



Nifurtimox for Treatment of Chagas Disease in Pediatric Patients: the Challenges of Applying Pharmacokinetic-Pharmacodynamic Principles to Dose Finding

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Abstract

The antiparasitic drug nifurtimox was approved in the USA in 2020 for the treatment of patients with Chagas disease aged less than 18 years and weighing at least 2.5 kg, based on outcomes from the phase 3 CHICO study. Accordingly, pediatric patients with Chagas disease take nifurtimox thrice daily with food at one of two body weight-adjusted dose ranges. We investigated possible relationships between pharmacokinetic (PK) data, and pharmacodynamic efficacy and safety data collected in an analysis population of 111 participants in CHICO, using a published population PK model to estimate nifurtimox exposure at the patient level. Pediatric exposure to nifurtimox was benchmarked against levels of nifurtimox exposure known to be effective in adults with Chagas disease. Given the complex dosing regimen for nifurtimox, we also modeled nifurtimox exposure associated with simpler dosing strategies. We found no relationship between exposure to nifurtimox and efficacy measures (e.g., serological response to treatment), or between exposure and safety outcomes (including typical adverse events, e.g., headache, decreased appetite, nausea/vomiting). The analysis population appeared to represent the overall CHICO population based on the similarity of their baseline characteristics and the profiles of adverse events in the two groups. Modeled exposure based on the dosing regimen in CHICO was within the reference range derived from phase 1 data in adults. The relationship between nifurtimox exposure and cure is complex; a simplified pediatric dosing regimen is unlikely to be beneficial.

Keywords efficacy · exposure · nifurtimox · pediatric · safety

Introduction

Chagas disease is a potentially life-threatening condition that manifests with acute and chronic phases and is caused by the parasite *Trypanosoma cruzi* (*T. cruzi*) (1). Two antitrypanosomal treatments are used to treat Chagas disease (2): nifurtimox, which has been used for approximately 50 years (3), and benznidazole, which has been used for more than

40 years (4). Since the 1970s, nifurtimox has been approved for treating adult and pediatric patients with Chagas disease in Latin American countries where the disease is endemic (5). Dose determination for nifurtimox in Chagas disease was conducted according to standards applicable at the time (6), and did not include phase 1 clinical development studies now routinely performed for new chemical entities. A recent population pharmacokinetic (popPK) model (7), which used pharmacokinetic (PK) data from a phase 3 study (CHICO) in patients with Chagas disease aged < 18 years (including newborns) (8), determined that both body weight and age can affect apparent oral clearance and apparent volume of distribution of nifurtimox in pediatric patients. This pediatric model also found that the PK characteristics of nifurtimox are unaffected by sex and that there was no indication of deviation from dose linearity across the pediatric dose range (7). The efficacy of nifurtimox was demonstrated in CHICO based on body weight-adjusted dosing stratified by age (participants aged < 12 years and weighing < 40 kg

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received a total daily dose in the range 10–20 mg/kg/day, and participants aged ≥ 12 years and weighing ≥ 40 kg received 8–10 mg/kg/day), determined at the judgement of the treating physician (8).

We wanted to examine possible relationships between nifurtimox exposure and efficacy and safety outcomes in a subgroup of pediatric patients from CHICO for whom PK data were available. We used the pediatric popPK model to simulate nifurtimox exposure at the patient level in this subgroup, comprising patients of all ages < 18 years, and benchmarked these values against an exposure reference range derived from non-compartmental PK analysis of adult data from phase 1 studies (9, 10). This comparison assumed that if plasma drug exposure at a certain level is known to be efficacious in adults, then the same level of exposure is likely to be efficacious in children.

The primary efficacy endpoint in CHICO was based on serological response to treatment using two conventional enzyme-linked immunosorbent assays (ELISAs) (8). At 12 months post-treatment follow-up, a substantial proportion of the study population showed either negative seroconversion or a reduction in optical density of at least 20% in conventional ELISAs (8). The currently accepted criterion to establish parasitological cure in Chagas disease is negative seroconversion measured by two types of assay: conventional ELISA and indirect hemagglutinin or immunofluorescence assay (11). However, treatment success is difficult to measure with current diagnostic tools because the parasite is extremely immunogenic and elicits a strong antibody response, which can persist long after the parasite has been cleared. Thus, many years of follow-up can be necessary, especially in chronically infected patients, to truly establish that an individual has been cured. In addition to serological endpoints, a quantitative polymerase chain reaction (qPCR) assay can be used to detect *T. cruzi* in blood. However, individuals with chronic disease are not permanently parasitemic, so the inability to detect parasite by qPCR is not considered evidence of cure. Both ELISA data (two conventional and one non-conventional assays) and qPCR data were available from CHICO, so we looked for evidence of a relationship between modeled plasma nifurtimox exposure and these different pharmacodynamic (PD) endpoints. We also looked for a possible link between exposure and treatment-emergent adverse events (TEAEs). Accordingly, we investigated whether the level of nifurtimox exposure in the CHICO population was associated with the occurrence or with the severity of specific TEAEs. We also obtained electrocardiogram (ECG) data to investigate possible relationships between nifurtimox exposure and its effects on the QT interval.

Finally, we wanted to examine the approved dosing strategy for nifurtimox more closely. Within the approved dose range (12), the total daily dose is divided into three equal

partial doses, each taken with food. Accordingly, we used the popPK model to simulate nifurtimox exposure at the patient level for a selection of theoretical dosing regimens to explore whether any alternative regimens could elicit acceptable levels of exposure.

Materials and Methods

Study Participants, Informed Consent, Ethics, Study Sites

The phase 3 CHICO study included 330 patients from all pediatric age groups < 18 years with a diagnosis of Chagas disease, which is described elsewhere (8). In brief, participants in CHICO were randomized either to a 60-day or a 30-day nifurtimox treatment regimen and plasma samples were obtained from approximately one-third of these individuals for PK analyses; patient characteristics in this PK population and the overall CHICO population are summarized in Table 1. Investigation of relationships between PK and PD outcomes was confined to the subgroup of the PK population randomized to the 60-day treatment regimen, which was the regimen tested in the primary efficacy endpoint of CHICO (8), and is the current guideline regimen duration for nifurtimox in Chagas disease (11). Full details of participants in CHICO, informed consent, design, and oversight of the study are reported elsewhere (8).

