dose utilizing the body surface area correction factor found in the 2005 US Food and Drug Administration (FDA) Guidance for Industry in selecting safe starting doses for clinical trials. Predictions were also tested by comparison with doses estimated by scaling up the LEffD in the model by the human to animal clearance ratio. Although initial proof-of-concept studies of oral fingolimod tested the efficacy and safety of 1.25 and 5 mg in treating RRMS, the EAE animal model predicted the approved dose of this drug, 0.5 mg daily. This approach would have also provided useful predictions of the approved human oral doses for cladribine, dimethyl fumarate, ozanimod, ponesimod, siponimod, and teriflunomide, drugs developed with more than one supposed mechanism of action. The procedure was not useful for i.v. dosed drugs, including monoclonal antibodies. We maintain that drug development scientists should always examine a simple allometric method to predict the therapeutic effective dose in humans. Then, following clinical studies, we believe that the animal model might be expected to yield useful predictions of other drugs developed to treat the same condition. The methodology may not always be predictive, but the approach is so simple it should be investigated.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Although it is well-recognized that experimental autoimmune encephalomyelitis (EAE) activity can predict clinical efficacy of potential multiple sclerosis (MS) drugs, the conversion of these data to predict effective doses in man has not been previously demonstrated.

WHAT QUESTION DID THIS STUDY ADDRESS?
Can EAE measurements of the lowest effective dose in animals be simply scaled-up to humans by employing the US Food and Drug Administration (FDA) body surface area conversion factor to convert animal doses to human doses?
WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
Quite reasonable and very simple predictions of the effective dose of oral drugs for MS can be obtained using animal EAE data.
HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
Sponsors of new oral MS drugs based on this publication will have a very good idea prior to beginning human studies of the upper and lower limits they might expect to find and can design their dose escalation studies during drug development based on this information.

INTRODUCTION

Prediction of the appropriate dose of a new molecular entity (NME) is a confounding task that is a major concern of the pharmaceutical industry. An enormous amount of time and expense in the drug development process is devoted to this effort. In January 2018, we reviewed approved drugs for treating relapsing remitting multiple sclerosis (RRMS) and were struck by the finding that the initial proof-of-concept study in 255 patients for fingolimod was carried out with 1.25 and 5.0 mg single oral daily doses for 6 months,[1] but that the US Food and Drug Administration (FDA) approved oral dose is 0.5 mg daily.[2] We could find nothing in the literature that provided the rationale for carrying out the initial proof-of-concept study at doses of 1.25 and 5.0 mg. The report of that study and a 227-patient extension of that study indicated that the 1.25 mg dose was as effective as the 5.0 mg dose related to relapse rate reduction, but that there were more adverse events with the higher dose.[1] The authors wrote that these findings "provide evidence of a need to explore the effects of a lower dose in future trials." However, we could find no evidence as to why the 0.5 mg dose was chosen. Because the final approved dose was at most 10-fold lower than that tested in the very expensive proof-of-concept study, we asked could there have been methodology that would have suggested that it would have been more appropriate to test the 0.5 mg oral daily dose?

In today's era of rational drug design, there would have been good reason for the sponsor of fingolimod, a sphingosine 1-phosphate (S1P) receptor modulator, to believe that this NME would be effective in treating RRMS, whereas basic mechanism scientists would always attempt to develop, if possible, an animal model of the disease. Experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model of multiple sclerosis (MS) and could be a predictor of effective drug dosing.[3] This led us, as we describe herein, to evaluate animal scale up models to predict the effective human dose. Finding that we could reasonably have predicted the efficacy of the 0.5 mg fingolimod oral daily dose based on EAE studies, we hypothesized that we could have reasonably predicted the human dose for other oral drugs developed for treating RRMS where animal EAE data were available. Since that time, we have been pleasantly surprised with the consistency of the predictions for oral MS drugs approved prior to early 2018 and especially for drugs approved since that date. Here, in this paper, we detail our continuing efforts.

METHODS

We reviewed data for all the MS drugs on the market through 2021, either indicated or empirically used (off-label) and determined the lowest effective dose (LEffD) from their published rat and mouse EAE studies. We then examined how these rodent EAE studies could be utilized to predict effective human doses. There are many complex methodologies to accomplish this end, but the easiest methodology to be tested is allometric scaling using a body surface area conversion factor (BSA-CF) to convert animal doses to human doses. We chose to use the FDA 2005 *Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers* for converting animal safety data to the maximum recommended starting dose for first-in-human studies.[4] The BSA-CF in mg/kg for mice is 0.08 and for rats 0.16 for a 60 kg human.[4] These mean conversion factors calculated across the mouse weight range of 0.018–0.033 kg, the rat weight range of 0.09–0.40 kg and a human weight range of 50–80 kg.[4] The human equivalent effective dose (HEffD) was calculated by multiplying the LEffD in published rat and mouse EAE studies by the

BSA-CF and compared to the recommended daily dose in the drug label.

We also collected, when available, animal and human clearance data, calculated this ratio for each drug, and divided it into the BSA-CFs. When studies used different EAE doses, we evaluated the prediction with respect to the dose range. The lowest effective EAE dose (LEffD) was converted by the BSA-CFs to the HEffD using the following equation: EAE dose (mg/kg) × BSA-CF × body weight (kg) = HEffD (mg).

