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ORIGINAL ARTICLE

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Effects of cladribine tablets on heart rate, atrio-ventricular conduction and cardiac repolarization in patients with relapsing multiple sclerosis

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Aims: Cladribine tablets have shown significant efficacy for the treatment of relapsing multiple sclerosis, a chronic and debilitating immune-mediated disorder. This study was conducted to examine acute and/or cumulative effects of cladribine tablets 10 mg (3.5 or 5.25 mg/kg cumulative dose over 2 years) on heart rate, AV conduction and cardiac repolarization in patients with relapsing–remitting multiple sclerosis (RRMS).

Methods: CLARITY was a 96-week, double-blind, placebo-controlled, multicentre trial which evaluated the safety and efficacy of cladribine tablets 3.5 and 5.25 mg/kg body weight in patients with RRMS. A total of 135 patients were included in the ECG substudy, providing a total of 1534 post-dose ECGs. ECG data were collected 15 minutes pre-dose and between 0.5 and 3 hours post-dose at pre-study evaluation, study Day 1 and Weeks 5, 9, 13, 48 and 52.

Results: For cladribine tablets 3.5 mg/kg, the maximum change in placebo-adjusted post-dose QTcF *vs*. visit-baseline (BL) was −0.42 ms (90% CI: −3.61–4.44) at Week 1 (acute effects), and 3.20 ms (90% CI: −0.08–6.33) for cladribine tablets 5.25 mg/kg. The greatest observed differences in post-dose QTcF *vs*. study BL occurred at Week 48 for both the 3.5 and 5.25 mg/kg doses of cladribine tablets with 5.99 ms (90% CI: 0.53–11.44) and 8.74 ms (90% CI: 3.18–14.31), respectively. No significant changes were observed in T-wave morphology in either treatment group.

Conclusions: Cladribine tablets 3.5 mg/kg (approved dose in Europe/other regions) did not confer clinically meaningful effects on heart rate, AV conduction and ventricular repolarization.

KEYWORDS

cardiac safety, cladribine, modelling, multiple sclerosis, QT/QTc interval

The principal investigator for the CLARITY study was Prof. Gavin Giovannoni. He had direct clinical responsibility for patients. Since we are reporting a secondary (sub-study) analysis, this PI is not included in the author list.

1 | INTRODUCTION

Multiple sclerosis (MS) is a chronic and debilitating immune-mediated disorder of the central nervous system, in which B and T cells are believed to play a major pathophysiological role.[1]

Cladribine tablets 10 mg (MAVENCLAD®) were recently approved in Europe[2] and other regions for the treatment of adults with relapsing MS (RMS), and were shown to have significant efficacy for the treatment of RMS in placebo-controlled Phase III trials.[1,3,4]

Cladribine is a nucleoside analogue of deoxyadenosine. The cladribine prodrug is phosphorylated intracellularly to its active product, 2-chlorodeoxyadenosine triphosphate (Cd-ATP), primarily by deoxycytidine kinase. In most cells this deoxynucleotide product is degraded, by **5'-nucleotidase**. Cells containing a high deoxycytidine kinase to 5′-nucleotidase activity ratio such as B and T lymphocytes accumulate deoxynucleotides to toxic concentrations, resulting in cell death. By this mechanism, cladribine tablets exert a selective mode of action on B and T lymphocytes.[5,6]

Assessing the potential for a new drug to affect cardiac ventricular repolarization, thereby causing life-threatening arrhythmias, is now an integral component of premarketing safety assessment in preclinical and clinical development.

The ICH E14 Guideline recommends a "Thorough QT Study" (TQT) as a default approach to assess clinical QT risk.[7] TQT studies aim for careful evaluation of drug effects on ventricular repolarization (i.e. the electrocardiographic QT/QTc interval) at multiples of therapeutic exposure together with a positive control to confirm the sensitivity of the study and methods employed. Yet for some drugs and diseases, including oncology and immunosuppressive drugs, elements of the TQT may be impractical or unethical (e.g. studies in healthy volunteers and administration of supra-therapeutic doses) due to the potential for posing unacceptable risk to healthy volunteers.[8,9] In these instances, alternative approaches to QT/QTc risk assessment are needed, and must be carefully considered and pursued. This has been acknowledged by the International Conference of Harmonisation and adopted by the European Medicines Agency by a recently issued question and answer (Q&A) document (R3) to the ICH E14 Guideline.[10]

This article presents the evaluation of potential QT/QTc risk of cladribine in patients with RMS by means of an electrocardiogram (ECG) substudy that was combined with a population pharmacokinetic/pharmacodynamic (PK/PD) approach as part of the multinational, multi-centre CLARITY Phase III trial.[1]

What is already known about this subject

- Cladribine is a nucleoside analogue of deoxyadenosine, and cladribine tablets were recently approved in Europe and other regions for the treatment of relapsing multiple sclerosis (RMS).
- Cladribine tablets are associated with targeted lymphocyte reduction and durable efficacy.

What this study adds

- This study presents the evaluation of the cardiac safety of cladribine tablets with particular emphasis on the potential QT/QTc risk in patients with RMS.
- Potential effects of cladribine tablets on the cardiac repolarization were examined by an ECG substudy as part of a Phase III trial (CLARITY) that was combined with a population PK/PD approach.
- There is no evidence of any cladribine-related effect on heart rate, atrio-ventricular conduction or cardiac repolarization.

2 | METHODS

2.1 | Introduction to the CLARITY trial

The CLARITY trial (ClinicalTrials.gov number, NCT00213135) was a 96-week, Phase III, double-blind, placebo-controlled, multicentre trial which evaluated the safety and efficacy of cladribine tablets 3.5 mg/kg and 5.25 mg/kg body weight (BW; cumulative dose) in patients with relapsing–remitting multiple sclerosis (RRMS). The cumulative dose of 3.5 mg/kg consisted of two annual courses of 1.75 mg/kg that each comprised two treatment weeks; one at the start of the first month and one at the start of second month of each year. Each treatment week consisted of 4 or 5 treatment days on which patients received 10 mg or 20 mg (one or two 10 mg tablets) as a single oral daily dose, depending on individual BW. The cumulative dose of 5.25 mg/kg consisted of a further two treatment weeks in the first year in addition to the 3.5 mg/kg dosing schedule.

CLARITY involved 1326 patients with a diagnosis of RRMS, according to the McDonald criteria. Patients were recruited from approximately 135 sites, located in, but not limited to, North America, South America, Europe, Russia and Australia. Patients were randomly assigned in an approximate 1:1:1 ratio to receive one of two cumulative doses of cladribine tablets, i.e., either 3.5 mg/kg or 5.25 mg/kg BW or matching placebo.

