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APPLICATION NUMBER:

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology Review

NDA or BLA Number	209321
Link to EDR	\\cdsesub1\evsprod\nda209321
Submission Date	06/15/2018
Submission Type	505(b)(1) Original NME NDA
Brand Name	RUZURGI
Generic Name	Amifampridine (3,4-Diaminopyridine, 3,4-DAP)
Dosage Form/Strength and Dosing Regimen	Immediate-release scored tablets/10 mg. (b) (4)
Route of Administration	Oral
Proposed Indication	(b) (4) Lambert-Eaton Myasthenic Syndrome (LEMS) in patients (b) (4)
Applicant	Jacobus Pharmaceutical Company, Inc.
Associated IND	IND 54313
OCP Review Team	Mariam Ahmed, Ph.D.; Katarzyna Drozda, Pharm.D., M.S.; Christian Grimstein, Ph.D.; Kevin Krudys, Ph.D.; Sreedharan Sabarinath, Ph.D.
OCP Final Signatory	Mehul Mehta, Ph.D.

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1. EXECUTIVE SUMMARY

Jacobus Pharmaceuticals, Inc. submitted a new drug application (NDA) seeking approval for amifampridine base (hereafter referred to as 3,4-Diaminopyridine or 3,4-DAP) tablets (RUZURGI™) for (b) (4) Lambert-Eaton Myasthenic Syndrome (LEMS) in patients (b) (4) LEMS is a rare autoimmune disease characterized by muscle weakness and fatigability. These symptoms are caused by the impairment of acetylcholine (ACh) release at the peripheral neuromuscular junctions, causing a loss of neuromuscular transmission.

3,4-DAP is a voltage-dependent potassium channel blocker, which causes depolarization of the presynaptic membrane and slows down or inhibits repolarization. This prolonged depolarization results in opening of slow voltage-dependent calcium channels and a subsequent influx of Ca^{2+} to increase ACh release and consequently, improve neuromuscular transmission. In the United States, 3,4-DAP has been available for the treatment of LEMS under a compassionate use program since the 1990s. 3,4-DAP phosphate tablets (FIRDAPSE®) were recently approved by FDA for symptomatic treatment of LEMS in adults. FIRDAPSE® is also approved for this indication by the European Medicines Authority (EMA) and has been marketed in the European Union by BioMarin Pharmaceutical, Inc. since December 2009.

In this NDA, the efficacy of RUZURGI was established in a randomized withdrawal, placebo-controlled, study (Study JPC 3,4-DAPPER or DAPPER). Thirty-two adult subjects with LEMS were enrolled, randomized, and analyzed in this study. Subjects were required to be on an adequate and stable dose of 3,4-DAP prior to entering the randomized discontinuation phase of the study. The primary measure of efficacy was the proportion of subjects with >30% deterioration in the triple timed up-and-go (3TUG) test scores at the end of the discontinuation period of the study compared with baseline.

As supportive evidence of efficacy, the applicant submitted a Duke University-sponsored, Phase II, randomized, double-blind, placebo-controlled, parallel-group study (JPC 3,4-DAP DUKE RCT, N=26). Additionally, the applicant submitted retrospective pharmacovigilance reviews of the compassionate use experience (i.e., patients who received 3,4-DAP through a compassionate use Investigational New Drug (IND)) to provide support for the long-term safety of 3,4-DAP. The submission also included information on pediatric clinical experience through the applicant's compassionate use program for RUZURGI and safety data from 22 pediatric patients (7 pediatric patients with LEMS and 15 pediatric patients with congenital myasthenia syndrome (CMS)).

The applicant also submitted clinical pharmacology studies that characterized the effect of intrinsic and extrinsic factors on the plasma exposure of RUZURGI (i.e. food effect, metabolizer phenotypes) and a thorough QT (TQT) study.

The primary objectives of this review are to evaluate:

1. The acceptability of the proposed starting dose, maximum single dose, and maximum daily dose [redacted] (b) (4)
2. The need for dose adjustments based on intrinsic and extrinsic factors.

1.1 Recommendations

The Office of Clinical Pharmacology has reviewed the information submitted under NDA 209321 and recommends approval of 3,4-DAP base tablets (RUZURGI) for [redacted] (b) (4) treatment of LEMS in patients [redacted] (b) (4)

Key review issues with specific recommendations and comments are summarized below:

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	The efficacy of RUZURGI was established in adults in a pivotal randomized withdrawal, placebo-controlled, study (DAPPER). The primary measure of efficacy was the proportion of subjects with greater than 30% deterioration in the 3TUG change from baseline at the end of the discontinuation period. Duke University-sponsored, Phase II, randomized, double-blind, placebo-controlled, parallel-group study (DUKE-RCT) provided additional evidence of effectiveness in adults. Clinical judgement on disease similarity between adults and pediatrics for LEMS, available safety data and clinical experience reported in pediatric patients (with LEMS and CMS) from the compassionate use program support the use of RUZURGI in pediatric patients 6 years of age and older.
General dosing instructions	The recommended oral dosage [redacted] (b) (4) is dependent on body weight. • Dosage should be increased based on clinical response and tolerability. Individual doses throughout the day do not need to be equal. • If a dose is missed, patients should not take double or extra doses. • RUZURGI can be administered without regard to food

	Recommended dose [redacted] (b) (4) ***weighing 45 kg or more:*** • The recommended starting dosage is 15 mg to 30 mg daily, in divided doses (2 to 3 times per day) • The dose can be increased to a maximum of 100 mg per day in 5 mg to 10 mg increments • The maximum single dose is 30 mg ***Recommended dose*** [redacted] (b) (4) ***weighing less than 45 kg:*** • The recommended starting dosage is 7.5 to 15 mg daily in divided doses (2 to 3 times per day) • The dose can be increased to a maximum of 50 mg per day in 2.5 mg to 5 mg increments • The maximum single dose is 15 mg
Dosing in patient subgroups (intrinsic and extrinsic factors)	Patients with renal impairment, hepatic impairment, or who are known N-acetyl transferase 2 (NAT2) poor metabolizers require a lower starting dose for treatment initiation. • The recommended starting dosage [redacted] (b) (4) weighing 45 kg or more is 15 mg daily taken in divided doses. • The recommended starting dosage for pediatric patients weighing less than 45 kg is 7.5 mg daily taken in divided doses. The recommended dose titration based on tolerability and efficacy is expected to allow for appropriate individualization of dose for all patients. For this reason, no change to the maximum daily dose is required.
Bridge between the to-be-marketed and clinical trial formulations	The 10 mg tablet formulation, scored on one side, used in the efficacy studies is identical to the to-be-marketed formulation and therefore no PK bridging is necessary. When patients require a dosage less than 5 mg, have difficulty swallowing tablets, or require feeding tubes, a 1 mg/mL suspension formulation can be prepared (e.g., by dissolving 10 mg tablet in 10 mL sterile water) just before use. This approach was used in the compassionate use program

	for RUZURGI. Please refer to the CMC review for additional details on the suspension formulation.

1.2 Post-Marketing Requirements and Commitments

- **Post-Marketing Requirements for a Hepatic Impairment Study:**

Amifampridine is predominantly metabolized by N-acetyltransferase 2 enzyme (NAT2) in the liver. Hepatic impairment can cause increase in exposure to amifampridine. There is no clinical experience with amifampridine in subjects with hepatic impairment. Therefore, a hepatic impairment study to evaluate the impact of hepatic function impairment on the exposure to amifampridine is required.

- **Post-Marketing Commitment for a Renal Impairment Study using reduced design:**

Renal excretion is one of the elimination pathways for amifampridine and there is limited clinical experience in subjects with renal impairment. Therefore, a renal impairment study using a reduced design is recommended to evaluate the potential increase in exposure to amifampridine in subjects with severe renal impairment (creatinine clearance 15-29 ml/min).

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

Mechanism of Action: 3,4-DAP is a voltage-gated potassium (K+) channel blocker. The blockade of K+ channels is believed to cause depolarization of the presynaptic membrane and slow down or inhibit repolarization. Prolonged depolarization results in opening of slow voltage-gated calcium (Ca^{2+}) channels and allows a subsequent influx of Ca^{2+}. The increased concentration of intracellular Ca^{2+} induces exocytosis of more synaptic vesicles containing acetylcholine (ACh), thus releasing ACh into the synaptic cleft. The influx of ACh into the presynaptic cleft enhances neuromuscular transmission, providing improved muscle function.

Absorption: 3,4-DAP is rapidly absorbed, and peak plasma concentration (C_{max}) is reached at about 0.5 to 1.5 hours after ingestion in a fasted state. Compared to administration of RUZURGI in the fasting state, administration of the 20 mg and 30 mg dose levels of RUZURGI with a standard high fat meal resulted in significant decrease in C_{max} (41% and 52%, respectively) and an increase in median T_{max} by 1.0 hour; AUC0-last was significantly reduced only for the 30 mg dose (by 23%).

Distribution: The estimated apparent volume of distribution of 3,4-DAP (V/F) ranged from 357-1383 L in different metabolizer phenotypes. In vitro human plasma protein binding of 3,4-DAP was about 25%.

Metabolism and Elimination: In vitro studies indicated that 3,4-DAP is metabolized by the N-acetyltransferase 2 (NAT2) enzyme to form a major but inactive metabolite, 3-N-acetyl 3,4-DAP (3-Ac-DAP). Acetylation of 3,4-DAP by NAT1 enzyme may also occur but at a much slower rate. The rate and extent of metabolism is affected by single nucleotide polymorphisms (SNPs) in the NAT2 gene. In the TQT study, poor metabolizers had 1.1 to 3.7 times higher AUC_{0-4h} and 1.3 to 3.7 times higher C_{max} than intermediate metabolizers, following the first dose. There were only three subjects with normal metabolizer status with PK data (N=3 enrolled in the TQT study) and based on this limited number of subjects, poor metabolizers had 6 to 8.5 times higher AUC_{0-4h} and 6.1 to 7.6 times higher C_{max} than normal metabolizers, following the first dose.

More than 65% of the administered dose is eliminated in the urine as 3,4-DAP or 3-Ac-DAP over 24 hours. Urinary excretion data in humans indicated that on average, 19% of the unchanged 3,4-DAP was eliminated in the urine in 24 hours. The average elimination half-life (t1/2) was 3.6 to 4.2 hours in healthy subjects.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

(b) (4) *weighing 45 kg or more:*

The recommended general starting dose of RUZURGI is 15 mg to 30 mg daily, taken orally in divided doses (2 to 3 times per day). The dose can be increased in 5 mg to 10 mg increments. The recommended maximum single dose is 30 mg. The recommended maximum total daily dose is 100 mg per day. RUZURGI can be taken without regard to food. If a dose is missed, patients should not take double or extra doses. This dosing/titration regimen based on tolerability and efficacy was tested in pivotal efficacy studies.

(b) (4) *weighing less than 45 kg:*

The recommended general starting dose of RUZURGI is 7.5 mg to 15 mg daily, taken orally in divided doses (2 to 3 times per day). The dose can be increased by 2.5 mg to 5 mg increments. The recommended maximum single dose is 15 mg. The recommended maximum total daily dose is 50 mg per day. Use of RUZURGI in this group is supported by evidence from adequate and well-controlled studies in adults with LEMS, clinical judgement on disease similarity between adults and pediatrics, clinical experience and safety data from 22 pediatric patients (7 pediatric patients with LEMS and 15 pediatric patients with CMS), pharmacokinetic data in adults, and pharmacokinetic modeling and simulation in pediatric patients.

2.2.2 Therapeutic individualization

The applicant conducted clinical pharmacology studies to evaluate dose proportionality, food effect with the commercial formulation and the influence of intrinsic factors on exposure. The food effect study suggested a reduction in C_{max} and AUC for RUZURGI with a high fat, high calorie meal. However, RUZURGI was administered without regard to food in efficacy studies and in the compassionate use program. Therefore, no specific instruction regarding food is required for RUZURGI.

In-vitro studies were conducted to evaluate potential drug interactions with major enzymes and transporters for both parent and the major inactive 3-N-acetyl amifampridine (3-Ac-DAP) metabolite. Results from in-vitro studies suggested that 3,4-DAP was not a substrate nor an inhibitor or inducer of major CYP enzymes or transporters. Also, 3-Ac-DAP was not an inhibitor or inducer of major enzymes or transporters. Therefore, drug-drug interaction (DDI) liability was considered low and no clinical DDI studies were conducted during the clinical development program.

Renal clearance is an elimination pathway for 3,4-DAP and 3-Ac-DAP. However, no renal impairment study was conducted with RUZURGI. Because of the potential for increased exposures in patients with renal impairment, a lower starting dose is recommended in such patients. (b) (4) patients weighing 45 kg or more with renal impairment are recommended to start dose titration with 15 mg daily in divided doses. Pediatric patients weighing less than 45 kg are recommended to start dose titration with 7.5 mg daily. As the dose is titrated based on efficacy and tolerability, there is no change to the recommended maximum single dose or daily dose.

The effects of hepatic impairment on RUZURGI have not been studied. However, hepatic impairment is expected to increase the plasma concentrations of 3,4-DAP. Therefore, as with renal impairment, a lower starting dose is recommended for patients with hepatic impairment. (b) (4) patients weighing 45 kg or more with hepatic impairment are recommended to start dose titration with 15 mg daily in divided doses. Pediatric patients weighing less than 45 kg are recommended to start dose titration with 7.5 mg daily. As with renal impairment, there is no change to the recommended maximum single dose or daily dose.

The metabolism of 3,4-DAP is affected by the polymorphic NAT2 enzyme activity. In the TQT study, poor metabolizers have 1.1 to 3.7 times higher $AUC_{0\text{-}4h}$ and 1.3 to 3.7 times higher C_{max} than intermediate metabolizers, following the first dose. There were only three subjects with normal metabolizer status with PK data (N=3 enrolled in the TQT study) and based on this limited number of subjects, poor metabolizers have 6 to 8.5 times higher $AUC_{0\text{-}4h}$ and 6.1 to 7.6 times higher C_{max} than normal metabolizers, following the first dose. The individualized dose

titration from a lower starting dose of 15 mg daily [redacted] (b) (4) patients weighing 45 kg or more) or 7.5 mg daily (pediatric patients weighing less than 45 kg) to a tolerated and effective dose in subjects with known poor metabolism status should account for individual differences in metabolism.

2.3 Outstanding Issues

None.

2.4 Summary of Labeling Recommendations

- [redacted] (b) (4) weighing 45 kg or more: the recommended general starting dose of RUZURGI is 15 mg to 30 mg daily, taken orally in divided doses (2 to 3 times per day). The dose can be increased in 5 mg to 10 mg increments. A lower starting dose (i.e. 15 mg daily) is recommended for patients who are known NAT2 poor metabolizers and for patients with any degree of renal/hepatic function impairment. The maximum single dose allowed is 30 mg and the recommended maximum total daily dose is 100 mg for all patients.
- [redacted] (b) (4) weighing less than 45 kg: the recommended starting dose is 7.5 mg to 15 mg daily, taken orally in divided doses (2 to 3 times per day). The dose can be increased in 2.5 mg to 5 mg increments. The low starting dose (i.e. 7.5 mg daily) is recommended for pediatric patients who are known NAT2 poor metabolizers or with renal/hepatic function impairment. The maximum single dose allowed is 15 mg and the recommended maximum total daily dose is 50 mg.
- RUZURGI can be administered without regard to food.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

RUZURGI is an immediate-release tablet formulation for oral administration that contains 10 mg of 3,4-DAP drug substance. The tablets are white to off-white, oval-shaped, with a functional score on one side. A 1 mg/mL suspension formulation can be prepared by dissolving the tablets in sterile water before use (e.g. one 10 mg tablet in 10 mL water), when a dosage less than 5 mg is required or when patients are unable to swallow intact tablets or use feeding tubes. The desired dosage can be drawn using a graduated oral syringe.

The applicant (Jacobus) received Orphan Drug designation for the use of 3,4-DAP for LEMS on December 18th, 1990. Since 1993, Jacobus has been supplying 3,4-DAP free base under a compassionate use program that provides drugs to treat patients under an expanded access IND application.

On October 20th, 1997, Jacobus submitted an initial IND application (No. 54313) to the agency for the development of 3,4-DAP. On September 7th, 2010, a Type B/pre-NDA meeting was held with the agency. At this meeting, Jacobus agreed to submit the DAPPER study protocol to FDA to solicit detailed feedback, including feedback on the primary endpoint. On June 17th, 2014, Jacobus updated the Division on the status of the overall development program for 3,4-DAP, shared the topline results of the DAPPER study, and discussed the components for an NDA (Ref. End-of-Phase 2 Meeting Minutes, June 17th, 2014). Subsequently, Jacobus received Fast Track designation for 3,4-DAP on September 19th, 2014.

During the pre-NDA meeting (on February 17th, 2016), Jacobus discussed the requirements for NDA submission with the agency. During this meeting, the agency indicated that in addition to data from the DAPPER study, source level data from Study JPC 3,4-DAP DUKE RCT could support an NDA filing for 3,4-DAP in LEMS from an efficacy perspective. On March 27th, 2017, the agency provided written responses agreeing with Jacobus's plan for a rolling NDA submission. The first part of the NDA was submitted on August 11th, 2017 (chemistry, manufacturing, and controls (CMC) and clinical modules), and the second part was submitted on December 7th, 2017 (labeling information, TQT study report, nonclinical and supplemental CMC information). However, it was determined that the application was not sufficiently complete to permit a substantive review of the adequacy of the CMC information. Thus, a refuse to file letter was issued on January 31st, 2018. On March 29th, 2018, a Type A meeting was held with the agency to achieve concurrence on the CMC section requirements. The NDA was subsequently resubmitted on June 15th, 2018.