ANALYSIS AND RESULTS

Apart from interferon, there are 16 MS drugs on the market, including five monoclonal antibodies, and two off-label usages (cyclophosphamide and rituximab). Daclizumab was withdrawn globally in March 2018 due to severe and fatal hepatic injuries and inflammatory encephalitis and meningoencephalitis. Of the 16 drugs, EAE data for alemtuzumab were not found. As we detail subsequently, eight drugs had mouse EAE data, and six drugs reported rat EAE data, ofatumumab reported marmoset data due to not binding to rodent CD20.[5] Glatiramer acetate was evaluated in a variety of species, through various routes of administration. Dalfampridine, a potassium channel blocker, showed no effect in both prophylactic and therapeutic treatment in the EAE model.[6] In Table 1, we list the eight oral MS drugs evaluated for which mouse or rat EAE studies were available, detailing each drug's hypothesized mechanism of action (MOA), the approved formulation, the LEffD in the EAE model, the calculated HEffD based on the BSA-CF, the approved label daily dose in humans and the ratio of HEffD/label doses. Table 2 lists the comparable information for the seven nonoral MS drugs for which EAE data were available. In Table 3, for the nine drugs where human and animal clearance data were available, we list the human approved dose, the HEffD/label value from Tables 1 or 2, the human type of patients for which clearance was determined, the human clearance value (CL_H) in units of L/h/kg, the EAE animal species and dose evaluated, the animal clearance value (CL_A) in units of L/h/kg, the clearance ratio (CL_H/CL_A) and the BSA-CF value from the FDA Guidance[4] divided by the clearance ratio. The detailed analysis information for each drug is summarized below.

Oral drugs (in order of FDA approval date)

Dalfampridine (2010)

Dalfampridine was the first MS oral drug approved by the FDA, but neither prophylactic nor therapeutic treatment altered disease incidence or disease course of EAE in mice.[6] Dalfampridine functions more like a neurofunctional modifier and acts through improving conduction in demyelinated axons, which may explain why dalfampridine showed no effect in the EAE model.[7,8] However, in Table 3, clearance measurements in humans versus rats were available with the ratio of the human clearance to animal clearance for rats and mice very close to the BSA-CF values in the Guidance.[4]

Fingolimod (2010)

Kataoka et al.[9] reports that 0.1 mg/kg was the lowest tested dose of fingolimod to have a therapeutic effect on EAE in mice and rats. However, we could not identify lower doses of fingolimod in rats that were not effective in the EAE model. However, in mice, Webb et al.[10] reported

TABLE 1 Human equivalent effective dose and label dose comparison for oral MS drugs

Drug	MOA	Formulation	EAE LEffD	HEffD from EAE	Label daily dose	HEffD/label
Cladribine	IMST	IR	9.6–16 mg/kg in mice	46.1–76.8 mg	10–20 mg	2.3–3.8
Dalfampridine	K+	Delayed release	No effect	/	/	/
Dimethyl fumarate		ER	100–200 mg/kg in mice	480–960 mg	Maintenance: 480 mg	1–2
Fingolimod	S1P	IR	0.1 mg/kg in mice	0.48 mg	0.5 mg	0.96
Ozanimod	S1P	IR	0.2 mg/kg mice	0.96 mg	0.92 mg	1.04
Ponesimod	S1P	IR	6 mg/kg b.i.d. in mice	57.6 mg	20 mg	2.88
Siponimod	S1P	IR	0.3 mg/kg in rats	2.88 mg	2 mg	1.44
Teriflunomide	DHO-DH	IR	3 mg/kg in rats	28.8 mg	7 or 14 mg	2.1–4.1

Abbreviations: DHO-DH, dihydroorotate dehydrogenase; EAE, experimental autoimmune encephalomyelitis; ER, extended release; HEffD, human equivalent effective dose; IMST, immunosuppressant; IR, immediate release; K+, potassium channel; LEffD, lowest effective dose; MOA, mechanism of action; MS, multiple sclerosis; S1P, sphingosine 1-phosphate.

TABLE 2 Human equivalent effective dose and label dose comparison for non-oral MS drugs

Drug	MOA	Animal RoA	EAE LEffD	HEffD from EAE (mg)	Label daily dose (mg)	HEffD/label
Cyclophosphamide[a]	IMST	i.v.	Rat: 5 mg/kg	48	400	0.12
Glatiramer acetate	Immune system modifier	i.v.	GP: 0.29 mg/kg (prevent)	3.82	20 s.c.	0.19
			GP: 2.9 mg/kg (suppress)	38.2		1.91
			Rabbit: 2.67 mg/kg	51.2		2.56
		p.o.	Rat: 4 mg/kg	38.4		1.92
			Mice: 4 mg/kg	19.2		0.96
			RM: 2 mg/kg	38.4		1.92
			CM: 2.22 mg/kg	42.7		2.14
Mitoxantrone	IMST	i.p.	Rat: 0.25 mg/kg	2.4	~20 (12 mg/m^2)	0.12
Natalizumab	IgG4κ	i.v.	Rat: 4–6.4 mg/kg	38.4–61.44	300	0.128–0.2
Ocrelizumab	CD20	i.v.	Mice: 200 μg	38.4	300	0.128
Ofatumumab	CD20	s.c.	Marmoset: 5 mg/kg	48	20	2.4
Rituximab[a]	CD20	i.v.	Mice: 4 mg/kg	19.2	~600 (375 mg/m^2)	0.03
		p.o., s.c.	Mice: 0.04–0.4 mg/kg	0.192–1.92		0.0003–0.003