The treatment weeks were started on the first day of the first month (Week 1, Day 1) and of the second month (Week 5, Day 1) of each of both years. The cumulative dose of 5.25 mg/kg BW consisted of six treatment weeks (Weeks 1, 5, 9, 13, 48 and 52). The 3.5 mg/kg dose group received matching placebo tablets in treatment Weeks 9 and 13, and patients assigned to placebo received their treatment according to the schedule of the 5.25 mg/kg BW treatment group. Accordingly, the weekly doses of the 3.5 mg/kg and 5.25 mg/kg BW groups were identical, and the higher total cumulative cladribine dose in the 5.25 mg/kg BW group was achieved by the employment of two additional treatment weeks (i.e., Weeks 9 and 13). Further details of the CLARITY trial have been published elsewhere.[1]

2.2 | ECG substudy of CLARITY

The ECG substudy in the target population of RRMS patients was designed to evaluate potential acute and/or cumulative effects of cladribine on the ECG intervals (RR, PR, QRS, QT, QTcB and QTcF) and T-wave morphology, with a particular emphasis on the heart rate corrected QT interval (QTcF, QTcB), as a well-accepted surrogate measure signifying delay or heterogeneity in cardiac ventricular repolarization which may possibly be associated with proarrhythmic characteristics of a drug. QTcF was the primary outcome variable. The timing of the ECG assessments within a day (between 0.5 and 3 hours after drug administration) and the day within a treatment week (Day 1) was selected to obtain ECGs at maximum exposure and to allow for proper characterization of the concentration–effect relationship. To allow for a concentration–effect analysis of QT/QTc-interval data (QTc/PK modelling), the entire ECG population (n = 135) formed part of the PK sub-population (n = 173) of the CLARITY study.

2.3 | ECG data collection

For the ECG substudy, 23 centres from nine countries were appropriately qualified, equipped and quality checked by a dedicated vendor, and central reading of all ECGs by expert cardiologists was applied (eResearchTechnology, Inc., Philadelphia, PA, USA).

Standard 12-lead ECGs were recorded at the study sites in patients randomized to the ECG population. These patients had ECGs collected at pre-study evaluation, study Day 1 and at Weeks 5, 9, 13, 48 and 52 at the following time points: three ECGs taken 15 minutes apart pre-dose, and two ECGs taken no longer than five minutes apart between 0.5 and 3 hours post-dosing. Recordings were made on Day 1 of each treatment week in each patient after at least 15 minutes' rest in the supine position. According to the ECG substudy protocol, a total of 31 ECGs (i.e., three baseline and two post-dose ECGs at each visit, plus one screening ECG) were to be recorded for each patient.

The timing of the ECG assessments within a day (between 0.5 and 3 hours after drug administration) and the day within a week (Day 1) was selected to obtain ECGs at maximum cladribine exposure and to allow for proper characterization of the concentration–effect relationship. Instead of pre-specifying a fixed post-dose time, a target time window of 0.5–3 hours post-dose was specified for random ECG assessments. This time window was selected because it covers the times of the expected C_{max} values of the population as well as the initial decline in plasma concentrations, which is important to allow for the characterization of the concentration–effect. The sampling strategy of capturing ECGs at random times within a pre-specified time window, also had the additional methodological advantage that ECGs in individual patients were captured at different post-dose times at each occasion of the six different study visits. Hence, this approach provided a set of six "serial" post-dose ECGs in each individual patient, that were obtained at random times within 0.5 and 3.0 hours post-dose, essentially following a "population approach" that is well accepted for PK analysis.

2.4 | ECG procedures and analysis

ECGs were recorded at the qualified sites on each patient and sent to a central laboratory (eResearch Technology, Inc., Philadelphia, PA, USA) for a high-resolution measurement of the cardiac intervals and morphological assessment by a central cardiologist blinded to the study treatment. ECG measurements were performed using digitization software with magnification of the ECG and on screen callipers by experienced technicians and a centralized cardiologist who was blinded to the tracings.

Manual measurements of the RR, PR, QRS and QT interval durations were performed and QTcB, QTcF and heart rate were derived from three consecutive complexes per single ECG. From these values, means were calculated and reported by treatment group. For the QT interval measurement, ECGs were measured in lead II. If lead II was not measurable, lead V5 was used.

The following calculations were made from the interval measurements: Heart rate (HR = 60/RR); heart rate corrected QT-interval using HR-correction by Fridericia's formula (QTcF = QT/3√RR); heart rate corrected QT-interval using HR-correction by Bazett's formula (QTcB = QT/√RR).

Abnormal morphology features present on any one of the post-dosing ECGs were compared with baseline. The occurrences of new T wave inversion and new U waves were documented by treatment group.

In line with the ICH E14 Guideline,[10] clinical relevance was defined as a drug-induced effect on QTc above the upper bound limit of 10 ms. A negative QT/QTc study outcome is one in which the upper bound of the one-sided 95% confidence interval of the ANOVA analysis for the largest time-matched mean effect of the drug on the QTc interval is below this threshold for all time-points. It needs to be acknowledged that the 10 ms threshold refers to acute effects *vs*. visit baseline in well-controlled settings without any confounders such as time and disease effects and concomitant medications. In longitudinal observations in Phase III studies, confounding factors such as disease-related QT effects and changes in concomitant medications have to be taken into account.

Besides this common ECG evaluation by treatment group/dose, a modelling and simulation analysis of the available PK and ECG data was applied to analyse the individual concentration–effect relationships by means of a population approach using NONMEM (Version VI, level 2; Icon Development Solutions, Ellicott City, MD, USA).

The primary outcome variable, the HR correction according to Fridericia (QTcF) was pre-specified in the study protocol. Use of an alpha parameter to account for individual HR correction (e.g., Friberg et al. 2006[11]) was explored based on the placebo data. Since there was no apparent improvement in describing the data with this approach, and inter-subject variability in alpha was not supported, QTcF was used as the dependent variable in the analysis.

2.5 | Pharmacokinetic assessments

To allow for a concentration–effect analysis of QT/QTc-interval data (QTc/PK modelling), the patients who underwent ECG recording also

took part in a pharmacokinetic substudy. Blood samples for quantification of cladribine and major cladribine metabolite (2-chloroadenine; M1) plasma concentrations were taken following a population pharmacokinetic (PopPK) approach. Samples were taken on the first day of the first treatment week in Year 2, with a first sample taken between 1 and 6 hours after dosing and a second sample 24 hours post dose, prior to intake of the dose on Day 2 of the respective treatment week. Further details on the PopPK analysis were published by Savic et al.[12]

Plasma concentrations were determined using a validated HPLC–MS/MS with a lower limit of quantitation (LLOQ) of 0.1 ng/mL for cladribine and for 2-chloroadenine (M1), respectively.

