

Pharmacokinetic/Pharmacodynamic Modelling of Receptor Internalization with CRTH2 Antagonists to Optimize Dose Selection

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Abstract

Background and Objective The chemoattractant receptor-homologous molecule expressed on T helper-2 cells (CRTH2) is a G-protein-coupled receptor for prostaglandin D₂ (PGD₂), a key mediator in inflammatory disorders. Two selective and potent CRTH2 antagonists currently in clinical development, ACT-453859 and setipiprant, were compared with respect to their (predicted) clinical efficacy. **Methods** Population pharmacokinetic (PK) and pharmacodynamic (PD) models were developed to characterize how plasma concentrations (PK) of ACT-453859, its active metabolite ACT-463036 and setipiprant related to their effect on blocking PGD₂-induced internalization of CRTH2 on eosinophils (PD). Simulations were used to identify doses and dosing regimens leading to 90 % of maximum blockade of CRTH2 internalization at trough. **Results** A combined concentration of ACT-453859 and its metabolite ACT-463036, with weights proportional to potency (based on an eosinophil shape change assay), enabled good characterization of the PD effect. The modelling and simulation results facilitated decision making by suggesting an ACT-453859 dose of 400 mg once daily (or

100 mg twice daily) for clinically relevant CRTH2 antagonism.

Conclusion Pharmacometric quantification demonstrated that CRTH2 internalization is a useful new biomarker to study CRTH2 antagonism. Ninety percent of maximum blockade of CRTH2 internalization at trough is suggested as a quantitative PD target in clinical studies.

Key Points

CRTH2 [chemoattractant receptor-homologous molecule expressed on T helper-2 cells] internalization is a useful new biomarker for CRTH2 antagonists, a new class of compounds with promising characteristics for treatment of allergic diseases.

This biomarker is of great help for designing future clinical studies and selecting efficacious doses of such drugs.

This biomarker can accelerate development of this class of drugs.

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1 Introduction

The chemoattractant receptor-homologous molecule expressed on T helper-2 cells (CRTH2) is a G-protein-coupled receptor expressed on the surface of blood-borne cells (T helper-2 lymphocytes, eosinophils and basophils) [1]. The receptor mediates the activation and chemotaxis of

these cell types in response to prostaglandin D₂ (PGD₂), the major prostanoid produced by mast cells [2, 3].

PGD₂ is released through mast cell degranulation in the initial phase of immunoglobulin E (IgE)-mediated reactions (e.g. upon encountering an allergen) and acts as an important mediator of allergic inflammation in diseases such as asthma and eosinophilic oesophagitis [4–6]. This process is suggested to occur at the site of inflammation, such as the oesophageal or bronchial mucosa. Through interaction with CRTH2, PGD₂ mediates recruitment and activation of CRTH2-bearing cell types, in consequence amplifying and maintaining the allergic inflammation [7–10]. In particular, CRTH2 antagonism has the potential to influence the inflammatory process at a high hierarchical level by reducing the recruitment of specific immune cells and inhibiting the production of pro-inflammatory cytokines [11].

ACT-453859 and setipiprant are both selective and orally active CRTH2 antagonists of human CRTH2 in clinical development [12–14]. Setipiprant and ACT-453859, as well as the latter's active metabolite ACT-463036, effectively block PGD₂-induced internalization of CRTH2 on eosinophils and basophils [15]. For the purpose of a phase I clinical trial, CRTH2 internalization was developed as a decision-making biomarker to monitor the pharmacodynamic (PD) effect in human whole blood [15].

This paper summarizes the pharmacokinetic (PK)/PD analysis of ACT-453859/ACT-463036 and setipiprant in healthy subjects from study AC-072-101, a three-part study to assess the tolerability, safety, PK and PD of single ascending doses (part A) of ACT-453859 in healthy male subjects, of multiple ascending doses of ACT-453859 in healthy male and female subjects (part B) and of multiple doses of setipiprant in healthy male and female subjects (part C) [14].

The objective was to guide the phase II dose selection and dosing regimen of ACT-453859 on the basis of population PK/PD models.

2 Methods

2.1 Study Design

Study AC-072-101 was a three-part study [14] approved by an independent institutional review board (Aurora, ON, Canada). Part A was a randomized, double-blind, placebo-controlled, single-ascending-dose design in healthy male subjects, with seven successive cohorts of eight healthy subjects. Six subjects in each cohort were dosed with 1, 3, 10, 30, 100, 300 and 800 mg of ACT-453859, while two subjects received matching placebo.