Abbreviations: CM, Cynomolgus monkey; EAE, experimental autoimmune encephalomyelitis; GP, Guinea pig; HEffD, human equivalent effective dose; IMST, immunosuppressant; LEffD, lowest effective dose; MOA, mechanism of action; MS, multiple sclerosis; RM, Rhesus monkey; ROA, route of administration.
[a]Off-label use.

that 0.3 mg/kg was effective in fingolimod EAE studies, but that a dose of 0.03 mg/kg was ineffective. Using the BSA-CF of 0.08 for mice, the 0.1 mg/kg lowest tested effective dose would convert to 0.08 mg/kg in humans or 0.48 mg using the 60 kg human basis for this BSA-CF. This value is almost exactly the label daily dose, yielding a HEffD/label dose ratio of 0.96 as listed in Table 1.

Because exposure is based on drug clearance, we compared the fingolimod human drug oral clearance reported by Kahan et al.[11] with the rat fingolimod oral clearance (CL/*F*) reported by Meno-Tetang et al.,[12] because no clearance data were available in mice. Kahan et al.[11] reported mean CL/*F* values at various oral doses to be 7.7, 23.7, 7.4, 9.5, 13.4, and 9.4 L/h. If we consider the 23.7 value as an outlier, then these values coalesce around a CL/*F* of 9 L/h for 80.7 ± 13.5 kg patients. Meno-Tetang et al.[12] provide the rat fingolimod data to estimate CL/*F* following oral dosing as 1.05 L/h/kg (calculated from the reported i.v. clearance of 0.748 L/h/kg divided by the rat bioavailability of 0.71). That is, the rat to human clearance conversion factor is 0.104 as given in Table 3, reasonably close to the 0.16 value of the FDA BSA-CF for rats.

Teriflunomide (2012)

A 3 mg/kg dose showed both prophylactic and therapeutic effects in the rat EAE model whereas 1 mg/kg did not in studies[13] published 3 years prior to teriflunomide FDA approval. The 3 mg/kg rat dose converts to a 28.8 mg daily oral dose using BSA-CF versus the label recommendation of 7–14 mg, a 2.1–4.1 overprediction, as shown in Table 1. However, if EAE studies had been run at doses between 1 and 3 mg/kg and shown to exhibit prophylactic and therapeutic effects, the overprediction would be decreased. The human/rat oral clearance ratio was very low and gave even greater overpredictions, as shown in Table 3.

Dimethyl fumarate (2013)

There are two mice studies reporting dimethyl fumarate (DMF) in EAE models. Kihara et al.[14] showed that DMF could only prevent the development and severity of EAE but when 100 mg/kg DMF was delivered b.i.d. near peak clinical scores, DMF treatment had no effect on therapeutic clinical scores even after 14 days of treatment. Whereas Schulze-Topphoff et al.[15] showed a 100 mg/kg q.d. oral dose could inhibit the development of spontaneous EAE. These mice 100 and 200 mg/kg daily dose EAE studies would translate to 480–960 mg in humans using BSA-CF versus the label recommended maintenance dose of 480 mg in humans, as shown in Table 1. DMF is essentially a prodrug for monomethyl fumarate and is not quantifiable in plasma due to rapid presystemic

TABLE 3 Clearance comparison

Drug	Label daily dose (mg)	HEffD/label	Human type	CL_H (L/h/kg)	EAE animal species	CL_A (L/h/kg)	Clearance ratio CL_H/CL_A	BSA-CF value/ (CL_H/CL_A)
Cladribine	10–20	2.3–3.8	MS	1.68[a–c]	Mouse (2 mg/kg MD)	13.51[d,e]	0.12	0.67
Cyclophosphamide	400	0.12	NA	0.067[c,f]	Rat (i.v. 25 mg/kg)	1[d]	0.067	2.4
			NA	0.093[c,f]			0.093	1.7
Dalfampridine	20	/	MS	0.53[a]	Rat (2 mg/kg SD)	3.66[b,d]	0.15	1.07
					Mouse (male 2 mg/kg MD)	7.04[d,g]	0.08	1.0
					Mouse (female 2 mg/kg MD)	11.90[d,g]	0.04	2
Fingolimod	0.5 mg	0.96	Renal transplant patients (0.125 mg −5.0 mg MD)	0.11 (11)	Rat	1.05 (12)	0.105	1.5
Mitoxantrone	~20 (12 mg/m^2)	0.12	Cancer patients	0.239[44]	Rat (i.v. 0.5 mg/kg)	7.08[45]	0.03	4.75
					Rat (i.v. 2.0 mg/kg)	7.08[45]	0.03	4.75
Ozanimod	0.92 mg	1.04	Healthy	2.43[b,h]	Mouse (male 0.4 mg/kg MD)	2.08[d,g]	1.17	0.07
					Mouse (female 0.4 mg/kg MD)	2.38[d,g]	1.02	0.08
					Mouse (male 4 mg/kg MD)	1.97[d,g]	1.23	0.07
					Mouse (female 4 mg/kg MD)	2.23[d,g]	1.09	0.07
					Mouse (male 80 mg/kg MD)	1.94[d,g]	1.25	0.06
					Mouse (female 80 mg/kg MD)	2.48[d,g]	0.98	0.08
Rituximab	~600 (375 mg/m^2)	0.0003–0.03	AAV patients (i.v. 375 mg/m^2)	0.000217[c,e,f]	Mouse (i.v. 1 mg/kg)	0.00035[46]	0.54	0.15
					Mouse (i.v. 40 mg/kg)	0.00058[46]	0.32	0.25
Siponimod	2 mg	1.44	MS	0.043[a]	Rat (male 3 mg SD)	0.803[b,d]	0.054	2.96
					Rat (female 10 mg SD)	0.106[b,d]	0.411	0.39
					Mouse (8 mg/kg SD)	0.125[b,d]	0.348	0.23

