# Non-Clinical Toxicology Evaluation of the Novel Non-ATP Competitive Oral PI3 Kinase Delta Inhibitor Roginolisib

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#### Abstract

Roginolisib (IOA-244) is a novel, non-ATP competitive phosphoinositide-3-kinase (PI3K) delta inhibitor that regulates Akt/mTOR signaling. Roginolisib was administered once daily to rats and dogs in dose-range finding (DRF) and 4-week GLP toxicology studies. Free plasma levels of roginolisib exceeded the cellular target engagement IC<sub>90</sub> for PI3K $\delta$  for  $\geq$ 12 hours at doses of 5 mg/kg, the IC<sub>90</sub> for PI3K $\beta$  for  $\geq$ 2 hours at doses  $\geq$ 15 mg/kg, and the IC<sub>50</sub> for PI3K $\alpha$  for  $\geq$ 2 hours at dose levels  $\geq$ 45 mg/kg. Toxicity in rats occurred at doses  $\geq$ 100 mg/kg. In dogs, we observed dose-dependent skin and gastrointestinal toxicity and doses  $\geq$ 30 mg/kg had a greater incidence of mortality. Lymphoid tissue toxicity occurred in both species. Toxicities in dogs observed at the  $\geq$ 15 mg/kg dose, affecting the digestive mucosa, liver, and skin, cleared after treatment cessation. Doses  $\leq$ 75 mg/kg were tolerated in rats and the no-observed-adverse-effect-level (NOAEL) in rats was 15 mg/kg. Due to mainly epithelial lesions of the skin at 5 mg/kg and necrotizing damage of the intestinal epithelia at  $\geq$ 15 mg/kg, no NOAEL was determined in dogs. However, the adverse effects observed in dogs at 5 mg/kg were considered monitorable and reversible in patients with advanced malignancies. Furthermore, the PK profile subsequently proved to be a decisive factor for achieving selective PI3K $\delta$  inhibition without the toxicities observed in dogs. As the result of the unique PK profile of roginolisib, patients were able to take daily roginolisib without dose modification and showed pharmacodynamic PI3K $\delta$  inhibition over several months without gastrointestinal or dermatologic toxicities.

#### **Keywords**

phosphoinositide-3 kinase delta, PI3K $\delta$ , toxicology, phosphoinositide-3 kinase inhibitors, roginolisib

#### Introduction

Phosphoinositide 3-kinases (PI3Ks) are heterodimeric lipid kinases that regulate protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling, which controls cell growth, motility, survival, and angiogenesis.<sup>1</sup> Aberrant Akt/mTOR signaling occurs in many treatment-resistant cancers.<sup>2,3</sup> High expression of the PI3K isoform *PI3KD*, which encodes the p110 catalytic subunit of PI3K $\delta$  (i.e., p110 $\delta$ ), promotes tumor growth and metastasis;<sup>4</sup> conversely, PI3K $\delta$  inhibition impairs tumorigenesis.<sup>5,6</sup> The PI3K $\delta$  isoform is expressed in leukocytes and modulates B- and T-cell activation, B-cell-driven proliferation, and neutrophil inflammatory responses.<sup>7,8</sup> Direct inactivation of PI3K $\delta$  in regulatory T cells and myeloid-derived suppressor cells promote tumor regression by increasing effector T-cell immunosurveillance.<sup>9,10</sup>

Inhibitors of PI3K and mTOR are used to treat various malignancies, but the therapeutic window is narrow. Most PI3K and mTOR inhibitors exhibit limited efficacies as monotherapies and cause dose-limiting toxicities (e.g., fatigue, diarrhea, rash, and hyperglycemia).<sup>11</sup> Inhibiting PI3K $\alpha$ , Akt and mTOR primarily affects insulin signaling and glucose metabolism,<sup>12</sup> the modulation of which is crucial in mitigating toxic adverse effects and increasing efficacy.<sup>13</sup> However, PI3K isoforms have specific functions and the impact of isoform specificity on the type of toxic adverse effects (AEs) experienced by patients remains to be fully elucidated. New isoformselective PI3K inhibitors aim to improve efficacy and minimize the frequency and severity of AEs. For example, alpelisib is a PI3K $\alpha$  inhibitor used in combination therapy

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to treat breast cancer<sup>14</sup>; tenalisib is a dual PI3K $\delta/\gamma$  inhibitor being investigated for treating relapsed/refractory T-cell lymphoma;<sup>15</sup> and idelalisib is a first-in-class PI3K $\delta$ inhibitor that was originally approved by the United States Food and Drug Administration (FDA) for treating relapsed chronic lymphocytic leukemia.<sup>16,17</sup> Recently, the FDA's public review of idelalisib and similar agents recommended their withdrawal from use in multiple indications based on unfavorable benefit:risk ratios.<sup>18</sup> Thus, safer PI3K $\delta$  inhibitors are needed to achieve their full therapeutic potential.

Roginolisib (IOA-244) is a novel, small molecule non-ATP competitive, and potentially a safer and more effective PI3K $\delta$  inhibitor, that is selective for the PI3K $\delta$  isoform.<sup>19</sup> Based on its potency, selectivity, and efficacy in non-clinical models,<sup>19,20</sup> roginolisib may provide a therapeutic option for wide ranging malignancies, including solid and hematological tumors with dysregulated PI3K/Akt/mTOR signaling. The non-clinical toxicology studies in both rats and dogs reported here provide insights into the toxicological profile of roginolisib. These findings have advanced roginolisib into clinical investigation and preliminary clinical data suggests this drug is propitious for treating malignancies, including uveal melanoma and lymphoma.<sup>21</sup>

#### Materials and Methods

#### Test Compound

A free-base or a hemi-fumarate salt form of roginolisib was used in these studies. The activity and pharmacokinetics (PK) of both salt forms were similar (data not shown).

Animals were administered with vehicle alone (.25% aqueous hydroxypropyl methylcellulose [Methocel® K4M Premium]) or specified doses of roginolisib (diluted in the vehicle solution). Throughout, samples of 2 dosing formulation preparations of roginolisib were collected at the beginning and end of usage, and sample concentrations were assessed using high performance liquid chromatography (HPLC). Vehicle or roginolisib was administered orally via gavage in accord with the intended administration route for roginolisib in humans. All treatment doses were defined as mg/kg of bodyweight and were administered once daily in a volume of 5 mL/kg bodyweight for rats and 10 mL/kg bodyweight for dogs.

#### Animals and Husbandry

Two hundred and seventy-four (137 male and 137 female) healthy Wistar rats aged approximately 8 weeks, whose body weights ranged from 145 to 254 g, were purchased from Charles River Laboratories (Germany and USA). Rats were housed in single-sex groups of 2-3 per cage with free access to food (Provimi Kliba 3433.0) and water, and the room was maintained at  $22^{\circ}C$  ( $\pm 2^{\circ}C$ ) with a relative humidity of 35%-78%, and the day/night cycle was 12:12 hours.