Part B was a randomized, double-blind, placebo-controlled, multiple-ascending-dose design in healthy male and female subjects of non-childbearing potential, with three successive cohorts of eight subjects. Six subjects in each cohort received 30, 100 or 800 mg of ACT-453859 once daily for 7 days, while two subjects received matching placebo.

Part C was an open-label part consisting of multiple oral doses of setipiprant in eight healthy male and female subjects of non-childbearing potential treated with 500 or 1000 mg of setipiprant twice daily for 7 days.

2.2 PK Sampling and Bioanalytical Method

Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Within 30 min of collection, the tubes were centrifuged at approximately 1500 g for 10 min at 4 °C, and the plasma was separated and frozen at -70 ± 20 °C, pending analysis.

In part A, plasma samples were taken pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 16, 24, 48 and 72 h after drug intake. In part B, plasma samples were collected immediately prior to the first dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12 and 16 h after the morning dose on day 1; pre-dose from day 2 to day 7; and 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 16, 24, 48, 72 and 96 h post-dose on day 7. In part C, plasma samples were collected immediately prior to the first dose on day 1 and at 1, 2, 2.5, 3, 4, 6, 9 and 12 h after the morning dose on day 1; pre-dose starting from the evening dose on day 1 up to the morning dose on day 7; and at 1, 2, 2.5, 3, 4, 6, 9, 12, 24, 48 and 72 h after the morning dose on day 7.

Analysis of ACT-453859 and ACT-463036 (its active metabolite) in plasma [14] and setipiprant in plasma [16] was performed using validated liquid chromatography coupled to a tandem mass spectrometry (LC–MS/MS) assay. The lower limits of quantification were 0.1 ng/mL for ACT-453859 and ACT-463036, and 1 ng/mL for setipiprant.

Plasma concentration data for ACT-453859 and its metabolite ACT-463036 were used from part B (multiple ascending doses, day 1 and day 7). Plasma concentration data for setipiprant were used from part C.

For correlation of PK with PD for ACT-453859 and ACT-463036, a combined concentration was calculated considering the respective potency of both compounds (ACT-463036 being approximately 50 % less potent than the parent drug in an eosinophil shape change assay [data on file]):

$$\text{Combined concentration} = [\text{ACT} - 453859] + [\text{ACT} - 463036]/2$$

Furthermore, plasma concentrations were corrected by the blood-to-plasma ratio (approximately 0.5 due to no penetration into blood cells) to account for the fact that PD were measured in whole blood.

$$\begin{aligned} &\text{Combined concentration (whole blood)} \\ &= ([\text{ACT} - 453859] + [\text{ACT} - 463036])/2/2 \end{aligned}$$

For setipiprant, the concentration estimate in whole blood used was:

$$\text{Concentration (whole blood)} = [\text{setipiprant}]/2$$

Since calculation of the combined concentration is an approximation, the small difference in molecular weight between ACT-453859 (389 g/mol) and its metabolite ACT-463036 (375 g/mol) was considered negligible.

One subject in cohort B2 was excluded from the PK/PD analysis for technical reasons.

2.3 CRTH2 Internalization (PD)

The CRTH2 internalization assay was conducted as previously described [15]. Blood samples were obtained from healthy subjects who received either placebo, ACT-453859 or setipiprant. Those blood samples were split into three aliquots and used as a baseline sample (MIN), as a maximum CRTH2 internalization sample (MAX) by stimulation with an excess concentration of the CRTH2-specific agonist 13,14-dihydro-15-keto-PGD₂ (dk-PGD₂) and as a sample to show antagonism (PD), which was stimulated with an intermediate dk-PGD₂ concentration of 300 nM. Flow cytometry was used to quantify the amount of CRTH2 on eosinophils in the three samples. The data were then normalized as follows, with (*t*) indicating the particular time point of sampling:

$$\text{PD effect (\%)} = 100(\text{PD}(t) - \text{MIN}(t))/(\text{MAX}(t) - \text{MIN}(t))$$

Finally, the PD effect represents the normalized percentage of CRTH2 present on the surface of eosinophils at a given time point.

2.4 Software

Data preparation, summary statistics and figures were generated using R software [17]. The population PK/PD analysis was performed using Monolix software [18].

2.5 Exploratory Data Analysis

The relationship between the concentration and the percentage of CRTH2 on eosinophils was explored graphically.