The phosphate salt of 3,4-DAP, FIRDAPSE®, was approved by the agency on November 28th, 2018 for the treatment of adult patients with LEMS. The approved maximum total daily dose is 80 mg for FIRDAPSE® and no single dose should exceed 20 mg. FIRDAPSE® is not approved for pediatric patients with LEMS. No approved treatment options are available for pediatric patients with LEMS.

Studies that evaluated the clinical pharmacology, efficacy and safety of 3,4-DAP in this NDA are summarized in Table 1.

Table 1: Summary of Individual Clinical Pharmacology and Efficacy Studies

Study	Type	Subject Population	Dosage Regimen and Route of Administration
Studies conducted by the applicant:			
PK1	Phase I	HVs	Single oral dose of 3,4-DAP (20 or 30 mg) or placebo in both fasting and fed states.
TQT	Phase I	HVs	3 study periods with at least a 5-day washout between treatments of: • 120 mg (four 30 mg doses administered every 4 hours) 3,4-DAP and placebo moxifloxacin; • moxifloxacin 400 mg and placebo 3,4-DAP; or • placebo 3,4-DAP and placebo moxifloxacin.
DAPPER	Phase II	LEMS	Total daily dose ranged from 30 to 100 mg in a minimum of 3 oral doses per day, with the single doses ranging from 10 to 30 mg administered 3 to 7 times per day, either continuous or tapered to placebo followed by resumption of original dose.
RPV162	RPVs of the compassionate use experience	LEMS (including special population such as pediatrics)	The average starting dose ranged from 5-30 mg (for equal dosing) and from 10-35 mg (unequal dosing) daily. The maximum total daily dose ranged from 10 to 175 mg and the total number of doses per day ranged from 1 to 10. There were 8 subjects receiving a total daily dose >100 mg with a median duration of 8.9 years (range: 1 to 21 years). Safety data from 22 pediatric patients receiving 3,4-DAP were included in this submission[1]. The age range at starting 3,4-DAP treatment was 13 months to 16 years

[1] In the initial submission, RPV162 included safety data from 6 pediatric patients (5 subjects with LEMS and 1 subject with CMS). Subsequently, the sponsor submitted safety data for 2 additional subjects with LEMS and 14 additional subjects with CMS.

			and the starting dose ranged from 5 mg/day to 120 mg/day.
Other Studies			
DUKE RCT	Duke University-sponsored, Phase II	LEMS	10 to 20 mg 3,4-DAP or placebo 3 or 4 times daily for 6 to 9 days followed by 24-hour washout followed by 3,4-DAP 10 mg single dose. Patients then entered an open-label period for up to 6 months.

Note: HVs is healthy volunteers; LEMS is patients with Lamberts Eaton Myasthenia Syndrome; RPVs is Retrospective Pharmacovigilance Reviews.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of Action	3,4-DAP is a voltage-gated potassium (K+) channel blocker. The blockade of K+ channels causes depolarization of the presynaptic membrane and slows down or inhibits repolarization. Prolonged depolarization results in opening of slow voltage-gated calcium (Ca2+) channels and a subsequent influx of Ca2+. The increased concentration of intracellular Ca2+ induces exocytosis of more synaptic vesicles containing acetylcholine (ACh), thus releasing ACh into the synaptic cleft. The influx of ACh into the presynaptic cleft enhances neuromuscular transmission, providing improved muscle function.
Active Moieties	There are no known active metabolites of 3,4-DAP. There is one major inactive metabolite reported (3-Ac-DAP). Under fasting conditions, the mean C_{max} and $AUC_{0\text{-}last}$ values for 3-Ac-DAP were approximately 4-fold and 7-fold as compared to the parent 3,4-DAP, respectively.
QT Prolongation	The effect of a therapeutic dose of 3,4-DAP on QTc interval prolongation was studied in a double-blind, randomized, placebo and positive controlled study in 52 healthy subjects, including 23 subjects with NAT2 poor metabolizer phenotype. Study participants were administered 120 mg RUZURGI in 4 equal doses of 30 mg at 4-hour intervals. RUZURGI did not prolong the QTc interval to any clinically relevant extent.
General Information	
Bioanalysis	In the food effect and TQT studies, 3,4-DAP was analyzed using validated HPLC-MS/MS methods. Please refer to Section 4.1 for a summary of bioanalytical method validation and its performance.
Accumulation	In the TQT study, a 30 mg of 3,4-DAP was administered every 4 hours in 4 divided doses (120 mg daily dose). There was no accumulation observed for

	C_{max} or AUC_{0-4h} at the fourth dose (i.e. R_{AUC} was 1.08 and R_{Cmax} was 0.79). This is expected given the short half-life of 3,4-DAP (range of median $t_{1/2}$ across different metabolizer phenotypes is 3.4-4.2 h).
Variability	There was significant inter-subject variability in 3,4-DAP levels. In the TQT study, the coefficients of variation (CV%) for C_{max} and $AUC_{0-\infty}$ were approximately 75% and 81%. The CV% for C_{max} were: 18%, 78%, and 25% for normal, intermediate, and poor metabolizers, respectively. The CV% for $AUC_{0-\infty}$ were: 26%, 117%, and 27% for normal, intermediate, and poor metabolizers, respectively.
Bioavailability	The absolute bioavailability of 3,4-DAP was not reported.
T_{max}	3,4-DAP was rapidly absorbed with a T_{max} ranging from 0.5 to 1.5 hours after dosing in a fasting state.
Volume of Distribution	In healthy volunteers, the apparent volume of distribution for plasma 3,4-DAP varied by metabolizer status; potentially because of variation in bioavailability (the estimated apparent volume of distribution across different metabolizer phenotypes was 357 - 1383 L).
Plasma Protein Binding	An in vitro human plasma protein binding study demonstrated that the plasma protein binding of 3,4-DAP was about 25%
Substrate transporter systems	3,4-DAP and its major metabolite are not substrates or inhibitors for major drug transporters based on in-vitro studies.
Metabolism	In vitro studies with cDNA expressed human NAT enzyme preparations indicate that 3,4-DAP is rapidly acetylated by the NAT2 enzyme.
Excretion	The mean recovery of 3,4-DAP and its metabolite in urine was greater than 65% with no differences observed between the fed and fasted states.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

The primary evidence of effectiveness of 3,4-DAP for the treatment of LEMS is from the DAPPER study. An individual dose-response relationship was observed in this study, providing

supportive evidence of effectiveness. Additionally, the DUKE study, a Phase II study sponsored by Duke University, provides additional evidence of effectiveness.

DAPPER study: This was a Jacobus-sponsored, pivotal, Phase II, randomized, double-blind, placebo-controlled, withdrawal study to evaluate efficacy and safety in subjects with LEMS (N =32). Eligible subjects were at least 18 years of age with known clinically active LEMS, who had been on a chronic stable regimen of 3,4-DAP and other LEMS related medications for at least 3 months and a stable daily regimen of other concomitant medications for at least 1 month. This study consisted of 3 stages.

During Stage I, 49 subjects were admitted to the in-patient unit and underwent 2 days of baseline assessments (i.e. Day 1 and Day 2) while continuing their usual pre-study dosing regimen of 3,4-DAP (ranged from 30 to 100 mg/day). Subjects were required to demonstrate a sufficient triple time up and go (3TUG) response in order to progress to randomization in Stage II.

Thirty-two subjects were confirmed to be eligible for Stage II (i.e., having a sufficient 3TUG response during Stage I) and were randomized in a 1:1 ratio to continuous 3,4-DAP (14 subjects) or to taper from their current 3,4-DAP dose to placebo (18 subjects) over a 3-day period (i.e. starting from the evening of Day 2 to the evening of Day 5). After up to 3.5 days of Stage II withdrawal assessments, the usual pre-study 3,4-DAP dosing regimen was reinstituted (Stage III; the morning of the Day 6).

During Stage III, the subjects were observed for 0.5 days or until deemed clinically stable, and then the subjects were discharged from the in-patient unit. The primary efficacy endpoint was the proportion of subjects with at least 30% deterioration of their 3TUG, evaluated at 2-h post dose, upon withdrawal of 3,4-DAP (i.e. at the evening of Day 5) in comparison to their time-matched baseline 3TUG. The results from the primary endpoint assessments are summarized in Table 2.

Table 2: Summary of Primary Efficacy Endpoints for DAPPER Study: Efficacy Population

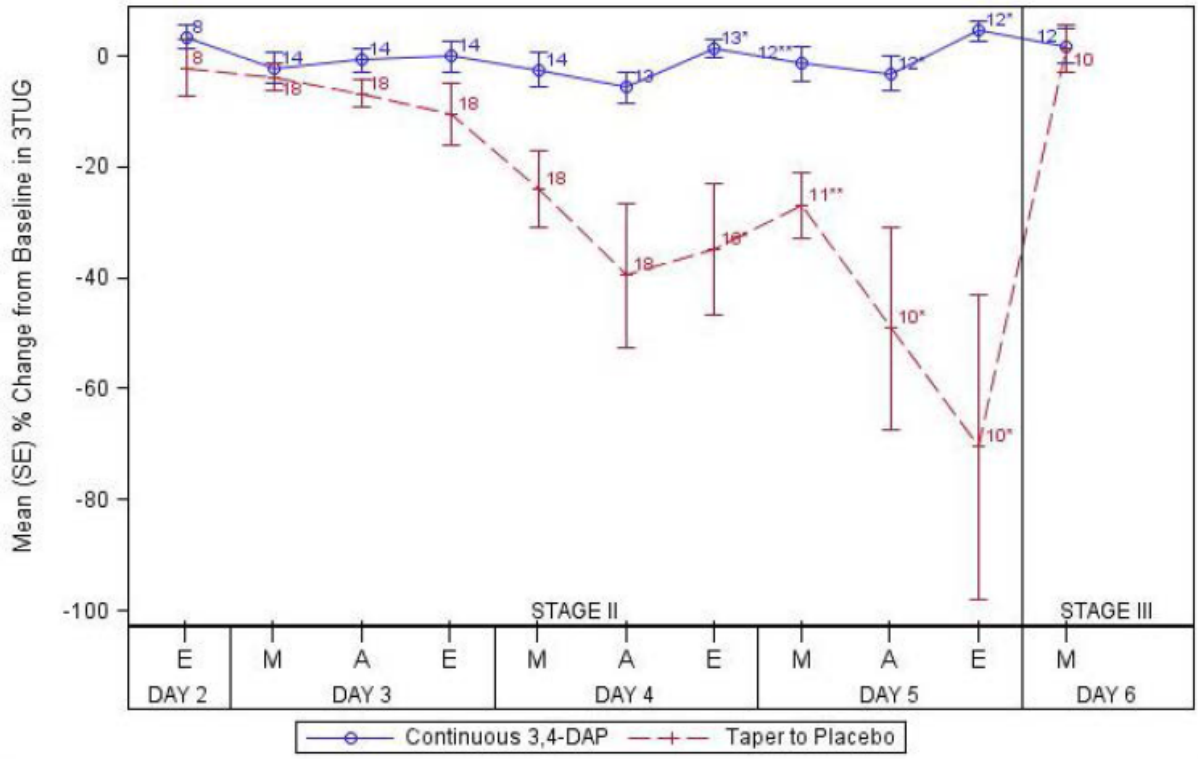
Triple Time UP and GO (3TUG)	**Taper to Placebo** **N = 18** **N (%)**	**Continuous 3,4-DAP** **N = 14** **N (%)**
No change or faster	5 (27.8)	14 (100)
>30% slower	13 (72.2)	0 (0.0)
p-value		<0.0001

Source: Study JPC 3,4-DAPPER CSR.

Individual Dose-Response Relationship in DAPPER:

The individual dose-response relationship observed during the withdrawal phase in Study DAPPER provided additional evidence of effectiveness. For those subjects randomized to taper to placebo, tapering was not done by reducing the dosing frequency, but by reducing the individual dose levels. On Day 2, the evening dose was reduced by 10% of the usual dose. Then, each subsequent dose was reduced so that subjects reached about 50% of their usual dose at the end of Day 3, 25% at the end of Day 4, and 0% (i.e. placebo) in the middle of Day 5. On the morning of Day 6, subjects reinstituted their usual fully active morning dose. The 3TUG was measured at pre-dose and at 2 hours post-dose at the morning, afternoon, and evening of every day. The 2-hour time point was chosen as it was assumed to have maximum effect of the 3,4-DAP on the 3TUG by that post-dose time. Figure 1 presents the percent change from time-matched baseline in 3TUG at 2 hours post-dose versus different time of the day. As the 3,4-DAP withdrawal was implemented in the taper to placebo group on the evening of Day 2, as well as on Days 3, 4 and 5, a deterioration in the 3TUG response was observed. When 3,4-DAP was stopped in the middle of Day 5, the 3TUG response further deteriorated through the evening. The post-dose 3TUGs in the taper to placebo group returned to baseline upon reinstitution of 3,4-DAP with the Day 6 morning dose in Stage III. Taken together, these observations suggest that within an individual, the effect of 3,4 DAP on the 3TUG response is closely related to the 3,4-DAP dose/concentrations.

Figure 1: Mean (±SE) Percent Change from Baseline in 3TUG at 2 Hours After Dosing Versus Time by Treatment Groups: Efficacy Population



M: Morning; A: Afternoon; E: Evening.
Source: Study JPC 3,4-DAPPER CSR.

DUKE Study: This was a prospective, randomized, placebo-controlled study to evaluate the effectiveness of 3,4-DAP in adult subjects with LEMS and to determine the acute and long-term side effects of 3,4-DAP. Eligible subjects were randomized to receive 3,4-DAP 10 mg to 20 mg three to four times per day (TID or QID) or placebo TID or QID in a double-blind phase. Subjects received study drug TID or QID for 6 to 9 days. The primary efficacy measure was the change from baseline Quantitative Myasthenia Gravis (QMG) score in the last 2 days of study drug administration. After the last day of study drug administration, all subjects received no study medication for 24 hours. Subjects then rolled-over to the 6-month open-label phase in which subjects were given 10 to 20 mg 3,4-DAP TID and observed in hospital for 24 hours. The 3,4-DAP dose was subsequently adjusted to produce the maximum symptomatic benefit. When the optimal dose of 3,4-DAP had been determined, pyridostigmine was added, and the optimal combination of 3,4-DAP and pyridostigmine was determined based on the symptomatic response. Results of the primary efficacy analysis is summarized in Table 3.

Table 3: Summary of Change from Baseline in Quantitative Myasthenia Gravis Scores for Study DUKE (Per Protocol Population)

Quantitative Myasthenia Gravis Scores	3,4-DAP N = 12	Placebo N = 13
Change from baseline	–2 (-3- 0)	-0.5 (-1.0 - 1)
p-value	0.015	

Source: JPC 3,4-DAP DUKE RCT Supplement.

In addition to the above two studies, the applicant submitted literature reports for four randomized, double-blind, controlled studies to provide supportive evidence of the efficacy of 3,4-DAP in LEMS.

Efficacy in the pediatric population was extrapolated from the adult data because the pathophysiology of LEMS is expected to be similar between adults and children. The response to therapy and the relationship between drug exposure and therapeutic response can also be assumed to be similar for LEMS (please refer to the clinical review for details). Additionally, clinical experience from the compassionate use program in pediatrics and available clinical safety data from 22 pediatric patients (combined experience in LEMS and CMS) support the use of RUZURGI in pediatrics.

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

The proposed regimen for RUZURGI is a dose titration based on efficacy and tolerability to optimal dose for each patient. The proposed starting dose (b) (4)

The proposed dose titration approach based on tolerability and efficacy is generally acceptable and is consistent with the regimen tested in adult patients in efficacy studies that support this NDA. The review team's proposed starting dose of 15 to 30 mg, maximum allowed single dose of 30 mg (i.e. unit dose) and maximum total daily dose of 100 mg administered in divided doses are based on the efficacy study DAPPER. There is very limited clinical experience beyond 100 mg dose per day in LEMS patients. Since 3,4-DAP has an elimination half-life of 3-4 hours and provides symptomatic relief for short duration, repeat administration of RUZURGI during the day as done in DAPPER study is necessary.

The rationale for the review team's recommendation to include (b) (4) body weight-based dosing scheme are described below:

Pediatric Dosing:

The applicant is seeking approval of RUZURGI (b) (4) This proposal is based on the clinical experience in 7 children with LEMS (age range 9-18 years) using RUZURGI in the compassionate use program. In this program, most children received weight-based doses. Based on this experience, the applicant proposed the following dosing regimen for pediatric patients with LEMS (b) (4)

(b) (4)

The applicant used an extemporaneous suspension formulation to achieve precision dosing in children. The suspension formulation is to be prepared by dissolving a 10-mg tablet in 10 mL sterile water. The appropriate dose is then drawn with a syringe and administered directly into the mouth or through a feeding tube (Please refer to the CMC review for details). There is clinical experience with the suspension formulation from the compassionate use program.

The efficacy data in pediatric patients with LEMS is limited. However, given the pathogenesis of LEMS, the disease is considered similar between adults and pediatric patients. Safety information on pediatric use of RUZURGI is available from 22 subjects (7 subjects with LEMS and 15 subjects with CMS). Please refer to the clinical review for details on disease similarity between adults and pediatrics and the adequacy of pediatric safety information. Based on the disease similarity assumption, matching the exposure in adults and children for efficacy is considered appropriate if available safety information justify such exposure levels in pediatrics. LEMS is reported in children as young as 13 months of age and no treatment options are available for pediatric patients at this time. Therefore, it would be beneficial to expand dosing recommendations (b) (4) if supported by available information.

The applicant submitted the dosing information for 7 pediatric patients with LEMS (age range 9-16 years at the time of starting treatment with 3,4-DAP). The applicant also submitted the dosing information and safety experience for 15 pediatric patients with CMS who were treated with 3,4-DAP and were as young as 13 months of age when they started the therapy. Table 4 summarizes the age and total daily dose when 3,4-DAP was initiated for these populations.