(Continues)

TABLE 3 (Continued)

Drug	Label daily dose (mg)	HEffD/label	Human type	CL_H (L/h/kg)	EAE animal species	CL_A (L/h/kg)	Clearance ratio CL_H/CL_A	BSA-CF value/ (CL_H/CL_A)
					Mouse (25 mg/kg SD)	0.223[b,d]	0.195	0.41
					Mouse (25 mg/kg MD)	0.177[d,g]	0.245	0.33
Teriflunomide	7 mg	2.1–4.1	MS (MD)	0.000216[a,g]	Rat (7.5 mg/kg SD)	0.00782[b,d]	0.028	5.71
	14 mg		MS (MD)	0.000185[a,g]			0.024	6.67

Abbreviations: AAV, adeno-associated virus; BSA-CF, body surface area conversion factor; CL_A, animal clearance; CL_H, human clearance; EAE, experimental autoimmune encephalomyelitis; HEffD, human equivalent effective dose; MD, multiple dose; MS, multiple sclerosis; NA, information not available; SD, single dose.

[a]Data from the US Food and Drug Administration (FDA) Clinical Pharmacology Review.

[b]Oral clearance (CL/F) = dose/$AUC_{0-\infty}$.

[c]Corrected to body weight (BW) of 60 kg.

[d]Data from the FDA Pharmacology Review.

[e]Calculated from available data.

[f]Data from the FDA label.

[g]CL/F = dose/$AUC_{ss,0-t}$.

[h]Data from the European Medicines Agency (EMA) Assessment Report.

hydrolysis, thus no human or mouse clearance data for DMF are available.[16]

Cladribine (2019)

Cladribine was approved by the European Medicines Agency (EMA) in 2017 and the FDA in 2019. The EAE dose of cladribine was reported by Musella et al.,[17] in which the investigators compared the oral dosing of 0.4 mg/mouse (16 mg/kg) and intracranial infusions of 0.24 mg/mouse (9.6 mg/kg). Both groups showed efficacy in the EAE model induced in mice. Here, the dose prediction based on EAE in mice was 2.3–3.8 greater than the label dose (Table 1). In contrast, comparing the mouse BSA-CF value to the human/mouse clearance ratio the resulting value (0.67) was close to unity (Table 3).

Siponimod (2019)

The second S1P modulator siponimod was approved for RRMS by the FDA in 2019. Gergely et al.[18] reported that the LEffD of siponimod in the EAE rat model was 0.3 mg/kg by oral administration, which could translate via the BSA-CF to a HEffD of 2.88 mg. This value is only 1.44 greater than the 2 mg label dose (Table 1). The oral clearance comparisons were highly variable. Male rats gave a ratio of BSA-CF/oral clearance ratio overprediction of almost 3, whereas female rats gave an underprediction of 0.39. Although no EAE data were available for oral dosing in mice, mice clearance values yielded underpredictions (Table 3).

Ozanimod (2020)

Ozanimod, a selective modulator of S1PR1 and S1PR5, was approved by both the FDA and the EMA in 2020. Ozanimod showed efficacy at 0.2 mg/kg in a mouse EAE model,[19] yielding an excellent prediction of a HEffD of 0.96 mg, only 1.04-fold the 0.92 mg label dose (Table 1). However, dosing predictions based on human and mouse clearance measurements were very poor (Table 3).

Ponesimod (2021)

Ponesimod is a selective S1PR1 modulator with high affinity to S1P receptor 1. It was approved both by the FDA and the EMA in 2021. According to the pharmacology review,

6 mg/kg b.i.d. is effective in the mouse EAE model.[20] The dose could be converted to 57.6 mg daily dose, which is 2.88-fold of the label dose (Table 1). No mice clearance data are available.