2.6 Population PK/PD Analysis

A combined population PK/PD model was developed to quantify model-predicted PK and PD, including variability in PK and PD measurements. PK parameters of ACT-453859 and its metabolite ACT-463036, as well as PD parameters, were estimated simultaneously.

Different structural PK and error models were tested for ACT-453859/ACT-463036 (one- and two-compartment models with and without an absorption lag time [*t*_{lag}] and proportional and exponential error models). The metabolism of ACT-453859 to ACT-463036 was modelled as a conversion rate, and the link between PK and PD was modelled with a maximum effect (*E*_{max}) model with a baseline level (receptor internalization in the absence of the drug due to the presence of endogenous agonists) with the combined concentration as the PK input.

The population PK/PD analyses were performed for the PD effect on eosinophils. The effect of a CRTH2 antagonist on eosinophils is considered clinically relevant because CRTH2 antagonists are developed for eosinophil-driven indications [19].

From clinical experience with setipiprant [data on file], a sustained target PD effect level of 90 % CRTH2 blockade was defined as clinically relevant [15].

The final population PK/PD model was subsequently used to conduct simulations to estimate for different doses and dosing regimens the percentage of subjects with blockade of receptors at trough of at least 90 % (equal to at least 90 % blockade throughout the dosing interval).

Doses from 100 to 800 mg once daily (in 50 mg steps), as well as twice-daily doses of 100, 150 and 200 mg, were simulated. Each dose was simulated for 100 subjects, the sample size considered for a potential phase II dose-finding study.

Subsequently, the percentage of simulated subjects above the threshold of 90 % of maximum blockade of receptor internalization at trough was determined for each simulated study data set. The confidence boundaries of the predicted percentages were derived by bootstrapping the data sets 100 times.

3 Results

3.1 Data and Exploratory Data Analysis

To build the population PK/PD models for ACT-453859/ACT-463036 and setipiprant, PK and PD data were used from 17 and 8 subjects (from part B [day 1 and day 7] and part C, respectively). Figure 1 shows the exploratory data analysis, including *E*_{max} regression model fits.

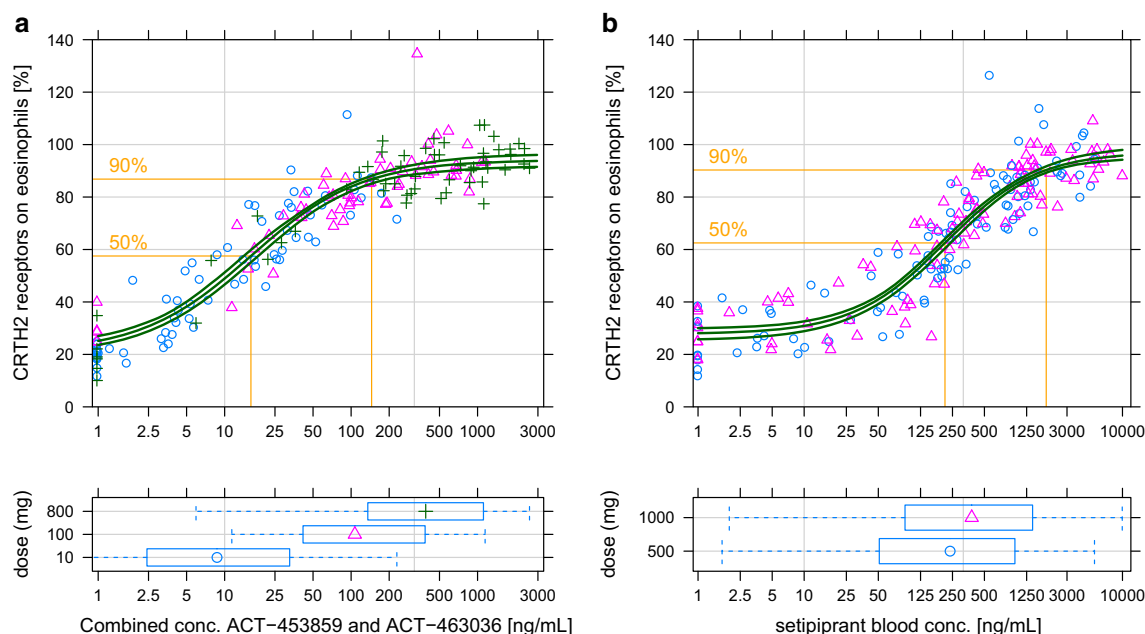


Fig. 1 **a** Combined concentration (conc.) of ACT-453859/ACT-463036 and **b** concentration of setipiprant versus percentage of CRTH2 [chemoattractant receptor-homologous molecule expressed on T helper-2 cells] receptors on eosinophils. In the maximum effect ($E_{\max}$) model (top graphs), green lines indicate the model fit and the 90 % confidence interval, and observed data are shown as symbols,