For the 14-day dose range finding (DRF) study, 4 healthy beagle dogs (2 males and 2 females; Marshall farms, US) with a mean age of 42 months and a body weight range from 7.6 to 10.4 kg (at treatment start) were used. These dogs had been used before in unrelated pharmacokinetic studies. For the 4week oral toxicology study, 36 naïve healthy beagle dogs (18 male and 18 female; Marshall farms, Italy and USA) with a mean age of 10.5 months and a body weight range of 5.9-9.8 kg (at study start) were included. All dogs were housed individually (DRF study) or in single-sex groups of 2-3 per kennel (4-week oral toxicology study), were individually fed daily, within a two-hour window, with a Provimi Kliba 3353.0 diet and given potable water ad libitum. The temperature remained between 14.3 and 28.8°C, with a relative humidity of 3.6%-72%. Table 1 summarizes the number of animals used, the doses administered, and the parameters investigated for each study.

All studies were conducted in compliance with the Organization for Economic Co-operation and Development Principles of Good Laboratory Practice (GLP) (as revised in 1997), with the Principles of Good Laboratory Practice according to Annex 1 German Chemicals Act, and the Principles of Good Laboratory Practice of the European Union. The GLP standards applied also included the requirements of Japanese, USA, and other non-European guidelines.

#### Experimental Procedures

*Rats.* In the 4-week DRF study, 40 rats (20 male and 20 female) were assigned to 4 treatment groups, each consisting of 10 animals (5 male and 5 female). Each group was assigned either vehicle alone or a specific dose of the free-base of roginolisib (10, 30, and 100 mg/kg) that was administered daily for 28 days. Based on the high bioavailability of roginolisib in rat,<sup>20</sup> only 2 additional males and 2 additional females were assigned to each group as "satellite animals" for use in the toxicokinetic investigation. The middle dose of 30 mg/kg was considered a therapeutically effective dose based on the calculated ED<sub>50</sub> value of 18 mg/kg in female Lewis rats with semi-established type II collagen arthritis (unpublished data).

In the 4-week oral toxicology study, 150 rats (75 male and 75 female) were assigned to 5 treatment groups, each consisting of 15 males and 15 females. Each group received hemi-fumarate salt roginolisib (0, 5, 15, 50 or 75 mg/kg) daily for 28 days and then the animals were monitored during an 8-week, treatment-free, recovery period. An additional 3-5 males and 3-5 females (68 animals [34 males, 34 females] in total) were assigned to each group as "satellite animals" for the toxicokinetic investigation.

**Dogs.** In the DRF study, 4 dogs (2 male and 2 female) were assigned to 2 treatment groups. In group 2, dosing started with 30 mg/kg of the free-base of roginolisib on Day 1, and then the roginolisib dose was increased to 100 mg/kg on Day 2,

Study	Total number of rats (group size) duration	Dose/dose regimen (mg/kg/day)	Duration	Parameters investigated
Rats				
Dose range-finding 4- week oral toxicology study	20 male, 20 female (5 m + f each dose)	0-10-30-100	28 days	Clinical signs, bodyweight, food consumption, mortality, hematology, clinical chemistry, pathology, histopathology, immunophenotyping, urinalysis, toxicokinetics
GLP 4-week oral toxicology study and 8-week recovery period	75 male, 75 female (15 m + f each dose)	0-5-15-50-75	28-day treatment	Clinical signs, bodyweight, food consumption, mortality, hematology, clinical chemistry, pathology, histopathology, immunophenotyping, urinalysis, toxicokinetics, eye examination, and biomarker investigation
Dogs				5
Dose range-finding study (dose escalation)	2 male, 2 female (1 m + f each group)	Group 1: 100 Days 5- 14 Group 2: 30- 100-300-1000; 300 Days 5-14	14 days	Clinical signs, bodyweight, food consumption, mortality, hematology, clinical chemistry, pathology, histopathology, immunophenotyping, urinalysis, toxicokinetics, electrocardiograms, body temperature, blood pressure
GLP 4-week oral toxicology study and up to 8-week recovery period (mid/high doses)	18 male, 18 female (3 m + f for vehicle group, 5 m + f per dose group)	0-5 (12 weeks) 15 (25 days) 45/30 (16 days)	Up to 12-week treatment. Mid/high doses recovery period started on Day 26/17	Clinical signs, bodyweight, food consumption, mortality, hematology, clinical chemistry, pathology, histopathology, immunophenotyping, urinalysis, toxicokinetics, electrocardiograms, body temperature, blood pressure, reflex tests, eye examination

#### Table I. Non-Clinical Toxicology Studies.

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Abbreviations: m = male; f = female; GLP = good laboratory practice.

300 mg/kg on Day 3, and 1000 mg/kg on Day 4 for both dogs. As the top dose was not tolerated in the group 2 female, the remaining group 2 male was treated with 300 mg/kg from Days 5 to 14. Group 1 dogs received a dose of 100 mg/kg of the free-base of roginolisib from Days 5 to 14.

In a 4-week oral toxicology study, 36 dogs (18 males and 18 females) were assigned to 4 treatment groups based on dose (0, 5, 15, 45 mg/kg roginolisib) daily. Each treatment group consisted of 10 dogs (5 male and 5 female), and the control group consisted of 6 dogs (3 males and 3 females). As there were no clinical signs of toxicity after 4 weeks of treatment, the treatment period for the vehicle and the low dose group (5 mg/kg) was extended from 4 to 12 weeks. However, dogs treated with 15 mg/kg (mid-dose group) were prematurely euthanized in Week 4 owing to severe clinical symptoms. Dogs treated with the high dose were initially administered 45 mg/kg for 14 days and then the dose was reduced to 30 mg/ kg until Day 21. For the mid- and high-dose groups, 2 animals per sex were investigated during the 8-week recovery period. Based on the nature of the vehicle (.25% aqueous hydroxypropyl methylcellulose in water), only 1 control group was included for both the end of dosing as well as the recovery group assessment.

A minimum of 3 males and 3 females, including all recovery animals (with the exception of 1 female in the highdose group that was euthanized during the recovery period), from each treatment group were used for the toxicokinetic investigation.

#### Toxicokinetic Investigations

For the toxicokinetic investigation, blood samples were collected from designated satellite animals, in their respective studies, on multiple days, including Days 1 and 28. Samples were taken before dosing and at 1, 3, 6, and 24 hours post-dose in rats and at .5, 1, 2, 4, 6, and 24 hours in dogs following roginolisib administration. Any variations from this dosing schedule in a study are reported alongside the results.

Whole blood samples were transferred into tubes containing a lithium-heparin anti-coagulation agent, centrifuged under cooled conditions (~4°C), and subsequently stored at  $-20^{\circ}$ C. Concentrations of roginolisib in the lithium-heparin plasma were determined using an Agilent 1100 Series HPLC system with a CTC Analytics HTC PAL Autosampler coupled to an AB Sciex API4000 mass spectrometer and bioanalytical methods validated according to the FDA Guidance for Industry: Bioanalytical Method Validation, May 2001. Toxicokinetic parameters assessed included the highest concentration value of roginolisib in blood plasma (C<sub>max</sub>), the time at which the  $C_{max}$  was determined  $(t_{max})$ , and the area under the plasma concentration vs time curve from dosing until 24 hours post-dose (AUC<sub>0-24h</sub>). The AUC<sub>0-24h</sub> corresponded to an entire dosing interval and was calculated by the log-linear trapezoidal rule (linear up to the Cmax, logarithmic after the  $C_{max}$ ). To determine the rate of accumulation, an accumulation ratio (Rac AUC<sub>0-24h</sub>) was used to compare the  $AUC_{0-24h}$  for the first dose and the  $AUC_{0-24h}$  on subsequent sampling days. The formula used for this calculation was:

$$Rac AUC_{0-24h} = \frac{AUC0-24h \text{ Timepoint 2}}{AUC0-24h \text{ Timepoint 1}}$$

In this equation *Timepoint 1* is Day 1 and *Timepoint 2* is the day when the subsequent sampling occurred (either Day 25 or Day 88).