Modeling Summary

Population PK analysis used a validated installation of NONMEM (version 7.3, ICON Development Solutions, Ellicott City, MD, USA). NONMEM-run execution, bootstrap, and visual predictive check (VPC) were performed using Perl-speaks-NONMEM (PsN), version 4.8.1. Methodological details of the development of the popPK model have been reported (7). Modeling used PK data from phase 1 studies of nifurtimox in adults with Chagas disease (9, 10) and the phase 3 CHICO study in pediatric patients with Chagas disease (8). An adult popPK model described the data in adults but could not adequately predict the sparse-sampled pediatric data; therefore, the pediatric data were modeled separately to obtain exposure estimates for comparison with adult data (7).

Explorative Assessment of Exposure–Response Relationships

For evaluation purposes, a reference exposure range was derived from phase 1 PK data in adult patients, based on the assumption that the antiparasitic efficacy of the drug would be similar if equivalent exposure was attained in the

Table 1 Baseline Characteristics in Pediatric Patients in the CHICO Population and for Whom PK Data Were Available, by Treatment Regimen

Characteristic	PK set			CHICO population		
	30-day treatment (<i>n</i> = 39)	60-day treatment (<i>n</i> = 72)	All (<i>N</i> = 111) ^a	30-day treatment (<i>n</i> = 111)	60-day treatment (<i>n</i> = 219)	All (<i>N</i> = 330) ^a
Age group						
0–< 1 month	2 (5.1)	2 (2.8)	4 (3.6)	–	–	–
0–27 days	–	–	–	3 (2.7)	4 (1.8)	7 (2.1)
1 month–< 2 years	2 (5.1)	11 (15.3)	13 (11.7)	–	–	–
28 days–< 2 years	–	–	–	12 (10.8)	25 (11.4)	37 (11.2)
2–< 18 years	35 (89.7)	59 (81.9)	94 (84.7)	96 (86.5)	190 (86.8)	286 (86.7)
Sex						
Female	23 (59.0)	40 (55.6)	63 (56.8)	59 (53.2)	119 (54.3)	178 (53.9)
Male	16 (41.0)	32 (44.4)	48 (43.2)	52 (46.8)	100 (45.7)	152 (46.1)
Race						
American Indian or Alaska Native	3 (7.7)	10 (13.9)	13 (11.7)	30 (27.0)	64 (29.2)	94 (28.5)
White	36 (92.3)	62 (86.1)	98 (88.3)	81 (73.0)	155 (70.8)	236 (71.5)
Ethnicity						
Hispanic or Latino	37 (94.9)	72 (100.0)	109 (98.2)	108 (97.3)	217 (99.1)	325 (98.5)
Not Hispanic or Latino	2 (5.1)	0 (0.0)	2 (1.8)	3 (2.7)	2 (0.9)	5 (1.5)
Weight, kg						
Mean (<i>SD</i>)	41.3 (18)	35 (20)	37 (20)	37 (19)	35 (20)	35 (19)
Median (range)	44 (2.5–71.0)	34 (2.9–94.0)	38 (2.5–94.0)	39 (2.5–72.8)	32 (2.9–95.0)	34 (2.5–95.0)

Data are *n* (%) unless stated otherwise

PK pharmacokinetic, SD standard deviation

^aIn total, 330 patients were randomized in CHICO; sampling for PK analyses was performed in 111 patients

target pediatric population. The basis for this was that PK characteristics are dose-proportional within the dose range usually seen during treatment. Therefore, exposure results from single-dose phase 1 studies of nifurtimox, administered in the range 30–240 mg, were scaled to provide a reference range of exposure (area under the curve [AUC]) that would be expected in adults receiving the lowest clinical dosing regimen of 8 mg/kg/day divided into three daily doses. Unlike AUC, there was no simple scaling method to derive C_{max} . Therefore, a simulation was run in the popPK model of the PK in all adults receiving 8 mg/kg total daily divided into three doses, from which C_{max} at steady state was calculated. The reference ranges used here are the 5th–95th percentiles of the scaled exposure and C_{max} values; exposure modeled at the individual level in the target pediatric population is assessed against the reference exposure range. Exposure–response relationships were explored in participants with PK data using the individual predicted exposure of plasma nifurtimox concentration *versus* time at steady state (AUC_{ss}), AUC within the dosing interval (0–8 h), and maximum concentration at steady state ($C_{max,ss}$) based on dosing records (7), with observed effects on efficacy (e.g., seroreduction measured by conventional ELISAs) and safety

parameters (TEAE occurrence and severity). We used R version 3.5.1 (13) to create forest plots and then diagnosed any correlation between PK and efficacy or between PK and safety parameters by visual inspection.

ELISA, qPCR Methodology

Blood samples were obtained for conventional ELISAs (recombinant ELISA and lysate ELISA), non-conventional ELISA using the *T. cruzi* F-29 flagellar protein (a member of the calcium-binding protein superfamily exclusively found in trypanosomatids and considered to be an early marker of therapeutic efficacy in chronic Chagas disease), and detection of *T. cruzi* DNA using qPCR immediately before treatment (baseline), during treatment, and up to 12 months post-treatment. Commercially available assay kits were used for conventional ELISAs, and the optical density was measured at 450/620–650 nm using a bichromatic microplate reader. Non-conventional ELISAs were performed as described elsewhere (14). The qPCR tests amplified the *T. cruzi* satellite DNA region. Serological response to treatment was assessed in conventional ELISAs and defined as either seroreduction (patients aged 8 months to < 18 years)

or negative seroconversion (all patients) at 12-month post-treatment follow-up. Seroreduction was defined as a $\geq 20\%$ reduction in mean optical density measured in the two conventional ELISAs compared with baseline. Seroconversion was defined as a negative anti-*T. cruzi* immunoglobulin G concentration by two conventional ELISAs (8).

Safety

Assessment of TEAEs was performed at each study visit; events were classified as mild, moderate, or severe. The following TEAEs were examined for a possible relationship between nifurtimox exposure and their incidence and severity: abdominal pain (including upper abdominal pain), diarrhea, nausea, vomiting, asthenia, fatigue, pyrexia, decreased appetite, myalgia, dizziness, headache, anxiety, pruritus, rash (including macular rash), and urticaria. All these TEAEs are known undesirable effects of nifurtimox. Standard 12-lead ECGs were obtained before, during, and after the end of treatment with nifurtimox and used to investigate possible effects of nifurtimox on the QT/QTc interval in the PK set.