which correspond to the dose groups in the box-and-whisker plots (bottom graphs), showing drug concentrations by dose group; symbols indicate medians, boxes indicate 50 % ranges around the medians, whiskers approximately indicate 95 % intervals and extreme values are shown individually

3.2 Population PK/PD Analysis

3.2.1 ACT-453859/ACT-463036

For ACT-453859 and ACT-463036, the joint population PK/PD model was identified as a two-compartment model with first-order absorption and t_{lag} for ACT-453859, a conversion rate from the parent drug to the metabolite, a two-compartment model for the metabolite ACT-463036 and an $E_{\max}$ model with a baseline effect (E_0) and the combined concentration as PK variable. Since the metabolite ACT-463036 was not dosed per se, its volume of distribution, bioavailability (F) and apparent total body clearance could not be estimated.

The population PK/PD parameters were t_{lag} for ACT-453859, absorption rate constant (k_a) for ACT-453859, apparent volume of distribution in the central compartment (V_1/F) for ACT-453859, apparent total body clearance (CL/F) for ACT-453859, apparent volume of distribution in the peripheral compartment (V_2/F) for ACT-453859, intercompartmental clearance (Q) for ACT-453859, transfer/metabolism rate constant (k_{13}) from ACT-453859 to ACT-463036, intercompartmental transfer rate constant (k_{34}) for ACT-463036, elimination rate constant (k_m) for ACT-463036, E_0 , effective concentration to reach half of $E_{\max}$ (EC_{50}) and $E_{\max}$. The population model structure is depicted in Fig. 2, and the parameter estimates are given in Table 1.

Goodness-of-fit plots for ACT-453859, ACT-463036 and the percentage of CRTH2 on eosinophils (Fig. S1 in the Electronic Supplementary Material [ESM]) show that the data were symmetrically scattered around the lines of identity. This suggests that the model fitted the observed data for ACT-453859 and ACT-463036, and for the percentage of CRTH2 on eosinophils.

3.2.2 Setipiprant

For setipiprant, the population PK model was identified as a two-compartment model with first-order absorption and t_{lag} , while the PD model was an $E_{\max}$ model with E_0 . The population parameter estimates are given in Table 1. Goodness-of-fit plots (Fig. S2 in the ESM) and visual predictive checks (Figs. S3 and S4 in the ESM) show that the model fitted the data adequately.

3.3 Simulations Based on the Population PK/PD Model

3.3.1 ACT-453859/ACT-463036

The simulation results based on the population PK/PD model for ACT-453859/ACT-463036 showed that 200 mg once daily would result in median effects that were above 90 % of maximum blockade of receptor internalization at