Table 4: Summary of the Available Dosing Information in LEMS and CMS Pediatric Patients

Patient Population	Age at the Start of 3,4-DAP (range)	Dose at the Start of 3,4-DAP (range)
LEMS (N=7)	9-16 years	40-95 mg/day[2]
CMS (N=15)	13 months-14 years	5-120 mg/day

As there was no PK data available in pediatric patients, modeling and simulation approach was utilized to predict the exposures in this population based on the adult PK data. At birth, the majority of clearance pathways are not mature and ontogeny of different clearance pathways vary widely with some pathways reaching adult activity as early as 6 months while others take about 7 years. Once maturation is reached, the differences in exposures between adults and children can be described according to body size, if body weight is a significant covariate for drug exposure. Failure to account for maturation could result in erroneous dosing predictions based only on body weight. Therefore, a systematic review was conducted to understand NAT2 maturation, the major elimination pathway of 3,4-DAP (please refer to Section 4.3 for details). Available information on NAT2 enzyme maturation suggested that by 6 years, NAT2 activity reaches similar levels to that in adults. Given the uncertainty with extent of maturation of NAT2 in pediatric patients less than 6 years of age, dosing instructions for RUZURGI can be provided only for pediatric patients 6 years and older.

The population PK model submitted by the applicant was revised to provide pediatric predictions after verifying the effect of weight on 3,4-DAP clearance from the observed data (please refer to Section 4.2; Figure 7 for details). As the acceptability of bioanalytical method validation for the DAPPER and PK1 studies was still pending during the initial stages of this review, the population PK model was revised to incorporate data from only the TQT study[3]. The TQT study was conducted in healthy volunteers, aged 18 to 62 years, and body weight ranging from 56 to 99 kg. The revised population PK model was then used to simulate the applicant's proposed doses as well as an alternate simplified dosing scheme. Please refer to Section 4.2 for more details.

The review team's revised dosing scheme was aimed at simplifying the dosing scheme. As the lowest weight observed in adults enrolled in the DAPPER study was 45 kg, it can be expected that following the same adult dosing in children weighing 45 kg or more (typically adolescents),

[2] Dosing information available for 6 subjects
[3] The applicant submitted a revised bioanalytical report of the PK1 and DAPPER study on 04/08/2019 in response to an information request sent on 11/21/2018.

the exposure will be similar to the exposures already observed in the adult population. Therefore, the same adult doses are recommended for this group of patients with body weight greater than 45 kg. This is supported by the fact that the labeled doses in adolescents and adults are the same for the majority of drugs with an approved adolescent indication[4].

(b) (4) weighing less than 45 kg, the review team recommends a fixed lower starting dose, maximum single dose and maximum total daily dose. These doses are half the corresponding adult dose. There are no changes to the dosing frequency per day. The recommended dosing scheme is listed in Table 5 below:

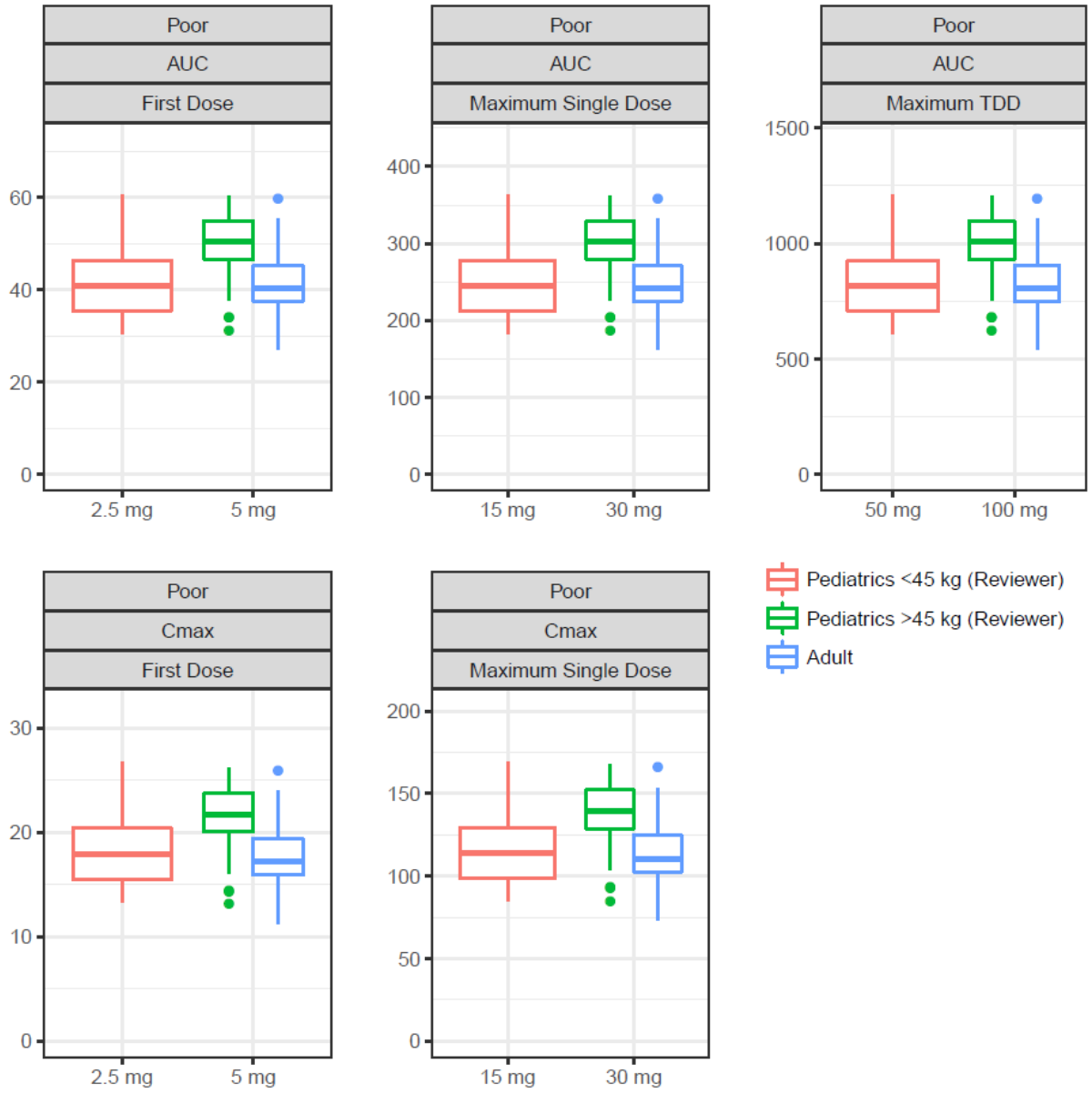
Table 5: Summary of the Recommended Dosing Scheme

Age & Body Weight	Initial dose	Titration Scheme	Maximum single dose	Maximum total daily dose
(b) (4) weighing 45 kg or more	15 mg to 30 mg daily, in divided doses (2 to 3 times per day)	Increase daily in 5 mg to 10 mg increments, up to 5 doses daily	30 mg	100 mg
(b) (4) weighing less than 45 kg	7.5 mg to 15 mg daily, in divided doses (2 to 3 times per day)	Increase daily in 2.5 mg to 5 mg increments, up to 5 doses daily	15 mg	50 mg

Figure 2 presents the simulated exposures (C_{max} and $AUC_{0-\infty}$) in adults and pediatric patients (6-17 year) after administering the doses recommended by the review team. The exposures presented here are for poor metabolizers (the most common phenotype in the Caucasian population). The same patterns are observed for other metabolizer phenotypes (i.e. intermediate and normal metabolizers) as well. As can be seen in Figure 2, the review team's recommended dosing strategy provides a reasonable match between adults and pediatric patients ≥ 6 years. The ratios of the median C_{max} and $AUC_{0-\infty}$ for children ≥ 6 years and <45 kg are 104% and 101%, respectively, relative to the adult median C_{max} and $AUC_{0-\infty}$. For children ≥ 45 kg receiving the adult dose, the ratio of the median C_{max} and $AUC_{0-\infty}$ in this group relative to that of adults is 126% and 125%, respectively.

[4] JAMA Pediatr. 2013 Oct;167(10):926-32.

Figure 2: Comparison of Exposure ($AUC_{0-\infty}$: ng·h/mL and C_{max}: ng/mL) in Pediatric and Adult Patients Following Review Team's Proposed Dosing in Pediatric Population: Poor Metabolizers (TDD is total daily dose)



The recommendation of weight band-based dosing is to simplify the dosing instructions. It should be noted that dose is titrated based on efficacy and tolerability for each subject and there exists an individual dose-response relationship (as illustrated previously in Section 3.3.1).

Table 6 presents the recommended weight band-based dosing versus the reported experience in the LEMS pediatric patients weighing <45 kg (N=3), submitted within this application.

Table 6: Comparison of the Reported Dose Ranges in the Pediatric Patients <45 kg with LEMS and the Recommended Simplified Dosing

Dose	Reported	Recommended
Starting Single Dose	2.5-5 mg [0.07-0.14 mg/kg]	2.5-5 mg [0.06-0.17 mg/kg]
Maximum Single Dose	10-20 mg [0.28-0.39 mg/kg]	15 mg [0.33- 1 mg/kg]
Maximum Total Daily Dose	40-140 mg [0.36-2.18 mg/kg]	50 mg [1.1-3.33 mg/kg]

As noted previously, there is experience with similar or even higher than the recommended 3,4-DAP doses in the pediatric population for other indications. The applicant submitted dosing information for 15 subjects (median age at treatment initiation [range]: 3 years [13 months-14 years]) receiving 3,4-DAP for CMS. The starting, maximum single, and maximum total daily dosing in this group was as high as 5 mg [in one 13 months old patient], 5 mg/kg [in the same 13 months old male], and 120 mg [in a 7 year old patient]. Please refer to the clinical review for more details.

In summary, the recommended dosing (See Table 5 above) in pediatric patients with LEMS (age range: 6- (b) (4) years) is supported by the following:

1) Combined safety data in LEMS and CMS pediatric patients in the recommended age range. There is clinical experience in pediatric patients as young as 13 months at treatment initiation with 3,4-DAP.
2) Clinical assessment on disease similarity between adults and pediatrics for LEMS
3) Available information allows verifying pediatric doses reported from clinical experience and for deriving simplified dosing based on body weight bands for exposure matching to adults, and
4) The ability to individualize therapeutic doses based on the efficacy and tolerability

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

The review team recommends initiating the dose titration with 15 mg daily for (b) (4) patients weighing at least 45 kg with renal/hepatic impairment or who are known NAT2 poor metabolizers. Similarly, the review team recommends initiating the dose titration with 7.5 mg daily for pediatric patients ≥ 6 years and weighing < 45 kg with renal/hepatic impairment or who are known NAT2 poor metabolizers. Individual dose titration based on tolerability and efficacy should account for the individual differences in 3,4-DAP exposure. The effect of intrinsic factors on the exposure of 3,4-DAP is explained below.

NAT2 Metabolizer Status: The pharmacokinetics and systemic exposure to 3,4-DAP is notably influenced by the overall metabolic acetylation activity of the NAT2 enzyme. The NAT2 gene is highly polymorphic and results in phenotypes with variable acetylation activity rates. In the healthy volunteer studies (i.e., Study PK1, and TQT), subjects were genotyped for NAT2 polymorphisms. Metabolizer phenotypes were defined based on genotyping test (refer to Appendix 4.4 for more details).

In the TQT study, poor metabolizers had 1.1 to 3.7 times higher $AUC_{0\text{-}4h}$ and 1.3 to 3.7 times higher C_{max} than intermediate metabolizers, following the first dose. There were only three subjects with normal metabolizer status with PK data (N=3 enrolled in the TQT study) and based on this limited number of subjects, poor metabolizers had 6 to 8.5 times higher $AUC_{0\text{-}4h}$ and 6.1 to 7.6 times higher C_{max} than normal metabolizers, following the first dose.

In the pivotal efficacy study DAPPER, 5 out of 11 genotyped patients were poor metabolizers and were stabilized at different steady state doses ranging from 60mg to 80 mg total daily dose. No specific safety event was noticed in the efficacy study DAPPER even for the 4 poor metabolizer patients stabilized at the 80 mg/day dose.

In conclusion, patients who are poor metabolizers are recommended to start dose titration with 15 mg daily for [redacted] (b) (4) patients ≥ 45 kg and with 7.5 mg daily for pediatric patients ≥ 6 years and <45 kg.

Renal Impairment:

Renal clearance is an elimination pathway for amifampridine and 3-N-acetyl amifampridine. No dose adjustment is recommended based on renal impairment status because individual dose titration is expected to provide optimal dose for each patient.

Although no specific dose adjustment is recommended, these patients should be started at the lowest starting dose when initiating treatment (i.e. 15 mg daily for [redacted] (b) (4) patients ≥ 45 kg and 7.5 mg daily for pediatric patients ≥6 years and < 45 kg)).

Hepatic Impairment: Although 3,4-DAP is eliminated through hepatic metabolism, the effect of hepatic impairment on the exposure of 3,4-DAP was not studied. Similar to patients who are known NAT2 poor metabolizers, patients with hepatic impairment are recommended to start dose titration slowly with the lowest dose (i.e. 15 mg daily for [redacted] (b) (4) patients ≥ 45 kg and 7.5 mg daily for pediatric patients ≥6 years and < 45 kg).

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

In humans, the rate and extent of absorption of 3,4-DAP is influenced by food. There was ~41%-52% decrease in C_{max} and ~9%-23% decrease in $AUC_{0-\infty}$, and a delay in T_{max} by ~1 hour when 3,4-DAP was administered with high fat meal as compared to the fasted state. However, REZURGI is administered multiple times a day and in the pivotal efficacy study (DAPPER), RUZURGI was given without regard to food. Therefore, we agree with the applicant's recommendation of administering RUZURGI tablets without regard to food.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

Plasma concentrations of 3,4-DAP were determined using liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. For studies PK1 and DAPPER, the method was validated (b) (4) The method utilized solid phase extraction with the final extract being analyzed by LC-MS/MS using positive ion electrospray. Calibration concentrations ranged from the lower limit of quantification (LLOQ) of 0.5 ng/mL to 250 ng/mL with a 0.3 mL human plasma aliquot for both 3,4-DAP and 3-Ac-DAP. 15N-3,4-DAP was used as the internal standard for both analytes.

Validation results showed acceptable precision (as determined by the percent coefficient of variation [%CV]) and accuracy at all quality control sample concentrations. The details of the bio-analytical method are presented in Table 7.

Plasma concentrations of 3,4-DAP for the TQT study were determined at Q Squared Solutions BioSciences LLC using a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method. The details of the bio-analytical method are presented in Table 8.

Table 7: Bioanalytical Assay Performance Characteristics: Studies PK1 and DAPPER

Analyte/Parameter	**Amifampridine**
Range (ng/mL)	0.500 – 250 ng/mL
Inter Batch Precision (% CV)	4.71%- 15.23%
Inter Batch Accuracy (%CV)	95.43%-102.73%
Internal standard (IS)	N15 labeled 3, 4-diaminopyridin
Reference standard	3, 4-diaminopyridin
Selectivity	No significant interfering chromatographic peaks were observed at the retention time of 3,4-DAP, or the internal standard
Stability	
Freeze/Thaw Stability (%CV)	3 Cycles (-80 °C) (3.1%-4.4%)
At room temperature (% CV)	2.7%-2.8%
Long-term storage (%CV)	Stable for 28 months at -80 °C (5.5%-7.4%)

Table 8: Bioanalytical Assay Performance Characteristics: Study TQT

Analyte/Parameter	**Amifampridine**
Range (ng/mL)	0.500 – 250 ng/mL
Inter Batch Precision (% CV)	4.71%- 15.23%
Inter Batch Accuracy (% CV)	95.43%-102.73%
Internal standard (IS)	3,4-DAP-d3 (3,4-diaminopyridine-d3)
Reference standard	3, 4-diaminopyridin
Selectivity	No significant interfering chromatographic peaks were observed at the retention time of 3,4-DAP, or the internal standard
Stability	
Freeze/Thaw Stability (%CV)	5 Cycles (-20 to -70°C) (1.2%-3.5%)
At room temperature (% CV)	1.3%-4.2%
Long-term storage (%CV)	Stable for 126 days at -20 °C (1.6%-7.4i %)

Reviewer's comments:

The bioanalyses satisfied the criteria for method validation and application to routine analysis set by the Guidance for Industry: Bioanalytical Method Validation, and hence is acceptable.

4.2 Pharmacometrics Assessment: Population PK Analyses

The applicant originally developed a sequential population PK/PD model based on data from the DAPPER study to analyze the concentrations of 3,4-DAP and 3-Ac-DAP and to describe the relationship between concentration and response (3TUG). During the review, the applicant was encouraged to revise their analysis to (1) include PK data from other studies (i.e., PK1 and TQT), (2) exclude the inactive metabolite 3-Ac-DAP from the analysis and (3) focus on pediatric dosing recommendations. An updated population PK analysis was submitted March 6, 2019. For this review, we focus on the updated analysis only.

Study report: Population Pharmacokinetic Modeling of Amifampridine Free Base (3,4-DAP) in Healthy Subjects with Lambert-Eaton Myasthenia and Pediatric Simulations Designed to Aid Dose Selection in Children

A brief description of the studies and data used in the analysis is provided in Table 9.

