Pharmacodynamics of Posaconazole in Experimental Invasive Pulmonary Aspergillosis: Utility of Serum Galactomannan as a Dynamic Endpoint of Antifungal Efficacy

Silke Gastine [1,2], William Hope [3], Georg Hempel [1], Ruta Petraitiene [4], Vidmantas Petraitis [4], Diana Mickiene [5], John Bacher [6], Thomas J. Walsh [7], and Andreas H. Groll [8 *]

[1] Institute of Pharmaceutical and Medical Chemistry – Dept. of Clinical Pharmacy, Westfälische Wilhelms-Universität Münster;
[2]Infection, Immunity & Inflammation Research & Teaching Dept., GOS Institute of Child Health, University College London, London, United Kingdom
[3]Centre of Excellence in Infectious Diseases Research, Department of Molecular and Clinical Pharmacology, University of Liverpool, United Kingdom
[4]Transplantation-Oncology Infectious Diseases Program, Division of Infectious Diseases, Department of Medicine, Weill Cornell Medicine of Cornell University, New York, NY, USA
5Laboratory Animal Sciences Program, SAIC-Frederick, Inc., Frederick, MD, USA
[6]Surgery Service, Veterinary Resources Program, Office of Research Services, National Institutes of Health, Bethesda, MD
[7] Departments of Pediatrics and Microbiology & Immunology, Weill Cornell Medicine of Cornell University, New York, NY, USA
[8] Infectious Disease Research Program, Center for Bone Marrow Transplantation and Dept. of Pediatric Hematology/ Oncology, University Children's Hospital Münster; Germany

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Corresponding Author:
Andreas H. Groll, M.D.
Infectious Disease Research Program
Center for Bone Marrow Transplantation and
Department of Pediatric Hematology and Oncology
University Children´s Hospital Münster
Albert-Schweitzer-Campus 1
D-48149 Münster, Germany
Phone -49-251-8347742
FAX -49-251-8347828
e-mail andreas.groll@ukmuenster.de

ABSTRACT

Background. *Aspergillus* galactomannan antigenemia is an accepted tool for the diagnosis of invasive pulmonary aspergillosis (IPA) in neutropenic patients. Little is known, however, about the utility of this biomarker to assess the efficacy of antifungal therapies.

Methods. The pharmacokinetics and pharmacodynamics (PK/PD) of posaconazole in treatment and prophylaxis were investigated in the persistently neutropenic rabbit model of *Aspergillus fumigatus* IPA at doses between 2 and 20 mg/kg and day. Sparse plasma sampling was used to obtain PK data at steady state, and the serum galactomannan index (GMI), as a dynamic endpoint of antifungal response, was obtained every other day in addition to conventional outcome parameters including survival and fungal tissue burden. Nonparametric PK/PD model building was performed using the Pmetrics Package in R.

Results. A one-compartment model with linear elimination best described the PK of posaconazole. The PD effect of posaconazole exposure in plasma on the GMI in serum was best described by a dynamic *Hill*-functions reflecting growth and kill of the fungus. Through calculations of the $AUC_{0\text{-}24h}$ at steady state, the exposure-response relationship between posaconazole and the GMI for treatment followed a sigmoidal function with an asymptote forming above an AUC0-24h of 30 mg*h/L. All prophylactic doses were able to control the fungal burden.

Conclusions. A nonparametric population PK/PD model adequately described the effect of posaconazole in prophylaxis and treatment of experimental IPA. An $AUC_{0\text{-}24h}$ greater than 30 mg*h/L was associated with adequate resolution of the GMI, which is well in support of previously suggested exposure-response relationships in humans.

61 INTRODUCTION

62

63 Posaconazole is a second generation antifungal triazole that is structurally related to itraconazole
64 and possesses broad spectrum antifungal activity *in vitro*, predictable pharmacokinetics, moderate
65 potential for drug-drug interactions, and an overall favorable safety profile (1-3). Based on a set of
66 carefully designed and well executed clinical trials performed during the past two decades (4-6), the
67 compound has evolved into an important option for prophylaxis and treatment of invasive
68 opportunistic fungal diseases (IFDs) in severely immunocompromised patients. Whereas the
69 usefulness of the initially approved oral suspension was limited by high intra- and interindividual
70 bioavailability (7, 8), the subsequently developed delayed release tablet formulation and the parenteral
71 cyclodextrin formulation allow for more controlled administration of the compound (9-12). Leading
72 international guidelines currently recommend posaconazole for primary antifungal prophylaxis in
73 patients with acute myeloid leukemia/myelodysplastic syndrome and prolonged neutropenia and in
74 patients with acute graft vs. host disease following allogeneic hematopoietic stem cell transplantation,
75 as well as second line therapy for treatment of invasive aspergillosis (13-15).

76 Posaconazole is predominantly metabolized via phase II glucuronidation and while CYP3A4
77 inhibition affects drug-drug interactions, only minor metabolization can be attributed to the CYP450
78 family. The compound is highly protein bound with serum albumin being the predominant binding
79 protein. Previous studies with the oral suspension have shown that posaconazole exhibits linear
80 elimination with a high apparent volume of distribution, a slow rate of absorption (8), and varying
81 bioavailability. Nevertheless, no distribution into deeper compartments was detectable and a one
82 compartment pharmacokinetic model was used in previous pharmacokinetic analyses (5). More recent

studies with the intravenous formulation revealed smaller volumes of distribution and higher peak concentrations relative to the oral suspension (2, 3, 16).