2.6 | Statistical analysis

All ECG interval parameters are presented as arithmetic means of two (post-dose ECGs) or three (baseline ECGs) measurements. ECG interval measurements (RR, PR, QRS, QT, QTcB and QTcF) and heart rate were summarized by treatment group at study and visit baselines, and Weeks 5, 9, 13, 48 and 52. The primary ECG variable was QTcF, that is QT interval corrected for heart rate according to Fridericia's formula. Abnormal morphology features present on any one of the post-dosing ECGs were compared with study baseline.

The ECGs used for defining the study baseline ECG values to define all changes from study baseline were the mean of three ECGs obtained at 15-minute intervals immediately prior to the first dose of cladribine. Accordingly, the mean of the three ECGs performed pre-dose at each visit were to define all changes from the visit baseline. The mean of the two post-dosing ECGs at each visit (two ECGs taken no more than 5 minutes apart within 0.5–3 hours after dosing) were used as the post-dose interval duration and were compared with both the study and the visit baseline ECG values, by visit.

The changes to study and visit baseline of the ECG parameters were analysed by means of an analysis of variance (ANOVA) on change to baseline at subject level with fixed effects for treatment and gender, separately at all defined time points. Based on the model, the differences between cladribine and placebo were estimated, and two-sided 90% confidence intervals were computed using residual errors. Point estimates and CI intervals for cladribine *vs.* placebo were presented graphically.

Additionally, the QTcF values were also analysed using an analysis of covariance (ANCOVA) model with treatment and gender as fixed effects and baseline as covariate, separately at all defined time points.

A categorical outlier analysis supplemented the central tendency analysis by determining if there were patients who had remarkable treatment-emergent values or changes *vs.* baseline on any ECG interval or HR that would not be revealed in a mean change from baseline central tendency analysis. The outlier analyses was performed based on number and percentage of patients exceeding the following limits for each time point and treatment: QTcF/QTcB interval >450 ms, >480 ms and >500 ms; QTcF/QTcB interval increase from visit baseline >30 ms and >60 ms; QTcF/QTcB interval increase from study baseline >30 ms and >60 ms; QT interval >500 ms; PQ interval >200 ms; QRS interval >120 ms; HR <50 or >100 beats per minute (bpm) (bradycardia); HR >100 bpm (tachycardia).

In addition, linear regression analyses according to Garnett et al.[13] and a modelling and simulation data analysis applying a population approach using NONMEM were conducted to analyse the individual cladribine/QTcF concentration–effect relationships.

2.7 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[14] and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18.[15]

3 | RESULTS

3.1 | Patient demographics

The population of the ECG substudy was well distributed across the three treatment groups with n = 49 and n = 48 patients in the cladribine 3.5 mg/kg and 5.25 mg/kg groups, respectively, and n = 46 patients in the placebo group (Table 1). In accordance with the known epidemiological characteristics of RRMS, more than 98% of patients in the overall ECG substudy population were of Caucasian ethnic origin (White), and about two-thirds of the patients (65.7%) were women. The mean age (± SD) was 42 ± 11 years, and the mean weight was 73 kg. While race and weight were equally distributed across treatment groups, the patients from the placebo group were on average about 5 and 1 years younger, and also had a 1.5 and 3.0 years shorter disease duration than patients in the cladribine 3.5 mg/kg and 5.25 mg/kg groups, respectively. Differences between the placebo group and the cladribine treatment groups were also apparent with regard to the gender distribution, with a distinctly higher proportion of male patients (50%) in the placebo group, while only 26.8% male patients were enrolled in both cladribine treatment groups.

3.2 | ECG data base and timing of assessment

The ECG substudy population provided a total of 1534 post-dose ECGs (1210 of which had complete timing information; 821 obtained from patients receiving cladribine treatment, and 389 obtained from patients receiving placebo). In general, patients provided a screening ECG and a series of longitudinal ECGs over a time period of 52 weeks (three pre-dose ECGs and two post-dose ECGs at each of six visits, i.e., 31 ECGs in total).

Analysis of the relative ECG times showed that the majority of ECGs were captured in the pre-specified time window. The great majority of patients (76 out of 97 in the active dose groups) had at least one ECG between 0.5 hour and 1 hour after cladribine administration, which is the time-window of the expected achievement of maximum cladribine plasma concentration for most patients. Figure 1 illustrates the individual distribution of ECG and PK sampling times

TABLE 1 Demographic characteristics of the ECG population

	Placebo	Cladribine tablets 3.5 mg/kg	Cladribine tablets 5.25 mg/kg	Overall
n	46	49	48	143
Age in years, mean (SD)	39.6 (11.7)	41.0 (10.3)	44.2 (9.4)	41.6 (11.0)
Disease duration in years, mean (SD)	8.7 (7.2)	10.2 (9.1)	11.7 (8.0)	10.2 (8.2)
Weight in kg, mean (SD)	73.2 (16.7)	71.2 (14.8)	73.7 (15.1)	72.6 (15.5)
Sex, *n* (%)				
Female	23 (50.0)	35 (71.4)	36 (75.0)	94 (65.7)
Male	23 (50.0)	14 (28.6)	12 (25.0)	49 (34.3)
Race, *n* (%)				
White	46 (100)	48 (98.0)	47 (97.9)	141 (98.6)
Asian	0 (0)	1 (2.0)	0 (0)	1 (0.7)
Other	0 (0)	0 (0)	1 (2.1)	1 (0.7)

SD, standard deviation.

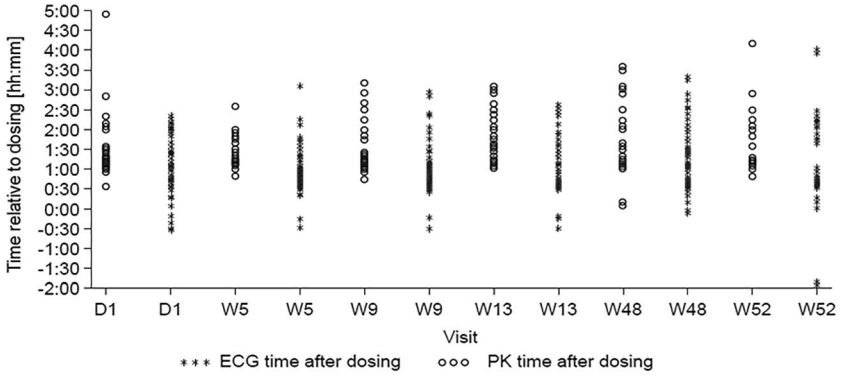

FIGURE 1 Times of ECG and PK assessments relative to dosing. Distribution of post-dose ECG (black stars, right columns of visits) and PK (open circles, left columns of visits) sampling times (ECG population). Negative times are before first dose in cycle, positive times are after first dose in cycle. Only observations with PK and ECG measurements on the same day are presented. D, day; ECG, electrocardiogram; PK, pharmacokinetic; W, week

across visits. Figure 2A shows modelled cladribine plasma concentration–time profiles of the ECG/PK substudy population from the PopPK analysis, Figure 2B shows the modelled distribution of the times of maximum cladribine plasma concentrations (t_{max}) in the ECG/PK substudy population, and Figure 2C displays the actual distribution of the times of capturing post-dose ECG data. Overall, the distribution of t_{max} data and ECG collection times were very consistent.