Table 9: Summary of the Characteristics of the Studies used for the Pop PK Analysis

Study ID	Subjects	Doses	Description of PK Sampling
PK1	Healthy volunteers (n=16)	Single doses of 20 mg or 30 mg on consecutive days	0, 0.5, 1, 1.5, 2, 3, 4, 7, 12, 16, and 24 hours post-dose
TQT	Healthy volunteers (n=56)	120 mg total dose in 4 equal doses of 30 mg administered at 0, 4, 8 and 12 hours	0, 0.5, 1, 1.5, 2, 3, 3.92, 4.5, 5, 6, 7, 7.92, 8.5, 9, 10, 11, 11.92, 12.5, 13, 14, 15, 16, and 24 hours post-dose
DAPPER	LEMS patients (n=49)	Stable dose of 3,4-DAP; at least 10 mg 3 times per day and up to 100 mg total daily dose	-30, +30, +90 and +150 minutes around each dose prior to randomization; -30 and +90 minutes around dose after randomization

Population PK analysis:

3,4-DAP concentrations were modeled using nonlinear mixed effects modeling software NONMEM (version 7.2, Icon Solutions, Ellicott City, MD, USA). The first-order conditional estimation method with interaction (FOCE-I) was implemented for all model runs. The original population PK analysis found that a two-compartment model was able to adequately describe the time-course of 3,4-DAP concentrations. In the current analysis one and three compartment models were also evaluated. Clinical covariates were explored to explain between-subject variability in the PK of 3,4-DAP using a forward inclusion and backward elimination approach. A forward inclusion ($p<0.05$ and objective function value [OFV] >3.8) and backward elimination

(p<0.01 and [OFV] >6.6) approach was used to evaluate statistical significance for inclusion of covariates in the model. In the population PK model, the following covariates were explored: NAT2 phenotype, body weight (WT), serum creatinine (SCR), age, gender and population type (healthy volunteers vs. LEM patients). The relationship between weight and PK parameters was characterized using an allometric relationship. Other continuous covariates were tested using a power model and centered using the median covariate value for the sample. The categorical covariates were tested using a multiplicative model. The effect of NAT2 phenotype on clearance was evaluated as both categorical and continuous covariate.

Results:

Patient demographics:

A total of 2919 samples (1649 in healthy volunteers and 1270 in LEMS patients) collected from 119 subjects were included in the population PK analysis. Pre-dose samples were excluded from the analysis. A total of 77 plasma samples collected after dose were BQL. Among 119 subjects in the database, 69 healthy volunteers and 11 LEM patients had NAT2 phenotype data. Subjects who had missing NAT2 phenotype data were included in the initial PK model development but excluded from the final model. A summary of clinical and laboratory covariates is provided in Table 10.

Table 10 : Summary of Clinical and Laboratory Covariates for All Patients.

	Healthy Volunteers		LEM patients		Total	
Covariate[a]	N[b]	Median (Range)	N[b]	Median (Range)	N[b]	Median (Range)
Age (years)	72	28 (18-62)	47	61 (23-83)	119	38 (18-83)
Weight (kg)	72	76.0 (50.8-97.2)	47	82.6 (45.8-131.5)	119	77.6 (45.8-131.5)
Serum creatinine (mg/dL)	72	0.9 (0.6-1.2)	47	0.8 (0.5-1.5)	119	0.8 (0.5-1.5)
BUN (mg/dL)	20	10.5 (5.0-17.0)	47	13.0 (3.0-23.0)	67	13.0 (3.0-23.0)
AST (U/L)	72	23.0 (12.0-82.0)	41	24.0 (10.0-50.0)	113	23.0 (10.0-82.0)
ALT (U/L)	72	23.0 (10.0-65.0)	41	22.0 (9.0-58.0)	113	22.0 (9.0-65.0)
Total bilirubin (mg/dL)	72	0.7 (0.3-1.6)	41	0.6 (0.2-1.9)	113	0.7 (0.2-1.9)
Direct bilirubin (mg/dL)	21	0.2 (0.1-0.4)	6	0.2 (0.1-0.5)	27	0.2 (0.1-0.5)
ALP (IU/L)	72	64.0 (36.0-108.0)	41	58.0 (38.0-215.0)	113	62.0 (36.0-215.0)
Albumin (g/dL)	20	4.4 (3.8-4.7)	41	4.0 (3.1-5.0)	61	4.1 (3.1-5.0)
Hemoglobin (g/dL)	72	14.3 (10.9-16.5)	47	14.1 (8.6-16.0)	119	14.2 (8.6-16.5)
Hematocrit (%)	72	41.2 (33.2-47.2)	47	42.3 (28.0-49.1)	119	41.5 (28.0-49.1)
Male[c]	72	47 (65)	47	21 (45)	119	68 (57)
Race[c]	72		47		119	
White		43 (60)		44 (94)		87 (73)
Black or African American		23 (32)		3 (6)		26 (22)
Asian		1 (1)		0 (0)		1 (1)
Other		5 (7)		0 (0)		5 (4)
NAT2 Phenotype[c,d]	69		11		80	
Slow acetylator		35 (51)		5 (45.5)		40 (5)
Intermediate acetylator		31 (45)		5 (45.5)		36 (45)
Rapid acetylator		3 (4)		1 (9)		4 (5)

[a]Descriptive statistics are calculated based on values at the time of first recorded dose.
[b]N signifies the number of subjects with a measurement available for each respective variable.
ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood Urea Nitrogen.
[c] Values for categorical variables were given as N (%).
[d] Three healthy volunteers and 36 LEM patients had missing phenotype data.

Source: DMPC-DAP-03A1, Table A1, Page 41

Population PK model:

A two-compartment model was selected for 3,4-DAP. The M6 (replace first BQL sample with LLOQ/2) was used for BLQ samples. Subjects without NAT2 phenotype were dropped from the final model. The final model included body weight on apparent oral clearance, apparent central volume of distribution, apparent inter-compartmental clearance and apparent peripheral volume of distribution as well as NAT2 phenotype and LEMS population on apparent oral clearance. LEMS patients had 52% lower apparent clearance of 3,4-DAP compared to healthy subjects. The apparent clearance of 3,4-DAP for a healthy adult weighing 70 kg with poor, intermediate and normal metabolizer status was estimated to be 112, 320 and 528 L/h, respectively. Population PK parameter estimates for the final model are summarized in Table 11.

Table 11: Population PK Parameter Estiamtes for the Final Model.

PARAMETER	Final Model		Bootstrap (n=500)[a]		
	Estimate	RSE[b] (%)	2.5th percentile	Median	97.5th percentile
Structural Model					
CL/F[c]=(θ_{CL0}+θ_{NAT}*n)*(θ_{LEMS})^{LEMS} (L/h, 70kg)					
θ_{CL0} (L/h, 70 kg)	112	4	102	112	122
θ_{NAT} (L/h, 70 kg)	208	15	149	211	276
θ_{LEMS}	0.483	16	0.329	0.475	0.652
Vc/F (L, 70kg)	40.6	15	26.5	41.0	54.3
Q /F (L/h, 70kg)	96.5	10	79.3	96.4	121
Vp/F (L, 70kg)	392	7	344	391	440
KA (1/h)	0.96	8	0.817	0.969	1.14
Inter-individual Variability (%CV)					
IIV[d] (CL/F)	42.3	26	29.9	41.5	50.8
IIV[d] (Vc/F)	62.6	46	31.2	63.5	108.3
IIV[d] (Q/F)	45.6	26	31.8	43.8	60.8
IIV[d] (Vp/F)	54.0	19	44.0	53.3	64.3
IIV[d] (KA)	36.2	43	20.6	33.6	50.7
Residual Variability					
Proportional error (%)	27.0	7	24.8	27.0	29.2

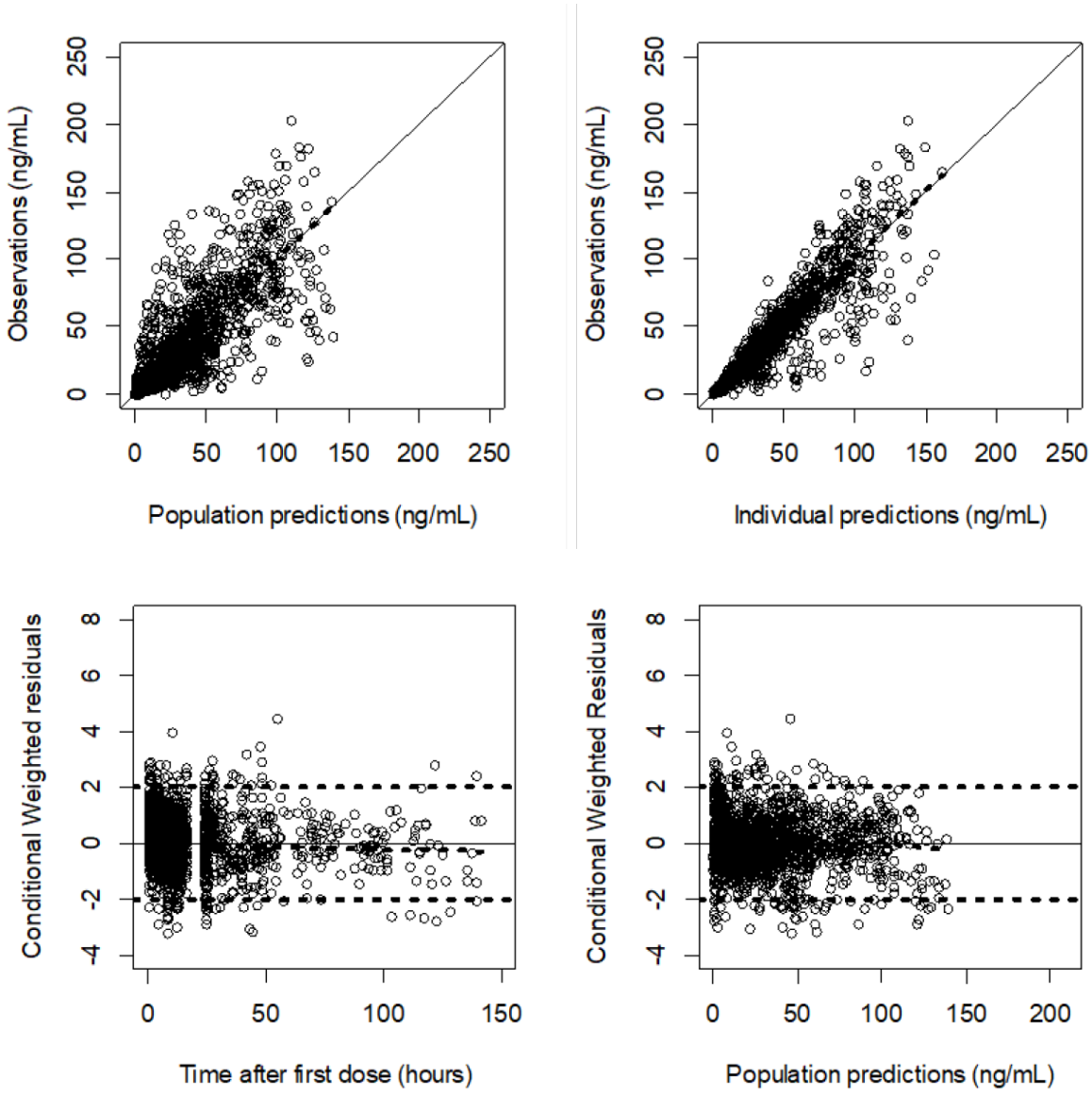
[a]81% of bootstrap datasets converged to >3 significant digits.
[b]RSE: relative standard error.
[c]CL/F=(θ_{CL0}+θ_{NAT}*n)*(θ_{LEMS})^{LEMS} L/h for a 70 kg adult, where n=0 for SLOW; n=1 for INTERMEDIATE; n=2 for RAPID; LEMS=0 for healthy volunteers; LEMS=1 for LEM patients
[d]IIV: inter-individual variability expressed as CV% (CV is the coefficient of variation).

Source: DMPC-DAP-03A1, Table 4, Page 29.

Model evaluations:

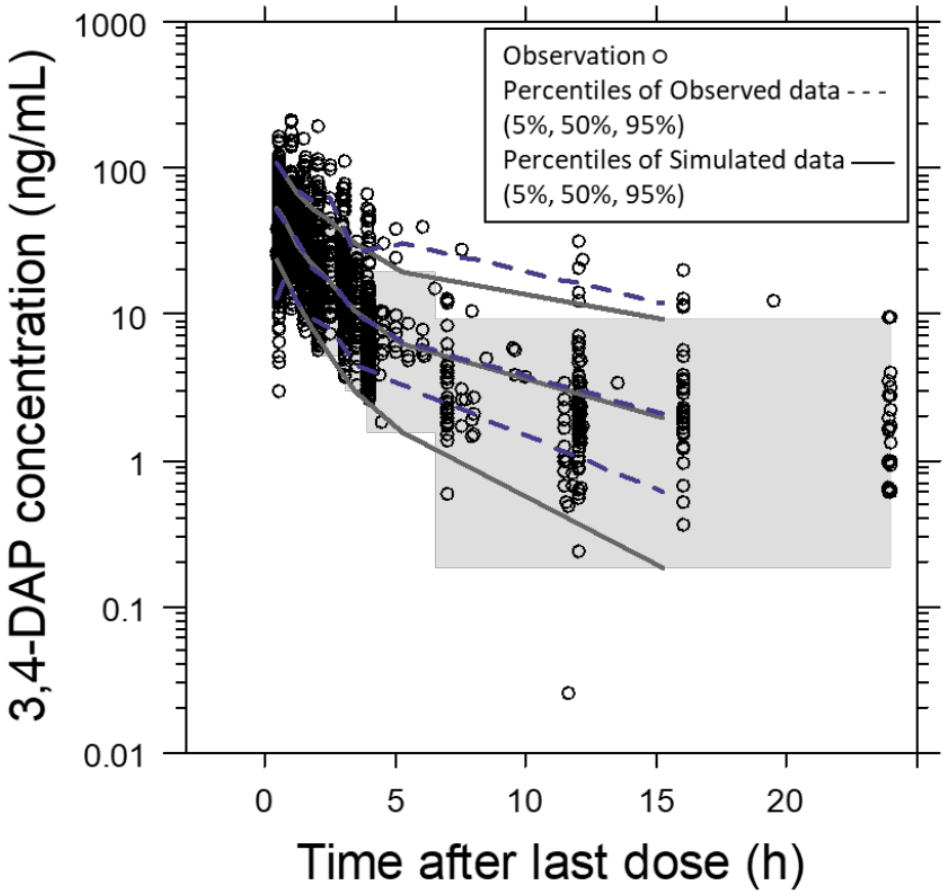
The goodness of fit plots for 3,4-DAP are presented in Figure 3. The visual predictive check is presented in Figure 4.

Figure 3: Goodness of Fit Plots for 3,4-DAP.



Source: DMPC-DAP-03A1, Figure 2 and Figure 3, Page 25.

Figure 4: Visual Predictive Check for 3,4-DAP.



Source: DMPC-DAP-03A1, Figure 4, Page 30.

Reviewer's comments:

The goodness of fit plots indicate that the model predicts the data reasonably well. While there is no information that suggest differences in clearances between patients with LEMS and healthy subjects because of the LEMS disease itself, the differences in CL/F estimation could be due to several factors. For example, patients with LEMS included in the study may have other concomitant diseases such as renal or hepatic impairment which could result in lower CL/F. Of note, there is no known PK based drug-drug interaction for 3,4-DAP. Therefore, the effect of concomitant medications administered in DAPPER is not expected to be a factor affecting exposure. While the analysis was being performed by the applicant, the review team did not have adequate data to confirm the adequacy of the assay validation for studies PK1 and

DAPPER. Therefore, the reviewers also performed an independent analysis using data only from the TQT study. See the reviewer's analysis section below for comments on the comparability of the two analyses.

Dosing Simulations

(b) (4)

For the virtual pediatric population, 500 patients were generated for 6 to < 12 years and 12 to < 18 years using the PK-Sim software. Height and weight for age were assigned according to the distributions from the National Health and Nutrition Examination Survey (NHANES) database. NAT2 phenotype was assigned based on the distribution observed in the merged dataset with healthy volunteers and LEMS patients. An adult virtual population was also generated using age and BMI ranges of 18-65 years and 18-30 kg/m^2, respectively. A summary of the demographics of the virtual populations is provided in Table 12.

Table 12: Demographic Data in Virtual Populations

	6-<12 years	12-<18 years	Adult
N	500	500	500
Age (years)	9.1 (6-11)	14.8 (12-17)	38 (18-62)
Body weight (kg)	33.7 (17.9-114.0)	60.7 (36.9-126.3)	70.9 (52.1-96.3)
Female (%)	55	55	55
Race N (%)			
White	460 (92)	460 (92)	460 (92)
Black	40 (8)	40 (8)	40 (8)
Asian	0 (0)	0 (0)	0 (0)
Other	0 (0)	0 (0)	0 (0)
Phenotype[a] N (%)			
Slow	245 (49)	245 (49)	245 (49)
Intermediate	230 (46)	230 (46)	230 (46)
Rapid	25 (5)	25 (5)	25 (5)

[a] Values for demographic data were given as median (range); N is number of subjects in each group.
[b] For all virtual populations, the NAT2 phenotype distribution was the same in white and black.

Source: DMPC-DAP-03A1, Table 7, Page 32.

The dosing regimen simulated for adults was based on the median total daily dose of 60 mg and the median number of doses per day of 4 (i.e. 15 mg 4x/day). Adult doses were used for simulations in the adolescent (12 to < 18 years) population. For children (6 to < 12 years), body weight based dosing (adult dose per 70 kg adult body weight or 0.214 mg/kg 4x/day) was rounded to 1 decimal place with a maximum single dose of 15 mg. PK samples at 0, 0.17, 0.33, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 6 hours after dose were created for each virtual patient. The area under the concentration-time curve over 24 hours at steady-state ($AUC_{0\text{-}24,SS}$) was calculated as the dose administered over 24 hours at steady-state divided by the apparent clearance of 3,4-DAP. Results are presented in Table 13 and Figure 5.