Exposure response relationships have been developed for most antifungal compounds (17, 18). For posaconazole, regulatory guidance in the process of dose finding studies for the tablet and the intravenous formulations (19) and a European Committee for Antimicrobial Susceptibility Testing (EUCAST) rationale (20) propose a dosing target of a minimum trough concentration of 0.7 mg/L for prophylaxis, corresponding to the required area under the curve/minimum inhibitory concentration (AUC/MIC) ratio of 167; an average concentration of 1.25 mg/L is suggested for salvage therapy. However, these relationships rely on observed outcomes linked to the drug exposure in clinical trials rather than a distinct pharmacodynamic criterion (21, 22).

The polysaccharide galactomannan is a major component of the cell wall of *Aspergillus* spp., and released into the systemic circulation during fungal degradation (23). It is detectable in the serum in some patients even before characteristic symptoms of invasive aspergillosis are present. Studies using galactomannan in serum as a diagnostic marker with a threshold of 0.5 for proven or probable disease status resulted in a test sensitivity of 82% with a specificity of 81% (24). The role of serum galactomannan as a surrogate marker of success or failure of antifungal interventions is an area of active investigation (25, 26). Nevertheless, it has been shown that a GMI-based response criterion, as aspergillosis specific marker in hematologic cancer patients, compares favorably with the EORTC/MSG invasive aspergillosis response definition, as well as survival outcomes and can be beneficial regarding earlier assessments of treatment response (27-29).

We therefore investigated the pharmacokinetics and pharmacodynamics (PK/PD) of posaconazole in experimental invasive pulmonary aspergillosis (IPA) using the galactomannan index (GMI) as a dynamic endpoint of antifungal response.

106 MATERIALS AND METHODS

107

108 **Study overview.** In order to develop a pharmacokinetic/pharmacodynamic (PK/PD) model of
109 posaconazole in prophylaxis and treatment of invasive pulmonary aspergillosis using galactomannan
110 as a dynamic endpoint of efficacy, raw data from experiments of previously published studies
111 investigating the pharmacokinetics and antifungal activity in normal and persistently neutropenic
112 rabbits was used. (30, 31).

113 The study included data from a total of 70 animals studied in four different experimental cohorts: 1)
114 Six healthy, non-infected rabbits who had received a single dose of 20mg/kg of posaconazole followed
115 by serial plasma sampling to explore the plasma pharmacokinetics of the compound in rabbits; 2) nine
116 neutropenic rabbits with experimental IPA who received posaconazole at 2, 6, and 20mg/kg QD as
117 prophylaxis starting four days prior to inoculation; 3) 16 neutropenic rabbits with experimental IPA
118 who received posaconazole at 2, 6, and 20mg/kg QD as treatment starting 24 hours after inoculation;
119 4) 22 rabbits neutropenic rabbits with experimental IPA who received posaconazole at 1, 2, and 3
120 mg/kg BID as treatment starting 24 hours after inoculation; and 15 rabbits with experimental IPA who
121 served as untreated controls in cohorts 2 and 3 (30, 31).

122 For development of the population PK model, data from the 53 posaconazole-treated animals included
123 in cohorts 1 to 4 were used. For investigation of the pharmacodynamics in experimental IPA, data
124 from 25 posaconazole-treated animals of cohorts 2 and 3 and 15 untreated controls were used for
125 whom serial QOD sampling of serum galactomannan was available. Cohort 4 was not included in the
126 PK/PD model, since only the last available serum galactomannan values were determined. An
127 overview of the study cohorts is provided in Figure 1 (Figure 1).

128

129 **Animals**. Healthy female New Zealand White rabbits weighing 2.6 to 3.7 kg (Hazleton, Deutschland,
130 PA) were used in all experiments. Rabbits were individually housed and maintained with water and
131 standard rabbit feed *ad libitum* according to National Institutes of Health (NIH) guidelines and in
132 fulfillment of the criteria of the American Association for Accreditation of Laboratory Animal Care
133 (NRC 1996). Vascular access was established in by placement of a silastic tunneled central venous
134 catheter (32).
135
136 **Organism and inoculation**. *Aspergillus fumigatus* (NIH isolate 4215; ATCC no. MYA1163)
137 obtained from a fatal case of pulmonary aspergillosis was used in all experiments. The minimum
138 inhibitory concentration (MIC) performed by NCCLS methods (33, 34) and the minimum fungicidal
139 concentration (MFC) for posaconazole was 0.125 µg/ml.

140 Pulmonary aspergillosis was established as previously described (31, 35). For each experiment, the *A.*
141 *fumigatus* inoculum was prepared from a frozen isolate that was subcultured onto Sabouraud dextrose
142 slants (BBL, Cockeysville, MD). Those slants were incubated for 24 h at 37°C and then kept at room
143 temperature for 5 days before use. Conidia were harvested under a laminar airflow hood with a
144 solution of 10 ml 0.025% Tween 20 (Fisher Scientific, Fair Lawn, NJ) in 0.9% NaCl (Quality
145 Biological, Inc., Gaithersburg, MD), transferred to a 50-ml conical tube, washed, and counted with a
146 hemocytometer. The concentration was adjusted to a predetermined inoculum of $1x10^8$ conidia of *A.*
147 *fumigatus* in a volume of 250 to 350 µl and confirmed by serial dilutions cultured on Sabouraud
148 glucose agar (SGA).