Results

Nifurtimox Exposure and Efficacy

In total, 111 pediatric patients for whom PK data were available in the phase 3 CHICO study were included in the popPK analysis. Baseline characteristics of these individuals are given in Table I. Possible associations between nifurtimox exposure and serological response to treatment were investigated in a subgroup of 72 patients who received a 60-day nifurtimox treatment regimen. No relationship was found between the level of nifurtimox exposure and serological response to treatment (by conventional ELISA using either recombinant or total purified antigen), or between exposure and non-conventional ELISA using F-29 or detection of parasite by qPCR (Fig. 1). Among children receiving the 60-day treatment regimen for whom ELISA data are available, 27 were in the ≥ 40 kg body weight category and 39 were in the < 40 kg body weight category (Table II). In the lower body weight category, 11 (27.5%) were seronegative at 12 months based on two different conventional ELISAs (purified antigen; recombinant antigen). Based on the modeled data, four of these children had plasma nifurtimox exposure that was below the 5th percentile in adults. In the higher body weight category, two children were seronegative by both conventional ELISAs and one of these children was underexposed to nifurtimox relative to the 5th percentile in adults.

Nifurtimox Exposure and Safety

Possible associations between the occurrence of specific TEAEs and exposure to nifurtimox were investigated in the overall group of 111 pediatric patients from CHICO who participated in PK evaluation. In total, 37 individuals experienced at least one TEAE. Of 67 events, none was severe, 14 were of moderate severity, and the remainder were mild. By organ system, the most frequent TEAEs ($n = 30$) were gastrointestinal (vomiting, 10; nausea, 8; diarrhea, 6; upper abdominal pain, 4; abdominal pain, 2). Otherwise, headache ($n = 13$), decreased appetite ($n = 9$), pyrexia and rash ($n = 3$, each), and asthenia ($n = 2$) were reactions occurring more than once in the group.

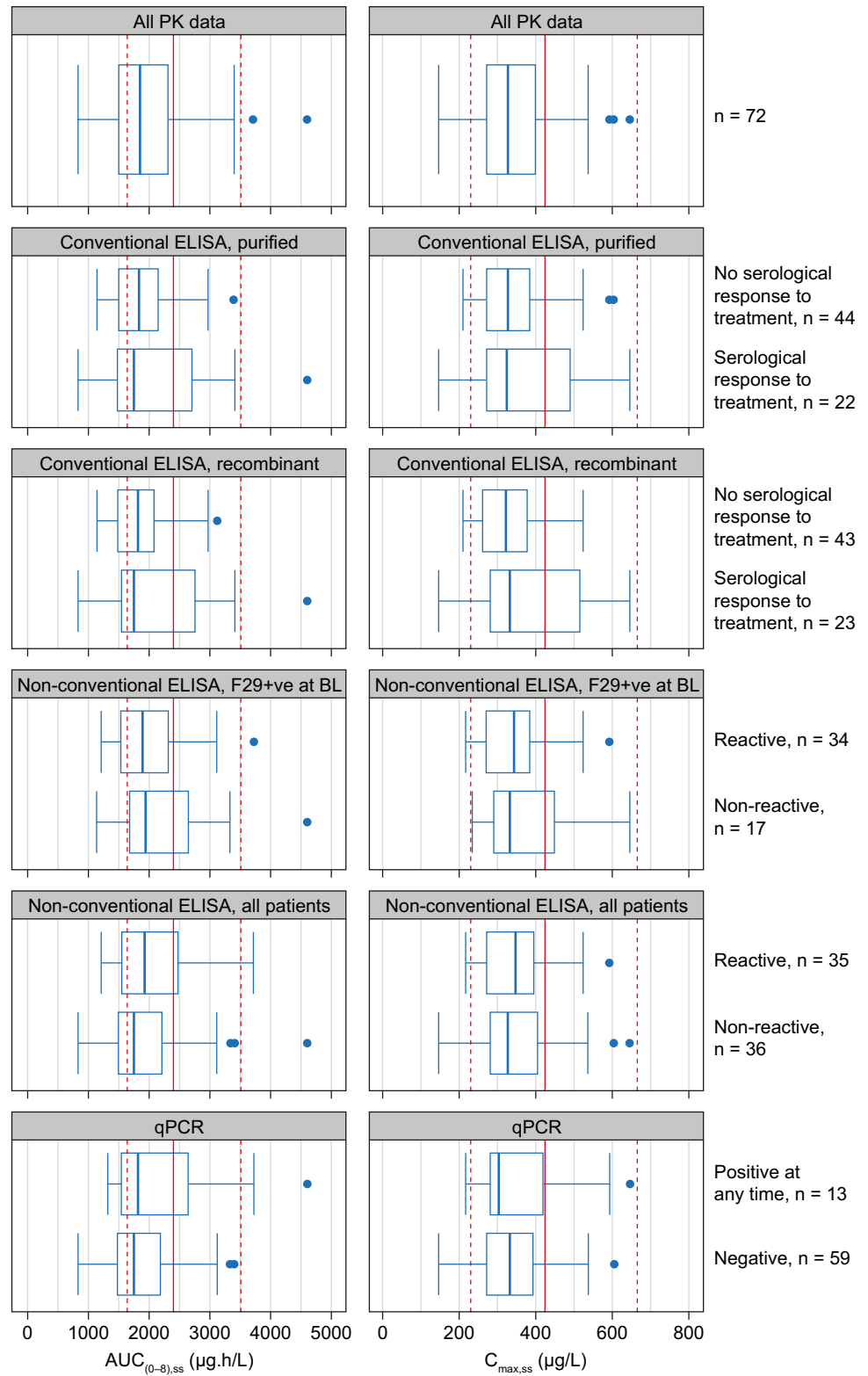
Overall, there were 213 nifurtimox-related and nifurtimox-unrelated TEAEs in the CHICO study. Analysis of the frequencies of given TEAEs in the group of individuals with PK data and in those without PK data found them to be highly correlated (R , 0.899), suggesting that with respect to the occurrence of TEAEs, those individuals with PK data were representative of the overall group. No apparent relationship between nifurtimox exposure and TEAEs was detected (Fig. 2a). Moreover, no apparent relationship between TEAEs or severity of TEAEs and body weight was found among pediatric patients (Fig. 2b).