# Clinical Symptoms, Bodyweight, and Food Consumption

General condition and behavior as well as food consumption were assessed daily. In addition, bodyweight was measured once per week.

# Electrocardiography, Arterial Blood Pressure, and Reflex Tests

In dogs, electrocardiograms (ECGs) were recorded with a MAC 1200ST electrocardiograph (GE Medical Systems, USA). Standard leads I, II, III, and the Goldberger leads aVR, aVL, and aVF were recorded and then standard lead II was examined. Heart rates and interval data were quantified. Van de Water's equation ( $V = QT - .087 \times [(60/HR) - 1]$ ) was used to dissociate the effects of heart rate on the QT-interval in standard lead II (Van de Water, 1989).

Systolic, diastolic, and mean arterial blood pressure values were measured indirectly using high definition oscillometry (Vet HDO Monitor, S + B medVet, Germany). The dogs were conscious during the measurement. The following reflexes in dogs were tested: pupillary light reflex, lid reflex, patellar reflex, flexor reflex, and anal reflex. All investigations were performed 3 weeks before starting the treatment and once in week 3 during the treatment period in all dogs before and 2 hours after dosing.

## Ophthalmology

In the 4-week GLP oral toxicology study in rats, eye examinations (pupillary reflex, anterior parts, and fundus) and further examinations by slit lamp were performed in nonsatellite rats before starting the treatment and at the end of treatment, in addition to at the end of the recovery period for rats in the control and the high-dose groups (75 mg/kg).

In the 4-week oral toxicology study in dogs, the anterior and posterior part of the eye was examined [handlamp (Heine Optotechnik, Germany), ophthalmoscope (Welch Allyn, USA), slit-lamp (Zeiss, Germany), fundus camera (KOWA Ltd, Japan)] 4 weeks before initiating treatment, and in weeks 5 and 10 during the treatment period and the recovery period, respectively.

## Hematology and Immunophenotyping

Blood samples were collected according to the schedule of each individual study. The following indexes were examined: red blood cells, platelets, immune cells, hemoglobin, and mean cell volume. For the studies using dogs, coagulation parameters including erythrocyte sedimentation rate and prothrombin time were also investigated. All measurements were generated using either ADVIA 120 Hematology System (Siemens Medical Solutions GmbH), FACS Canto Flow Cytometer (BD), and recorded in the LIM-System; Westergren method (erythrocyte sedimentation); or Coasys Plus– Thrombolyzer (Disasys-Greiner, Germany). Samples were also examined visually via a light microscope (ZEISS).

For immune cell phenotyping to identify the absolute number of each lymphocyte population (e.g., CD4<sup>+</sup> and CD8<sup>+</sup> T cells) the lyse-no-wash method was used.<sup>22</sup> The relative numbers of lymphocytes subpopulations refer to the number of lymphocytes analyzed via Forward/Side scatter measured with a NAVIOS<sup>TM</sup> flow cytometer (Beckman Coulter GmbH, Germany). The absolute numbers were determined by using flow-count fluorospheres. Here, a known volume and concentration of flow-count fluorospheres were mixed with an identical volume of the biological sample. After analysis on the flow cytometer, the absolute number was calculated by the instrument software.

#### Clinical Chemistry

Blood and urine samples were collected according to the schedule of each individual study. The following clinical chemistry parameters were investigated in blood: electrolytes, phosphate, iron, glucose, urea, creatinine (CREA), bilirubin (BIL), cholesterol, triglycerides, protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), and glutamate dehydrogenase (GLDH). All appropriate tests were conducted using either ADVIA 1650 Autoanalyser (Siemens Medical Solutions Diagnostics GmbH), InfiniTE M200 EIA/ELISA-System (TECAN Deutschland GmbH), or COBRA II Gamma Counter (Canberra Packard).

Urinalysis focused on glucose, bilirubin, protein, urobilinogen, blood, ketone, sediment and pH value using Clinitek ADVANTUS Reflection Spectrophotometer (Siemens Medical Solutions Diagnostics GmbH) or Olympus BX40F Microscope (Olympus Optical CO, LTD).

#### Pharmacodynamic Biomarker

In the 4-week oral toxicology study in rats, the pharmacodynamic effects of roginolisib were evaluated by measuring Akt phosphorylation (pAkt) in whole blood B cells using flow cytometry (BD FACSCanto). For this purpose, blood samples were collected on Days 5, 20, and 28, as well as 1 hour after treatment. Anti-immunoglobulin M (IgM)-induced pAkt (Ser473; Cell signaling Technology, Danvers, MA, US) was measured as previously described [19B].

#### Pathology

**Rat.** On the day of necropsy, all rats were anesthetized with a carbon dioxide  $(CO_2)$  air mixture and terminated via abdominal exsanguination on the scheduled last day of study or sampling (satellite animals). In the event of an animal's death, from any cause, a necropsy was performed to assess gross pathological alterations, organ weight, and histopathology.

**Dog.** On the day of necropsy, all dogs were anesthetized by an intravenous injection with thiopental (initial phase: 10 mg/kg; maintaining phase: dose depended on the corneal and flexor reflexes) in the vena saphena or vena cephalica. Afterward, an approximately 10-cm skin incision was performed besides the trachea (mid-cervical region), and the external carotid arteries were prepared bluntly and were cut. In the event of an animal's death, from any cause, a necropsy was performed to assess gross pathological alterations, organ weight, and histopathology were performed.

#### Statistical Analyses and Evaluation

In the rat studies, all parameters were analyzed separately for each time. To take the number of dose groups into account, all the test procedures maintained a multiple significance level of .05. Body weight, body weight gain, food consumption, and organ weights of the dose groups were compared with those of the control, using the multiple two-sided Dunnett's test.<sup>23,24</sup> For the hematological, clinical chemistry, and immunophenotyping parameters, the Wilcoxon rank-sum test<sup>25</sup> was used to make pairwise comparisons with the control group. The correction for multiple testing was in accordance with Bonferroni-Holm.<sup>26</sup>

Due to the small number of dogs used (3 males and 3 females per main group without recovery animals), no statistical test procedure was applied. Additionally, the animals of the mid- and high-dose groups were prematurely killed and the vehicle and low dose group were treated for 12 weeks. These factors further confound the relevance of

formal statistical analysis. Hence descriptive statistical analysis was performed and mean values and standard deviations were calculated for all parameters per group for each sex and time point. Body weight, body weight gain, food consumption, hematological and clinical chemistry parameters, and ECG and blood pressure parameters, were recorded and evaluated using the Merck's proprietary laboratory information management (LIM)-system. The LIM-system was also used to record pathological and histopathological findings.