149 Inoculation was performed on day 2 of the experiments under general anesthesia. Each rabbit was
150 anesthetized with 0.8 to 1.0 ml of a 2:1 mixture (vol./vol.) of IV ketamine (100 mg/ml) obtained as
151 Ketaset® (Phoenix Scientific, Inc., St. Joseph, MO) and xylazine (20 mg/ml) (Bayer Corp.,

Agriculture Division, Animal Health, Shawnee Mission, KS) obtained as Rompun®. A Flagg O straight-blade laryngoscope (Welch Allyn Inc., Skaneateles Falls, N.Y.) was inserted in the oral cavity until the vocal cords were clearly visualized and the inoculum was administered intratracheally with a tuberculin syringe attached to a 5 1/4-inch 16 gauge Teflon catheter (Becton Dickinson Infusion Therapy Systems Inc., Sandy, UT).

Immunosuppression and maintenance of neutropenia. Profound and persistent neutropenia (neutrophil count of <100/µl) was achieved by an initial course of 525 mg of cytarabine (Ara-C; Cytosar-U; The Upjohn Company, Kalamazoo, MI) per m^2 for 5 consecutive days starting one day before endotracheal inoculation. A maintenance dose of 484 mg of Ara-C per m^2 was administered for 4 additional doses on days 8, 9, 13, and 14 of the experiment. Methylprednisolone (Abbott Laboratories, North Chicago, IL) at 5 mg/kg of body weight was administered on days 1 and 2 of the experiment in to facilitate establishment of infection.

Ceftazidime (Glaxo, Inc., Research Triangle Park, N.C.) (75 mg/kg given IV twice daily), gentamicin (Elkins-Sinn, Inc., Cherry Hill, NJ) (5 mg/kg given IV every other day), and vancomycin (Abbott Laboratories, North Chicago, IL)) (15 mg/kg given IV daily) were administered from day 4 of immunosuppression until study completion to prevent opportunistic bacterial infections during neutropenia. To prevent antibiotic-associated diarrhea due to *Clostridium spiroforme*, rabbits continuously received 50 mg of vancomycin per liter of drinking water.

Antifungal compound. Posaconazole was provided by the Schering-Plough Research Institute. Drug stock solution (30 mg/ml) was prepared by dissolving the antifungal powder in solution of distilled water and Tween 80 (Fisher Scientific, Fair Lawn, NJ) according to manufacturer's instructions.

Treatment regimen. Study groups consisted of either untreated controls or animals treated with posaconazole administered orally (po) at dosages of 2, 6, and 20 mg/kg QD (cohort 3) or 1, 2, and 3 mg/kg BID (cohort 4), respectively. Antifungal therapy was started after 24 h after endotracheal inoculation and continued throughout the course of the experiments for a maximum of 12 days in surviving rabbits.

Prophylaxis regimen. The prophylaxis experiments used the same methods as described above with the following exceptions. Rabbits received the same dosages of posaconazole (2, 6, or 20 mg/kg/day) administered for 4 days before endotracheal inoculation. On the day of inoculation, posaconazole was administered in the morning and the endotracheal inoculum was administered approximately 4 h later. Posaconazole was then continued for a maximum of 12 more days after inoculation. To simulate the setting of antifungal prophylaxis, the administered inoculum was 5×10^7 conidia.

Outcome variables. Surviving rabbits were euthanized by intravenous (IV) administration of sodium pentobarbital (The Butler Company, Columbus, OH) (65 mg (1 ml) /kg of body weight) at 24 h after administration of the last dose of antifungal drug or vehicle (controls). In the primary experiments, a panel of outcome variables was used to assess antifungal efficacy. These variables included survival in days post inoculation, lung weight and pulmonary infarct score as measure of organism-mediated pulmonary injury, microbiological clearance from lung tissue in log CFU per gram. Blood was collected every other day from each rabbit and the serum galactomannan index determined with the exception of cohort 4, where only the last available specimen was determined.

Pharmacokinetic sampling. A sparse sampling strategy was employed to obtain key pharmacokinetic parameters in each individual infected animal and to correlate pharmacodynamic parameters with endpoints of antifungal efficacy. The time points for sparse plasma sampling were determined using optimal sampling theory implemented by the ADAPT II computer program (36) and full concentration-vs. time profiles from six healthy rabbits following p.o. administration of a single dose of 20 mg/kg with dense sampling prior to dosing and at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours post dose (cohort 1) (37). The plasma profiles of these rabbits fitted to a 1-compartment pharmacokinetic model with first order input, no lag time, and first order elimination. The model fitted the data well with a mean r^2 value of 0.964. The selected time points for sparse sampling were immediately before dosing, and 1, 4, 8, and 24 hours post dosing (30). Plasma sampling in infected rabbits of cohorts 2, 3, and 4 was performed 6 days after inoculation. Blood samples were immediately centrifuged, and plasma was stored at -80°C until assayed.