Non-oral drugs (in order of FDA approval date)

Glatiramer acetate (1996)

Glatiramer acetate (Copolymer 1, Cop 1, and Copaxone) is a synthetic amino acid copolymer composed of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine.[21] Glatiramer acetate has been studied for more than 30 years, tested in several species and through various route of administration, including oral, intramuscular, intraperitoneal, and intravenous. The approved route of administration is subcutaneous. Although oral dosing was studied in monkeys, and the HEffD calculated from the oral dosing studies fell around two-fold label dose (Table 2), clinical trials of oral doses failed to show significant positive effects on the clinical and magnetic resonance outcomes in patients with RRMS.[22] The authors suggest that oral dosing may not reach an effective bioactive form to achieve clinical efficacy,[22] because pharmacokinetic (PK) studies with glatiramer acetate do not give relevant measurable parameters.

According to the FDA's Clinical Pharmacology and Biopharmaceutics review, glatiramer acetate PK studies in humans have not been performed, and clinical trial data showed that only nine out of 17 subjects who received the drug exhibited quantitatively detected concentrations, with areas under the concentration-time curves varying from 1644 to 67,532 ng min/ml. The results indicate that a small fraction of glatiramer acetate is absorbed from the site of injection and rapidly removed from the circulation. Therefore, clearance data are not available.

Mitoxantrone (2000)

Mitoxantrone is a synthetic antineoplastic cytotoxic drug that also has immunosuppressive effects. It has been shown in vitro to decrease the proliferation of damaging B cells, T cells, and macrophages.[23] It has been tested in various species and showed efficacy in different types of EAE models, with 0.25 mg/kg intraperitoneal administration shown to suppress acute EAE in rats.[24,25] The approved dosage in humans is 12 mg/m^2 given as a short intravenous infusion every 3 months. Mitoxantrone was 10–20 time more potent than cyclophosphamide in the suppression of EAE.[26] The HEffD/label given in Table 2 is 0.12, the CL_H/CL_A ratio is 0.03 yielding a ratio of BSA-CF/(CL_H/CL_A) even more disparate from the HeffD/label ratio than cyclophosphamide (Table 3).

Alemtuzumab (2001)

Alemtuzumab has not been examined in EAE studies due to the lack of cross-reactivity between human and mouse CD52.[27,28] However, the effect of an anti-muCD52 was evaluated in an EAE model, exhibiting efficacy.[29]

Natalizumab (2004)

Natalizumab was first tested in a rat EAE model, in which 1.0–1.6 mg/rat showed comparable efficacy in preventing paralysis.[30] Taking 250 g as average rat weight, the rat dosage equivalent is 4–6.4 mg/kg, as cited in later reviews.[31,32] The ratio of human equivalent dose (HED) to the label dose is 0.128–0.2 (Table 2). Rat clearance data were not found.

Ocrelizumab (2017)

Ocrelizumab is a humanized anti-CD20 antibody and is the first agent approved for the use in patients with primary progressive MS. Weber et al.[33] tested ocrelizumab in hCD20 transgenic mice and showed that 200 μg/mouse could prevent or reverse EAE induced by mouse myelin oligodendrocyte glycoprotein. Taking 25 g as the average mouse weight, the 8 mg/kg dose could convert to 38.4 mg HEffD, which is 12.8% of the 300 mg label dose (Table 2). No mice clearance data are available for ocrelizumab.

Ofatumumab (2020)

Ofatumumab is a full recombinant human anti-CD20 antibody, first approved in 2009 for the treatment of patients with chronic lymphocytic leukemia, and more recently approved for the treatment of relapsing forms of MS. As with ocrelizumab, ofatumumab binds only to human and nonhuman primate CD20[5] and was not developed from an EAE model. However, experiments with marmoset EAE models have been reported.[34,35] In those studies, a 20 mg/kg initial dose followed by 5 mg/kg weekly maintenance regimen was applied, which significantly prevented the development of clinical signs. The translated HeffD of the initial dose is 48 mg, 2.4-fold of the label dose (Table 2). However, no lower doses were identified for the determination of LeffD. No marmoset clearance data are available for ofatumumab.

Drugs utilized but not presently approved for MS by the FDA

Rituximab

Rituximab is a chimeric human/mouse IgG1 antibody, which has been tested in a mouse EAE model. Rituximab 4 mg/kg administration (route not mentioned) rapidly depleted peripheral B cells and strongly reduced EAE severity in a mouse EAE model.[36] In addition, Brod et al.[37] studied the oral administration of rituximab, 1 μg (0.04 mg/kg) and found that this dose exhibited the most effect and reduction of disease severity, better than 10 μg (0.4 mg/kg) oral and 1 μg (0.04 mg/kg) subcutaneous dosing. As shown in Table 2, very low HeffD/label ratios result.

Cyclophosphamide

Cyclophosphamide was tested and compared with mitoxantrone.[26] Doses of 5 mg/kg cyclophosphamide suppressed the clinical and histological lesions associated with developing EAE. Although the HeffD/label is only 0.12, the CL_H/CL_A ratio is 0.067–0.093, about 42%–58% of the BSA-CF.