3.3 | Heart rate

The mean placebo-corrected change from baseline for the cladribine 3.5 mg/kg and 5.25 mg/kg groups was −1.6 bpm and −1.5 bpm, respectively. There were no significant imbalances in either the bradycardic or tachycardic outliers compared with placebo.

3.4 | PR- and QRS-interval duration

The mean placebo-corrected change in PR interval duration from baseline was 3 ms for both the cladribine 3.5 mg/kg and 5.25 mg/kg groups which is of no clinical significance. There was a slight increase in the number of outliers in the 5.25 mg/kg dose group; in that group 1–4 patients per visit showed PR interval duration of >200 ms *vs*. no patients in the placebo group and one patient in the cladribine 3.5 mg/kg group.

The mean placebo-corrected change in QRS interval duration from baseline was 0 ms and 1 ms for the cladribine 3.5 mg/kg and 5.25 mg/kg dose groups, respectively; there was one outlier (QRS > 120 ms) in the 5.25 mg/kg dose group, and no outlier patients in the placebo and cladribine 3.5 mg/kg groups.

3.5 | Analyses of central QTcF tendencies

Comparison of mean visit baseline QTcF values *vs*. post-dose placebo-adjusted QTcF data (i.e., ΔQTcF) did not show remarkable post-dose QTcF differences for either of the cladribine treatment groups compared with baseline. At several visits and across all treatment groups including the placebo group, there were consistently minor, transient

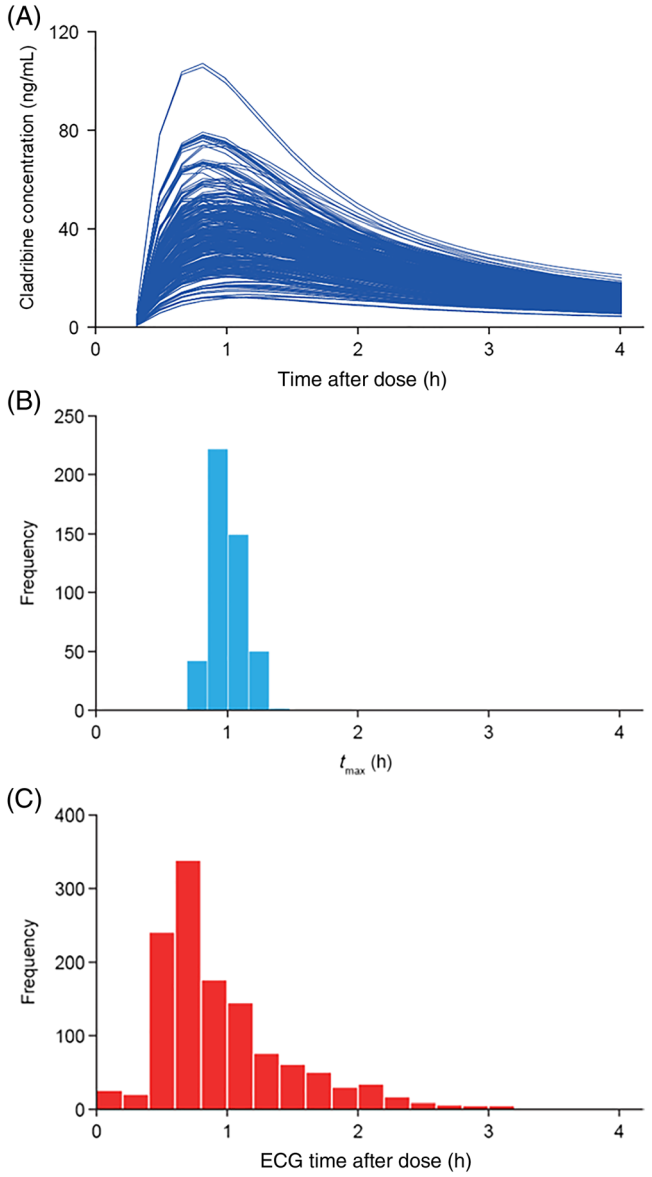

FIGURE 2 A, Cladribine plasma concentration–time profiles simulated for the ECG/PK subpopulation of the CLARITY trial. Estimated by population PK model. B, Estimated distribution of the maximum observed cladribine plasma concentrations (t_{max}) in the ECG/PK subpopulation of the CLARITY trial. C, Observed distribution of post-dose ECG data in the ECG/PK subpopulation of the CLARITY trial. ECG, electrocardiogram; h, hour; PK, pharmacokinetic

and clinically insignificant mean post-dose QTcF increases between 1 and 4 ms *vs*. QTcF visit baseline. However, the point estimates and the upper limits of 90% CI for placebo-corrected post-dose QTcF changes from visit baseline were at all occasions and for both cladribine treatment groups less than 5 ms (point estimates) and less than 7 ms (upper limit one-sided of 90% CI), respectively. These results are illustrated in Figure 3.

Placebo-adjusted ANOVA results (point estimates and 90% CI) for the time averaged QTcF changes from visit baseline are summarized for all study visits and both cladribine treatments in Table 2. These data indicate lack of a clinically relevant acute QTcF prolongation by both employed oral cladribine treatments. The findings were consistent across visits over the entire trial duration.

Placebo-adjusted ANOVA results (point estimates and 90% CI) for the differences to placebo of QTcF changes from study baseline are summarized for all study visits and both doses of cladribine in Table 3. In the cladribine 3.5 mg/kg treatment group, post-dose QTcF increases were below 6 ms at all time points, and less than 3.2 ms at five of the six visits (Table 3). The upper 90% CI data were below 8.2 ms at five of the six visits, and below 11.5 ms at one visit (Week 48). There was no apparent monotonic time-effect over the study duration.

In the cladribine 5.25 mg/kg treatment group, however, there was a non-clinically significant potential time-effect noted with more distinct post-dose QTcF increases that appeared at Week 9 and persisted, but did not increase, through Week 52. These mean post-dose QTcF increases starting from Week 9 were below 9 ms at all time points, with upper 90% CI data below 15 ms. The population PK/PD modelling and simulation analysis indicated that the additional QTcF increment starting at Week 9 amounted on average to about 4.3 ms, and that this effect was only apparent in the female population of the 5.25 mg cladribine dose group (for details, see Section 3.8 below).