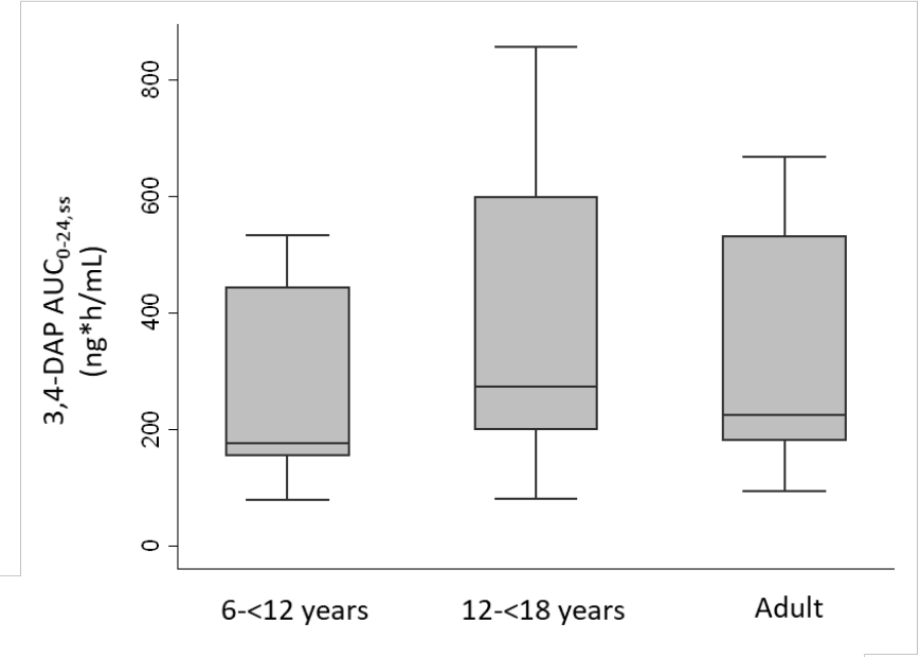
Table 13: Simulated Steady-State $AUC_{0\text{-}24,SS}$ (ng h/mL) in Pediatric and Adult Populations

	6-<12 years	12-<18 years	Adult
All	177.65 (79.92-532.72) N=500	273.13 (80.87-855.60) N=500	225.60 (94.51-668.28) N=500
Acetylator phenotype subgroups			
Rapid acetylators	98.21 (79.92-105.28) N=25	129.10 (80.87-152.56) N=25	115.57 (94.51-130.92) N=25
Intermediate acetylators	155.87 (130.06-185.59) N=230	206.29 (126.22-303.08) N=230	183.66 (147.65-233.11) N=230
Slow acetylators	444.84 (381.21-532.72) N=245	600.80 (344.15-855.60) N=245	533.80 (423.56-668.28) N=245

[a] Values for steady-state $AUC_{0\text{-}24,ss}$ were given as median (range); N is number of subjects in each group.

Source: DMPC-DAP-03A1, Table 9, Page 34.

Figure 5: Steady-State $AUC_{0\text{-}24,SS}$ (ng h/mL) in Pediatric and Adult Populations



Source: DMPC-DAP-03A1, Figure 7, Page 35.

Reviewer's comments:

The applicant's PK simulations suggest that at the proposed doses, adolescents have similar, but slightly higher (median within 20%) exposure and children 6 to < 12 years have similar but slightly lower (median within 20%) exposure compared to adults. It is worth noting that dosing is defined by age (6 to 12 years of age) in addition to weight, even though weight is the relevant variable for exposure. Also, the applicant assumed the pediatric dose was rounded to 1 decimal place, which may be an ideal scenario. We note that the choices used for comparison (healthy vs. LEMS patients, NAT2 phenotype and dose level) are not critical because 3,4-DAP exhibits dose proportional PK and relative, rather than absolute AUC measures, are more relevant for the simulation exercise.

Reviewer's Analysis:

While awaiting additional details regarding the bioanalysis of the PK1 and DAPPER studies, the reviewer performed an independent analysis including only the PK data from the TQT study. The revised model evaluated the inclusion of weight using an allometric scaling approach. The model also accounted for differences in both clearance and relative bioavailability in different NAT2 phenotypes. The reviewer also intended to evaluate the effect of weight-corrected creatinine clearance (estimated using Cockcroft Gault equation; C-G), gender, and age on 3,4-

DAP exposures. Simulations also considered Cmax as well as other PK metrics following the first dose and the maximum single dose.

Objectives:

1) To perform an analysis using PK data from the TQT study only.
2) To use the model to propose dosing recommendations in the pediatric population.

Method:

Dosing and PK sampling:

A summary of the TQT study design, dosing, number of subjects included in the PK dataset and PK sampling plans is included in Table 14.

Table 14: Summary of TQT Study Design and PK Data Included in the Model

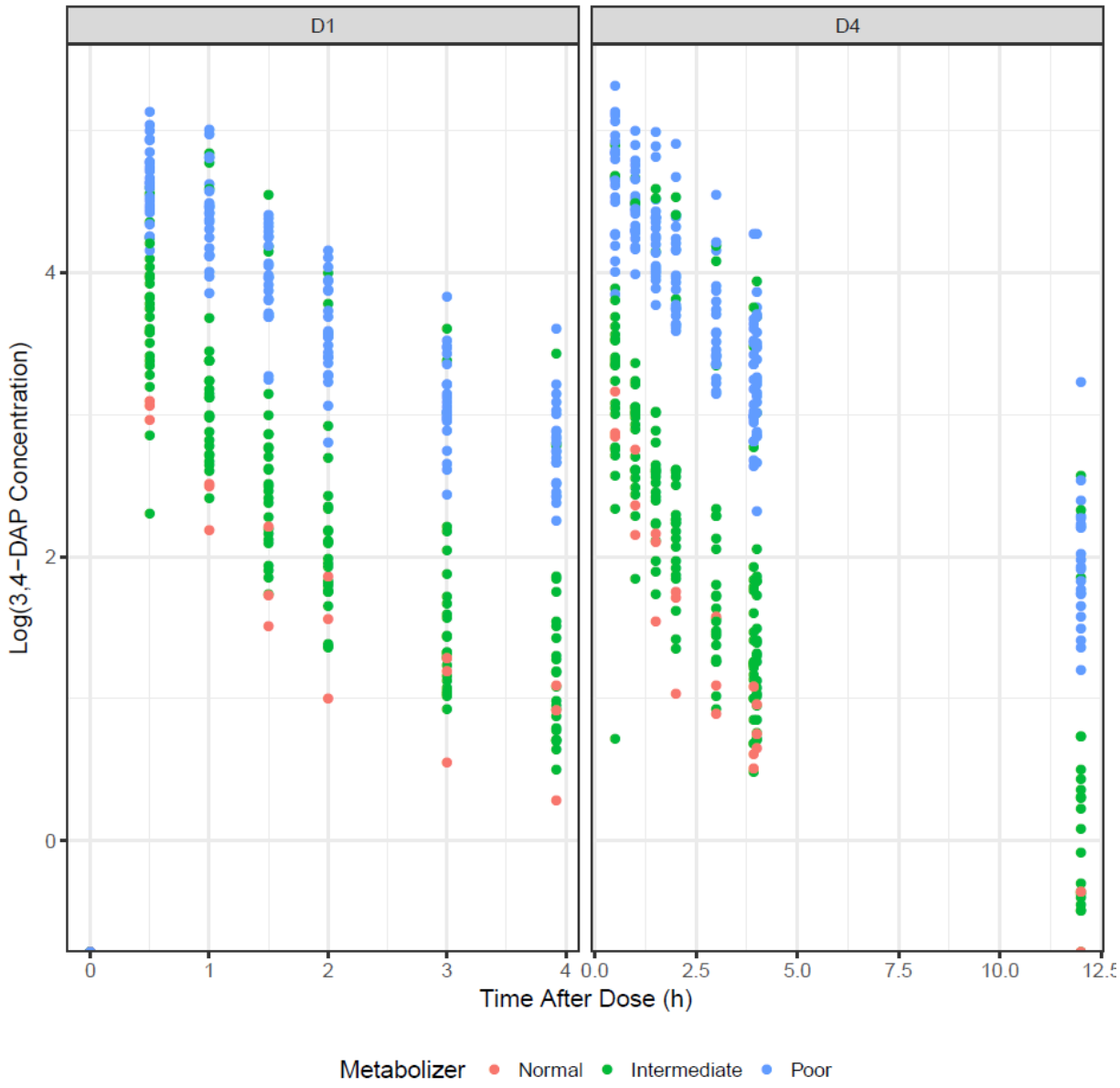
Study	Design	N	Dose	PK Sampling Points
TQT	A Phase I, double-blind, double-dummy, placebo and positive controlled (moxifloxacin 400 mg single dose, oral formulation), 3-way crossover TQT study. In each period, subjects received 1 of the following treatments: • 3,4-DAP placebo in 4 divided doses Q4h. • Active moxifloxacin (400 mg) was administered with the first dose of the day (positive control). • Active 3,4-DAP (120 mg total dose) in 4 equally divided doses administered Q4h.	52[5]	120 mg total dose in 4 equally divided doses[6]	Blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 3.92, 4.5, 5, 6, 7, 7.92, 8.5, 9, 10, 11, 11.92, 12.5, 13, 13.5, 14, 15, 16, 24 hours following the first study medication administration for each subject to measure both 3,4-DAP and 3-Ac-DAP

[5] For 3,4-DAP and 3-Ac-DAP, the PK analysis set included 52 subjects for Dose 1 (51 subjects for 3-Ac), 46 subjects for Dose 2, 45 subjects for Dose 3, and 43 subjects for Dose 4.
[6] 9 patients who are poor and intermediate metabolizers received <120 mg doses (dose range: 30-110 mg). One discontinued after taking only a single 30 mg dose of 3,4-DAP. Eight other subjects received less than the full 120 mg during the 3,4-DAP treatment arm: 5 subjects skipped one or two doses. Six subjects had a dose reduction during the 3,4-DAP treatment arm. Skipped and reduced doses were not unique to the 3,4-DAP treatment arm.

Data visualization:

The concentration time profile of 3,4-DAP, stratified by NAT2 metabolizer phenotypes, was plotted (Figure 6). The profile is consistent with the applicant's proposal of a 2-compartment model. Therefore, the structural model was kept as a two-compartment model.

Figure 6: Plasma PK Profile Plotted on a Log Scale After the First (D1) and Fourth (D4) Dose Administration in the TQT Study

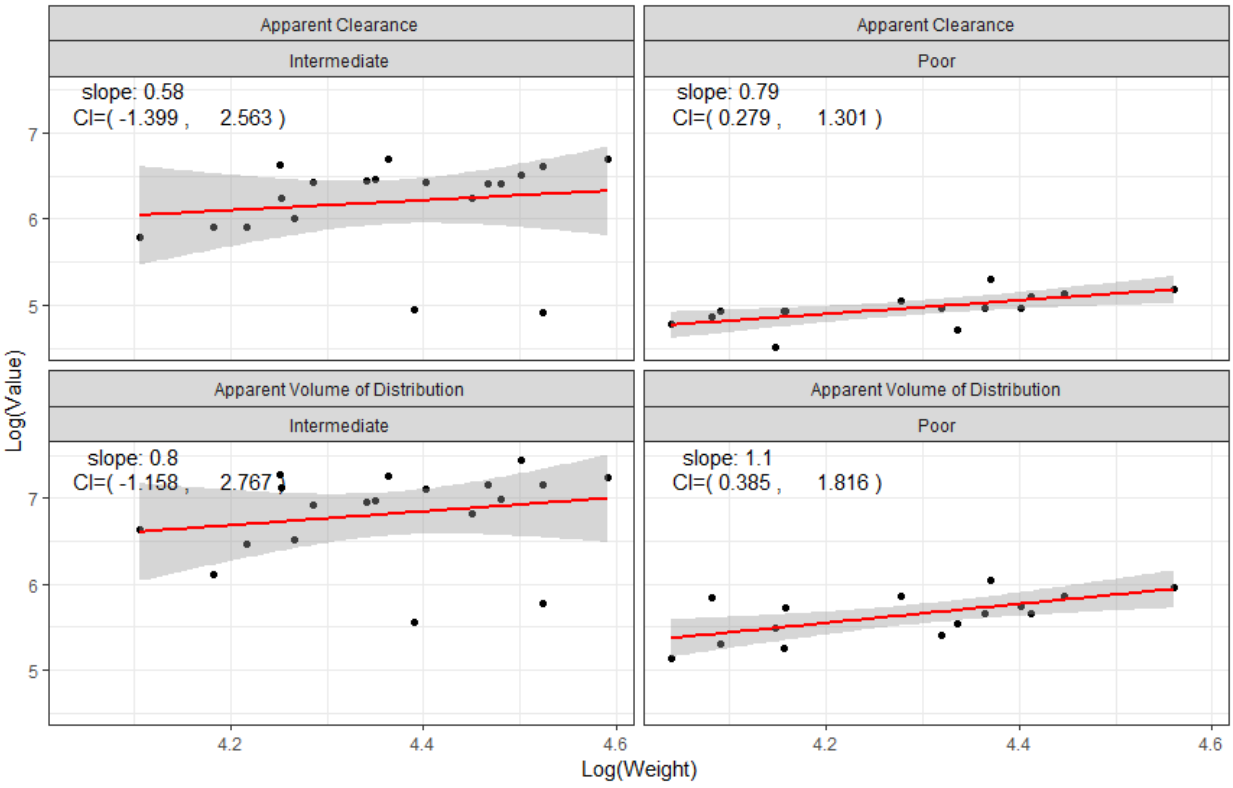


D1: first dose; D4: 4th dose. Note that rich PK sampling occurs after the first and 4th doses in the TQT study.

Relationship between 3,4-DAP PK parameters and body weight:

The relationship between CL/F and V/F obtained from the non-compartmental analysis from the TQT study versus body weight (range: 57.6 kg to 98.3 kg) on a log-log scale and stratified by NAT2 phenotypes is presented in Figure 7. Data were only plotted for poor and intermediate metabolizers as the CL/F and V/F were only available from 2 normal metabolizers enrolled in this study. The slope of the relationship appears to be consistent with allometric scaling principles.

Figure 7: Relationship Between 3,4-DAP PK Parameters and Weight



CI is the 95% confidence interval around the slope.

Structural and covariate model development:

Population PK analysis using the nonlinear mixed-effect modeling approach was conducted with the NONMEM software, Version 7.3 (ICON, Ellicott City, MD, USA). The first-order conditional estimation with the eta-epsilon interaction method in NONMEM was employed for all model runs. Concentrations that were below the limit of quantification occurred late in the PK profile

(i.e., 24 hours or 12 hours post last dose[7]) or at pre-dose time and thus, they were dropped from the analysis.

Structural model

A two-compartment model was chosen based on the previous model developed by the applicant and data visualization. All interindividual error terms were described by an exponential error model. An attempt was made to define a full-block covariance matrix for the interindividual random effects. Combined (i.e. proportional and additive) and proportional error models were tested for the residual error model.

Covariate model

Covariates investigated in this analysis included body weight, NAT2 phenotypes (poor, intermediate and normal), age, weight-corrected creatinine clearance estimated using C-G equation, and gender. Empirical Bayes estimates (EBEs) from the base model were graphically examined for trends between covariates and PK parameters.

Age, gender and body weight-corrected creatinine clearance were tested on CL/F after adjusting for body weight and NAT2 phenotypes using a forward addition/backward deletion approach. During the forward addition step, a covariate was considered significant based on a p-value of 0.01 using the likelihood ratio test. Following the identification of the full model, model reduction based on backward deletion was implemented whereby covariates were eliminated from the full model. The statistical significance was individually assessed during the stepwise deletion phase at a p-value < 0.001 level, i.e., only covariates associated with an increase of at least 10.83 (with 1 degree of freedom) in OFV were retained in the model.

The power model was used for continuous covariates. A separate fixed effect parameter for each level of a categorical covariate was used for categorical covariates. The exponent on body weight was estimated in one run to evaluate if the confidence intervals include the theoretical exponent values for the clearance and volume. Once verified, it was fixed to 0.75 for clearances, and a value of 1 for volumes.

Model diagnostics and evaluations:

The final model performance was evaluated by visual inspection of the basic goodness of fit plots and the prediction-corrected visual predictive checks (pc-VPC; N=200). Although most subjects received the same dose (120 mg administered as 4 equally divided dose every 4 hours), some subjects received lower doses.

[7] 5 subjects (3 with IM and 2 with NM phenotypes had BLQ at 12 hours post the last dose of the day).

Simulations:

The objective of the simulations is to evaluate the proposed dosing for pediatric patients. Once the final model was developed, the final fixed effect parameter estimates were used for simulations. The random effect parameter estimates were fixed to zero. Given the objective of the simulation and for simplicity of decision making, this approach is considered reasonable.

The dataset for adults was developed by sampling with replacement (N= 100 adults) from all adult subjects included in the 3 studies submitted within this application (i.e. PK1, DAPPER, and TQT). The pediatric dataset (N=200, age=6-18 years) was created utilizing the dataset cdc.wt in library AGD. Different percentiles of weights were calculated using the LMS transformation equation[8].

The adult data was simulated using 15 mg total daily dose in 3 equally divided doses (i.e. 5 mg Q8h) for the first day, followed by 90 mg total daily dose in 3 equally divided doses (i.e. 30 mg Q8h) for 2 days, followed by 100 mg total daily dose for 2 days. For the purposes of simulation and to calculate AUC, the 100 mg dose was given as a single dose on those days. AUC was defined as steady-state AUC and was calculated as Dose/Clearance. Two simulation scenarios for pediatric dosing were performed as shown in Table 15 to evaluate both the applicant's body weight-based dosing proposal as well as a weight band flat dosing.