Daclizumab (May 2016–June 2018)

Daclizumab was approved for the treatment of adult patients with RRMS. Because of its safety profile, the recommended usage was limited to patients who had an inadequate response to two or more drugs.[38] However, it was withdrawn in 2018 due to reports of serious inflammatory brain disorders associated with the drug use.[39] Although many studies investigated the role of anti-CD25 antibodies on the disease progression, we could not identify useful EAE studies with daclizumab. The animal EAE data showed either no effect or an exacerbated effect.[24,40]

DISCUSSION

The initial human dose selection is one of the most important steps in the clinical development of investigational new drugs. Different approaches are applied and tend to generate the most conservative starting dose.[41–43] However, better estimation of the potential HEffD would be helpful in proposing dose escalation in clinical trials and provide a target reference in selecting phase II/III doses.

Assuming that the pharmacodynamic (PD) model used in animals correlates well with PK/PD relationship in humans, the basis of dose conversion between species is believed to be a function of the differences of metabolic rate of an animal that is reflected in its size. Therefore, predicting human drug clearance is an important task, and a commonly used method for predicting human clearance is the simple allometric approach, $Y = aW^b$, where a is a constant, W the animal/human body weight, and b is the allometric exponent.[4] In Table 1, for approved oral MS drugs, the HEffD provided remarkably consistent predictions for dimethyl fumarate, fingolimod, ozanimod, and siponimod, with predictions for the remaining three drugs falling between two- and four-fold over prediction. EAE measurements did not provide a meaningful therapeutic under prediction for any of the seven oral RRMS drugs. There was no indication in any of the drug development histories that the sponsor was aware of a potential useful allometric approach. Of the eight oral drugs, dalfampridine showed no efficacy in an EAE model. It could be due to the MOA of dalfampridine, as a potassium channel blocker, that no anti-inflammatory effect is observed in the EAE model.

Although the allometric relationship presented above is based on animal body weight, it might be expected that human versus animal clearance values would also provide a useful human dose prediction. Such data when available are presented in Table 3. For the oral RRMS drugs, good predictions are obtained for cladribine, dalfampridine (where no EAE effect could be measured), and fingolimod. However, very poor predictions were observed using the clearance data for ozanimod, siponimod, and teriflunomide. We suspect that this may be due to comparing CL/F versus CL in making the predictions, because bioavailability is known to differ between animals and humans and not necessarily correlating with clearance differences.

For the seven non-oral drugs, including four monoclonal antibody-based therapeutics, as listed in Table 2, the BSA-CF prediction does not work well and perhaps we should rather conclude that the methodology does not appear useful for antibody therapeutics. This suggests that for antibodies the PD model in animals does not correlate with the PK/PD relationship in humans. For the three nonoral drugs where comparable human and animal clearance data are available, poor predictions are found for mitoxantrone and rituximab, but a 1.7 to 2.4-fold overprediction is observed for cyclophosphamide, where the HEffD/label prediction in Table 2 for cyclophosphamide exhibited marked underprediction. It is interesting to see that for glatiramer acetate, the acetate salts of synthetic polypeptides, the HEffD does appear to be well-predicted for oral doses in EAE studies in rat, mice, rhesus, and cynomolgus monkeys compared to the human 20 mg s.c. dose, but clinical studies show that glatiramer acetate is not effective orally.

From the data available to us in the literature, we found supportive results for our hypothesis that an animal model of a disease that usefully predicts the dose of an effective therapeutic agent in humans may have the potential to predict the human dose of other drugs using this animal model when actual effective doses differ by almost 1000-fold. However, we are not recommending or suggesting that sponsors immediately test the EAE/BSA-CF derived dose in clinical studies for new oral MS drugs, rather than follow the accepted methodology of beginning with a very low dose and escalating doses to determine the effective and safe regimen. None of the HEffD/label ratios are less than one so that sponsors of new oral MS drugs based on this publication have a very good idea prior to beginning human studies of the upper and lower limits they might expect to find and can design their dose escalation studies based on this information. We believe having a reasonable expectation of the efficacious dose prior to beginning the dose escalation would provide significant advantages.

Readers should recognize that the FDA HED, as published in the FDA Guidance,[4] predicts the translation of animal toxicity to potential human toxicity. We hypothesized that if the FDA HED reasonably predicted toxicity, might it also be able to predict efficacy. There is no expectation in the FDA Guidance[4] that the HED methodology can be used to predict efficacy. That is why we believe this approach is unique and holds potential for examination in other drug classes. It is well-recognized that therapeutic ratios (toxic dose/effective dose) differ markedly from drug to drug. Thus, knowing the HED from animal toxicity studies provides no prediction of the efficacious dose until one identifies an animal model efficacy predictor and then test whether the HED will correctly predict HEffD. The FDA expects the sponsor to begin human studies with at least a 10-fold lower dose than the animal toxicity HED and then dose escalate following a protocol proposed by the sponsor and approved by the FDA to determine the efficacious dose. Here, we show for oral drugs approved for treating RRMS that the methodology provides very reasonable predictions.

We recognize there are many limitations for our study. First, there are several drugs for which we could not identify the LEffD. Only dimethyl fumarate and teriflunomide have been tested at a lower dose that exhibited insufficient efficacy, whereas for the other drugs, we had to use the lowest dose tested. Second, there is very little quality control of PK/PD studies in terms of dose and dosing regimen selection. Except for fingolimod, we are unsure if the other MS drugs were tested to determine the LEffD. However, because none of our HEffD/label values for the oral MS drugs were markedly <1.0, the analysis here would suggest that this was not an issue. Third, models can always be developed that fit the available data. However, the potential usefulness of the relationship, we believe, is very intriguing, and could markedly benefit drug development timelines and costs, as well as assist regulatory evaluations.