Finally, we investigated whether nifurtimox exposure was associated with QTc prolongation in patients included in the PK set. At baseline in the CHICO study, 5 individuals had absolute QTcB > 480 ms and 2 had absolute QTcB > 500 ms; one had absolute QTcF > 480 ms, and none had absolute QTcF > 500 ms. At end of treatment, one individual had absolute QTcB > 480 ms, but none had absolute QTcB or QTcF > 500 ms. The same prevalence was observed at 12-month post-treatment follow-up. No changes in QT/QTc interval led to treatment discontinuation. Regression analysis of scatter plots of peak plasma nifurtimox concentration post-dose *versus* change from baseline in QTcB (Fig. 3a) and QTcF (Fig. 3b) both showed a small negative slope, providing no evidence of an association between increased exposure to nifurtimox and changes in QT/QTc interval.

Nifurtimox Dosing and Exposure

Applying the developed pediatric popPK model for nifurtimox (15), the exposure in all pediatric patients of the PK subgroup was re-simulated for age- and body weight-based dosing scenarios. As the PK of nifurtimox showed dose linearity (15), exposure at steady state was predicted from a single-dose simulation based on the superposition principle (linear PK over time). For each dosing scenario, the dosage for each individual is calculated as such that the resulting dosage would be a combination of available dose strengths

Fig. 1 Relationship between exposure, maximum plasma nifurtimox concentration, and pharmacodynamic measures of efficacy in individuals randomized to the 60-day nifurtimox treatment regimen in CHICO. Dotted red lines show the 5th ($AUC_{(0-8),ss}$, 1688 $\mu\text{g h/L}$; $C_{max,ss}$, 231 $\mu\text{g/L}$) and 95th ($AUC_{(0-8),ss}$, 3573 $\mu\text{g h/L}$; $C_{max,ss}$, 667 $\mu\text{g/L}$) percentiles, and the solid red line the median ($AUC_{(0-8),ss}$, 2441 $\mu\text{g h/L}$; $C_{max,ss}$, 426 $\mu\text{g/L}$) of plasma nifurtimox in adults based on an 8 mg/kg daily dose. The level of nifurtimox exposure did not correlate with either serological response to treatment (by conventional ELISA or non-conventional ELISA [response to *T. cruzi* F-29 flagellar protein]) or detection of parasite by qPCR. $AUC_{(0-8),ss}$ area under the curve at steady state within a dosing interval of 0–8 h; BL, study baseline; $C_{max,ss}$ maximum plasma nifurtimox concentration at steady state; ELISA, enzyme-linked immunosorbent assay; PK, pharmacokinetic; qPCR, quantitative polymerase chain reaction



(30 mg and 120 mg), selecting the first available dose that would end up between, e.g., 8–10 mg/kg/day or 10–20 mg/kg/day. The resulting exposures ($AUC_{0-8 h,ss}$) were visualized to investigate the difference in exposure between different

dosing scenarios in these children. Using a regimen based on the two approved dosing levels administered for 60 days, exposure to nifurtimox in most pediatric patients was within the same range as that shown to be effective in treating

Table II Number (%) of Subjects with a Serological Response to Treatment by Conventional ELISA Type and by Body Weight in Patients with PK Data Who Participated in CHICO and Received the 60-Day Nifurtimox Treatment Regimen

Assay	Weight	
	< 40 kg (<i>n</i> = 39)	≥ 40 kg (<i>n</i> = 27)
Conventional ELISA, purified Ag	18 (46.2)	4 (14.8)
Conventional ELISA, recombinant Ag	15 (38.5)	8 (29.6)
Both	11 (28.2)	2 (7.4)
Both, and exposure < 5th percentile of reference range	4 (10.3)	1 (3.7)

ELISA enzyme-linked immunosorbent assay, PK pharmacokinetic

Chagas disease in adults. The exposure profiles were similar whether a body weight threshold (40 kg) or an age threshold (12 years) was used to mark the transition between the two dosing levels (Fig. 4).

Simulating the PK of nifurtimox after a flat dosing regimen showed that only a relatively small subset of patients would have exposure levels within the reference range. The regimen was refined using body weight–adjusted dosing at the two extremes of the dose levels applied in the CHICO study (i.e., 8 and 20 mg/kg/day). At nifurtimox 8 mg/kg/day (the lowest dosing level used in patients weighing ≥ 40 kg), pediatric patients of all ages were often underexposed to drug, and at 20 mg/kg/day (the highest dosing level used in patients weighing < 40 kg), about half of the modeled population were overexposed to nifurtimox (Fig. 5). Refining the dosing regimen further by using approved, body weight–adjusted dose ranges across all body weights showed how exposure in younger children is close to the reference range if using a different dose range (10–20 mg/kg/day) from that used in older patients (8–10 mg/kg/day). Including a transition from the higher dose range to the lower dose range at a body weight of 40 kg aligns exposure with the reference range across most of the pediatric patients modeled (Fig. 6).