Table 15: Simulation Scenarios in Pediatric Population

Scenario	Initial Dose	Maximum Single dose	Maximum total daily dose
1*	0.07 mg/kg Q8h for the 1st day	0.3 mg/kg Q8h for 2 days	1.4 mg/kg Q24h for 2 days
2	2.5 mg for pediatric patients < 45 kg and 5 mg for pediatric patients ≥ 45 kg Q8h for the 1st day	15 mg for pediatric patients < 45 kg and 30 mg for pediatric patients ≥ 45 kg Q8h for 2 days	50 mg for pediatric patients <45 kg and 100 mg for pediatric patients ≥ 45 kg Q24h for 2 days

*The dose in scenario 1 for pediatric patients was rounded to the nearest 1 mg. The maximum single dose was not allowed to exceed 20 mg and the maximum total daily was not allowed to exceed 100 mg.

The exposure parameters (C_{max} and $AUC_{0-\infty}$) were stratified for the pediatric population based on body weight (i.e. < 45 kg and ≥ 45 kg). The lower age bound of 6 years was chosen based on

[8] https://www.cdc.gov/nchs/data/series/sr_11/sr11_246.pdf

the maturation of NAT2 enzymes in the pediatric population. The 45 kg weight cut-off was chosen based on the observed minimum weight in the adult dataset.

Results:

Patient demographics at baseline for the TQT study included in the analysis are summarized in Table 16.

Table 16: Summary of Covariates of Subjects in the Population Pharmacokinetic Analysis

Study	Acetylation Status	CRCL mL/min (Median, Range)	Weight (Median, Range) Kg	Age (Median, Range) Years
TQT	PM: 23 (44.2%) IM: 26 (50%) NM: 3 (5.77%)	115 (73.3-189)	77.2 (55.9-98.5)	27.5 (18-62)

PM: Poor metabolizer, IM: Intermediate metabolizer, NM: Normal metabolizer

Population PK modeling results:

A two-compartment model with first-order absorption described the data reasonably well. Because no reference intravenous data were available, the model was parameterized in terms of CL/F, V/F and absorption rate constant (Ka). V/F and CL/F were allometrically scaled by weight using a power model, with the exponents fixed to 0.75 for CL/F and 1 for V/F. In addition, the effect of NAT2 phenotypes on clearance and bioavailability were included in the final model. The effect of the NAT2 phenotypes on the bioavailability were modeled by assuming bioavailability of 1 for poor metabolizers and estimating 2 separate thetas for the intermediate and normal metabolizers. The final parameter estimates are included in Table 17.

Based on these parameter estimates, the terminal phase elimination half-life is estimated to be 4.7, 3.9, and 3.9 in poor, intermediate, and normal metabolizers, respectively. The model also predicts 3.1, and 6.4 times higher AUC in intermediate, and normal metabolizers as compared to poor metabolizers. The predicted Vss/F values are 357, 695, and 1383 L for poor, intermediate, and normal metabolizers, respectively. These predictions are in agreement with the results from non-compartmental analyses. The predicted CL/F values are 115, 361 and 740 L/h for poor, intermediate and normal metabolizers. These estimates compare favorably to the ones derived from the applicant's analysis (112, 320 and 528 L/h for poor, intermediate and normal metabolizers in healthy volunteers), even though the applicant included different data and used a different covariate modeling strategy. Differences in clearance estimates for normal metabolizers in the two analyses (740 L/h vs. 528 L/h) are not surprising given the limited patient numbers. Overall, the reviewer's analyses provides an independent check of the

applicant's analyses and suggests that any differences in pediatric dosing simulations between the applicant and reviewer are due to the selected dosing regimen, and not because of the modeling strategy.

Table 17: 3,4-DAP Fixed- and Random-Effect Parameter Estimates for the Final Model

Fixed Effect			Random effect		
Description	Estimate	RSE	Description	Estimate	RSE
CL (L/h) for PM= $\theta 1*(WT/70)^{0.75}$	115	5%	ω_{CL}, CV%	44%	19%
CL (L/h) for IM= $\theta 6*(WT/70)^{0.75}$	185	9%	$\omega_{CL,Vc}$, CV%	-0.06%	76%
CL (L/h) for NM= $\theta 7*(WT/70)^{0.75}$	191	18%	ω_{Vc}, CV%	80%	19%
F for PM	1	Fixed	ω_{Q}, CV%	33%	
F for IM ($\theta 8$)	0.513	13%	$\omega_{Q,Vp}$, CV%	0.09%	41%
F for NM ($\theta 9$)	0.258	24%	ω_{VP}, CV%	31%	24%
Vc (L)= $\theta 2*(WT/70)$	36.7	9%	ω_{KA}, CV%	27%	17%
Q (L/h)= $\theta 3*(WT/70)^{0.75}$	82	7%	$\varepsilon_{proportional}$, CV%	24%	5%
Vp (L)= $\theta 4*(WT/70)$	320	6%			
KA (1/h)= $\theta 5$	1.13	7%			

RSE is the relative standard error. CV% for the between subject variability for the parameters were calculated as sqrt(exp(ω^2)-1). CV% for the residual variability was calculated as sqrt(ε^2).

Model evaluations:

The basic goodness of fit plots for the final model are included in Figure 8. The prediction corrected visual predictive checks (pc-VPC), pc-VPC stratified by both acetylation phenotypes, and by weight band (WTB; ≤70 kg and >70 kg) are presented in Figure 9 and Figure 10, respectively. As the stratification reduced the number of subjects per strata, we were primarily concerned with evaluation of the model's ability to capture the central tendency of the data across different strata.

Figure 8: Goodness of Fit Plots for the Final Model

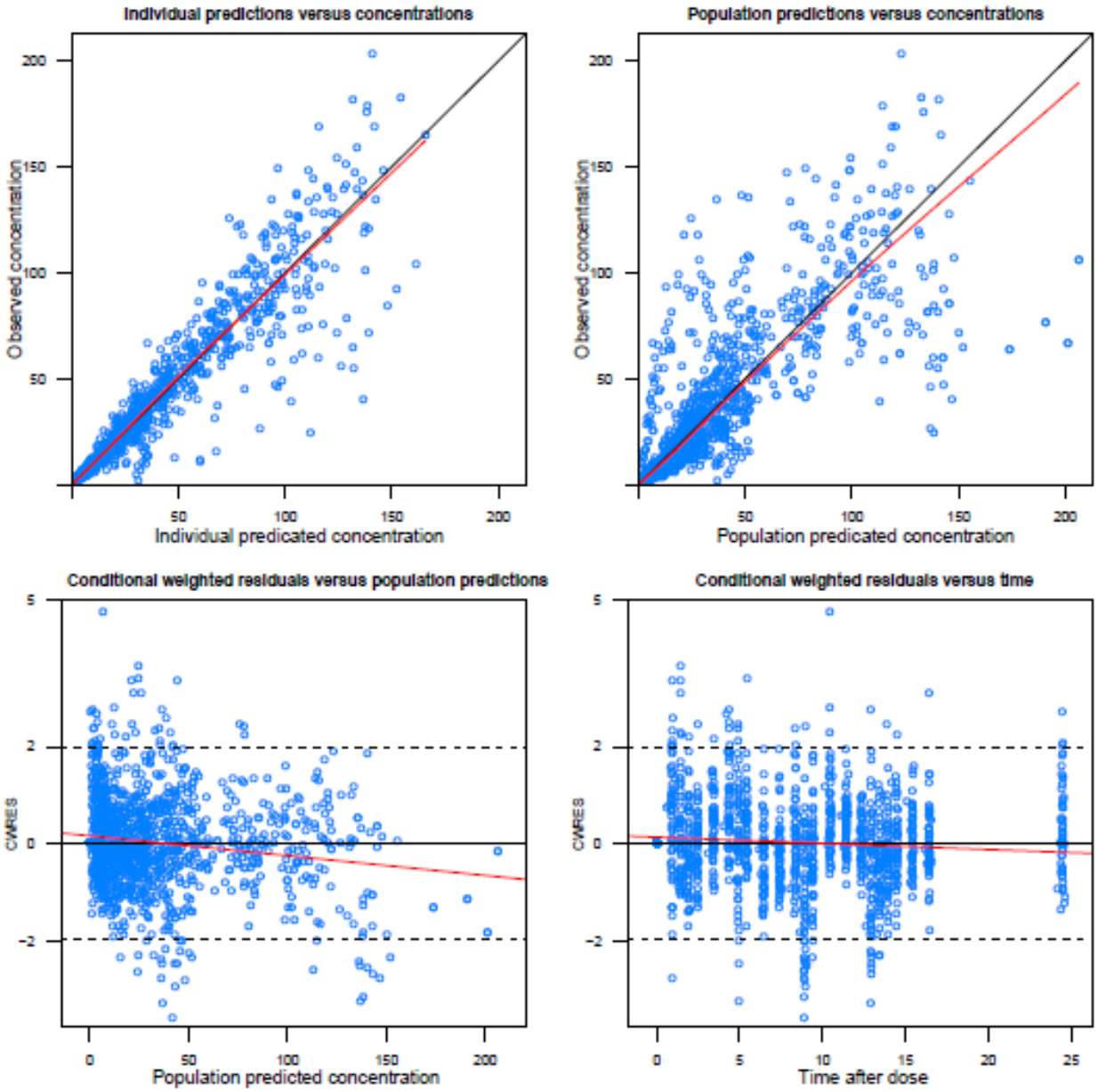
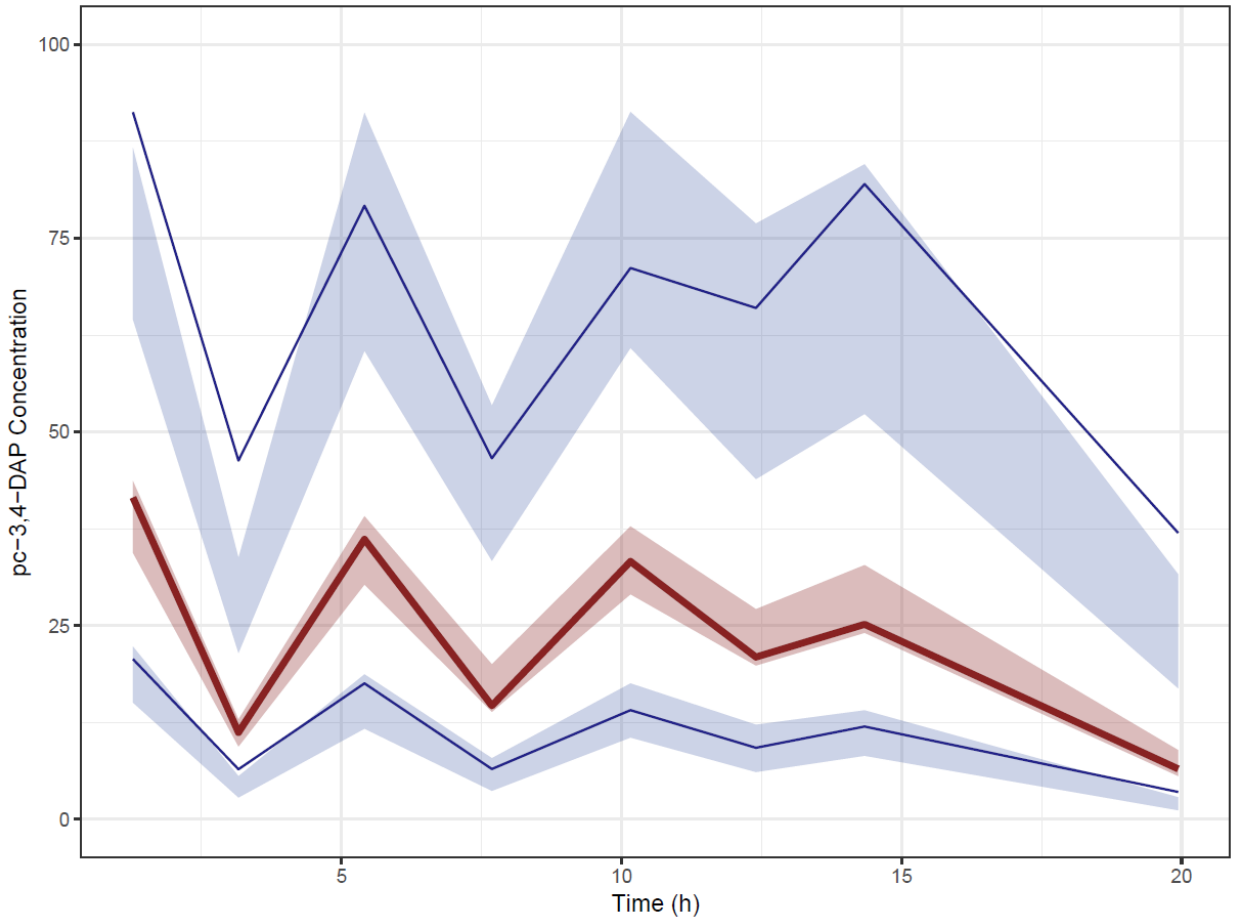
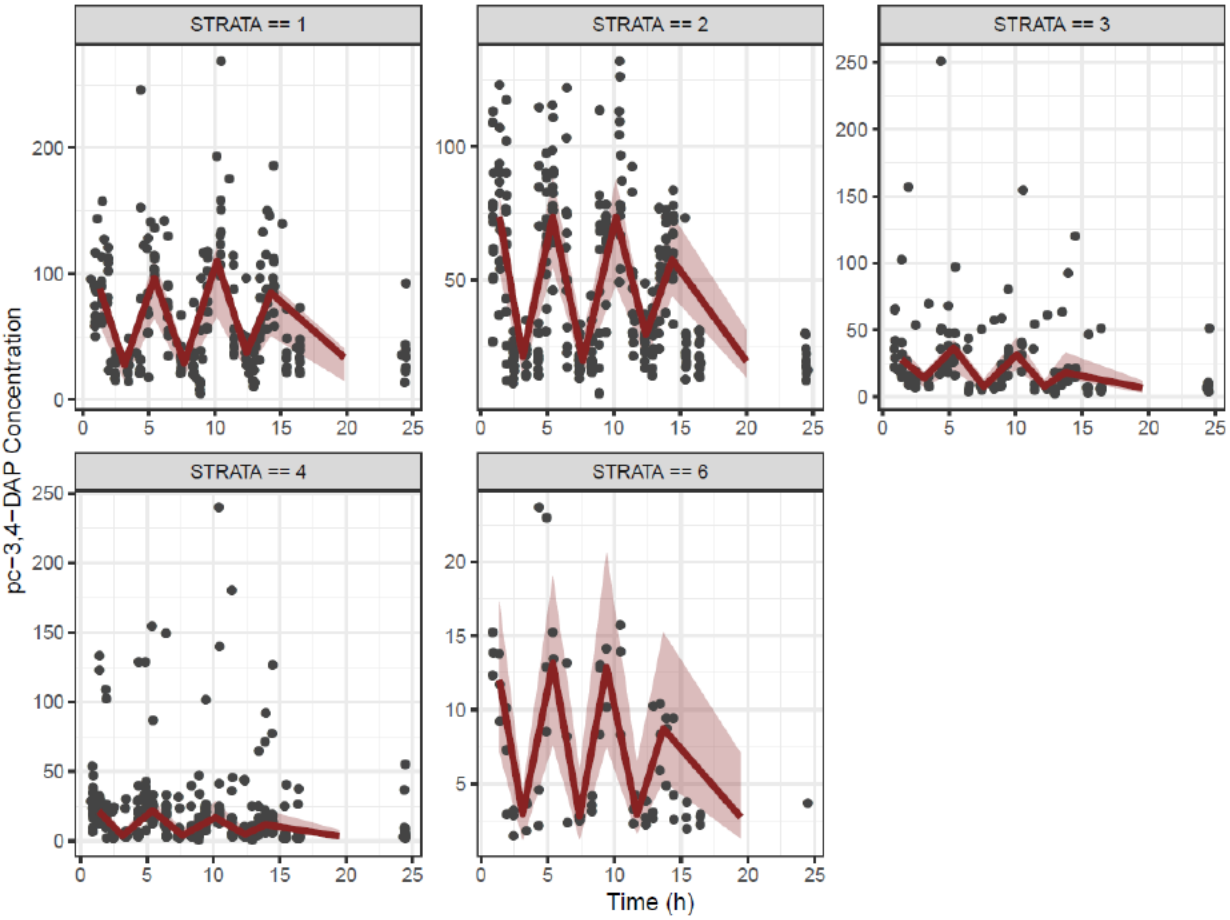


Figure 9: Prediction-Corrected VPC



The solid red and blue lines present the median and 90th percentiles, respectively of the observed prediction-corrected concentrations. The shaded areas present the 90th prediction intervals for each percentile (i.e. 5th, 50th, and 95th percentiles) based on 200 simulated datasets.

Figure 10: Prediction and Variability Corrected VPC Stratified by Acetylation Phenotype and Weight Band



The solid red line presents the median of the observed concentrations. The shaded areas present the 90th prediction interval of the median based on 200 simulated datasets. The solid points are the observed prediction-corrected 3,4-DAP concentrations.

Strata		N of Subjects (%)
1	PM with body weight less than or equal 70 kg	10 (19%)
2	PM with body weight greater than 70 kg	13 (25%)
3	IM with body weight less than or equal 70 kg	7 (13.5%)
4	IM with body weight greater than 70 kg	19 (36.5%)
5	NM with body weight less than or equal 70 kg	0 (0%)
6	NM with body weight greater than 70 kg	3 (6%)

Simulations:

Results of the simulation scenarios (described in Table 15) are presented below. Figure 11 and Figure 12 present the predicted exposures in adults and pediatric patients ≥ 6 years and stratified by body weight (i.e. <45 kg and ≥45 kg) for simulation Scenarios 1 and 2, respectively. Because the main aim is to show the exposures in children relative to adults, only the exposures in the most common phenotype in US (i.e., poor metabolizers) are shown. The relative exposures between adults and children in the 2 other phenotypes (i.e. intermediate and normal metabolizers) follow the same trend and therefore, are not presented.