CONCLUSION

The present study showed that BSA-CF could have a potential role in predicting effective dose for MS drugs, especially when orally dosed, indicating the BSA-CF could also be used in predicting HEffDs, to provide references in dose selection in drug development.

AUTHOR CONTRIBUTIONS

W.L. and L.Z.B. wrote the manuscript. L.Z.B. and E.L.W. designed the research. W.L. and Z.Y. performed the research. W.L., Z.Y., Z.-Y.W., and S.Z. analyzed the data.

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The authors dedicate this paper to Professor Margareta Hammarlund-Udenaes upon the occasion of her retirement in appreciation for her outstanding contributions to our discipline.

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CONFLICT OF INTEREST

Dr. Benet first became aware of the success of the methodology reported here while serving as an expert witness in a lawsuit concerning the validity of the fingolimod patent. All other authors declared no competing interests for this work.

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REFERENCES

1. Kappos L, Antel J, Comi G, et al. Oral fingolimod (FTY720) for relapsing multiple sclerosis. *N Engl J Med*. 2006;355:1124-1140.

2. Fingolimod (GILENYA) Drug Label, Washington (USA). https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022527s000lbl.pdf. Accessed March 28, 2022.
3. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol*. 2011;164:1079-1106.
4. Guidance for Industry: Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers, 2005, Rockville, MD. https://www.fda.gov/media/72309/download. Accessed March 28, 2022.
5. Ofatumumab Pharmacology Review(s), Washington (USA). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/125326s000_PharmR.pdf. Accessed March 28, 2022.
6. Göbel K, Wedell JH, Herrmann AM, et al. 4-Aminopyridine ameliorates mobility but not disease course in an animal model of multiple sclerosis. *Exp Neurol*. 2013;248:62-71.
7. Dunn J, Blight A. Dalfampridine: a brief review of its mechanism of action and efficacy as a treatment to improve walking in patients with multiple sclerosis. *Curr Med Res Opin*. 2011;27:1415-1423.
8. Dalfampridine (AMPYRA) Drug Label, Washington (USA). https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022250s000lbl.pdf. Accessed March 28, 2022.
9. Kataoka H, Sugahara K, Shimano K, et al. FTY720, sphingosine 1-phosphate receptor modulator, ameliorates experimental autoimmune encephalomyelitis by inhibition of T cell infiltration. *Cell Mol Immunol*. 2005;2:439-448.
10. Webb M, Tham CS, Lin FF, et al. Sphingosine 1-phosphate receptor agonists attenuate relapsing-remitting experimental autoimmune encephalitis in SJL mice. *J Neuroimmunol*. 2004;153:108-121.
11. Kahan BD, Karlix JL, Ferguson RM, et al. Pharmacodynamics, pharmacokinetics, and safety of multiple doses of FTY720 in stable renal transplant patients: a multicenter, randomized, placebo-controlled, phase I study. *Transplantation*. 2003;76:1079-1084.
12. Meno-Tetang GM et al. Physiologically based pharmacokinetic modeling of FTY720 (2-amino-2[2-(−4-octylphenyl)ethyl] propane-1,3-diol hydrochloride) in rats after oral and intravenous doses. *Drug Metab Dispos*. 2006;34:1480-1487.
13. Merrill JE, Hanak S, Pu SF, et al. Teriflunomide reduces behavioral, electrophysiological, and histopathological deficits in the dark agouti rat model of experimental autoimmune encephalomyelitis. *J Neurol*. 2009;256:89-103.
14. Kihara Y, Groves A, Rivera RR, Chun J. Dimethyl fumarate inhibits integrin α4 expression in multiple sclerosis models. *Ann Clin Transl Neurol*. 2015;2:978-983.
15. Schulze-Topphoff U, Varrin-Doyer M, Pekarek K, et al. Dimethyl fumarate treatment induces adaptive and innate immune modulation independent of Nrf2. *Proc Natl Acad Sci USA*. 2016;113:4777-4782.
16. Dimethyl Fumarate (TECFIDERA), Washington (USA). https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/204063lbl.pdf. Accessed March 28, 2022.
17. Musella A, Mandolesi G, Gentile A, et al. Cladribine interferes with IL-1β synaptic effects in experimental multiple sclerosis. *J Neuroimmunol*. 2013;264:8-13.
18. Gergely P, Nuesslein-Hildesheim B, Guerini D, et al. The selective sphingosine 1-phosphate receptor modulator BAF312 redirects lymphocyte distribution and has species-specific effects on heart rate. *Br J Pharmacol*. 2012;167:1035-1047.
19. Scott FL, Clemons B, Brooks J, et al. Ozanimod (RPC1063) is a potent sphingosine-1-phosphate receptor-1 (S1P1) and receptor-5 (S1P5) agonist with autoimmune disease-modifying activity. *Br J Pharmacol*. 2016;173:1778-1792.
20. Ponesimod (PONVORY) Non-clinical Reviews, Washington (USA). https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/213498s000lbl.pdf. Accessed March 28, 2022.
21. Teitelbaum D, Arnon R, Sela M. Immunomodulation of experimental autoimmune encephalomyelitis by oral administration of copolymer 1. *Proc Natl Acad Sci U S A*. 1999;96:3842-3847.
22. Teitelbaum D et al. Oral glatiramer acetate in experimental autoimmune encephalomyelitis: clinical and immunological studies. *Ann N Y Acad Sci*. 2004;1029:239-249.
23. Martinelli V, Radaelli M, Straffi L, Rodegher M, Comi G. Mitoxantrone: benefits and risks in multiple sclerosis patients. *Neurol Sci*. 2009;30(Suppl 2):S167-S170.
24. Mitoxantrone Pharmacology Review(s), Washington (USA). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2000/21120.pdf_Novantrone_Pharmr_P1.pdf. Accessed March 28, 2022.
25. Lublin FD, Lavasa M, Viti C, Knobler RL. Suppression of acute and relapsing experimental allergic encephalomyelitis with mitoxantrone. *Clin Immunol Immunopathol*. 1987;45:122-128.
26. Ridge SC, Sloboda AE, McReynolds RA, Levine S, Oronsky AL, Kerwar SS. Suppression of experimental allergic encephalomyelitis by mitoxantrone. *Clin Immunol Immunopathol*. 1985;35:35-42.
27. Friese MA, Montalban X, Willcox N, Bell JI, Martin R, Fugger L. The value of animal models for drug development in multiple sclerosis. *Brain*. 2006;129(Pt 8):1940-1952.
28. Robinson AP, Harp CT, Noronha A, Miller SD. The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. *Handb Clin Neurol*. 2014;122:173-189.
29. Turner MJ, Pang PT, Chretien N, et al. Reduction of inflammation and preservation of neurological function by anti-CD52 therapy in murine experimental autoimmune encephalomyelitis. *J Neuroimmunol*. 2015;285:4-12.
30. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. *Nature*. 1992;356:63-66.
31. Steinman L, Zamvil SS. Virtues and pitfalls of EAE for the development of therapies for multiple sclerosis. *Trends Immunol*. 2005;26:565-571.
32. Steinman L, Zamvil SS. How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. *Ann Neurol*. 2006;60:12-21.
33. Weber MS, Prod'homme T, Patarroyo JC, et al. B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. *Ann Neurol*. 2010;68:369-383.
34. Kap YS, van Driel N, Blezer E, et al. Late B cell depletion with a human anti-human CD20 IgG1kappa monoclonal antibody halts the development of experimental autoimmune encephalomyelitis in marmosets. *J Immunol*. 2010;185:3990-4003.
35. Kap YS, Bauer J, Driel N, et al. B-cell depletion attenuates white and gray matter pathology in marmoset experimental