Discussion

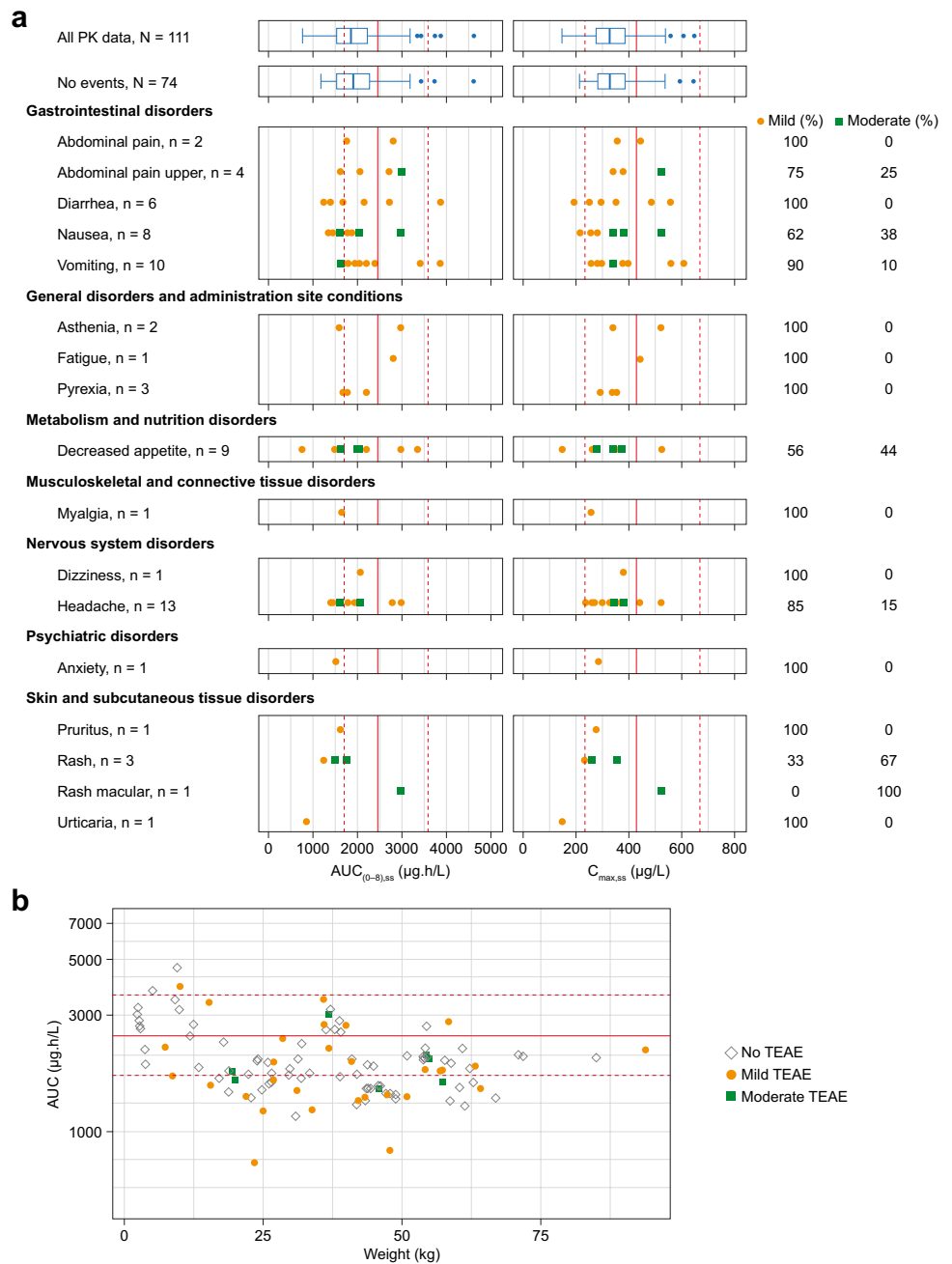
The aim of the work presented here was a retrospective exploration to find a PK/PD correlation based on the PK and PD data of the CHICO study in pediatric patients aged from birth to 17 years (8). In CHICO, the efficacy and safety of nifurtimox administered using the treatment regimens described herein were confirmed in pediatric subjects with Chagas disease by the primary efficacy parameter (i.e., at least 20% reduction in optical density or reversion of serological response to negative measured by two conventional ELISA tests) (8). However, post-treatment follow-up in

CHICO was limited to 12 months. In chronic Chagas disease, conversion of serological response to negative occurs several years or even decades after antiparasitic treatment in most individuals. Given the limitations resulting from the time of post-treatment follow-up, we refrained from defining a target exposure range based on these study results. Instead, we decided to build our assessment on phase 1 studies in adults. In the absence of data from controlled clinical studies in a corresponding patient population, we derived a reference exposure range for which antitrypanosomal efficacy can be assumed based on the experience from decades of clinical application. We explicitly defined this as the reference rather than the target range to account for the limitations of this approach.

Our investigations included a subgroup of patients enrolled in the phase 3 CHICO study who had consented to participate in the popPK part of the trial and for whom exposure data were collected. In terms of efficacy, we found no relationship between level of exposure to nifurtimox and serological measures associated with parasite clearance. In terms of safety, neither the overall level of exposure to nifurtimox nor peak plasma nifurtimox concentration was associated with the occurrence or severity of specific TEAEs. Modeling exposure in response to different treatment regimens supported the body weight–adjusted, age-stratified dosing regimen used in CHICO and demonstrated the limitations of using simpler dosing regimens in pediatric patients with Chagas disease. Comparison of demographic characteristics demonstrated that the subgroup of patients analyzed was representative of the overall CHICO population; therefore, findings within the subgroup can reasonably be extrapolated to the wider pediatric patient population.

To contextualize the efficacy and safety findings in pediatric patients reported here, we benchmarked the modeled levels of exposure in our analysis population against a 5th–95th percentile reference range derived from non-compartmental PK analysis of adult plasma nifurtimox data. This range was scaled from levels of exposure seen in subtherapeutic, single-dose, phase 1 studies of nifurtimox in adults with Chagas disease to provide an indication of the exposure range that would be expected in adults receiving nifurtimox at the approved therapeutic dose. Owing to a lack of adult clinical data (and of specific immunological or clinical markers) to validate the range as the therapeutic window of drug exposure, it is not intended to define fixed boundaries below which the antiparasitic efficacy of nifurtimox is compromised or above which TEAEs become more frequent or severe. The observation that median exposure and median maximum plasma nifurtimox concentration were similar in individuals who responded to treatment and in those who did not (Fig. 1) supports the assertion that the reference range does not define the efficacy limits of nifurtimox. In addition, Fig. 2b shows that individuals with mild TEAEs were