#### Results

#### Dose Range-Finding Studies in Rats and Dogs

Toxicokinetic Investigations. The toxicokinetic properties of roginolisib were determined after its oral administration to rats and dogs. Rapid absorption of the compound occurred through the gastrointestinal system in both species with the C<sub>max</sub> typically being reached within 1 hour of treatment administration, irrespective of dose. The increase in mean Cmax was roughly proportionate to the dose increase at all time points tested and was reflected in the AUC<sub>0-24h</sub>. The R<sub>ac</sub> AUC<sub>0-24h</sub> further demonstrated no accumulation of roginolisib within the blood plasma, with similar AUC<sub>0-24h</sub> values observed at each time point. Since D28 samples were not available for the female rats treated at 100 mg/kg, accumulation of roginolisib at this dose level cannot be excluded. Both female rats and dogs showed a tendency towards higher exposure compared to males especially at the highest dose levels. The Cmax and AUC<sub>0-24h</sub> values obtained from toxicokinetic investigations in each study are presented in Table 2.

#### Mortality and Clinical Symptoms

Mortality was seen in 4 of 5 female rats that received 100 mg/ kg between Days 7 and 9 as well as 1 female that was prematurely sacrificed on Day 9. In contrast, all male rats treated with the same dose survived. No toxicokinetic samples were obtained from the diseased animals to enable correlation of mortality with compound exposure.

Female rats did not tolerate the highest tested dose of 100 mg/ kg and showed clinical signs including incomplete/complete eyelid closure, pallor, lowered predose body temperature, piloerection, diarrhea, and reduced spontaneous activity.

In dogs, the female that received the highest tested dose of 1000 mg/kg was euthanized as a consequence of severe clinical symptoms on Day 4. The main clinical signs observed in at least one of the dogs that received a dose of  $\geq$ 300 mg/kg included vomiting, stool changes, salivation, hyperesthesia, tremor, hypoactivity, and genital discharge. In addition, increased body temperature was seen in the male treated at 300 mg/kg and the female treated at 100 mg/kg on some days.

Study	Species	Dose (mg/kg)	Sex	Study day	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	h × ng/mL
DRF study	Rat	10	Male	28	2,820	I	12,150
			Female	28	3,100	I	18,400
		30	Male	28	8,045	I	44,450
			Female	28	8,965	I	57,150
		100	Male	28	18,800	I	149,500
			Female	la	21,800	I	181,500
	Dog	100	Male	11	42,600	I	306,000
	•		Female	11	36,000	I	276,000
		300	Male	11	63,400	2	654,000
		1,000	Male	4	42,800	2	399,000
			Female	4	192,000	2	790,000
4-week GLP toxicology study	Rat	5	Male	28	1,646 ± 307	I	81,54 ± 2,089
0, ,			Female	28	1,940 ± 145	I	9,832 ± 637
		15	Male	28	2,869 ± 820	I	16,693 ± 2,420
			Female	28	4,680 ± 702	I	32,252 ± 3,965
		50	Male	28	,033 ±  ,949	I	70,822 ± 11,410
			Female	28	3,7   <sup>b</sup>	I	, 2  <sup>b</sup>
		75	Male	28	18,781 ± 4,739	<sup> -3</sup>	138,275 ± 40,363
			Female	28	23,562 ± 1,678	<sup> -3</sup>	157,744 ± 29,662
	Dog	5	Male	88	3,729 ± 945	I	23,199 ± 7,622
	•		Female	88	4,793 ± 2,419	I	31,245 ± 21,117
		15	Male	I	9,362 ± 3,160	I	55,694 ± 23,666
			Female	I	8,686 ± 1,846	I	46,797 ± 13,215
		45/30 <sup>°</sup>	Male	I	17,705 ± 1,870	I	107,920 ± 23,265
			Female	I	20,826 ± 4367	I	138,583 ± 54,556

**Table 2.** Mean Toxicokinetic Parameters of Roginolisib After Single and Repeated p.o. Administration to Male and Female Rats and Dogs in the DRF and 4-Week GLP Toxicology Studies (Summary Table).

<sup>a</sup>No data for Day 28 is available for female rats that received the highest doses of 100 mg/kg, as these were removed from the study due to sex specific toxicities; <sup>b</sup>n = 2;

<sup>c</sup>The treatment dose for this group was lowered on Day 15 from the original 45 mg/kg to 30 mg/kg. Treatment was stopped on Day 21.

## Body Weight and Food Consumption

In the DRF study, male rats administered 100 mg/kg showed a decrease in relative body weight gain of 16% on Day 28 compared with that of control male rats. Male rats exhibited slight changes in food consumption, but it was without any dose-dependency and therefore not treatment-related. However, in female rats treated with 100 mg/kg, a treatment-related decrease of 18% in food consumption was observed on Day 7.

In dogs, body weight was only slightly reduced compared to the pre-treatment values (range: -2.9 to -7.2%). Food consumption in all dogs was impaired, that is, marked reduction or no food intake during the treatment period.

#### Hematology

In the rat DRF study, the most prominent treatment-related alteration was a slight, but statistically significant ( $P \le .05$ ) drop in the white blood cell count (WBC) in males, reflected in absolute counts of lymphocytes (100 mg/kg = 3.91/nL; 30 mg/kg = 4.36/nL; 10 mg/kg = 4.1/nL; control = 5.7/nL). All changes were within the internal laboratory range and occurred without any dose dependency. Females did not show any changes in white blood cells; however, no hematology

values of group 4 females (100 mg/kg) were available as animals died or were terminated prematurely between Days 7 and 9. Slight changes in neutrophils, basophils, monocytes, reticulocytes, and platelets were also observed; however, all were within internal laboratory range. Immunophenotyping revealed a slight dose-dependent and statistically significant decrease ( $P \le .05$ ) in the relative percentage of B cells in males, and an associated increase in the relative percentage of T cells (mainly in males). Data for immunophenotyping in rats are reported in Table 3.

In the dog DRF study, the female dog that received the escalating dose had an increase in red blood cells at Day 4 (9.76/pL) compared with Day 5 (7.85/pL), as well as elevated hemoglobin levels (22.3 g/dL at Day 4 compared with 17.6 g/dL at Day 5). Immunophenotyping, including T cell subsets, did not indicate a global immune deficiency.