Analytical method. Concentrations of posaconazole were determined after solid phase extraction by liquid chromatography-tandem mass spectrometry (LC-MS) at the Schering-Plough Research Institute (Kenilworth, NJ, USA). The analytical procedure involved dilution of the samples in controlled plasma prior to extraction. The quantifiable range of the assay was 4 to 1000 ng/ml. Accuracies (bias) and intra- and inter-day variability (precision) were within ± 15 % and ± 20% at the lower limit of quantitation (LLQ) (30, 31).

Pharmacodynamic sampling. To describe the pharmacodynamics of posaconazole in prophylaxis and treatment of experimental invasive pulmonary aspergillosis, the galactomannan index (GMI) was used as a dynamic endpoint of antifungal efficacy. Blood was collected from each infected rabbit prior

to inoculation and every other day thereafter, and the serum GMI was determined. In cohort 4, the GMI was determined from each rabbit only in the last available specimen (30, 31).

Galactomannan assay. Serum galactomannan was determined by the Platelia Aspergillus EIA (Genetic Systems/Sanofi Diagnostic Pasteur, Redmond, WA) immunoenzymatic sandwich microplate assay method as previously described (31). EIA data were expressed as a serum galactomannan index (GMI). The GMI for each test serum is equal to OD (optical density determined by microplate spectrophotometer) sample divided by OD threshold serum.

Population PK/PD modeling. PK/PD model building was performed using the nonparametric adaptive grid (NPAG) approach with the Pmetrics software package in R (Version 1.5.1, Laboratory of Applied Pharmacokinetics, Los Angeles, CA, USA) (38). The additive Lambda approach was chosen to describe the residual error. Model building consisted of a two-step process. First the population PK Model was created. In a second step the model was extended to a full PK/PD model.

An overview of the PK/PD analysis study setup is given in Figure 2.

Pharmacokinetic model. To explore the pharmacokinetics of posaconazole in rabbits, an initial model was built on the basis of dense concentration data of six healthy rabbits after a single p.o. dose of 20 mg/kg of posaconazole (cohort 1). In a next step, the steady state concentration data obtained from infected rabbits receiving posaconazole as prophylaxis or treatment was added (cohorts 2, 3, and 4).

During the model building process, different structural options were tested. Models consisting of one or two compartments with either linear or nonlinear Michaelis-Menten type elimination were taken into consideration. To compare the different models, the log-likelihood profile was used on nested models. Non-hierarchical models were compared using the *Akaike* information criterion (AIC). In

addition to statistical criteria, graphical output was used for model evaluation, including goodness-of-fit plots comparing individual and population predictions with observed plasma concentrations, as well as graphical evaluations of the residuals.

Pharmacodynamic model. The pharmacodynamic effect of posaconazole in prophylaxis and treatment of experimental invasive pulmonary aspergillosis reflected by the serial assessment of the GMI (cohorts 2 and 3) was added to the final PK model. Since the GMI for each rabbit was determined only in the last available specimen, data from rabbits of cohort 4 were not included in this analysis. The previous PK modelling thus informed PK support point distributions for the full PK/PD model, where PK samples where only available at steady-state.

In a first step, adequate functions to represent the evolution of the GMI with and without antifungal treatment were explored. For this purpose, a subset including only the treatment data was formed. The pharmacodynamic effect was modelled linearly, with power functions as well as sigmoidal Hill functions. The type of function that best depicted the pharmacodynamics of posaconazole was then expanded to reflect the prophylaxis arm. During the model building process, the selection of the most appropriate model was guided by the inspection of the AIC and goodness of fit plots, as well as residual plots.

Exploration of PK/PD relationships. The final PK/PD model was used to calculate the individual Bayesian posterior for each parameter and was subsequently used to determine the area under the concentration-time-curve for posaconazole and for the GMI. To determine the effectiveness of the treatment regimens, the $AUC_{0\text{-}24\,h}$ for both variables was compared. For posaconazole plasma concentration AUC calculations, day 5 of the study was chosen, as posaconazole was thought to be at steady state and the GMI to be reasonably evolved at this time point. The prophylactic regimen was

evaluated by comparing the posaconazole trough level on the day of the inoculation with the GMI on day 5 after inoculation, to allow for a reasonable time frame for IPA evolution under prophylaxis.

RESULTS

Antifungal therapy. There was a significant improvement in survival post inoculation of rabbits treated with posaconazole compared to that of untreated controls. Through the entire study, 29 (76%) of 38 rabbits treated with posaconazole survived in cohorts 3 and 4, and none of the eight untreated controls survived ($p<0.001$ by Fisher Exact Test). There also was a significant quantitative reduction in the growth of *A. fumigatus* in lung tissues from rabbits treated with posaconazole in comparison to that of untreated controls as measured by the mean log CFU per gram ±SEM at the end of the experiment (0.28 ±0.07 vs. 1.49 ±0.17; $p<0.001$ by Mann Whitney U Test). Consistent with the improvements in survival and organismal clearance from lung tissue, rabbits treated with posaconazole had a significantly lower mean ±SEM GMI relative to untreated controls in the last of serial (QOD) measurements obtained during the experiments (1.78 ±0.37 vs. 4.66 ±0.54; $p=0.002$ by Mann Whitney U Test) (Figure 3).