Fig. 2 a Relationship between exposure or maximum plasma nifurtimox concentration and incidence and severity of TEAEs and **b** relationship between plasma nifurtimox exposure and severity of TEAEs by body weight. Dotted red lines show the 5th ($AUC_{(0-8),ss}$, 1688 $\mu\text{g h/L}$; $C_{max,ss}$, 231 $\mu\text{g/L}$) and 95th ($AUC_{(0-8),ss}$, 3573 $\mu\text{g h/L}$; $C_{max,ss}$, 667 $\mu\text{g/L}$) percentiles, and the solid red line the median ($AUC_{(0-8),ss}$, 2441 $\mu\text{g h/L}$; $C_{max,ss}$, 426 $\mu\text{g/L}$) of plasma nifurtimox in adults based on an 8 mg/kg daily dose. No correlation was seen between nifurtimox exposure and TEAEs (a) or between TEAEs or severity of TEAEs and body weight (b); $AUC_{(0-8),ss}$, area under the curve at steady state within a dosing interval of 0–8 h; $C_{max,ss}$, maximum plasma nifurtimox concentration at steady state; TEAE, treatment-emergent adverse event



exposed to nifurtimox across the breadth of the adult reference range and often at levels below the 5th percentile, so the upper limit of the adult reference range certainly does not define a threshold beyond which TEAEs become more frequent. Thus, the degree to which the range can be used to interpret pediatric exposure data has limitations, but it does provide a bracket in which there is some precedent for the efficacy and safety performance of nifurtimox, and outside of which the risk of safety and efficacy issues are unknown.

Despite the absence of a clear relationship between nifurtimox exposure and efficacy parameters measured, both parasite susceptibility *in vitro* and clinical study data confirm

that nifurtimox is an effective treatment for Chagas disease. However, the findings do show that the relationship between exposure and available PD measures of clinical efficacy is not straightforward. Having a reference range for exposure is helpful because of the challenge of demonstrating parasitological cure in Chagas disease and, therefore, of characterizing efficacious dosing levels in pediatric patients based on what is effective in adults. Treatment success in Chagas disease is difficult to quantify based on negative seroconversion measured by conventional serologic tests, because *T. cruzi* elicits a strong antibody response which persists even after successful treatment and parasitic clearance. Indeed,

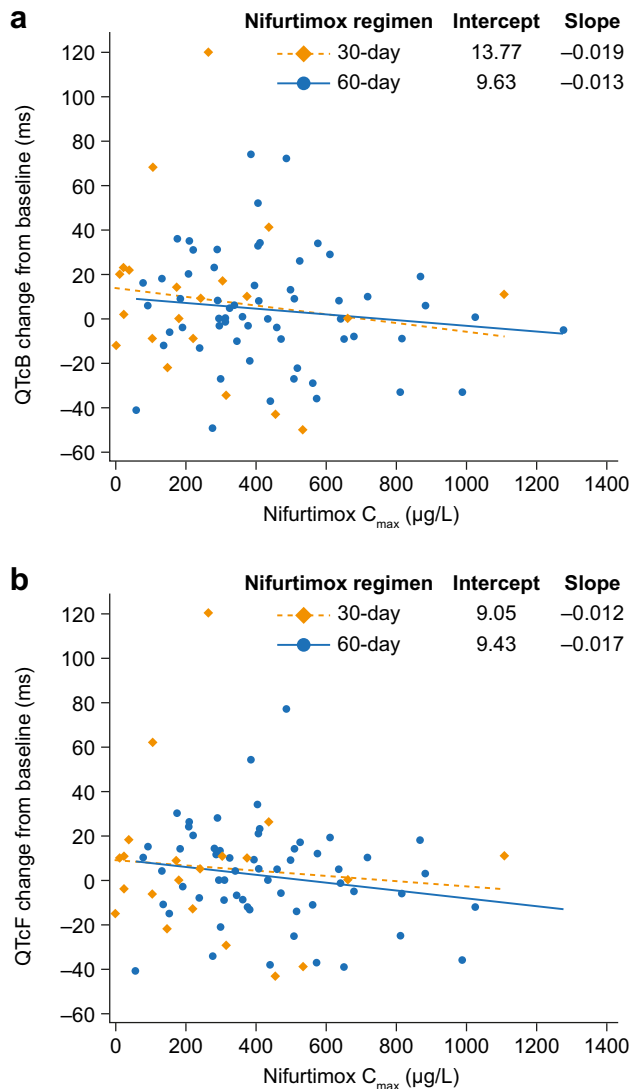


Fig. 3 Change from baseline in **a** QTcB interval and **b** QTcF interval versus nifurtimox maximum plasma concentration (C_{max}) after single dose and at steady state. No association was seen between increased exposure to nifurtimox and change in QTc interval. Nifurtimox plasma concentrations were taken at 2–4 h post-dose, which corresponds to the expected C_{max} . Values < lower limit of quantitation (LLOQ) are plotted as $0.5 \times \text{LLOQ}$

it can take 10 to 20 years for chronically infected adults to become seronegative following treatment. In a recent study in children aged 2–14 years at the time of antitrypanosomal treatment initiation, the average (*SD*) time to negative seroconversion by conventional serology was 15.7 (8.7) years post-treatment (18). Thus, the lack of any direct relationship between efficacy endpoints and nifurtimox exposure in our analysis may be attributable to the limited duration of follow-up post-treatment in CHICO (12 months).

The qPCR test also has limitations; a positive result can confirm treatment failure by detecting parasites in blood, but

a negative result is not a reliable indicator of parasitological cure. Further complications include the fact that different strains of *T. cruzi* have been shown to have variable levels of susceptibility to antiparasitic therapy (19, 20). It is also unknown whether children experience any drug concentration-dependent sensitivity to nifurtimox that is not observed in adult patients.

The TEAEs that were observed were representative of those known to be associated with nifurtimox. In CHICO, all but one of the TEAEs were classified as mild or moderate severity (8), and the few individuals studied here who had moderate TEAEs were generally below the median level of nifurtimox exposure in adults. Both the number of individuals who experienced a TEAE and the overall number of TEAEs experienced were relatively small, but we found no evidence of an association between safety outcomes and the levels of nifurtimox to which these patients were exposed. Drug-induced delay in cardiac repolarization is of particular interest in safety terms, but our analysis provided no evidence that nifurtimox caused QTc prolongation of particular concern (Fig. 3). In accordance with findings elsewhere (21), we found no evidence that nifurtimox delays cardiac repolarization. It cannot be concluded that no relationship exists between TEAEs and nifurtimox exposure, but it is unlikely that such a relationship would be seen among individuals receiving the age- and body weight-adjusted dosing used in CHICO.