#### Clinical Chemistry

Analyses of the clinical chemistry parameters in rats revealed slight changes in some serum substrates and enzymes, in particular CREA, total bilirubin (TBIL), triglyceride, total protein (TP), inorganic phosphorus, and GLDH mainly at 30 and 100 mg/kg. All changes were within the internal

			DRF s	tudy					4-v	eek GLP tox	kicology stu	γþ			
			Wee	k 5				Day 29					Day 85		
Treatment dose (mg/kg/day)		0	9	30	100ª	0	ъ	15	50	75	0	ъ	15	50	75
Total n (m/f) CD45RA <sup>+</sup> cells		5/5	5/5	5/5	5/5	01/01	01/01	01/01	01/01	01/01	5/5	5/5	5/5	5/5	5/5
Counts   (/nL)	Mean (m/f) 3D (m/f)	<u>+</u> +	<u>+</u> +	-/-		1.18/1.17 .37/.36	1.08/1.04 .37/.36	.80°/.76 .24/.27	.89/.85 .34/.36	.71 <sup>d</sup> /.79 .21/.35	1.00/1.08 .35/.25	1.52/1.12 .30/.54	1.28/1.15 .45/.22	1.02/.75 .18/.19	1.09/1.20 .28/.29
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Mean (m/f) SD (m/f)	37.1/ 31.3 2.72/	28.1°/ 25.9 6.89/3.71	26.5°/ 31.2 7.88/4.34	23.6°/- 2.30/-	32.6/34.3 5.73/6.04	30.2/32.9	30.0/30.0 5.33/7.59	25.0 <sup>c</sup> /22.6 <sup>d</sup> 6.20/5.06	19.3°/21.8 <sup>d</sup> 3.69/6.55	34.4/37.0 6.76/4.14	41.8/38.3 2.46/7.91	35.1/44.5° 3.24/1.71	34.2/30.3 5.67/5.31	34.7/38.5 5.63/5.74
CD3 <sup>+</sup> T cells	Ann (m/A	20.C	-	-	-	2 01/1 95	I 96/1 97	ו בז/ו בל	1 78/7 E4	) 45/7 51		64/1 53		77/  5	54/1 47
(/nL)	SD (m/f)					.38/.75		.36/.47		1 C. 7 / CO. 7 (1 . 03).	.54/.32	.43/.75	.87/.30	1.00.1/00.1	
- ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	∀ean (m/f)	54.8/ 41.4	64.3/48.7	65.6/49.3	69.3 <sup>c</sup> /-	56.2/56.3	58.2/58.0	57.5/62.0	64.7 <sup>c</sup> /70.0 <sup>d</sup>	70.9 <sup>d</sup> /70.5 <sup>d</sup>	51.4/53.8	46.5/51.6	53.2/46.5 <sup>c</sup>	54.7/61.1	49.2/52.3
• /	SD (m/f)	2.88/ 12.7	8.64/8.34	8.16/5.99	3.82/-	6.39/6.41	5.78/11.4	5.29/7.54	8.22/5.79	4.42/6.09	9.23/3.66	3.86/8.99	4.98/1.64	5.86/6.04	6.56/7.36
CD4 <sup>+</sup> T cells															
Counts   (/nL)	Mean (m/f) SD (m/f)			-/-		l.44/l.42 .28/.60	1.39/1.46 .25/.44	1.10/1.14 .26/.37	1.69/1.85 .43/.62	1.94/1.83 .55/.72	1.10/1.12 .40/.25	1.08/1.06 .17/.49	1.39/.64 <sup>c</sup> .54/.31	1.15/1.02 .15/.18	.98/1.17 .30/.32
°,	√ean (m/f)	39.3/ 37 3	46.8/38.4	49.5/35.I	51.3 <sup>c</sup> /-	40.1/40.9	43.3/43.3	42.1/46.3	47.3 <sup>c</sup> /51.2 <sup>d</sup>	51.3 <sup>d</sup> /51.5 <sup>d</sup>	36.6/37.4	32.6/37.2	36.5/34.I	37.4/41.6	31.9/36.3
.,	SD (m/f)	2.29/ 12.0	7.65/6.08	9.10/4.58	2.82/-	5.11/4.88	6.45/8.61	4.30/6.96	4.68/4.67	4.14/5.07	9.08/2.72	I.84/6.83	3.91/1.63	4.55/4.38	4.45/5.00
CD8 <sup>+</sup> T cells															
Counts	Mean (m/f)			-/-	- <u>'</u> -	.55/.50	.47/.56	.40°/.39	.62/.72 20/20	.71/.68	.42/.48	.46/.41	.68/.32	.51/.46	.51/.51
(יוור) %b	Vean (m/f)	-/- 12.5/	-/- 15.7/10.9	-/- 15.9/13.6	-/- 16.6 <sup>c</sup> /-	.12/.10 15.3/14.7	.11/.22 14.5/16.2	.00/.17 15.3/15.9	.20/.27 17.3/19.6 <sup>d</sup>	.17/.32 19.2 <sup>c</sup> /18.8 <sup>c</sup>	.17/.11 13.9/16.0	.17/.22 13.5/14.0	16.7/12.3		.17/.17 16.8/15.5
	SD (m/f)	10.4 1.23/ 3.89	3.93/2.50	3.06/4.64	2.11/-	2.29/2.49	2.80/3.45	I.84/3.77	3.73/3.17	2.34/3.63	2.48/2.33	3.84/2.59	4.71/1.31	I.39/2.82	2.50/3.92
															continued)

Table 3. Immunophenotyping of Rats Treated with Roginolisib.

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			DRF s	ttudy					4-4	veek GLP to	xicology stu	dy			
			Wee	k 5				Day 29					Day 85		
Treatment d (mg/kg/day)	ose	0	01	30	100 <sup>a</sup>	0	ß	15	50	75	0	ß	15	50	75
CD161a <sup>+</sup> N	K cells														
Counts	Mean (m/f)	-/-	-/-	-/-	-/-	.17/.13	.18/.12	.20/.12	.23/.18	.22/.16	.16/.11	.18/.12	.19/.09	.15/.10	.21/.12
(/u/)	SD (m/f)	-/-		-/-	-/-	.04/.03	.06/.03	.09/.05	.10/.07	90'/90'	.05/.03	.04/.04	.08/.03	.02/.01	.04/.06
۹%	Mean (m/f)	-/-	-/-	-/-	-/-	4.6/3.8	5.3/3.9	7.3 <sup>d</sup> /4.7	6.6 <sup>c</sup> /5.2	5.9 <sup>c</sup> /4.8	5.7/3.9	5.1/4.6	5.4/3.5	4.9/4.0	6.7/3.9
	SD (m/f)	-/-	-/-	-/-	-/-	.83/.89	l.69/.74	1.75/1.92	2.20/1.73	1.10/1.60	2.50/1.11	.93/2.12	2.05/.76	.69/.48	1.76/2.53
Abbreviations:	DRF = dose rang	e-finding; (	GLP = good	laboratory	practice; N	JK = natural	killer.			-					
remales with	n this group were	removed	Trom study h	orematurely	V as a cons	equence or t	Sither morts	ality or eutris	inasia on weil.	are grounds;					

weltare gr 5 lasia euthar Б tallty b either 5 emales within this group were removed from study prematurely as a consequence

<sup>b</sup>ercentage of total lymphocytes;

<sup>c</sup>Wilcoxon rank sum test with Bonferroni-Holm: 2 sided P < .05;

Bonferroni-Holm: 2 sided P < .01test with rank sum <sup>d</sup>Wilcoxon

test with Bonferroni-Holm: 2 sided P < .00Wilcoxon rank sum International Journal of Toxicology O(0)

laboratory historical control range. Clinical chemistry parameters showing significant treatment-related changes compared to control are reported in Table 4.