Antifungal prophylaxis. Similar to antifungal therapy, in comparison to untreated controls, rabbits receiving antifungal prophylaxis with posaconazole had significant improvements in survival (8/9 vs. 0/9; $p<0.001$ by Fisher Exact Test), a significant reduction of the residual pulmonary fungal burden at the end of the experiments (0.13 ±0.08 vs. 1.40 ±0.72; $p<0.001$ by Mann Whitney U Test), and a significantly lower mean ±SEM GMI in the last of serial (QOD) measurements obtained during the experiments (0.34 ±0.08 vs. 4.28 ±0.83; $p<0.001$ by Mann Whitney U Test) (Figure 3).

Antimicrobial Agents and Chemotherapy

Population pharmacokinetic model. For development of the population pharmacokinetic model, 270 concentration-time points from the 53 posaconazole-treated animals included in cohorts 1 to 4 (Figure 1) were used. A one-compartment pharmacokinetic model with first-order oral absorption and linear elimination best described the pharmacokinetics of posaconazole in the densely sampled healthy rabbits studied after administration of a single dose of 20 mg/kg (cohort 1). Additional compartments or implementing a non-linear elimination did not improve the model in terms of statistical or graphical criteria.

The final model developed in the healthy rabbits was transferred to the infected rabbits receiving posaconazole as treatment or prophylaxis, in whom sparse sampling was performed at presumed steady/state after multiple daily doses ranging from 2 to 20 mg/kg. In this step, the absorption rate constant was not estimated sufficiently and thus fixed to 0.35 h^{-1}, the mean estimate derived from the data obtained in the group 4 pre-analysis.

For the final pharmacokinetic model component, a linear regression of the individual predictions through utilization of the Bayesian posterior versus the observed values resulted in a mean (95% confidence interval) intercept of -0.018 (-0.11 - 0.075). A mean slope of 1.04 (0.99 - 1.09) and a correlation coefficient of 0.934 were determined.

Pharmacodynamic model. For development of the pharmacodynamic model, data from 25 posaconazole-treated animals of cohorts 2 and 3 and 15 untreated controls were used for whom serial QOD sampling of serum galactomannan was available (Figure 2). The combined group 2 and 3 data set included a total of 125 posaconazole concentration-time points and a total of 211 individual GMI measurements.

The effect of posaconazole on the GMI was implemented via integration of an effect compartment. In a first step, only data from the treatment cohort was used. Data from the prophylaxis cohort was added consecutively.

The evolution of the GMI was best described via sigmoidal Hill functions:

$$dGMI/dt = Kgmax * (1 - (\frac{POC}{Vc})\text{^}Hg/((C50g)\text{^}Hg + (\frac{POC}{Vc})\text{^}Hg)) * (1 - CEFF/POPmax)$$

$$* CEFF - Kkmax * (\frac{POC}{Vc})\text{^}Hk/((C50k)^{Hk} + \frac{POC}{Vc})\text{^}Hk \ * CEFF$$

where POC, amount Posaconazole in the PK compartment at time *t*; CEFF, GMI in the effect compartment at time *t*, POPmax is population maximal growth reflected by galactomannan index; Hg, Hill coefficient for growth; Hk, Hill coefficient for kill; Kgmax, maximum rate of growth; Kkmax, maximum rate of kill; C50g, concentration for half maximal growth; C50k, concentration for half maximal kill ; Vc, central volume of distribution.

The implemented Hill functions were able to describe the GMI decline in treatment and prophylaxis together with the GMI increase in the control group. Due to the non-parametric modelling approach, only one set of parameters was necessary for the entire population, with the respective support point distribution estimated. A summary of the estimated PK/PD model parameters is shown in table 1 (Table 1).

323 The linear regression of the individual GMI predictions through utilization of the Bayesian posterior
324 versus the observed values resulted in a mean (95% confidence interval) intercept of 0.11 (-0.002 -
325 0.224). A mean slope of 0.933 (0.882 - 0.983) and a correlation coefficient of 0.864 were determined.

326 The goodness of fit for individual posaconazole and GMI predictions is shown in Figures 4 and 5
327 (Figures 4 and 5).

328 **Exploration of pharmacokinetic/pharmacodynamic relationships.** The final PK/PD model was
329 used to explore the relationship between posaconazole exposure and the GMI. For this purpose, the
330 AUC $_{0\text{-}24\ h}$ was calculated for posaconazole and for the GMI in rabbits included in the treatment group
331 (cohort 3).

332 To quantify the relationship and to derive a pharmacodynamic threshold, a function was fit to the
333 derived AUCs (Figure 6). A sigmoidal function best described the relationships of both AUCs:

$$\text{AUC } GMI = \alpha - \frac{Emax * AUC\ POC^{\gamma}}{EC_{50}{}^{\gamma} + AUC\ POC^{\gamma}}$$

334 where AUC is the area under the concentration-time curve; POC, posaconazole; GMI, galactomannan
335 antigen index; γ, Hill coefficient.

336 α= 118, Emax, maximal GMI depicting fungal effect; EC50, AUC POC for half maximal effect.
337 Emax= 93.7 GMI, EC50= 11.6 mg/L/h, γ= 2.3.

338 In the prophylaxis group (cohort 2), the posaconazole trough concentration was determined at the time
339 of inoculation and compared to the AUC $_{0\text{-}24h}$ for the GMI on day 5 post inoculation. When
340 posaconazole prophylaxis was administered, the calculated GMI AUC with day 5 post inoculation did
341 not exceed 24, suggesting the GMI did not cross the threshold of 1 with this 24h time window,

whereas in the control group (visible as AUCs at posaconazole concentration of 0 mg/L) show high GMI AUC values.