3.6 | Categorical QTcF outlier analyses

No evident outlier incidents were noted in any of the treatment groups, which include absolute QT duration >500 ms, QTcF duration >500 ms, QTcF duration >480 ms, a >60 ms change from study or visit baseline QTcF, and findings of abnormal U waves.

The less specific outlier criteria include a QTcF duration >450 ms and a 30–60 ms change (QTcF) from study or visit baseline. None of the patients in the placebo and cladribine 3.5 mg/kg groups, and one (Week 1 and Week 52) to two (Week 9) patient(s) in the cladribine 5.25 mg/kg group showed a slightly prolonged QTcF duration of >450 ms. QTcF post-dose changes from study baseline ranging between 30 and 60 ms were noted in one (placebo, Week 13) to two patients in each treatment group (cladribine 3.5 mg/kg, Week 9; cladribine 5.25 mg/kg, Week 13). No QTcF changes from visit baseline values ranging between 30 and 60 ms were noted in the placebo group, and only single occasions in both cladribine treatment groups (cladribine 3.5 mg/kg one patient in Weeks 5 and 48; cladribine 5.25 mg/kg one patient in Weeks 1, 9 and 48). There were no notable dose or time dependencies (i.e., apparent relationship to the total cumulative cladribine dose) in the pattern of the less specific outlier criteria in the cladribine treatment groups.

3.7 | T- and U-wave morphology changes

The morphological changes upon both cladribine treatment dose regimens did not show any significant changes in T- or U-wave morphology compared with visit or study baseline ECGs, and no

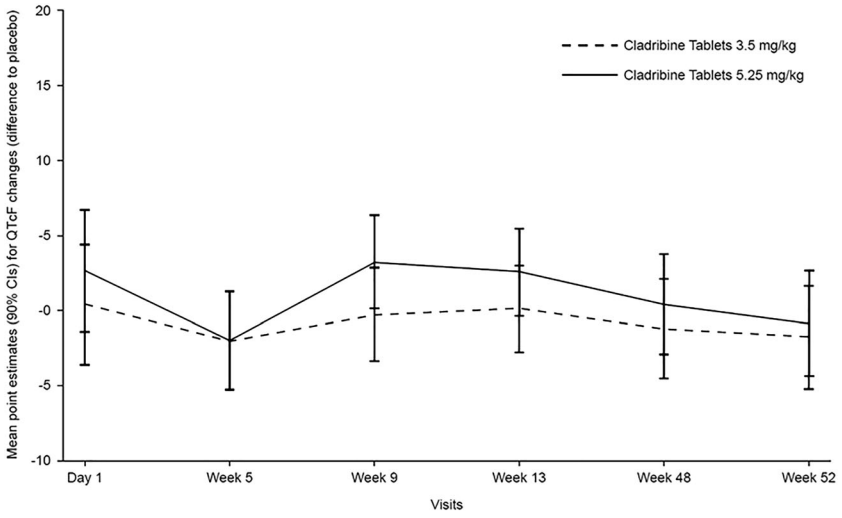

FIGURE 3 Point estimates and 90% CIs of QTcF changes (difference to placebo) from visit baseline by cladribine treatment group (ECG population). CI, confidence interval; ECG, electrocardiogram

TABLE 2 ANOVA results for the time averaged QTcF change from visit baseline, placebo adjusted (ECG population)

Treatment	Visit	Diff. To placebo (ms)	Std. error (ms)	Lower 90% CI (ms)	Upper 90% CI (ms)
Cladribine tablets 3.5 mg/kg	Week 1	0.42	2.43	−3.61	4.44
	Week 5	−2.01	1.98	−5.28	1.26
	Week 9	−0.27	1.88	−3.38	2.84
	Week 13	0.13	1.75	−2.77	3.02
	Week 48	−1.23	1.99	−4.53	2.06
	Week 52	−1.77	2.07	−5.20	1.66
Cladribine tablets 5.25 mg/kg	Week 1	2.65	2.44	−1.40	6.69
	Week 5	−1.95	1.97	−5.20	1.31
	Week 9	3.20	1.88	0.08	6.33
	Week 13	2.58	1.75	−0.32	5.49
	Week 48	0.42	2.04	−2.95	3.80
	Week 52	−0.85	2.11	−4.35	2.65

TABLE 3 ANOVA results for the time averaged QTcF change from study baseline, placebo adjusted (ECG population)

Treatment	Visit	Diff. To placebo (ms)	Std. error (ms)	Lower 90% CI (ms)	Upper 90% CI (ms)
Cladribine tablets 3.5 mg/kg	Week 1	0.42	2.43	−3.61	4.44
	Week 5	2.24	2.87	−2.51	6.99
	Week 9	3.16	3.02	−1.85	8.17
	Week 13	1.47	2.84	−3.24	6.17
	Week 48	5.99	3.29	0.53	11.44
	Week 52	−0.79	3.88	−7.23	5.65
Cladribine tablets 5.25 mg/kg	Week 1	2.65	2.44	−1.40	6.69
	Week 5	3.96	2.86	−0.78	8.71
	Week 9	7.27	3.03	2.24	12.29
	Week 13	8.52	2.85	3.80	13.24
	Week 48	8.74	3.35	3.18	14.31
	Week 52	6.10	3.93	−0.44	12.64

differences compared with placebo. A single patient in the cladribine 3.5 mg/kg group showed an inverted T-wave on three occasions (Weeks 1, 5 and 9).

3.8 | Population PK/PD modelling and simulation analysis

The results of the PK/QTcF modelling and simulation analysis were overall highly consistent with the results of the standard descriptive and ANOVA data analyses by treatment groups as detailed above. The pre-dose QTcF was estimated to be 405 ms for women and 385 ms for men. The inter-individual and inter-occasion variability (expressed as coefficients of variation) in the baseline QTcF were estimated to be 3.5% and 1.7%, respectively, and the additive residual error variability was 8.7 ms. There was a minor, clinically insignificant (albeit statistically significant) mean post-dose increase of 2.0 ms in QTcF 0.5–4 hours after treatment administration that was similar for placebo- and cladribine-treated patients. There was no other significant increase with time or a relationship between QTcF and concentration of cladribine, concentration of 2-chloroadenine (M1) or cladribine dose, except a 4.3 ms QTcF increase that was only observed in female patients of the 5.25 mg cladribine group, starting at Week 13 and remaining essentially unchanged throughout the following visits, despite continued cladribine treatment at Weeks 48 and 52.