autoimmune encephalomyelitis. *J Neuropathol Exp Neurol*. 2011;70:992-1005.
36. Monson NL, Cravens P, Hussain R, et al. Rituximab therapy reduces organ-specific T cell responses and ameliorates experimental autoimmune encephalomyelitis. *PLoS One*. 2011;6:e17103.
37. Brod SA. Ingested (oral) rituximab inhibits EAE. *Cytokine*. 2016;85:177-183.
38. Daclizumab (ZINBRYTA) Drug Label, Washington (USA). https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/761029s000lbl.pdf. Accessed March 28, 2022.
39. FDA. FDA working with manufacturers to withdraw Zinbryta from the market in the United States. Drug Safety and Availability. March 14, 2018. https://www.fda.gov/drugs/drug-safety-and-availability/fda-working-manufacturers-withdraw-zinbryta-market-united-states. Accessed March 28, 2022
40. Kohm AP, McMahon JS, Podojil JR, et al. Cutting edge: anti-CD25 monoclonal antibody injection results in the functional inactivation, not depletion, of CD4+CD25+ T regulatory cells. *J Immunol*. 2006;176:3301-3305.
41. Le Tourneau C, Stathis A, Vidal L, Moore MJ, Siu LL. Choice of starting dose for molecularly targeted agents evaluated in first-in-human phase I cancer clinical trials. *J Clin Oncol*. 2010;28:1401-1407.
42. Viala M, Vinches M, Alexandre M, et al. Strategies for clinical development of monoclonal antibodies beyond first-in-human trials: tested doses and rationale for dose selection. *Br J Cancer*. 2018;118:679-697.
43. Sachs JR, Mayawala K, Gadamsetty S, Kang SP, de Alwis DP. Optimal dosing for targeted therapies in oncology: drug development cases leading by example. *Clin Cancer Res*. 2016;22:1318-1324.
44. Savaraj N, Lu K, Manuel V, Loo TL. Pharmacology of mitoxantrone in cancer patients. *Cancer Chemother Pharmacol*. 1982;8:113-117.
45. Yang X, Morris ME. Pharmacokinetics and biliary excretion of mitoxantrone in rats. *J Pharm Sci*. 2010;99:2502-2510.
46. Kagan L, Zhao J, Mager DE. Interspecies pharmacokinetic modeling of subcutaneous absorption of rituximab in mice and rats. *Pharm Res*. 2014;31:3265-3273.

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