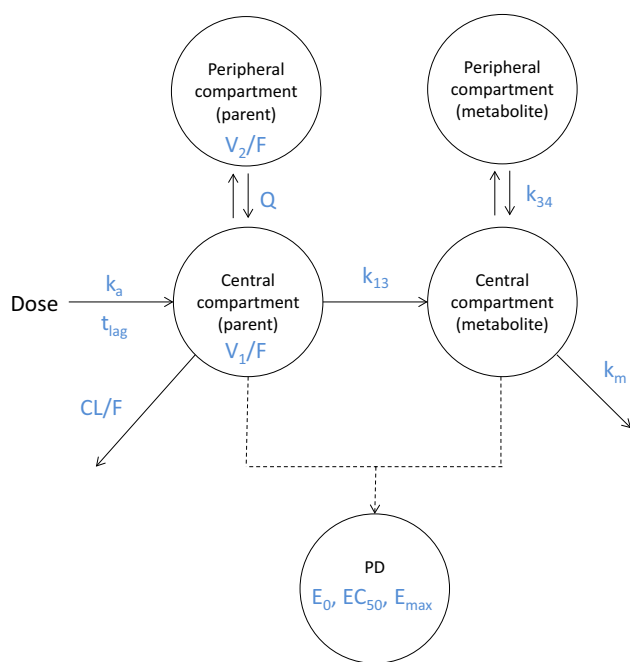


Fig. 2 Schematic illustration of the population pharmacokinetic/pharmacodynamic (PK/PD) model, showing its compartments (circles) and model parameters (blue text). CL apparent total body clearance, E_0 baseline effect, EC_{50} effective concentration to reach half of E_{max} , E_{max} maximum effect, F bioavailability, k_{13} transfer/metabolism rate constant, k_{34} intercompartmental transfer rate constant, k_a absorption rate constant, k_m elimination rate constant, Q intercompartmental clearance, t_{lag} absorption lag time, V_1 apparent volume of distribution in the central compartment, V_2 apparent volume of distribution in the peripheral compartment

trough (given as $E_0 + 0.9 E_{max}$) for almost the entire dosing interval (Fig. 3a, b). The quantiles of the simulated subjects showed that because of inherent variability in PK and PD, a substantial number of subjects were predicted to still be below this threshold at trough.

The simulations for eosinophils at steady-state trough (at 168 h in Fig. 3a) showed that doses above 100 mg were predicted to yield only marginally more subjects above 90 % of maximum blockade of receptor internalization at trough (Fig. 3b).

Figure 4a shows that the median percentage of subjects above 90 % of maximum blockade of receptor internalization at trough increased with higher doses, e.g. with 100 mg, about 20 % of subjects were above 90 % of maximum blockade of receptor internalization, whereas with 350 mg, approximately 60 % of subjects were predicted to be above this threshold. With doses above 500 mg, the effect threshold was reached in 70–80 % of subjects.

Simulations of twice daily dosing (instead of once daily) showed that the median percentage of subjects above 90 % of maximum blockade of receptor internalization at trough could be increased substantially by dosing twice daily, compared with dosing once daily, with an equivalent daily

dose: while fewer than 50 % of subjects were predicted to achieve 90 % of maximum blockade of receptor internalization with 200 mg once daily (Fig. 4a), approximately 65 % of subjects would reach the threshold with 100 mg twice daily (Fig. 4b).

3.3.2 Setipiprant

The simulation results based on the population PK/PD model for setipiprant showed that 1000 mg twice daily would result in effects that were above 90 % of maximum blockade of receptor internalization at trough for a few hours only (Fig. 3c). The quantiles of the simulated subjects showed that because of inherent variability in PK and PD, a substantial number of subjects were predicted to be below this threshold for most of the time during a dosing interval at steady state.

The simulated steady-state PD effect based on the population PK/PD model for setipiprant on eosinophils at trough (at 84 h in Fig. 3c) with 500 and 1000 mg twice daily showed that the effect at trough was slightly greater with 1000 mg than with 500 mg, but with both doses, most of the subjects were predicted to be below 90 % of maximum blockade of receptor internalization at trough (Fig. 3d).

Figure 4c shows that the median percentage of subjects above 90 % of maximum blockade of receptor internalization at trough was higher with 1000 mg twice daily than with 500 mg twice daily, but the median percentage of subjects above 90 % of maximum blockade of receptor internalization was low (<30 %) with both doses.

4 Discussion and Conclusion

The PK/PD relationships of the combined plasma concentration of ACT-453859/ACT-463036 and the concentration of setipiprant to the percentage of CRTH2 on eosinophils could be characterized adequately by E_{max} models.

Combined population PK/PD models were successfully developed, and population parameters could be estimated with good precision. Parameter estimates with low overall variability could be achieved because of the good quality of the available data for both PK (plasma concentration data) and PD (CRTH2 internalization assay). This showed that the procedures and assays for both PK and PD are suitable for use in further trials during clinical development of CRTH2 antagonists.

The desired PD effect at trough was defined as 90 % throughout the entire dosing interval, to achieve a clinically relevant effect. The threshold was selected on the basis of phase II clinical trial data in adult patients with seasonal allergic rhinitis [20]. An E_{max} model was established for the primary endpoint of the daytime nasal symptom score