In both dogs treated at the highest dose of 1000 mg/kg serum electrolytes (potassium, calcium, chloride, inorganic phosphorus) and triglycerides were decreased on Day 4, whereas glucose was increased. Sodium was decreased in the female only. On Day 9, serum electrolytes were unchanged in the male dog treated at 300 mg/kg. Sodium, inorganic phosphorus, and chloride (male only) were slightly increased in both dogs at the dose of 100 mg/kg. ALT, AST and GLDH were clearly increased in both males, most pronounced in the male dog treated at 300 mg/kg when compared to pre-test values. Urinalysis, performed on test Day 10, did not reveal any toxicological relevant changes. Selected clinical chemistry parameters are reported in Table 5.

# Pathology

In the rat DRF study, gross pathology, and organ weights of all animals were inconspicuous. Histopathology of the deceased females (100 mg/kg) revealed depletion of the lymphatic system sometimes combined with cell necrosis, ulceration of the forestomach, necrosis of the intestinal mucosa, and skin hyperkeratosis. In female rats treated with 10 and 30 mg/kg as well as in male rats treated with 10, 30, and 100 mg/kg, morphological alterations were mainly restricted to the B-cell compartments of lymph nodes and the spleen, demonstrating a decrease of germinal center formation and a depletion of the marginal zone. The observed histopathological findings including the respective severity grade are given in the form of a heatmap in Figure 1 (increasing color intensity in red matches the degree in severity for males represented in upper triangle, females in lower triangle).

Gross pathology revealed a red and white discoloration of the intestinal mucosa in the female dog at 100 mg/kg. The female dog that was euthanized prematurely at 1000 mg/kg showed a completely empty lumen of the small and large intestine. Histopathology revealed a massive lymphoid depletion in all lymphatic tissues, in particular the thymus and spleen. Beside the pronounced pharmacodynamically mediated atrophy of the lymphatic system, secondary focal alterations were observed in the liver in almost all dogs (small necroses and microgranuloma). Single observations were additionally noted in the stomach (hypersecretion), intestine (hyperemia), adrenals (hemorrhage), bone marrow (necrosis), and prostate (inflammation).

# Pivotal Oral Repeat Dose Toxicology Studies in Rats and Dogs

Toxicokinetic Investigations. In rats, Cmax and AUC0-24h increased approximately dose proportional and concentrations of roginolisib in the blood plasma were similar between repeated doses, suggesting effective clearance of the compound from the body (see Table 2). In general, females were slightly more exposed than males on both sampling occasions, the male/female AUC<sub>0-24h</sub> ratios being .6-.8 on Day 1 and .5-.9 on Day 28. A moderate accumulation was determined after repeated dosing at the higher dose levels, that is, the Rac AUC<sub>0-24h</sub> ranged from .9 at 15 mg/kg to 1.4 at 75 mg/kg in male rats and from 1.2 at 15 mg/kg to 1.3 at 75 mg/kg in female rats on Day 28.

In dogs,  $C_{max}$  and  $AUC_{0-24h}$  showed an approximately dose proportional increase in males and a less than dose proportional increase in females (see Table 2). A moderate accumulation was determined after repeated dosing, that is, the  $R_{ac}$  $AUC_{0-24h}$  ranged from 1.5 at 5 mg/kg to 2.2 at 15 mg/kg in male dogs and from 1.3 at 5 mg/kg to 1.6 at 15 mg/kg in female dogs on Day 25.

### Mortality and Clinical Signs

In contrast to the rat DRF study, no treatment-related mortality was detected in rats treated with 5, 15, 50, and 75 mg/kg in the 4-week oral toxicology study. Furthermore, no treatment-related clinical signs were observed in rats treated with doses of  $\leq$ 50 mg/kg. Only short and transient episodes of salivation (<5 minutes) were seen in 8 rats receiving 75 mg/kg.

In the pivotal toxicology study in dogs, premature euthanasia was performed in dogs at 5 mg/kg (1 dog, Day 65), 15 mg/kg (2 dogs, Day 19), and 45/30 mg/kg (1 dog, Day 12; 4 dogs, Day 16; 1 dog, Day 36). In addition, mortality was seen in 1 dog at 15 mg/kg during the premature euthanasia on Day 15 and 1 dog at 45/30 mg/kg on Day 16. Dogs receiving 5 mg/ kg had shown very few clinical symptoms up to Day 25, and therefore, it was decided to extend the treatment of both that dose group and the control group to 12 weeks.

At 5 to 45/30 mg/kg, epithelial lesions of the skin (whole body including paws) and mucosa as well as discharge from the eyes and genitals (all males, occurring around Days 9-11) were seen during the treatment period and/or recovery period (especially paw lesions). In addition, at 15 and 45/30 mg/kg gastrointestinal symptoms like vomiting, soft stool with yellowish-mucous admixtures, soft stool with bloody admixtures, and diarrhea as well as bloody urine, salivation, hypothermia, photophobia, tremor, hypoactivity, apathy, and lateral and prone positions were detected during the treatment period.

## Body Weight and Food Consumption

In the pivotal toxicology study in rats, body weight did not differ between treatment and control males at the end of the treatment and recovery period. In females, body weight was slightly lower in group 4 (50 mg/kg, statistically significant) and group 5 (75 mg/kg) animals compared to control by 4.9 and 3.6%, respectively, on Day 28. The body weight gain was therefore slightly lower in these animals. No treatment-related

changes of food consumption during the treatment or recovery period were observed in either sex.

In dogs at 15 mg/kg (1 female) and 45/30 mg/kg (2 females), significantly reduced body weight during the treatment period was related to bad clinical condition. All other roginolisib treated dogs showed only minor changes in bodyweight. Short incidences (1-4 days) of reduced or no food consumption were observed in some dogs of either sex that received  $\geq$ 15 mg/kg.

# Electrocardiography, Arterial Blood Pressure, and Reflex Tests

In dogs, the ECG parameters PQ-, QRS-, and QT-interval as well as heart rate showed no relevant change within or among the groups during the treatment period. In addition, the heart rate-corrected QT-intervals (according to Van de Water) did not change relevantly during treatment within 1 group, and no relevant differences between the groups were observed. No treatment-related effect was seen on mean-, systolic-, and diastolic arterial blood pressure. Furthermore, there were no changes in reflex tests which are considered to be related to the treatment.

#### Ophthalmology

In rats, ophthalmological examinations including pupillary reflex, and the examination of anterior and posterior eye segment did not reveal any treatment-related effects. However, in dogs a corneal erosion (ulcer) was seen in 1 animal at 5 mg/kg and central vitreous floater, hyper-reflectivity, and foci formation in the tapetal-non tapetal junction in a dog at 45/30 mg/kg in week 10. These findings were considered likely related to a spontaneous event (such as housing conditions or animal behavior) and not to roginolisib.

## Hematology Parameters

A dose-dependent increase of neutrophils was observed in both male and female rats, accompanied by a decrease of relative lymphocyte count. Differentiation of lymphocyte populations by immunophenotyping identified a clear decrease of relative B-cell count (males: 75 mg/kg = 19.3%; control = 32.6%, P < .001; females: 75 mg/kg = 21.8%, control = 34.3%; P < .01) and an increase of relative T cells (males: 75 mg/kg = 70.9%, control = 56.2%; P < .01; females 75 mg/kg = 70.5%, control = 56.3%; P < .01) consisting of Thelper and T-suppressor cells in both sexes at 50 and 75 mg/ kg. The effects proved to be reversible. Data for immunophenotyping in rats is reported in Table 3.