3.9 | Correlation of cladribine plasma concentration and QTcF

Available cladribine plasma concentration data and QTcF interval data were subjected to two different linear regression analyses by using QTcF study and visit baseline data. For the analyses, all observations with a time difference of less than 1 hour between PK sampling and ECG assessment were included, and the correlation between the placebo-adjusted post-dose QTcF differences to baseline QTcF (ΔΔQTcF) with the corresponding cladribine plasma concentrations was analysed according to the approach proposed by Garnett et al.[13] For the placebo adjustment, the mean of the post-dose QTcF values within the placebo group was calculated for each visit. The analysis was performed by using both study and visit baselines. The results of both analyses are highly consistent and illustrated in Figure 4 for the study baseline analysis, and Hermann et al.[16] for the visit baseline analysis.

Both linear regression analyses of placebo-adjusted post-dose QTcF data (ΔΔQTcF) to study and visit baseline QTcF values and the corresponding actual cladribine plasma concentrations resulted in slopes very close to zero and corresponding non-significant *p*-values for both baseline scenarios (Figure 4 and Hermann et al.[16]). Accordingly, the linear regression analyses indicate that there is no significant correlation between the QTcF interval duration and cladribine plasma concentrations.

4 | DISCUSSION

Some non-cardiac drugs have been found to have undesired proarrhythmic effects which go along with a prolongation of the QT/QTc interval in the ECG and are associated with a specific polymorphic form of a ventricular tachycardia denoted as Torsade de Pointes (TdP). TdP can degenerate into ventricular fibrillation in about 20% of cases, and an overall mortality between 10 and 17% has been reported.[17] Over the last two decades, the single most common cause for the withdrawal or restriction of the use of marketed drugs has been the prolongation of the QT/QTc interval associated with TdP.[18] Furthermore, many of these drugs were not thought to have adverse cardiac effects based on their respective mechanisms of action, highlighting the importance of evaluating cardiac safety during clinical development.[19]

Regulatory authorities have established a framework of preclinical and clinical development requirements for characterization of all new chemical entities (NCEs) for their effect on cardiac repolarization. These are defined by the International Conference on Harmonisation (ICH) Guidelines SB7 (preclinical) and E14 (clinical).[7,20] Guideline ICH E14 describes the clinical evaluation of drug-induced QTc prolongation via a well-controlled clinical study, the TQT study, typically conducted in healthy volunteers with employment of supra-therapeutic doses and positive control treatments, as the primary method of

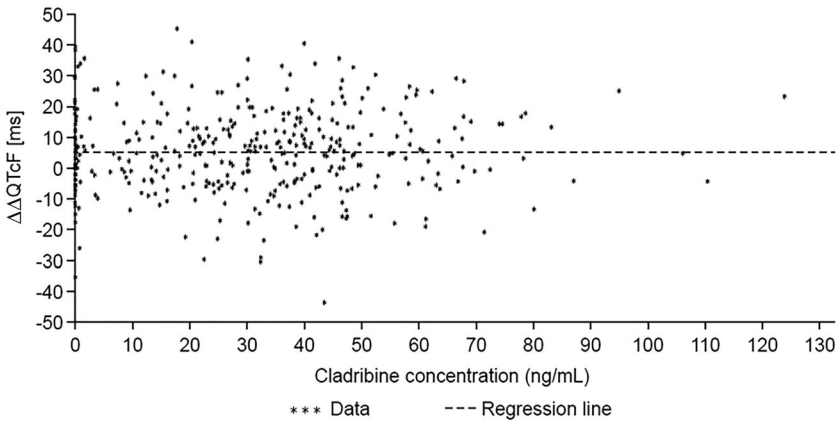

FIGURE 4 Cladribine plasma concentration *vs.* ΔΔQTcF using study baseline in the ECG/PK subpopulation of the CLARITY trial. ECG, electrocardiogram; PK, pharmacokinetic

evaluating the QT liability of NCEs during their development. As science develops, these guidelines become subject to updates and amendments such as the recently issued Q&A document R3 to the ICH E14 Guideline.[10] Therein it is acknowledged that in certain cases conventional TQT studies might not be feasible, and suggests a variety of study design and data analysis considerations—including concentration effect analyses and modelling—to address the clinical development needs for cardiac safety testing in such cases. The intense ECG substudy of cladribine tablets presented here applied many of the suggested methodological approaches of ICH E14 (R3) before the document was issued.

Repeat-dose TQT studies in healthy subjects were conducted for other recently approved orally acting RMS disease-modifying drugs with shorter duration of immune-suppressive effects, such as fingolimod and teriflunomide. While the teriflunomide TQT study did not show potential for prolonging the QTcF interval at mean therapeutic steady-state concentrations compared with placebo,[21] with fingolimod a mild QTc prolongation was seen in a dedicated TQT study.[22] However, this study has not yet been published. The only quantitative information on the QTc prolongation potential of fingolimod can be found in the Summary of Product Characteristics (SPC)[23] where it is stated that doses of 1.25 mg or 2.5 mg fingolimod at steady-state resulted in a prolongation of QTc with the upper limit of the 90% CI $\leq$13.0 ms.

Cladribine tablets 10 mg have recently been approved in Europe, Australia, Canada and several other countries and regions for the treatment of adult patients with certain types of relapsing MS (RMS). The recommended dose is 3.5 mg/kg BW, consisting of two annual courses, each comprising two treatment weeks one month apart. Cladribine tablets exert a selective mode of action on B and T lymphocytes which goes along with a long-lasting reduction in lymphocyte count.[5,6] Given this mode of action, the examination of cardiac safety by means of a TQT study was not feasible. Therefore, an intense ECG substudy as part of a multinational, large-scale Phase III study (the CLARITY trial) has been designed as an alternative approach to detect and characterize possible drug effects on the QT/QTc interval of the ECG. Such an alternative approach has also been suggested by others.[8,9]

The data collection in the intense ECG substudy applied important methodological prerequisites to enable accurate measurement and quantification of the time intervals of the ECGs. These measures included appropriately qualified and trained personnel at selected study centres, validated 12-lead ECG machines provided, maintained and quality checked by a dedicated vendor, replicate ECG collection (i.e., triplicate at baseline and duplicate for all post-dose ECGs), and fully manual central reading of all ECGs by expert cardiologists (eResearchTechnology, Inc., Philadelphia, PA, USA). ECGs were always captured on the first day of each treatment week, in accordance with the BW-adjusted dose regimen. All patients with a BW of $\geq$60 kg received the maximum recommended daily dose of 20 mg cladribine tablets on the first day of each treatment week, and no accumulation of cladribine plasma concentrations occurred upon once-daily repeated dosing.