In figures 6 and 7 (Figure 6 and 7), the GMI AUCs for treatment and prophylaxis are compared to posaconazole exposure, showing the effect of posaconazole treatment and prophylaxis on *A. fumigatus* infections throughout the tested posaconazole dosing range. The quantification of these relationships was facilitated through the PKPD modelling approach, which enables extrapolation of Posaconazole concentration and GMI to the necessary time points and thus is able to display more dynamically the pharmacodynamic effect in prophylaxis and treatment.

DISCUSSION

In this well-established persistently neutropenic rabbit model, posaconazole was highly effective in prophylaxis and treatment of experimental IPA, as documented by endpoints of survival, residual fungal burden in lung tissue, and suppression of the GMI in serum at end of treatment. The PK/PD relationship between posaconazole exposure in plasma and the evolution of the GMI during prophylaxis and treatment was best described by dynamic Hill-functions reflecting growth and kill of the fungus. The exposure-response relationship for treatment followed a sigmoidal function with an asymptote forming above an $AUC_{0\text{-}24h}$ of 30 mg*h/L.

Whereas a link between drug exposure and observed outcomes has been documented in animal models (31, 39) and in clinical studies (6, 40, 41), to the best of our knowledge, this is the first published PK/PD modelling study investigating the effects of posaconazole against IPA taking the effects of treatment and prophylaxis into account. In a murine kidney target model of invasive candidiasis using non-compartmental PK, the $AUC_{0\text{-}24h}$ /MIC ratio was most predictive of treatment success (42) which

365 is in accordance to previous PK/PD assessments of antifungal triazoles in invasive candidiasis. In
366 neutropenic murine models of disseminated aspergillosis and mucormycosis, AUC_{0-24h}/MIC ratios of
367 >100 were predictive of successful treatment with posaconazole (39). Likewise, in experimental
368 murine IPA, other investigators found an AUC_{0-24h}/MIC ratio of at least 94 to be strongly associated
369 with success in antifungal prophylaxis (43).

370 Using a PK/PD modeling approach, Howard et al. investigated the exposure-response relationship of
371 posaconazole in an inhalational murine model. Here an AUC:MIC ratio of 167 was associated with
372 half-maximal antifungal effect (22).
373
374 Apart from a robust assessment of the efficacy of posaconazole against experimental IPA, the
375 persistently neutropenic rabbit model is well suited to establish the link between posaconazole
376 exposure and surrogate markers for pharmacodynamic effects (44, 45). In the present study, we used
377 the GMI as biomarker to monitor the decline of the fungal burden in lung tissue and linked it to the
378 plasma concentration time profile of posaconazole in each infected rabbit. Given the notorious
379 problems is assessing the effects of antifungal interventions in immunocompromised patients by
380 clinical and radiographic methods, the existence of a validated and readily available biomarker would
381 be a major advance to steward treatment in clinical practice and to guide treatment decisions in clinical
382 trials (25, 26).

383 The one compartment model with linear elimination found to best describe the PK of posaconazole in
384 the rabbits is well in accordance with previously published PK models of the compound in human
385 subjects (40, 46) and emphasizes the usefulness of this species for PK/PD bridging studies with
386 antifungal agents (47). The final PK/PD individual Bayesian posteriors accounted for 93% of the
387 observed variability in plasma concentrations and for 86% of the observed variability in the GMI.

Linking the GMI to the posaconazole exposure in the prophylaxis cohort showed the strong ability of posaconazole to control tissue invasion after inoculation and to prevent invasive disease in the model. In the treatment cohort, a clear exposure-response relationship was detectable. Depending on the intensity of the posaconazole treatment started at 24 h after inoculation, the GMI was able to evolve in the rabbits as a marker of uncontrolled or controlled disease. A sigmoidal function was found to best describe this relationship. The detected function turned asymptotic at $AUC_{0\text{-}24h}$ values greater than 30-35 mg*h/L indicating this AUC value to be the threshold for fungal suppression and treatment success. This value corresponds well with the previously reported $AUC_{0\text{-}24h}$ that was associated with a 75% response rate in invasive aspergillosis salvage therapy (6). Assuming a common ECOFF MIC value of 0.25 mg/L for *Aspergillus* spp., effective antifungal treatment in the model corresponded to a posaconazole $AUC_{0\text{-}24h}$/MIC ratio of 120 -140. Of note, the magnitude of this PK/PD target value is identical to current recommendations for therapeutic drug monitoring, which suggest a target AUC 0-24h /MIC ratio of between 100 and 200 for treatment and of at least 94 for sufficient antifungal prophylaxis (19, 20).

Whereas previously published studies linking PK to antifungal efficacy used non-compartmental PK to estimate key pharmacokinetic parameters including AUC, clearance rate, half-life and volume of distribution (30, 39, 42, 43), nonlinear mixed effects modelling was used in this study to estimate individual and population based pharmacokinetic parameters, allowing to also assess inter-subject variability and time-dependence. The established model was then able to predict plasma concentration time profiles for each rabbit at the exact time points of individual GMI measurements. This approach allowed for more flexibility in the study setup and the ability to connect the population PK model with a second pharmacodynamic biomarker model. While previous PK/PD investigations linked the fungal burden in target sites such as the kidney or the lungs at the end of the intervention or observation

period to the observed PK profiles, the model presented here was able to connect the population PK model with a time-varying marker measurement to actually observe the suppression of fungal growth during the intervention.