Male and female dogs treated with 5 to 45/30 mg/kg showed a non-dose-dependent increase in platelet counts on Day 23/24. For example, the pre-test vs Day 23/24 platelet

					-									
		DRF	study					4- 2	eek oral to	xicology stu	ф			
		Wee	sk 5				Day 29					Day 85		
Treatment dose (mg/kg/day)	0	01	30	100 <sup>a</sup>	0	5	15	50	75	0	5	15	50	75
Total n (m/f)	5/5	5/5	5/5	5/5	01/01	01/01	01/01	10/10	10/10	01/01	01/01	01/01	01/01	01/01
ALT (U/L) Mean (m/f)	30/21	29/22	24/24	34/-	31/21	29/21	30/18	30/26	38 <sup>b</sup> /35 <sup>d</sup>	57/36	34/24	38/24	35/24	37/24
SD (m/f)	6/3	2/I	2/1	7/-	4.9/3.4	3.1/2.2	4.7/4.7	4.5/6.3	3.7/5.9	26.1/23.6	4.3/3.I	11.5/6.1	6.0/2.8	6.0/5.1
AST (U/L)														
Mean (m/f)	<i>61/11</i>	77/83	18/06	95/-	93/75	87/81	87/86 <sup>b</sup>	93/90 <mark>b</mark>	98/98 <mark>c</mark>	115/96	89/73	90/76	80 <sup>b</sup> /65	82/75
SD (m/f)	12/5	4/8	8/5	-/11	8.6/9.4	8.6/13.9	5.5/11.2	7.1/12.6	7.5/10.5	21.0/17.2	7.6/2.2	32.8/8.1	7.0/6.2	12.4/10.5
CREA (umol/L)														
Mean (m/f)	18/23	18/22	14 <sup>b</sup> /17 <sup>b</sup>	13 <sup>b</sup> /-	19/23	18/20	18/19 <mark>0</mark>	16 <sup>c</sup> /16 <sup>c</sup>	20/19 <mark>b</mark>	25/28	29/28	23/27	26/29	30/29
SD (m/f)	2/2	2/2	1/4	-/	2.2/2.5	2.0/2.8	2.8/3.4	1.6/2.9	1.0/2.5	2.9/1.9	3.6/4.5	2.9/2.8	4.4/3.4	5.1/2.8
GLDH (U/L)														
Mean (m/f)	9.8/5.3	8.9/9.1 <sup>b</sup>	8.6/10.3 <sup>b</sup>	9.2/-	6.2/5.3	6.3/5.7	6.8/7.4 <sup>b</sup>	7.1/7.6 <sup>b</sup>	7.6/8.5 <sup>c</sup>	11.5/6.5	7.5/7.1	11.0/7.2	6.6/4.9	9.7/5.8
SD (m/f)	5.2/.6	1.3/3.8	1.2/4.4	-/0.1	1.34/1.63	.78/.82	1.12/2.37	.68/2.19	1.53/1.72	8.43/4.24	2.13/5.29	7.42/3.97	1.12/1.37	5.55/3.77
TBIL (µmol/L)														
Mean (m/f)	.4/.9	.5/1.4	<mark>6.1/6</mark> .	.4 <sup>b</sup> /-	1.2/1.9	1.2/2.2	1.5 <sup>b</sup> /2.1	I.8 <sup>c</sup> /3.0 <sup>b</sup>	1.7 <sup>d</sup> /2.4	.8/2.1	.8/2.0	.8/2.3	.8/1.6	.9/2.0
SD (m/f)	.4/.3	.1/.5	.2/.4	.3/-	.17/.79	.36/.52	.34/.49	.45/.89	.28/.47	.29/.19	.21/.76	.37/.59	.35/.38	.11/.40
TP (g/L)														
Mean (m/f)	59.5/60.5	59.5/61.3	57.8/57.8	55.9 <sup>b</sup> /-	60.0/61.0	59.9/62.9	59.8/60.0	60.0/59.1	60.7/57.9 <sup>b</sup>	64.5/65.6	63.2/66.6	62.3/67.6	63.1/67.2	62.6/66.1
SD (m/f)	2.2/3.2	1.7/2.0	3.6/2.2	-/1.1	2.19/2.10	1.93/2.77	2.01/3.98	2.04/4.53	I.45/2.63	1.96/5.28	1.55/3.81	2.22/2.74	2.28/2.40	1.24/3.11
Abbreviations: ALB = albumin: AL	.T = alanine	transaminas	e: AST = aspa	rtate trans	aminase: CRI	EA = creatin	ine; DRF = d	ose range-fin	ding; f = fema	ile: GLDH = 5	glutamate de	hydrogenase	: m = male: T	-BIL = total
bilirubin; TP = total protein.			-					D	ò			D		
<sup>a</sup> Females within this group were	removed fro	om study pre	ematurely as a	a conseque	ence of eithe	r mortality c	or euthanasia	on welfare g	grounds;					
<sup>c</sup> Milcovon rank sum test with Bc	nilei roili-m	olini. 2 sided												
		רייין ביווור אווור	, 10, / 1											
VIICOXON FANK SUM TEST WILL DE	UTELFOIL-IN	OIM: 2 SIGEU	r < .uui.											

Table 4. Clinical Chemistry Parameters in Rat Toxicology Studies Showing Significant Treatment-Related Changes Compared to Control.