Table 1 Population pharmacokinetic/pharmacodynamic (PK/PD) parameter estimates

Parameter	ACT-453859/ACT-463036		Setipiprant	
	Estimate	CV (%)	Estimate	CV (%)
t_{lag} (h)	0.327	8	0.791	5
k_a (1/h)	0.206	6	1.13	26
V_1/F (L)	9.27	18	53.1	18
CL/F (L/h)	10.4	9	19.2	14
V_2/F (L)	56.6	13	46	14
Q (L/h)	2.01	18	4.44	20
k_{13} (1/h)	0.0174	29	–	–
k_{34} (1/h)	0.069	8	–	–
k_m (1/h)	0.375	10	–	–
E_0 (%)	20.4	8	28.5	6
EC_{50} (ng/mL)	17.3	12	286	17
E_{max} (%)	74.3	2	70.4	4
$\Omega_{\text{Omega}_t t_{lag}}$ ^a	0.289	20	0.0939	60
$\Omega_{\text{Omega}_k k_a}$	0.217	22	0.899	21
$\Omega_{\text{Omega}_V V_1}$	0.695	19	0.588	24
Ω_{Omega_CL}	0.356	18	0.555	18
$\Omega_{\text{Omega}_V V_2}$	0.508	19	0.369	29
Ω_{Omega_Q}	0.691	19	0.461	36
$\Omega_{\text{Omega}_k k_{13}}$	1.17	18	–	–
$\Omega_{\text{Omega}_k k_{34}}$	0.0685	269	–	–
$\Omega_{\text{Omega}_k k_m}$	0.376	19	–	–
$\Omega_{\text{Omega}_E E_0}$	0.073	137	0.0752	112
$\Omega_{\text{Omega}_E EC_{50}}$	0.272	39	0.38	35
$\Omega_{\text{Omega}_E E_{max}}$	0.0121	520	0.0259	348
Proportional error PK	0.376	3	0.532	4
Proportional error PK (metabolite)	0.302	3	–	–
Constant error PD	8.08	6	11.3	5

CL apparent total body clearance, CV coefficient of variation, E_0 baseline effect, EC_{50} effective concentration to reach half of E_{max} , E_{max} maximum effect, F bioavailability, k_{13} transfer/metabolism rate constant, k_{34} intercompartmental transfer rate constant, k_a absorption rate constant, k_m elimination rate constant, Q intercompartmental clearance, t_{lag} absorption lag time, V_1 apparent volume of distribution in the central compartment, V_2 apparent volume of distribution in the peripheral compartment

^a Ω values denote standard deviations of the corresponding random effect distributions

(DNSS) change versus the trough concentrations of setipiprant at weeks 1 and 2. A clinically relevant change in the DNSS was defined in the protocol as -0.15 , which corresponded to a minimum effective plasma concentration of setipiprant of 3200 ng/mL, corresponding to 90 % CRTH2 blockade [15].

This desired PD effect level of 90 % provides the means for decision making but needs confirmation in future clinical trials. Receptor internalization was used in previous studies to investigate CRTH2 antagonists in clinical studies; however, a clinically relevant cut-off was not defined [21, 22]. Further studies in the targeted patient population could provide more insight into the relationship between receptor internalization and clinical efficacy, strengthening decision making.

The simulations of the PK/PD relationships for ACT-453859/ACT-463036 for once daily dosing showed that the median percentage of CRTH2 on eosinophils was above 90 % during the entire dosing interval for most subjects with doses above 300 mg. To define the dose range for a future clinical study, it was of interest to conduct further simulations to determine the percentage of subjects showing >90 % of the maximum PD effect, since this would be more relevant than the median effect. These simulations showed that the median percentage of subjects reaching this threshold increased substantially with higher doses, e.g. with 100 mg once daily, fewer than 20 % of subjects were predicted to have >90 % of the maximum PD effect, whereas with 400 mg, approximately 60 % of subjects were predicted to be above this threshold.