The differences in the median exposures (C_{max} and $AUC_{0-\infty}$) in children ≥ 6 years and < 45 kg as well as in children ≥45 kg versus adults following different doses under the previously mentioned simulation scenarios are presented in Table 18.

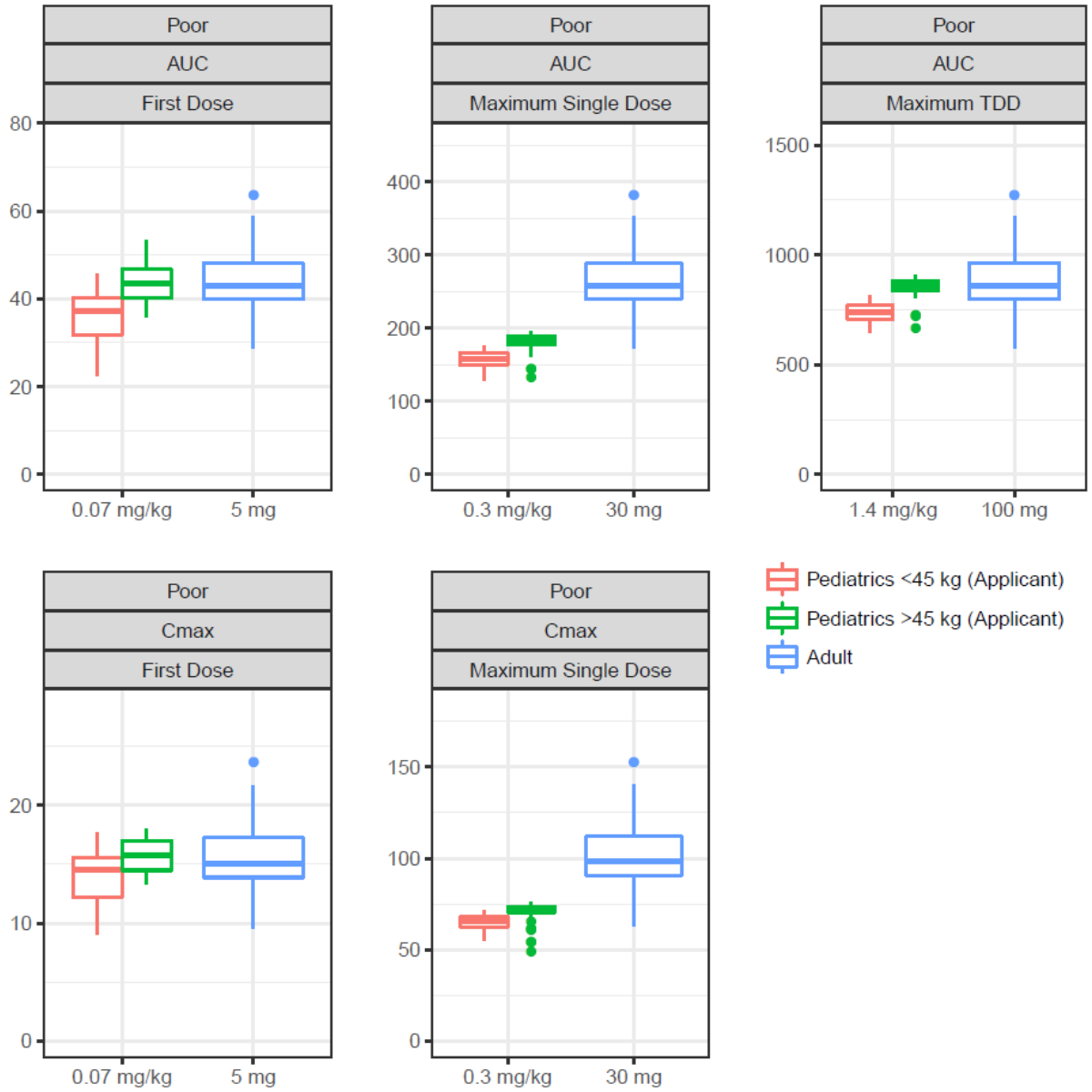
It can be seen that the recommended simplified regimen (Scenario 2) results in similar exposures between adults and children. The need for easy to implement/remember dosing regimen is important given that the 3,4-DAP is administered multiple times per day.

Table 18: Relative Median Exposures in Children versus Adult Following Different Dosing Scenarios

Scenario	Dose	Children≥6 years and <45 kg versus Adult		Children ≥45 kg versus Adult	
		C_{max} (Child/Adult) %	$AUC_{0-\infty}$ (Child/Adult) %	C_{max} (Child/Adult) %	$AUC_{0-\infty}$ (Child/Adult) %
1	First Dose	97	87	104	102
	Maximum Single Dose	67	61	73	71
	Maximum TDD	NA	86	NA	100
2	First Dose	104	101	126	125
	Maximum Single Dose	103	101	126	125
	Maximum TDD	NA	101	NA	125

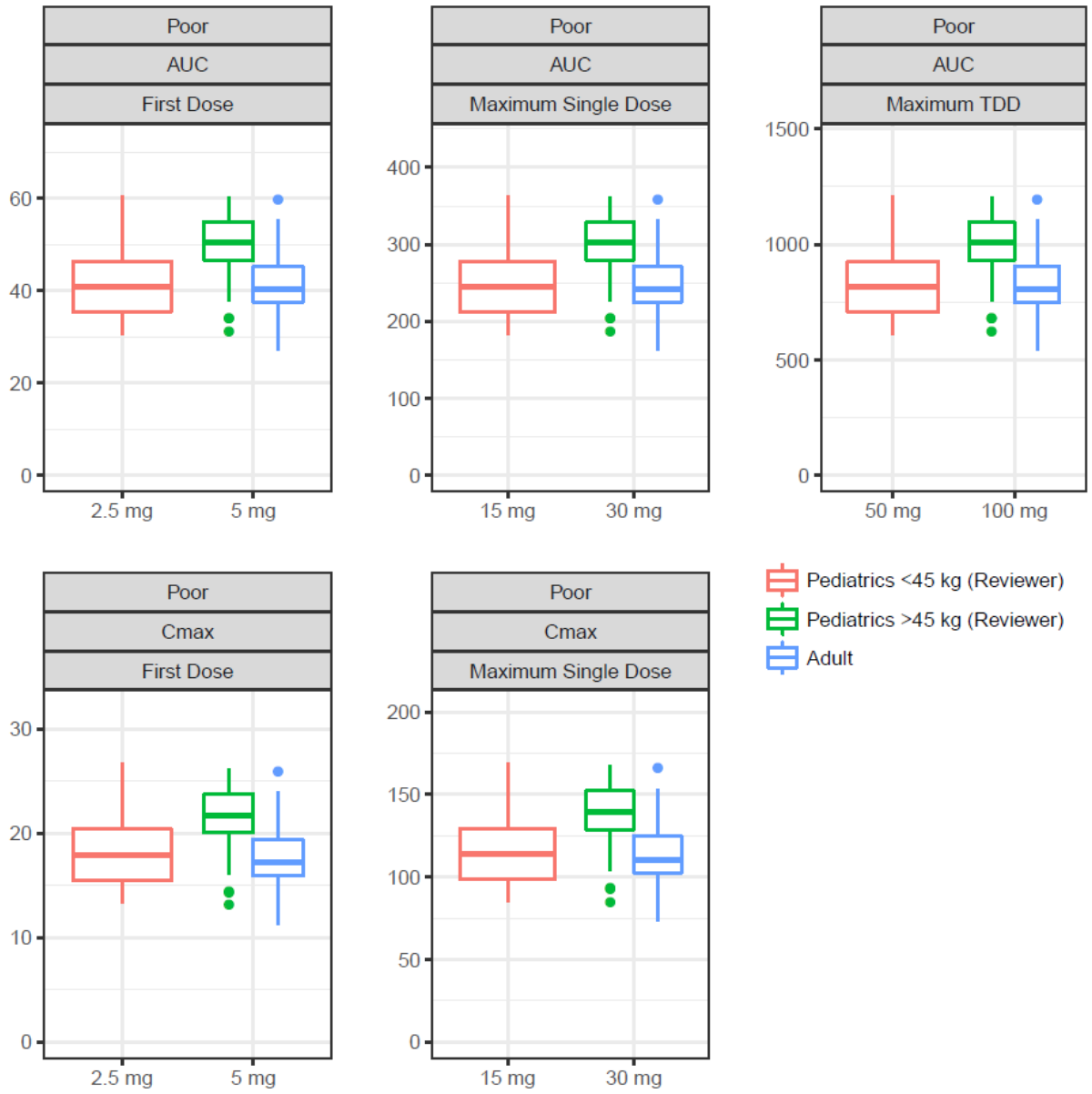
Note: Scenario 1 is 0.07 mg/kg Q8h for the 1st day, 0.3 mg/kg for Q8h for 2 days, and 1.4 mg/kg Q24h for 2 days. Scenario 2 is 2.5 mg for pediatric patients < 45 kg and 5 mg for pediatric patients ≥ 45 kg Q8h for the 1st day, 15 mg for pediatric patients < 45 kg and 30 mg for pediatric patients ≥ 45 kg Q8h for 2 days, and 50 mg for pediatric patients <45 kg and 100 mg for pediatric patients ≥ 45 kg Q24h for 2 days.

Figure 11: Comparison of Exposure ($AUC_{0-\infty}$: ng·h/mL and Cmax: ng/mL) in Pediatric and Adult Patients Following Scenario 1 Simulation Stratified by Weight Groups in Pediatric Population: Poor Metabolizers (TDD is total daily dose)



Note: Scenario 1 is 0.07 mg/kg Q8h for the 1st day, 0.3 mg/kg for Q8h for 2 days, and 1.4 mg/kg Q24h for 2 days.

Figure 12: Comparison of Exposure ($AUC_{0-\infty}$: ng·h/mL and Cmax: ng/mL) in Pediatric and Adult Patients Following Scenario 2 Simulation Stratified by Weight Groups in Pediatric Population: Poor Metabolizers (TDD is total daily dose)



Note: Scenario 2 is 2.5 mg for pediatric patients < 45 kg and 5 mg for pediatric patients ≥ 45 kg Q8h for the 1st day, 15 mg for pediatric patients < 45 kg and 30 mg for pediatric patients ≥ 45 kg Q8h for 2 days, and 50 mg for pediatric patients <45 kg and 100 mg for pediatric patients ≥ 45 kg Q24h for 2 days.

Summary

- The final population PK model predicts the data reasonably well
- The use of allometric scaling approach to predict pediatric exposure appears to be supported by the empirical data
- The exposure in pediatric patients with the proposed pediatric dosing regimen is within the range of adult exposure after administering the adult recommended dose

4.3. NAT2 Enzyme Maturation

Background and Aim:

NAT is a cytosolic enzyme widely distributed in mammalian tissues. It is a constitutive enzyme and is not inducible[9]. Two isoforms of the cytosolic enzymes have been identified in humans: NAT1 and NAT2 enzymes. These enzymes differ considerably in their substrate specification and their tissue distribution. NAT2 expression is mostly limited to the gastrointestinal tract and liver, whereas NAT1 is widely expressed. Additionally, genetic polymorphisms are more frequent in NAT2. In vitro studies indicate that 3,4-DAP is predominantly metabolized by the NAT2 enzyme to the inactive 3-Ac-DAP metabolite.

Genetic polymorphisms in the expression of NAT2 have been identified resulting in normal, intermediate and poor metabolizers phenotypes[10]. In adults, genotype is highly correlated with phenotypes [11]. However, genotype is not related to phenotype at birth but develops upon maturation of the enzyme.

The ontogeny of NAT2 is not adequately studied[12]. Studies that used fetal human liver, obtained from aborted fetuses, to evaluate NAT2 activity were reported for several NAT2 substrates[13,14,15]. However, it is not possible to evaluate NAT2 activity in children using liver biopsy for ethical reasons.

The aim of this systematic review is to summarize the current understanding of NAT2 developmental expression in humans after birth. We specifically aim to evaluate the age at which NAT2 reaches activity similar to adults. The results of this review will help inform pediatric dosing of RUZURGI.

Methods:

We used the following keywords: (“acetyl transferase” or “acetyltransferase” or "N-acetyltransferase" or “NAT2”) AND (“pediatric” or "ontogeny" or "age" or “Children” or “allometry” or “neonate” or “infant” or “development” or “mature” or “maturation”) using PubMed search engine. The search results were then screened using titles and abstracts to identify relevant articles. Once relevant articles were identified, they were checked for

[9] Indian Pediatr. 1989 Feb;26(2):197-8.

[10] Drug Metab Rev. 1999 May;31(2):489-502.

[11] Pharmacogenomics. 2012 Jan; 13(1): 31–41.

[12] J Pharmacol Exp Ther. 2002 Feb;300(2):361-6.

[13] Biol Res Pregnancy Perinatol. 1986;7(2):74-6.

[14] Pharmacology. 1986;32(5):283-91.

[15] Pediatr Pharmacol (New York). 1984;4(3):155-9.

additional references. Other than limiting species to humans, no specific filter was implemented.

Results and Discussion:

The preliminary search using the above-mentioned keywords revealed 2827 articles. However, majority of these articles were not relevant to the objective of this research. In general, several of the irrelevant articles can be classified into 3 categories:

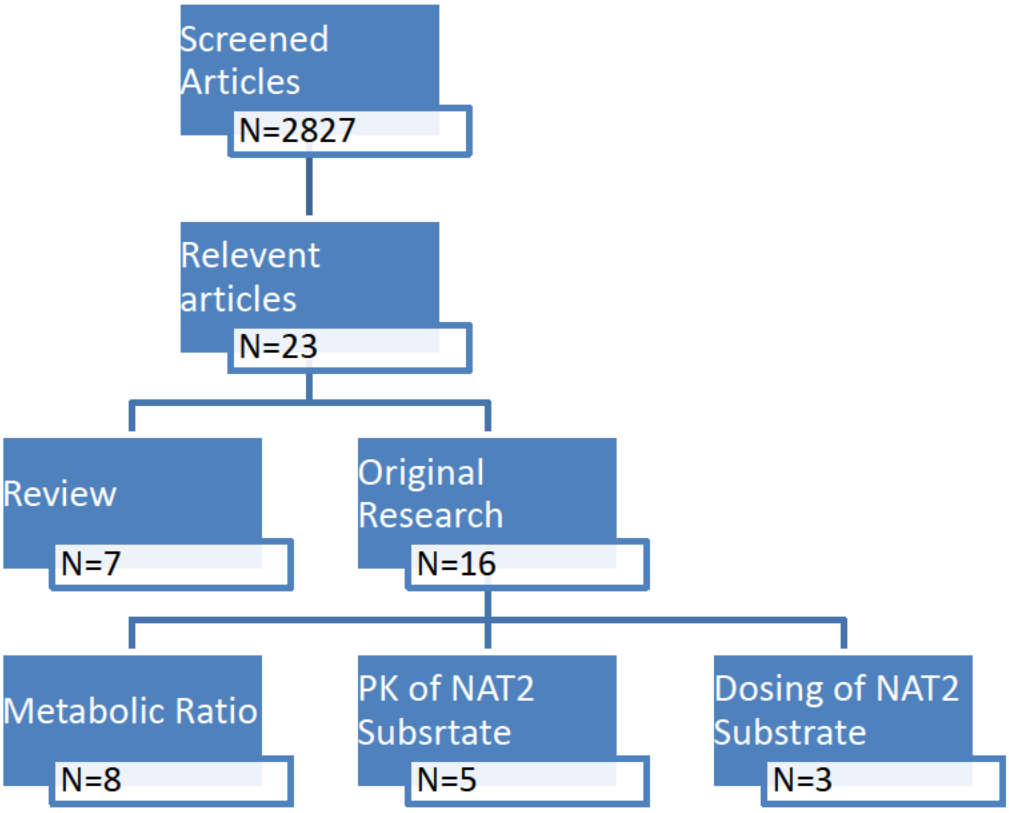
1) articles focused on the association of NAT2 phenotypes and risk for diseases such as Down syndrome, asthma, Psoriasis Vulgaris, Parkinson's disease, lung cancer, head and neck squamous-cell cancer, prostate cancer, urinary bladder cancer, diabetes mellitus, pediatric acute lymphocytic leukemia, hearing impairment, endometriosis;
2) articles focused on the association of polymorphisms and development of liver induced hepatoxicity or induced systemic lupus erythematous as a result of drug administration, and
3) articles focused on the frequency of NAT2 polymorphisms in different ethnic groups such as Kazakh, Turkish, Chinese, Japanese, kwashiorkor.

Twenty-three articles were eligible, including 7 review articles and 16 original research articles. The original research articles were divided into three categories:

1) articles that assessed enzyme maturation through assessment of the metabolic ratio of NAT2 (using NAT2 probe substrate);
2) articles that assessed enzyme maturation through assessing the PK of NAT2 substrate; and
3) articles that assessed the adequacy of dosing in the pediatric population as compared to adults for a NAT2 substrate.