In conclusion, posaconazole was highly effective in treatment and prophylaxis of experimental invasive aspergillosis in persistently neutropenic rabbits at exposures comparable to those achieved in human subjects. All prophylactic dosing regimens were able to suppress the surrogate marker GMI below 1.0 throughout the experiment. In the treatment experiments, a sigmoidal exposure-response relationship was detected leading to an asymptote at an $AUC_{0\text{-}24h}$ greater than 30 mg*h/L was associated with significant resolution of the GMI and maximum fungal eradication of *A. fumigatus* in lung tissue.

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429 posaconazole

FIGURE LEGENDS

Figure 1: Overview of the study cohorts and their disposition in the analysis.

For development of the population PK model, data from the 53 posaconazole-treated animals included in cohorts 1 to 4 was used. For investigation of the pharmacodynamics in experimental IPA, data from 25 posaconazole-treated animals of cohorts 2 and 3 and 15untreated controls was used for whom serial QOD sampling of serum galactomannan was available. Cohort 4 was not included in the PK/PD model, since only the last available serum galactomannan values were determined.

IPA, invasive pulmonary aspergillosis; PK, pharmacokinetics; QOD, every other day; GMI, galactomannan index; EOT, last available sample before EOT

Figure 2: Overview PK/PD study setup

Observed data and interventions are depicted against time. Dotted lines, start of posaconazole therapy in treatment and prophylactic group – time first dose was applied; dashed line, time point inoculation with $1x10^8$ conidia of *A. fumigatus;* black connected dots, galactomannan index; grey connected dots, posaconazole plasma concentration;

Figure 3: Effects of treatment and prophylaxis with posaconazole on invasive pulmonary aspergillosis in persistently neutropenic rabbits as measured by the residual fungal burden in lung tissue (log CFU/g) at the end of the experiment and the last available galactomannan index.

Dosage groups of posaconazole (2, 6, and 20 mg/kg/day) were combined (light columns) and compared to untreated controls (dark columns). Survival in animals receiving posaconazole was 76% (29/38) in treatment and 89% (8/9) in prophylaxis. For comparison, none of the 8 and 9 animals in the control cohorts survived through the end of the experiment ($p<0.001$ by Fisher Exact Test).

A: Residual fungal burden in lung tissue in log CFU/g: Treatment and prophylaxis with posaconazole resulted in highly significant reductions in the mean ± SEM residual fungal tissue burden versus untreated controls (0.28 ±0.07 vs. 1.49 ±0.1 and 0.13 ±0.08 vs. 1.40 ±0.72, respectively; $p<0.001$ by Mann Whitney U Test)

B: Last available serum galactomannan index: Concordant with the residual fungal burden, there was a significant reduction in the GMI in animals receiving posaconazole for treatment and prophylaxis (0.78 ±0.37 vs. 4.66 ±0.54 and 0.34 ±0.08 vs. 4.28 ±0.83, respectively; $P= 0.002$ and $p<0.001$ by Mann Whitney U Test).

Figure 4: Pharmacodynamic model: Goodness of fit plot for individual posaconazole predictions

Black dots, observed and individual predicted values; solid line, line of identity; dashed line, Loess-Fit across predictions

Figure 5: Pharmacodynamic model: Goodness of fit plot for individual galactomannan index predictions

Black dots, observed and individual predicted values; solid line, line of identity; dashed line, Loess-Fit across predictions

Figure 6: Pharmacokinetic/Pharmacodynamic relationship between posaconazole exposure and the galactomannan index in treatment of experimental invasive pulmonary aspergillosis as assessed by the $AUC_{0\text{-}24\ h}$ of posaconazole and the $AUC_{0\text{-}24\ h}$ of the index.

Both AUCs are calculated at day 5 after inoculation. Grey line, fitted curve from regression analysis, resulting in displayed equation

Figure 7: Pharmacokinetic/Pharmacodynamic relationship between posaconazole exposure and the galactomannan index in prophylaxis of experimental invasive pulmonary aspergillosis as assessed by the posaconazole trough and the AUC 0-24 h of the index.

Calculated at the day of inoculation.

484 **Table 1: Summary table of estimated pharmacokinetic and pharmacodynamic parameters of**
485 **the final model describing the relationship between posaconazole exposure and the**
486 **galactomannan index**

487

Parameter	Mean	SD
CL (L/h)	0.60	0.56
V (L)	117	98.6
Kgmax (GMI/h)	0.03	0.02
Hg	196	98.4
Hk	55.8	88.2
POPmax (GMI)	6.58	1.73
Kkmax (GMI/h)	1.97	1.69
C50g (mg/L)	0.19	0.13
C50k (mg/L)	3.99	1.95

488

489 POPmax, population maximal growth reflected by galactomannan index; Hg, Hill coefficient for
490 growth; Hk, Hill coefficient for kill; Kgmax, maximum rate of growth; Kkmax, maximum rate of kill;
491 C50g, concentration for half-maximal growth; C50k, concentration for half-maximal kill