A fixed-dose simulation (data not shown) was performed to better understand the relationship between physiological development of the child and exposure to nifurtimox. The PK data modeled and reported here demonstrate the limitations of using flat dosing regimens compared to established body weight-adjusted regimens in pediatric patients with Chagas disease. Furthermore, a flat-dose nifurtimox regimen seems to be ill-suited for the whole pediatric population owing to the very broad range of body weights seen from newborns to 18-year-olds. Thus, any flat-dose strategy would lead to extremes of exposure somewhere in the pediatric age range. Abrupt changes in exposure can also be seen on transition between different dose levels. This would not necessarily preclude the approach, but the maximum dose level at which body weight-adjusted dosing is applied would have to be considered carefully. The dose limit in humans is not well characterized; dose-limiting toxicity was reported in patients with neuroblastoma aged less than 21 years, receiving nifurtimox at more than 30 mg/kg/day (22). Toxic effects have also been reported in animal studies of nifurtimox at high doses (up to 150 mg/kg/day in rodents and up to 120 mg/kg/day in dogs for 28 days) (23).

Moving to a body weight-adjusted dosing regimen at a fixed level (for example, 8 mg/kg/day) with no defined body weight threshold at which the dose level changes (e.g., 40 kg) was also associated with exposure to nifurtimox

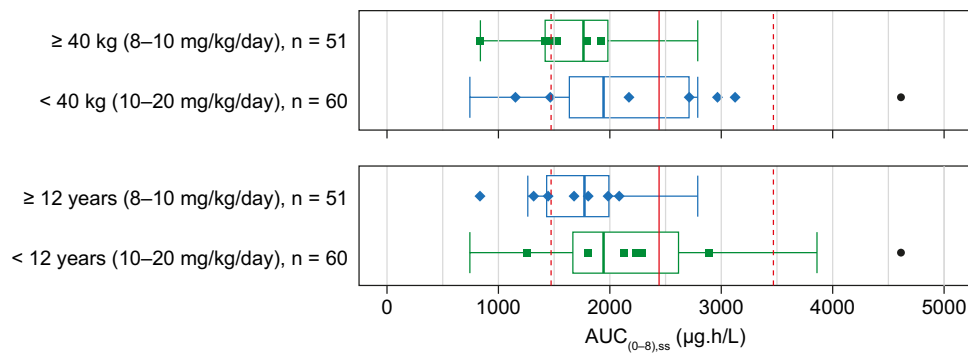


Fig. 4 Simulated pediatric exposure to nifurtimox estimated in a popPK model (7) of patients with Chagas disease for age- and body weight-based dosing scenarios. Dotted lines show the reference range (5th [1688 µg h/L] and 95th [3573 µg h/L] percentiles) of plasma nifurtimox exposure in adults; the solid line is median adult exposure (2441 µg h/L). Green rectangles represent patients < 12 years with ≥ 40 kg body weight, and blue diamonds represent patients ≥ 12 years with < 40 kg body weight. The threshold of 40 kg

is the median body weight in boys and girls aged 12 years based on population norms (16, 17). In most pediatric patients, body weight-adjusted exposure to nifurtimox based on the two dosing levels shown is within the same range of exposure known to be effective in treating Chagas disease in adults. The exposure profiles are similar whether a body weight threshold (40 kg) or an age threshold (12 years) marks the transition between the two dosing levels. $AUC_{(0-8),ss}$, area under the curve at steady state within a dosing interval of 0–8 h

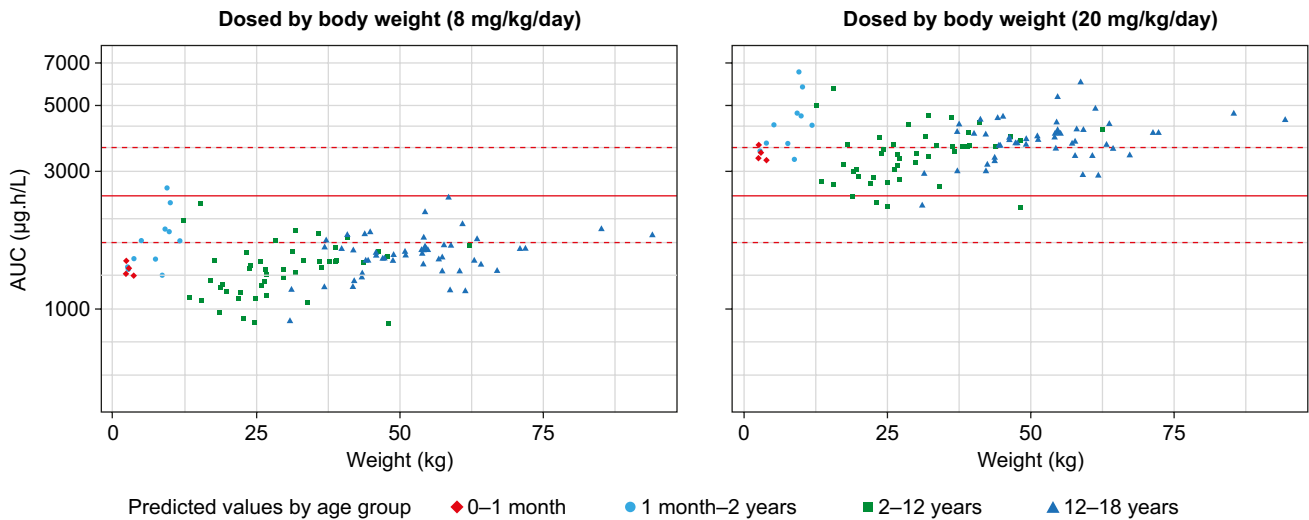


Fig. 5 Estimated pediatric exposure to nifurtimox based on body weight adjustment using two fixed-dose levels. Dotted red lines show the 5th (1688 µg h/L) and 95th (3573 µg h/L) percentiles, and the solid red line shows the median (2441 µg h/L) of plasma nifurtimox exposure in adults scaled based on a 500 mg daily dose. Underexpo-

sure to nifurtimox is often seen in pediatric patients at 8 mg/kg/day, and overexposure is seen in approximately 50% of pediatric patients at 20 mg/kg/day, compared with the adult reference range. AUC , area under the curve

outside of the adult reference range (Fig. 5). Selecting a low dose may prioritize safety in terms of reducing the risk of TEAEs, but this must be balanced against possible impact on effectiveness. As shown in Fig. 4, population norms indicate that children aged 12 years have a median body weight of approximately 40 kg (16, 17). Most of the pediatric population had a level of exposure below the adult reference range when modeled at 8 mg/kg/day, but nearly all children aged less than 12 years were below the range at this dosing level. It is hard to justify such a strategy when a higher dose would

have no safety implications. Similarly, prioritizing efficacy by using the higher dose of 20 mg/kg/day subjected most children aged over 12 years to a level of exposure that may put them at increased risk of TEAEs (Fig. 5).