The CLARITY study protocol aimed to comply with the MS-specific medical needs of the participating patients throughout the duration of the trial. Therefore, only a few medications were excluded by the study protocol for safety reasons (i.e. essentially immunomodulatory/immunosuppressive therapies, cytokine or anti-cytokine therapy, and concomitant usage of medications that could affect gastrointestinal motility and absorption of cladribine, including proton pump inhibitors and H_2 antagonists). All other medications that were considered necessary for the subjects' welfare and deemed unlikely to interfere with the study medication were allowed to be given at the discretion of the investigators. This implies that the study did not restrict the use of concomitant medications that may have had effects on the QTc interval. As there were only a few individual QTcF outlier incidences observed in the study population and these were well balanced across the treatment groups, concomitant medications were not considered as a covariate in the analysis.

The additional employment of PK blood samplings for the examination of the relationship between drug concentration and QT/QTc interval provided important additional and robust information for the overall interpretation of the captured longitudinal ECG data in a heterogeneous RMS patient population. For the ECG- and PK-sampling strategy, a sparse data population approach was chosen with application of random PK and ECG sampling times in a time-window of the expected maximum drug exposure, i.e., 0–3 hours post-dose (PK samples at 0–6 hours and at 24 hours post-dose). Although the acquisition of only one post-dose ECG time point (with duplicate ECG acquisition) on each visit may be seen as a limitation, the repeated performance of this procedure over six visits in 143 patients participating in the ECG substudy provided a total sample of 1534 post-dose ECGs, which can be considered a solid database for the evaluation of drug effects on the QT/QTc interval and the examination of concentration effects. Recently it was explicitly acknowledged in ICH E14 (R3)[10] that concentration–response analyses, in which all available data across all doses are used to characterize the potential for a drug to influence QTc, can serve as an alternative to the by-time-point analysis or intersection–union test as the primary basis for decisions to classify the risk of a drug. In either case this result is an important component of the totality of evidence assessment of the risk of QT prolongation.

Another aspect of this design feature is that this approach was associated with minimal discomfort and PK sampling burden of the patients, but allowed ECG and PK collection over a wide range of plasma concentrations despite the low sampling burden. As shown in Figure 4, a broad range of cladribine plasma concentrations was indeed covered. The repeated acquisition of ECG and PK data at all study visits further allowed a longitudinal analysis and demonstration of the cardiac safety of cladribine tablets over the entire treatment duration. However, the interpretation of serial long-term ECG data in patients with MS may be challenging and complicated, because progression of the disease itself may lead to cardiac repolarization abnormalities (i.e. time effects), due to disease-related dysfunctions of the autonomic nervous system (ANS), probably based on axonal lesions either at the level of spinal

cord or the ANS nuclei that are located in the periventricular region of the fourth ventricle.

Various studies have shown evidence of QTc prolongation in MS patients over time with progression of the disease.[24-26] A recent MRI study[27] confirmed these results in RMS patients by three-dimensional quantification of brain lesion load at the level of the structures involved in superior autonomic control (insula, cingulate cortex and amygdala-hippocampus). Overall, 16% of the patients had an increased QTc interval. The QTc interval length was correlated with disease duration, cortical insular lesion volume and grey matter lesion volume in the three examined areas and inversely correlated with global and insular cortical thickness. As the distribution of MS-related alterations of ANS functions across treatment groups is likely to occur by chance, and randomization cannot account for these events, disease-related QTc changes with time have to be taken into account as a confounding factor in the interpretation of the long-term serial ECG data. In this context, a limitation of the study was that the randomization of the ECG population resulted in a larger proportion of women and of patients with a longer history of illness in the cladribine treatment groups as compared with placebo (Table 1). Importantly, the largest proportion of women (75% *vs*. 50% in the placebo group) and the longest disease duration (11.7 years *vs*. 8.7 years in the placebo group) were noted in the cladribine 5.25 mg/kg group. Based on this, it cannot be excluded that these randomization imbalances for important population factors may have contributed to the apparent time-effects on QT/QTc in the cladribine 5.25 mg/kg group, rather than reflecting cumulative drug effects. However, the ANOVA results of both cladribine treatment groups for the placebo-adjusted QTcF changes (ΔΔQTcF) from visit baseline indicated that post-dose QTcF means were consistently below 5 ms across all visits, and that the respective upper 90% CIs were consistently below 10 ms, suggesting a lack of clinically relevant effects of oral cladribine treatment on cardiac repolarization (Table 2). For the 3.5 mg/kg cladribine group, the same holds true for the comparisons to study baseline, with the exception of a single occasion slightly above these limits (Week 48 with mean QTcF of 5.99 ms and a corresponding 90% CI of 11.44 ms; Table 3). These results are confirmed by the linear regression concentration–effect analysis of ΔΔQTcF according to Garnett et al.[13] (Figure 4 and Hermann et al.[16]), which indicate that there is no significant correlation between the placebo-adjusted post-dose QTcF interval duration and cladribine plasma concentrations regardless of whether study or visit baseline comparisons are considered.

Finally, the categorical outlier analyses also did not indicate a clinically meaningful potential of cladribine to cause QT/QTc prolongation. No cladribine- or placebo-treated patients met the criteria for evident outliers (i.e., QTcF duration >480 ms and/or a post-dose change of >60 ms from baseline), and only a few incidents met the nonspecific outlier criterion of a 30–60 ms QTcF change from study baseline when the 3.5 mg/kg dose regimen (approved in Europe and other regions) is considered. The slightly higher number of 30–60 ms QTcF changes in the 5.25 mg/kg dose group appears compatible with the modest time-effect on QT/QTc duration in this particular group.

5 | CONCLUSIONS

In conclusion, the ECG results presented here indicate that cladribine showed no evidence of any effect on heart rate, AV conduction or cardiac depolarization as measured by the PR and QRS interval durations. No significant changes in T-wave morphology were observed in either of the cladribine treatment groups.

Overall, the data showed no evidence of an acute cladribine concentration-dependent effect on cardiac repolarization. Considering the challenges associated with the long duration of the trial, and the possible contributing factors of the subjects' underlying condition, the QTcF outcomes were found to be highly consistent over time *vs*. visit baseline data, indicating that cladribine does not confer a clinically meaningful long-term effect on cardiac repolarization. Considering the recent marketing approval of cladribine tablets for MS in Europe and other regions, and the up-to-date assessment methodology used for cardiac safety evaluation, this report is highly relevant for the practising community, and also to clinical researchers involved in cardiac safety testing of novel products.