Antimicrobial Agents and Chemotherapy

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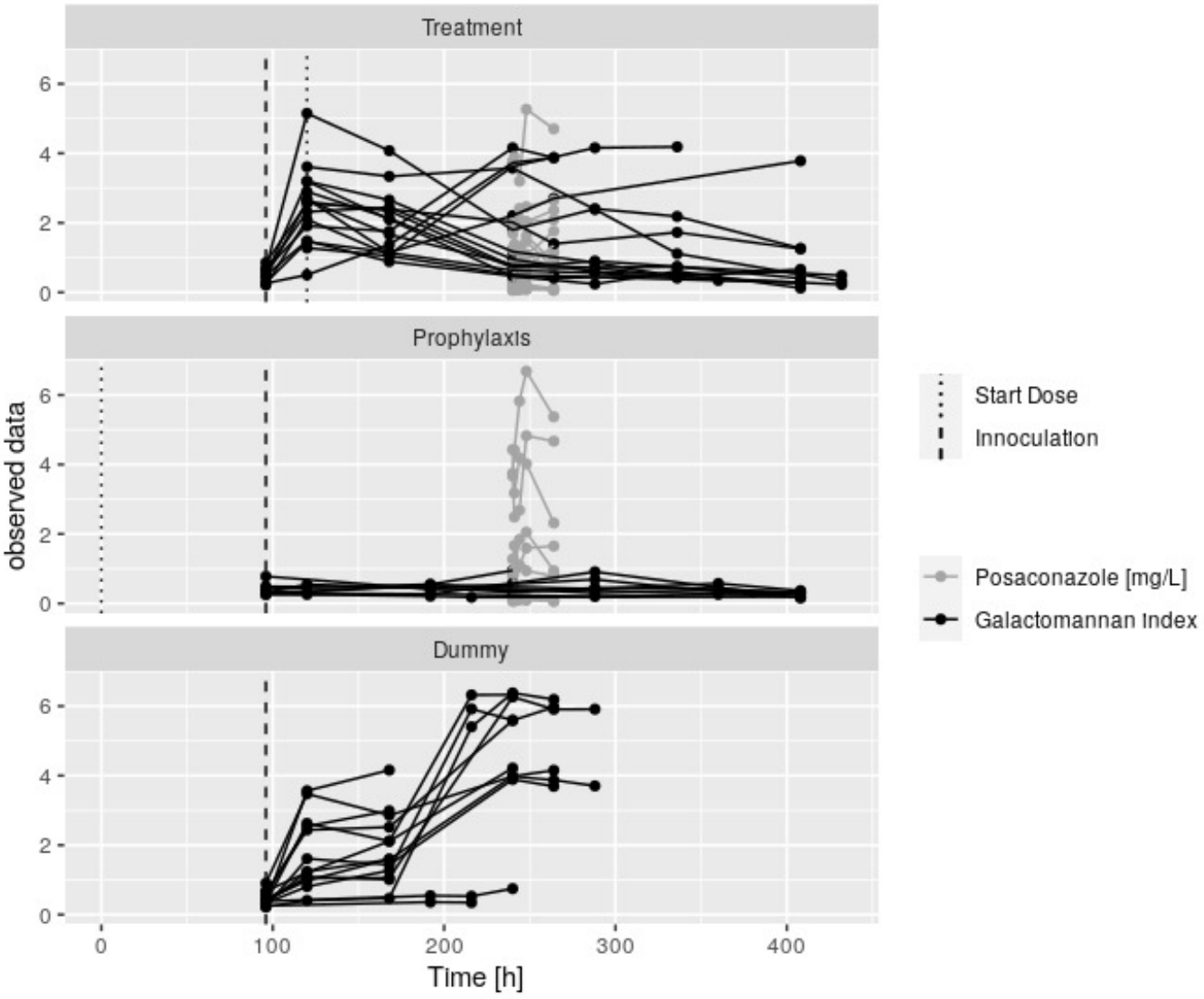
Cohort 1	Cohort 2	Cohort 3	Cohort 4
6 non-infected healthy rabbits Single dose of 20 mg/kg Dense PK sampling Dense PK sampling No GAI assessment	9 neutropenic rabbits with experimental IPA Repeat doses of 2, 6, and 20 mg/kg once daily as prophylaxis Sparse PK sampling QOD GAI assessments 8 untreated controls	16 neutropenic rabbits with experimental IPA Repeat doses of 2, 6, and 20 mg/kg once daily as treatment Sparse PK sampling QOD GAI assessments 9 untreated controls	22 neutropenic rabbits with experimental IPA Repeat doses of 1, 2, and 3 mg/kg twice daily as treatment Sparse PK sampling GAI assessment at EOT



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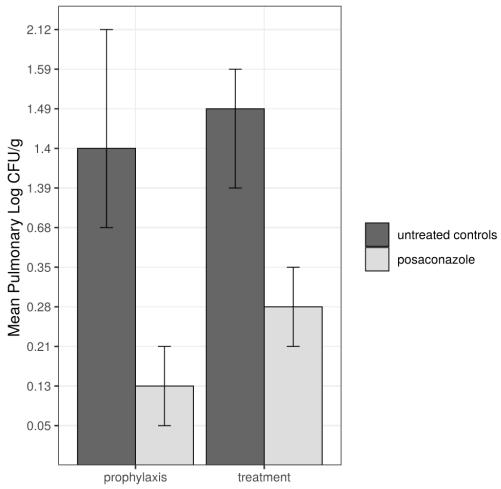

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