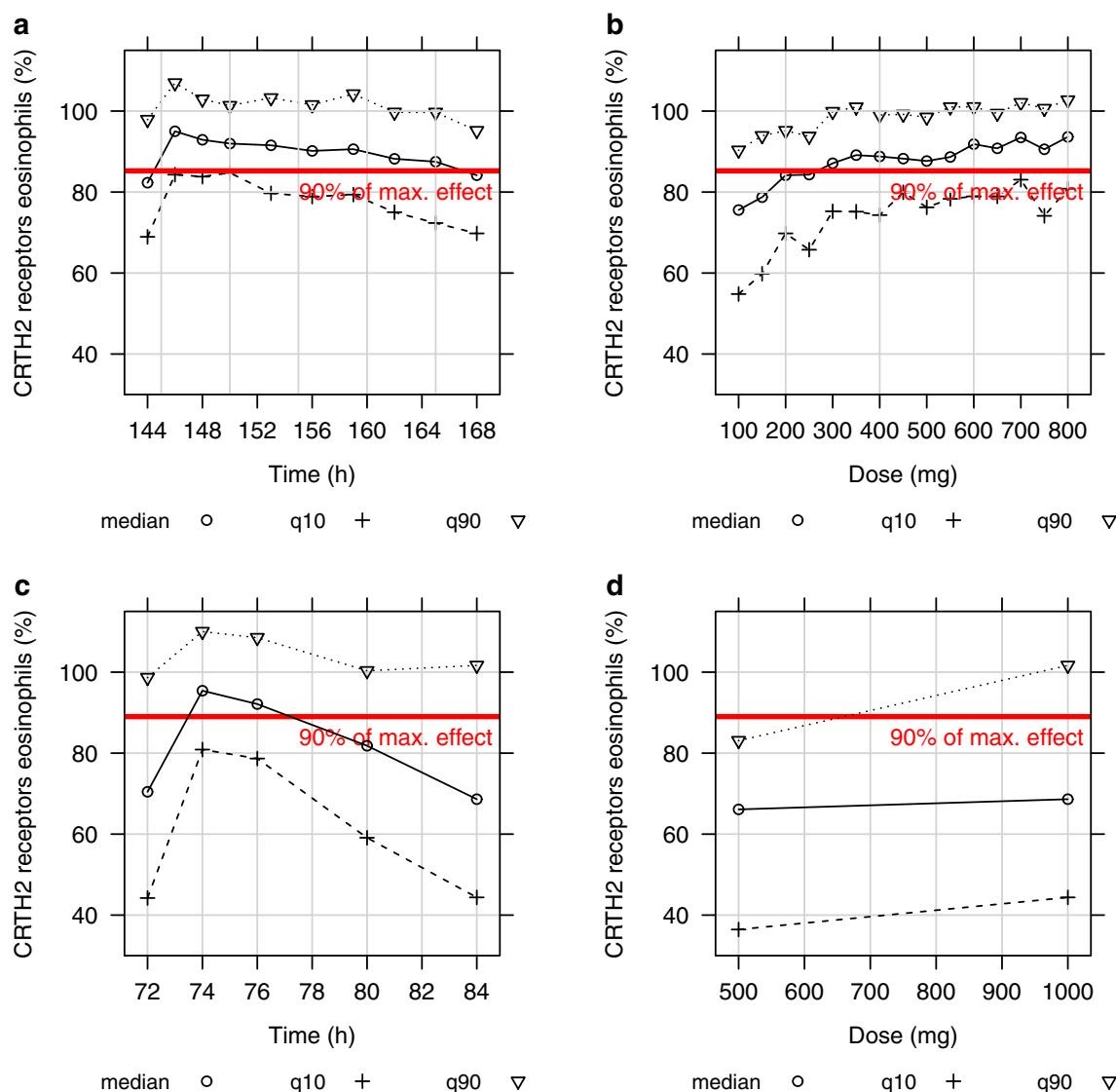


Fig. 3 Pharmacodynamic effect on eosinophils of **a** and **b** ACT-453859/ACT-463036 (**a** over a dosing interval at steady state after 200 mg once daily; **b** at trough with different doses) and **c** and

d setiprant (**c** over a dosing interval after 1000 mg twice daily; **d** at trough with 500 and 1000 mg twice daily), based on 100 simulated subjects. Lines indicate medians and 10th and 90th percentiles

With doses above 450 mg, the effect increased only marginally in that 70–80 % of subjects were predicted to reach the threshold. With higher doses (above 450 mg), the corresponding plasma concentrations elicited effects close to the maximum effect, i.e. the respective PK/PD relationship was described by the upper part of the $E_{\max}$ model curve, close to the maximum effect (a plateau). Accordingly, the simulation results showed that the percentage of subjects above 90 % of the maximum effect could not be substantially increased further with higher doses, since the achievable maximum effect had already been reached.