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COMPETING INTERESTS

R.H. served as external Clinical Pharmacology expert advisor for various aspects of several cladribine studies, and received financial support for research, consulting and training services from Merck KGaA, Darmstadt, Germany. J.S.L. was employed by ERT (the ECG core lab for this study) when this study was conducted. L.F. is an employee of Uppsala University. Uppsala University has performed contractual research for Merck KGaA, Darmstadt, Germany. F.D. is an employee of EMD Serono Research & Development Institute Inc., a business of Merck KGaA, Darmstadt, Germany. A.M. is an employee of Merck Serono SA, Switzerland, an affiliate of Merck KGaA, Darmstadt, Germany.

CONTRIBUTORS

J.S.L. was involved in the design of the ECG substudy, acquisition, analysis and interpretation of the data. A.M. was involved in the design of the ECG substudy, analysis and interpretation of the data. R.H., L.F., and F.D. were involved in the analysis and interpretation of the data. R.H. drafted the manuscript and all other authors critically revised the manuscript. All authors gave final approval of the manuscript to be published.

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REFERENCES

1. Giovannoni G, Comi G, Cook S, et al. A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis. *N Engl J Med*. 2010;362(5):416-426.
2. Merck Europe B.V. MAVENCLAD 10 mg tablets Summary of Product Characteristics; 2018 [online]. Retrieved from https://www.ema.europa.eu/documents/product-information/mavenclad-epar-product-information_en.pdf (last accessed February 2019).
3. Leist TP, Comi G, Cree BA, et al. Effect of oral cladribine on time to conversion to clinically definite multiple sclerosis in patients with a first demyelinating event (ORACLE MS): a phase 3 randomised trial. *Lancet Neurol*. 2014;13(3):257-267.
4. Giovannoni G, Soelberg Sorensen P, Cook S, et al. Safety and efficacy of cladribine tablets in patients with relapsing-remitting multiple sclerosis: results from the randomized extension trial of the CLARITY study. *Mult Scler*. 2018;24(12):1594-1604.
5. Beutler E. Cladribine (2-chlorodeoxyadenosine). *Lancet*. 1992;340(8825):952-956.
6. Comi G, Hartung HP, Kurukulasuriya NC, Greenberg SJ, Scaramozza M. Cladribine tablets for the treatment of relapsing-remitting multiple sclerosis. *Expert Opin Pharmacother*. 2013;14(1):123-136.
7. European Medicines Agency. CHMP/ICH/2/04—ICH E14 Clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs; 2005 [online]. Retrieved from http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002879.pdf (last accessed February 2019).
8. Morganroth J, Shah RR, Scott JW. Evaluation and management of cardiac safety using the electrocardiogram in oncology clinical trials: focus on cardiac repolarization (QTc interval). *Clin Pharmacol Ther*. 2010;87(2):166-174.
9. Rock EP, Finkle J, Fingert HJ, et al. Assessing proarrhythmic potential of drugs when optimal studies are infeasible. *Am Heart J*. 2009;157:827-36, 36 e1.
10. European Medicines Agency. ICH Guideline E14: The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs (R3)—Questions and answers (Step 5). Committee for Human Medicinal Products, EMA/CHMP/ICH/2008; 2016 [online]. Retrieved from https://www.ema.europa.eu/documents/scientific-guideline/ich-guideline-e14-clinical-evaluation-qt/qtc-interval-prolongation-proarrhythmic-potential-non-antiarrhythmic-drugs-r3-questions-answers-step_en.pdf (last accessed February 2018).
11. Friberg LE, Isbister GK, Duffull SB. Pharmacokinetic-pharmacodynamic modelling of QT interval prolongation following citalopram overdoses. *Br J Clin Pharmacol*. 2006;61(2):177-190.
12. Savic RM, Novakovic AM, Ekblom M, Munafo A, Karlsson MO. Population pharmacokinetics of cladribine in patients with multiple sclerosis. *Clin Pharmacokinet*. 2017;56(10):1245-1253.
13. Garnett CE, Beasley N, Bhattaram VA, et al. Concentration-QT relationships play a key role in the evaluation of proarrhythmic risk during regulatory review. *J Clin Pharmacol*. 2008;48(1):13-18.
14. Harding SD, Sharman JL, Faccenda E, et al. The IUPHAR/BPS guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Res*. 2018;46(D1):D1091-D1106.
15. Alexander SP, Fabbro D, Kelly E, et al. The Concise Guide to PHARMACOLOGY 2017/18: enzymes. *Br J Pharmacol*. 2017;174(Suppl 1):S272-S359.
16. Hermann R, Karlsson MO, Novakovic AM, Terranova N, Fluck M, Munafo A. The clinical pharmacology of cladribine tablets for the treatment of relapsing multiple sclerosis. *Clin Pharmacokinet*. 2019;58(3):283-297.
17. Shah RR. The significance of QT interval in drug development. *Br J Clin Pharmacol*. 2002;54(2):188-202.
18. Roden DM. Drug-induced prolongation of the QT interval. *N Engl J Med*. 2004;350(10):1013-1022.
19. Kannankeril P, Roden DM, Darbar D. Drug-induced long QT syndrome. *Pharmacol Rev*. 2010;62(4):760-781.
20. European Medicines Agency. CHMP/ICH/423/02—ICH Topic S 7 B The nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals; 2005 [online]. Retrieved from http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002841.pdf (last accessed February 2019).
21. Sanofi-Aventis Groupe. Aubagio Summary of Product Characteristics; 2019 [online]. Retrieved from https://www.ema.europa.eu/documents/product-information/aubagio-epar-product-information_en.pdf (last accessed February 2019).
22. Camm J, Hla T, Bakshi R, Brinkmann V. Cardiac and vascular effects of fingolimod mechanistic basis and clinical implications. *Am Heart J*. 2014;168(5):632-644.
23. Novartis Europharm Limited. Gilenya Summary of Product Characteristics; 2018 [online]. Retrieved from https://www.ema.europa.eu/documents/product-information/gilenya-epar-product-information_en.pdf (last accessed February 2019).
24. Drouin E, Nataf S, Lande G, Louboutin JP. Abnormalities of cardiac repolarization in multiple sclerosis: relationship with a model of allergic encephalomyelitis in rat. *Muscle Nerve*. 1998;21(7):940-942.
25. de Seze J, Stojkovic T, Gauvrit JY, et al. Cardiac repolarization abnormalities in multiple sclerosis: spinal cord MRI correlates. *Muscle Nerve*. 2000;23(8):1284-1286.
26. Mahovic D, Lakusic N. Progressive impairment of autonomic control of heart rate in patients with multiple sclerosis. *Arch Med Res*. 2007;38(3):322-325.
27. Turri G, Calabrese M, Pancheri E, Monaco S, Gajofatto A, Marafioti V. QTc interval in patients with multiple sclerosis: an inference from the insula of Reil? *Eur J Neurol*. 2017;24(3):491-496.

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