Figure 13 presents a summary of identified articles with relevant information on NAT2 maturation

Figure 13: Summary of Identified Articles with Relevant Information on NAT2 Maturation



Phenotyping by probe substrates of NAT2 enzymes is widely used to assess the in vivo activity of NAT2. Caffeine has been used as a model drug in acetylation phenotype studies. It's main metabolite, formed in a reaction catalyzed by NAT2, is 5- acetylamino -6- formyl amino -3- methyl uracil (AFMU). Urinary metabolic ratio of AFMU to 1-mehtylxanthine (1X) is used for the evaluation of NAT2 activity[16,17]. Additionally, phenotyping with isoniazid (INH) using a single-sample of INH and its metabolite at 2 or 3 hours post INH dose[18] to calculate the metabolic ratio have been also described and used in literature for phenotyping purposes. These methods suffer from some limitations, such as the instability of the metabolites that require freezing condition for sample storage. Moreover, concomitant illness could modify the acetylation phenotypes resulting in disconcordance between the phenotypes and genotypes[19].

[16] Br J Clin Pharmacol. 2004 Dec;58(7):S788-93; discussion S794-5.
[17] Br J Clin Pharmacol. 1984 Apr;17(4):459-64.
[18] Br J Clin Pharmacol. 1986 Sep;22(3):343-5.
[19] Pharmacogenetics. 2000 Mar;10(2):171-82.

Nonetheless, several groups were able to show that there is good concordance between these phenotyping methods and genotyping in adults[20,21,22,23]. On the other hand, during enzyme maturation, in the pediatric population, the metabolic ratio changes with age and no correlation exists between phenotyping and genotyping. The majority of infants show poor metabolism phenotype regardless of their genotype. Once full enzyme maturation is reached, the distribution of the poor and normal metabolizers matches between adults and pediatrics.

Articles that assessed enzyme maturation through assessment of the metabolic ratio of NAT2 (N=8)[24,25,26,27,28,29,30,31] were investigated to identify the age at which the metabolic ratio is no longer related to age and to identify the age at which the distribution of different phenotypes is similar to adults. In some studies, patients were followed longitudinally, albeit for short amount of time (i.e. less than 15 months)[32,33,34]. It is important to note that the majority of these articles compared the metabolic ratio across different subjects in different age groups without accounting for genotype differences. Nonetheless, collectively, these articles support the fact that NAT2 maturations occurs as fast as 1 year and as late as 4 to 6 years[35, 36].

NAT2 is the primary metabolizing enzyme for several drugs such as procainamide, hydralazine, dapsone, INH, phenelzine, sulphonamides, and other drugs. The PK of INH is largely studied in adults and to some extent in children. Through assessing the PK of INH in children, one may be able to understand the NAT2 maturation in infants and young children. It is expected that after maturation, body weight corrected clearance, is faster in young children. Therefore, the exposure following the same body weight normalized dose should be lower in young children. However, before enzyme full maturation, the exposure is expected to be higher following the same body weight adjusted dosing. We therefore, assessed the articles that focused on the INH PK in children (N=5) to see at which age children achieve similar or lower exposure following

[20] Arch Toxicol. 2005 Apr;79(4):196-200.
[21] Am J Hum Genet. 1995 Sep;57(3):581-92.
[22] Eur J Clin Pharmacol. 2000 Dec;56(9-10):689-92.
[23] Am J Respir Crit Care Med. 1997 May;155(5):1717-22.
[24] Pharmacogenomics. 2014 Feb;15(3):285-96.
[25] Int J Clin Pharmacol Ther. 2014 Apr;52(4):292-302.
[26] Clin Ther. 2008 Sep;30(9):1687-99.
[27] Pediatr Res. 1999 Mar;45(3):403-8.
[28] Clin Pharmacol Ther. 1997 Oct;62(4):377-83.
[29] Pediatr Res. 1991 May;29(5):492-5.
[30] Biol Res Pregnancy Perinatol. 1987;8(1 1ST Half):23-5.
[31] Fundam Clin Pharmacol. 1989;3(6):589-95.
[32] Pediatr Res. 1991 May;29(5):492-5.
[33] Clin Ther. 2008 Sep;30(9):1687-99
[34] Fundam Clin Pharmacol. 1989;3(6):589-95.
[35] Pharmacogenomics. 2014 Feb;15(3):285-96.
[36] Int J Clin Pharmacol Ther. 2014 Apr;52(4):292-302.

the same adult INH body weight corrected dose. These articles were also evaluated for information on weight-corrected clearance and drug exposures in children and were compared to the reported clearance in adults.

The PK parameters in these articles were estimated using different methodology such as naïve pooling[37], 2-Stage approach[38], population PK modeling[39,40], or multivariate regression techniques[41]. The population PK models estimated the time to reach maturation according to a sigmoidal function described previously[42]. These estimates were used to calculate the time at which maturation occurs. While most of these articles included a relatively wide age range of children, one of these articles included infants only. In this article, a population PK model was developed based on an age range of 3-24 months and therefore, model estimates of the population PK model were interpreted with caution[43].

Table 19 summarizes the main conclusions of the original research papers that assessed the PK or NAT2 metabolic ratio in pediatrics.

Table 19: Summary of Relevant Literature on NAT2 Maturation

Main Conclusion	Reviewer Comments	References
Maturation is reached by ≥ 1 year	The first article assessed the agreement between phenotype and genotype in 82 children (1 month-17 years; 45% were infants). Even though the children were not followed longitudinally, the authors showed that there is a linear correlation between age and metabolic ratio of AMFU/1X in children <1 year. The second article included 10 infants (1-19 months) receiving caffeine for apnea. One infant was serially studied and the metabolic ratio of AFMU/1X significantly increased between 6 and 12 months and seemed to reach a plateau by 12	1) Pediatr Res. 1999 Mar;45(3):403-8. 2) Fundam Clin Pharmacol. 1989;3(6):589-95.

[37] BMC Infect Dis. 2016 Apr 2;16:144.
[38] Arch Dis Child. 2005 Jun;90(6):614-8.
[39] J Clin Pharmacol. 2012 Apr;52(4):511-9.
[40] J Antimicrob Chemother. 2014 May;69(5):1339-49
[41] EBioMedicine. 2016 Sep;11:118-126.
[42] Drug Metab Pharmacokinet. 2009;24(1):25-36.
[43] J Clin Pharmacol. 2012 Apr;52(4):511-9.

	months suggesting maturation by this age.	
Maturation is reached by≥ 15 months	This prospective study in infants showed that during the first year of life in >60% of the studied infants, a change from poor to normal acetylation phenotype, using caffeine, occurred. However, limited number of subjects that were followed longitudinally for change in the acetylation phenotypes in the first 15 months of life.	1) Pediatr Res. 1991 May;29(5):492-5.
Maturation is reached by 2 years	The authors of the first article showed that the frequency of poor metabolizer phenotypes (based on urinary sulfadimidine) is the highest in neonates. They also showed that the poor metabolizer phenotype is similar in children (2-23 years) and adult (19-59 years). In the second article, based on PopPK model parameter estimates, NAT2 activity reaches 90% of adult activity by 2 years.	1) Biol Res Pregnancy Perinatol. 1987;8(1 1ST Half):23-5. 2) J Antimicrob Chemother. 2014 May;69(5):1339-49.
Maturation is reached by 4 years	Several articles suggest the same findings of NAT2 maturations by 4 years.	1) Pharmacogenomics. 2014 Feb;15(3):285-96. 2) Clin Ther. 2008 Sep;30(9):1687-99[44]. 3) Pharmacogenomics. 2014 Feb;15(3):285-96. 4) Fundam Clin Pharmacol. 2001 Oct;15(5):355-9.
Maturation is completed by 5.3 years	The authors applied non-parametric regression approach (multivariate adaptive regression splines; MARS) to model INH PK in children (age range: 0.25-10.5 years). Based on this approach, the authors describe an age of about 5.3 years when the non-linear modification of the	1) EBioMedicine. 2016 Sep; 11:118-126.

[44] NAT2 didn't change significantly over 6 months for 11 slow metabolizers (based on caffeine testing) child (4.5-14.6 years) receiving recombinant growth hormone.

	contribution of the NAT2 genotype to the INH metabolism ends.	
Maturation rate depends on Phenotypes with 50% of mature NAT2 activity reached at 11 and 15 months post menstrual age[45].	Data from this study is limited as the model was based on 3 months to 24 months old only	1) J Clin Pharmacol. 2012 Apr;52(4):511-9.
There is a tendency to increase metabolic ratio after 4 years of age which may reflect "less intense" maturation after 4 years	Potential confounding of acetylation genotype cannot be eliminated. None of the children were followed longitudinally. In addition, no adult data were included.	1) Int J Clin Pharmacol Ther. 2014 Apr;52(4):292-302[46].

Articles that assessed the adequacy of dosing in the pediatric population as compared to adults for a NAT2 substrate (N=3) [47,48,49] were investigated to understand the similarity of exposure following the weight-corrected dosing in children and adults. It is expected that similar or even higher body weight-corrected doses is required for children when full enzyme maturation is achieved to achieve the same target exposure. However, before full enzyme maturation, a lower body weight-corrected dose would be required to achieve similar exposures in adults.

The current WHO dosing recommendations of INH for adults ≥ 30 kg is 300 mg (resulting in doses up to 10 mg/kg in adults). In infants and children, the WHO revised its recommended dose and increased it from 5 mg/kg to 10 mg/kg. The previous and current dosing recommendations were evaluated in children to investigate if they achieve target C_{max} ≥3 mg/L. Even in children < 2 years, the previous WHO dosing recommendations of 5 mg/kg did not achieve its target C_{max}[50]. The use of higher weight corrected dosing in infants and children as compared to adults agrees with the allometric scaling theory and suggests that maturations of NAT2 occurs as early as <2 years.

[45] Based on data from 3-24 months and therefore, parameter estimates are not reliable.
[46] Metabolizers phenotype showed positive correlation with age, with a significant change around the 4th year of life. There was tendency to increase metabolic activity after 4 years of age but "less intense".
[47] Pediatr Infect Dis J. 2018 Jan;37(1):43-51.
[48] Antimicrob Agents Chemother. 2011 Dec;55(12):5560-7.
[49] Ther Drug Monit. 2012 Aug;34(4):446-51.
[50] Antimicrob Agents Chemother. 2011 Dec;55(12):5560-7.

Conclusion

Information on the activity of the NAT2 enzyme in children is limited. However, accumulated evidence suggests that maturation can occur by 6 years of age. . Additionally, body weight-corrected doses for drugs eliminated by NAT2, such as INH and dapsone, in children is the current standard[51,52,53,54,55].

[51] Pharmacotherapy. 2009 Jun;29(6):680-90.
[52] Semin Pediatr Neurol. 2010 Dec;17(4):208-13.
[53] Pediatr Infect Dis J. 2018 Jan;37(1):43-51.
[54] J Antimicrob Chemother. 2015 Apr;70(4):1115-23.
[55] Antimicrob Agents Chemother. 2016 Mar 25;60(4):2171-9.

4.4 Pharmacogenomics Review

BACKGROUND

NAT2 polymorphisms are known to result in variable acetylation activity (i.e., slow, also referred to as "poor metabolizers" vs. intermediate vs. rapid/fast, also referred to as "normal metabolizers") within and across different racial and ethnic populations. Published frequencies for NAT2 phenotypes are listed below (Table 20). Frequencies reported in different studies may vary depending on 1) the performance characteristics of the NAT2 genotyping assay used, 2) which alleles were genotyped, and 3) on how the genotypes were grouped into phenotype categories.

Table 20: Frequency of NAT2 Phenotypes Across Racial/Ethnic Populations

Racial/Ethnic Population	NAT2 Phenotype Frequency (%)		
	Normal	Intermediate	Poor
Caucasian	6-7	35-40	56-59
African American	14-19	44-45	37-42
U.S. Hispanic	14	55	33
Japanese	44-45	46-49	7-10
U.S. Korean	46	40	13
Chinese	23-27	48-53	21-27

Source: Modified based on PMID: 20183529. Normal metabolizer (2 normal function alleles)= homozygous or heterozygous for NAT2*4, *12A, *13A; Poor metabolizer (2 reduced function alleles)= homozygous or heterozygous for NAT2*5-*7, *14; Intermediate metabolizer (1 normal and 1 reduced function alleles).

The purpose of this review is to determine if the NAT1 and NAT2 phenotype assignments as performed by the applicant are acceptable.

CONTENTS

The overall genotype data for the NAT2 gene performed in subjects from 3 clinical trials are shown in Table 21.

Table 21: Listing of Clinical Studies with Available NAT2 Metabolizer Status Information

Study Identifier	Number of Subjects (n)	NAT2 Metabolizer Status (n)		
		Normal	Intermediate	Poor
JPC 3,4-DAP.PK2[#]	17	0	5	12
JPC 3,4-DAP.TQT	52	4	29	23
DPMC-DAP-01A2[†]	49	1	5	5

3,4-DAP= 3,4-Diaminopyridine free base; [#]Genotyping addendum to clinical study JPC 3,4-DAP.PK1;[†]DPMC-DAP-01A2 was a PK/PD study associated with clinical study JPC 3,4-DAPPER.

CLINICAL STUDIES

Study JPC 3,4-DAP.PK2 was a genotyping addendum to the clinical study JPC 3,4-DAP.PK1. Only subjects who participated in the JPC 3,4-DAP.PK1 study and were known to received active drug were eligible to participate in the JPC 3,4-DAP.PK2 study.

Methods

Genotyping test:

Blood samples were analyzed for NAT2 and NAT1 genotypes as described in the study protocol. Metabolizer phenotypes were inferred using seven human NAT2 and four human NAT1-specific single nucleotide polymorphisms (SNPs) as described in Table 22. According to the Clinical Study Report, no statistical analysis of the genotyping status was planned. The genotype results were treated as a descriptive variable to help clarify the metabolizer phenotype.

Table 22: Assessed NAT1 And NAT2 Variants and Assigned Metabolizer Status

NAT1		NAT2	
Genotyped Variant	Metabolizer Status	Genotyped Variant	Metabolizer Status
*5, rs55793712	N/A	*5D, rs1801280	Poor
*11, rs4986783	N/A	*6B, rs1799930	Poor
*14, rs4986782	Poor[‡]	*7A, rs1799931	Poor
*17, rs56379106	Poor[‡]	*11A, rs1799929	Normal
		*12A, rs1208	Normal
		*13A, rs1041983	Normal
		*14A, rs1801279	Poor

N/A= Not available; NAT1*4= reference; [‡]Lower than NAT1*4; NAT1 phenotypes assigned by the reviewer based on the Arylamine N-acetyltransferases (NATs) database (http://nat.mbg.duth.gr/). NAT2 phenotypes assigned by the applicant: Normal metabolizer (2

normal function alleles)= homozygous or heterozygous for NAT2*11A, *12A, *13A; Poor metabolizer (2 reduced function alleles)= homozygous or heterozygous for NAT2*5D, *6B, *7A, *14A; Intermediate metabolizer (1 normal and 1 reduced function alleles).

Reviewer's comments:
*The variant selection seems adequate to cover the most common and relevant haplotypes of the NAT2 gene to adequately determine NAT2 phenotype. The applicant's classification of phenotype based on genotype seems reasonable based on the Arylamine N-acetyltransferases (NATs) database (http://nat.mbg.duth.gr/). Of note, per the NATs database, carriers of the rs1799931 variant (i.e., *7A haplotype) should be assigned poor metabolizer status; however, poor metabolizer status based on *7A haplotype may be substrate dependent. Also, the variants listed in* Table 22 *are present in multiple haplotypes. Some of the NATs database listed haplotypes do not have assigned phenotype status based on limited data in available literature. According to the applicant, acetylation of 3,4-DAP by NAT1 may also occur but at a much slower rate. NAT1 phenotype assignments were not provided by the applicant and were not verified by the reviewer.*

Study JPC 3,4-DAP.TQT was a randomized, thorough QT study performed in healthy volunteers.

Methods
Genotyping test:
Blood samples were analyzed for NAT2 and NAT1 genotypes as described in the study protocol. The same genotyping approach was utilized as in study JPC 3,4-DAP.PK2 (see Table 22). Metabolizer phenotypes were inferred using seven human NAT2 and four human NAT1- specific SNPs. The inferred NAT2 phenotypes were correlated with the PK findings from the previously completed 3,4-DAP PK study to ascertain whether the observed acetylation patterns for 3,4-DAP correspond to the genotypically inferred phenotypes.

Reviewer's comments:
The variant selection seems adequate to cover the most common and relevant haplotypes of the NAT2 gene to adequately determine NAT2 phenotype. Also, performed NAT2 phenotype assessment seems to be adequate.

Study DPMC-DAP-01A2 was a PK/PD study associated with clinical study JPC 3,4-DAPPER in patients diagnosed with Lambert-Eaton Myasthenia Syndrome (LEMS).

Methods
Genotyping test:

Blood samples were analyzed for NAT2 and NAT1 genotypes as described in the study protocol. The same genotyping approach was utilized as in the Study JPC 3,4-DAP.PK2 (see Table 22). Metabolizer phenotypes were inferred using seven human NAT2 and four human NAT1- specific SNPs.

Reviewer's comments:

Similarly to the studies JPC 3,4-DAP.PK2 and JPC 3,4-DAP.TQT, the variant selection seems adequate to cover the most common and relevant haplotypes of the NAT2 gene to adequately determine NAT2 phenotypes. Also, NAT2 phenotype assessment performed by the applicant seems to be adequate.

SUMMARY OF FINDINGS

The approach and methodologies utilized by the applicant for determination of the metabolizer status based on the NAT2 genotype are generally acceptable. Of note, in some instances genotype assignments could not be verified. For example, no information about phenotype assignments based on the NAT1 genotype results were provided in all studies described above.

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/s/

MARIAM A AHMED
04/16/2019 03:23:37 PM

KATARZYNA DROZDA
04/16/2019 03:30:25 PM

CHRISTIAN GRIMSTEIN
04/16/2019 03:33:53 PM

KEVIN M KRUDYS
04/16/2019 03:34:54 PM

SREEDHARAN N SABARINATH
04/16/2019 03:57:05 PM

MEHUL U MEHTA
04/16/2019 04:18:32 PM