The simulations of the PK/PD relationships for ACT-453859/ACT-463036 with the different twice-daily dosing regimens showed that the percentage of subjects above

90 % of the maximum effect was >60 % at the 100 mg dose level. A twice-daily dosing regimen maintained higher plasma concentrations for a longer time during the day, resulting in a higher percentage of subjects with >90 % of the maximum PD effect. A dosing regimen of 100 mg twice daily was predicted to have effects similar to those achieved by once daily doses of 400 mg and higher. In conclusion, on the basis of the models and the simulation results, a dose of 400 mg once daily (or 100 mg twice daily) was predicted to be the lowest dose of ACT-453859 leading to sustained CRTH2 blockade during the entire dosing interval. The choice between the two dosing regimens might need to be further evaluated in patient studies. While the drug load using twice daily dosing would result

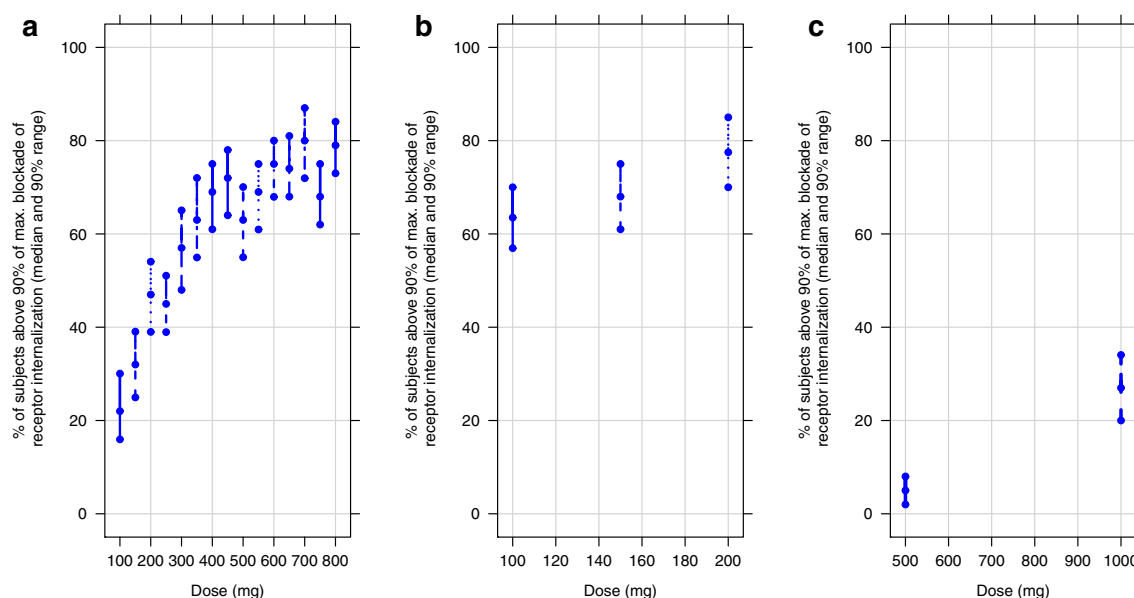


Fig. 4 Percentage of subjects showing at least 90 % of maximum (max.) blockade of receptor internalization at trough with **a** ACT-453859 once daily, **b** ACT-453859 twice daily and **c** setipiprant twice

daily, based on 100 simulated subjects per dose. Medians and 90 % confidence intervals are shown

in lower area under the plasma concentration–time curve (AUC) values, and consequently increased safety margins, a once daily dosing regimen is expected to lead to better compliance, particularly for a chronic treatment.

The simulations of the PK/PD relationships for the two doses of setipiprant showed that the median percentage of subjects with >90 % of the maximum PD effect was higher with 1000 mg than with 500 mg but remained below 30 %. These results confirmed that ACT-453859 with its active metabolite ACT-463036—in terms of blockade of CRTH2—is a substantially more potent antagonist in humans than setipiprant.

The CRTH2 internalization assay is as a robust, sensitive and therefore clinically useful biomarker [15]. This paper demonstrates the added value of this biomarker to characterize the PK/PD relationship of CRTH2 antagonists in a clinical setting. Moreover, in combination with the 90 % PD effect defined as being clinically relevant, the data provide the basis for dose selection in clinical studies. The applied modelling and simulation techniques enabled best use of the PK and PD data to influence the selection of doses and dosing regimens for future studies and to impact clinical decision making by suggesting that a dose of 400 mg once daily (or 100 mg twice daily) is predicted to be the lowest dose of ACT-453859 leading to sustained CRTH2 blockade during the entire dosing interval.

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Compliance with Ethical Standards

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Conflict of interest Andreas Krause, Jochen Zisowsky, Daniel S. Strasser, Martine Gehin, Patricia N. Sidharta, Peter M.A. Groenen and Jasper Dingemans were employees of Actelion Pharmaceuticals Ltd at the time when the study was conducted and may receive stock or stock options as part of their compensation.

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