Modeling of the two standard dose ranges used in pediatric patients (Fig. 6) showed how younger children are generally best served using a dose range that is different to that used in older patients in terms of balancing efficacy and safety by smoothing changes in exposure as body weight increases. However, there was also a noticeable inflexion

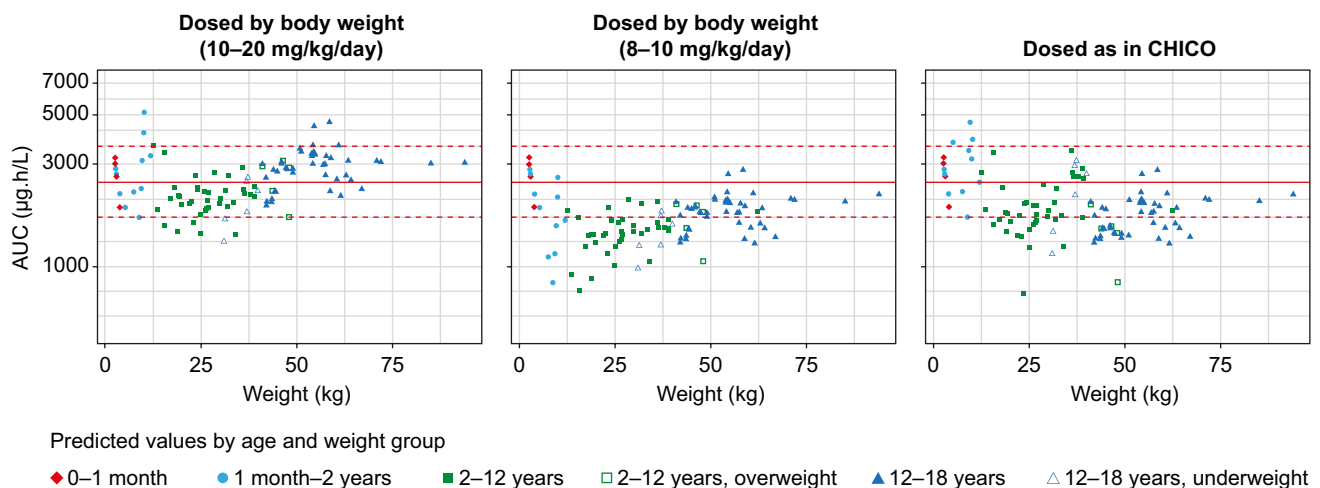


Fig. 6 Estimated pediatric exposure to nifurtimox based on body weight dose adjustment using single-dose ranges (10–20 mg/kg/day and 8–10 mg/kg/day) and with dosing used in CHICO, in which patients transitioned from the 10–20 mg/kg/day dose range to the 8–10 mg/kg/day range at age 12 years. Dotted red lines show the 5th (1688 µg h/L) and 95th (3573 µg h/L) percentiles, and the solid red line shows the median (2441 µg h/L) of plasma nifurtimox exposure

in the data modeled at the 10–20 mg/kg/day dose that coincides with the threshold of body weight of 40 kg and age 12 years. Comparison with modeled data in which pediatric patients switch from the higher to the lower dose range at age 12 years shows that such a regimen reduces the likelihood of excessive nifurtimox exposure in adolescents. The regimen could be refined even further by introducing another threshold and more than two dose ranges, but this would increase the complexity of administration. Figure 6 also shows that modeled levels of exposure based on the dosing used in CHICO were generally below the median exposure level in adults. Thus, the efficacy of nifurtimox in CHICO was not attributable to drug overexposure, and in safety terms, levels of exposure were conservative.

Conclusions

Our study could not define a relationship between nifurtimox exposure in pediatric patients with Chagas disease and PD measurements of serological response to treatment, nor could we find any relationship between plasma nifurtimox exposure and the occurrence of selected TEAEs. A lack of clear and early markers which correlate with clinical outcomes following antitrypanosomal therapy poses a drug development challenge, limiting standard dose-finding methodologies. Based on our modeled data, the approved pediatric nifurtimox dosing regimen would be expected to

in adults scaled based on a 500 mg daily dose. The actual dose for each patient was calculated based on the combination of available tablet sizes (30 or 120 mg) that would fall within the specified dose range (8–10 mg/kg/day, or 10–20 mg/kg/day). Exposure increases steeply in infants and children younger than 12 years. *AUC*, area under the curve

achieve similar or lower levels of plasma nifurtimox exposure to those seen in adults with Chagas disease, and in general, attempts to simplify the pediatric dosing regimen were generally associated with greater deviations from exposure ranges known for adults. Further refinements to the dosing regimen would be unlikely to offer efficacy benefits, and any additional complexity may indeed be counterproductive in terms of impeding compliance.

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Author Contribution Heino Stass: conception of the work, interpretation of the data, drafting and critical revision of the work.

Ibrahim Ince: analysis and interpretation of the data, drafting and critical revision of the work

Ulrike Grossmann: analysis and interpretation of the data, drafting and critical revision of the manuscript

Boris Weimann: interpretation of the data, critical revision of the manuscript

Stefan Willmann: conception of the work, analysis and interpretation of the data, critical revision of the manuscript

All authors approve the version of the manuscript submitted for publication. All authors agree to be accountable for all aspects of the work and its accuracy and integrity and agree to investigate and resolve any questions relating to the work.

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Declarations

Conflict of interest Heino Stass, Ibrahim Ince, Ulrike Grossmann, and Stefan Willmann are employees of Bayer AG. Boris Weimann is the owner of Chrestos Concept GmbH & Co. KG, an organization that received funding from Bayer AG for undertaking parts of the work reported here.

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