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	DRI						4-w	eek oral to	oxicology st	Арп				
	Day	6		Day -I	3/-12			Day	23/24			Day 8	32/85	
Treatment dose (mg/kg/day)	100 <sup>a</sup>	300 <mark>6</mark>	0	ъ	15	45/30 <sup>c</sup>	0	ß	15	45/30 <sup>c,d</sup>	9 0	പ്പ	15 <sup>d</sup>	45/30 <sup>c,d,f</sup>
Total n (m/f) ALB (g/L)	1/1	1/1	3/3	5/5	5/5	5/5	3/3	5/5	3/4	2/2	3/3	4/5	2/2	2/I
Mean (m/f)	34.4/31.8	30.9/-	33.6/31.8	32.6/33.3	32.8/34.9	31.7/32.4	31.2/31.9	30.6/32.8	26.6/27.7	25.1/30.5	30.7/31.3	30.1/31.9	32.7/32.6	31.7/31.0
SD (m/f)	I	I	.93/3.20	.83/1.66	.94/.87	1.28/1.06	1.64/3.35	I.09/2.33	I.69/3.75	.21/1.27	1.97/3.40	I. I 6/2.42	I.56/.7I	I.I3/-
Mean (m/f)	64/35	-/611	56/34	46/35	39/62	49/42	53/39	89/79	134/106	207/82	60/33	114/115	56/59	60/45
SD (m/f)			20/7.6	7.1/9.4	7.4/43.8	18.6/17.2	11.5/12.2	29.4/41.7	24.9/76.1	75.0/9.2	20.2/12.9	20.4/74.8	7.8/8.5	3.5/-
AP (U/L)														
Mean (m/f)	58/55	-//6	97/95	99/126	66/26	79/105	163/106	171/207	387/284	528/166	203/120	164/208	158/110	115/112
SD (m/f)	I		45.8/46.8	18.7/54.7	37.2/36.4	27.2/35.5	131/64.8	34.2/126	230/213	421/34.6	178/43.3	8.5/107	116/15.6	5.7/-
AST (U/L)														
Mean (m/f)	57/28	65/-	32/31	37/35	33/37	34/35	39/33	44/46	98/71	67/51	36/31	60/53	35/45	54/38
SD (m/f)	I		I.7/5.I	4.4/9.4	6.1/6.2	3.8/6.8	6.1/4.2	5.3/9.1	29.1/25.0	3.5/31.1	7.0/1.2	7.2/11.6	6.4/14.8	I 4.8/-
CHOL (mmol/L)														
Mean (m/f)	3.56/3.49	2.73/-	4.36/3.22	3.98/4.53	3.47/3.78	3.60/4.18	3.80/3.42	2.54/2.73	1.65/1.72	2.76/2.79	3.98/3.17	2.27/2.96	3.29/3.52	2.61/2.18
SD (m/f)	I		.14/.40	.8/.90	.51/.56	.37/.92	.20/.79	.65/.43	.44/.53	.78/.84	.31/.59	.36/.94	.30/.74	-/60.
GLDH (U/L)														
Mean (m/f)	16.1/6.8	39.9/-	8.37/5.83	5.84/6.24	5.60/12.7	6.96/9.34	7.37/6.70	11.1/13.3	12.6/9.40	7.2/5.60	6.57/6.37	16.4/20.0	7.10/10.2	9.55/7.60
SD (m/f)	I		4.87/2.17	.56/2.12	.49/11.5	3.27/5.98	.61/2.10	5.08/11.9	5.99/4.35	1.98/.57	.55/3.37	8.06/16.5	1.41/.50	3.47/-
TP (g/L)														
Mean (m/f)	61.0/57.4	55.8/-	54.7/51.9	55.4/54.6	54.5/56.8	53.2/53.5	52.9/52.4	53.6/52.9	49.2/48.I	48.6/54.I	54.5/52.9	53.7/54.6	56.1/55.4	55.3/52.5
SD (m/f)			.31/3.98	1.09/2.77	1.42/1.33	2.19/2.03	.95/4.74	1.27/4.11	3.51/3.50	.71/.42	.95/3.82	1.41/5.05	2.19/.49	2.12/-
Abbreviations: ALB = albumin; /	ALT = alanin	e transar	ninase; AST :	= aspartate t	cransaminase	; AP = alkali	ne phosphat	ase; CHOL	= cholesterc	l; DRF = do	se range-finc	ling; f = fem	ale; GLDH =	- glutamate
dehydrogenase; m = male; TP = a A :	total protein				001	-								
bThe female dog receiving the esc	veu no muai alating dose v	uose oi vas eiitha	u eaunenu ioi nized due to v	r + uays, wiu velfare conce	Prins followin	administrat	ays ⊃- 1-t, ion of the ma	ximal 1000 r	nø/kø dose or	Dav 4. The r	male dog was	dosed with 3	800 mø/kø dog	ses on Davs
1110 10111110 400 100011116 410 000	מומנוויוק בכייי	1000 000				2 משוווווייים של			18/15 4000	רמין וי וייי	11alc 405 1140		~~ 9~ /9 · · · 0	

Table 5. Clinical Chemistry Parameters in Dog Toxicology Studies Showing Treatment-Related Changes Compared to Control.

5-14; <sup>C</sup>The treatment dose for this group was lowered on Day 15 from the original 45 mg/kg to 30 mg/kg. Treatment was stopped on Day 21; <sup>d</sup>Remaining animals at this timepoint were "recovery" animals and were no longer receiving treatment with roginolisib; <sup>e</sup>The treatment period for these groups was extended to 12 weeks; <sup>f</sup>Day 71.



**Figure 1.** Heat map shows the histopathological findings including the severity grades of sites of toxicity in rats in a 4-week DRF study. Females that received 100 mg/kg of roginolisib resulted in premature mortality due to either clinical symptoms or euthanasia due to welfare concerns on Days 7-9. Male findings are represented in the upper colored triangles and female findings in the lower colored triangles. Coloring is matched with the severity grade assigned to the finding with increasing intensity of red being associated with severe grade toxicity.

count in the 45/30 mg/kg group increased from 324 to 440/nL in male dogs, and from 290 to 473/nL in female dogs. Furthermore, an increase in WBC, mainly due to an increase in neutrophils, was seen at 15 mg/kg (WBC in males = 8.84/nL at pre-test vs 15.45/nL on Day 23; WBC in females = 9.16/nL at pre-test vs 17.24/nL on Day 24) and at 45/30 mg/kg (WBC in males = 10.15 at pre-test vs 20.45/nL on Day 23; WBC in females = 8.71/nL at pre-test vs 17.24/nL on Day 24). At the highest dose of 45/30 mg/kg males additionally showed a slight reduction in red blood cell counts, hemoglobin values only, and a concurrent slight increase of reticulocytes. Thrombin and prothrombin time were slightly prolonged in males as well. Box plots of platelet, WBC, neutrophil, and lymphocyte counts are shown in Figure 2.

Immunophenotype data in dogs revealed substantial decreases in all immune subsets, however only at the highest dose of roginolisib. Immunophenotyping data are reported in Table 6.

## **Clinical Chemistry**

Only a slight dose-dependent and reversible increase in ALT, AST (females only), GLDH, TBIL, and TP values as well as a slight dose-dependent decrease in CREA were seen in male and female rats (see Table 2). However, within the pivotal toxicology study in dogs treated with 5 to 45/30 mg/kg, a mild to severe dose-dependent increase in the liver enzymes ALT, AST, AP, and GLDH as well as a slight to moderate decrease in the liver substrates CHOL, ALB, and TP were observed (see Table 5 and Figure 2). These findings indicate an impairment of liver cell

function and liver cell damage; however, the effects proved to be reversible in the recovery animals.

#### Pharmacodynamic Biomarker

In rats, pAkt was inhibited by roginolisib at all dose levels on Days 5, 20, and 28 (see Figure 3). On Day 5, the range of inhibition was variable especially in female rats (between 15% and 73%) and no clear dose response could be observed. The pAkt inhibition was more consistent on Day 20 with approximately 65% of inhibition at the dose levels of 5 and 15 mg/kg and approximately 80% at the dose levels of 50 and 75 mg/kg. On Day 28 pAkt was inhibited >95% at all dose levels.

## Pathology

10^3/µL

10^3/µL

week -2

In the pivotal toxicology study in rats, gross pathology and organ weight determination revealed several findings that

WBC

week 4

LYMPH

week 10-12

were considered to be spontaneous. The spleen weight in females at 50 and 75 mg/kg were decreased, which might be caused by depletion of marginal zone cells.

Histopathology showed treatment-related effects in lymph nodes and spleen. The mandibular lymph node showed a decrease of germinal centers in some rats at 50 mg/kg and in most rats at 75 mg/kg. The doses of 5 and 15 mg/kg were not affected. In the mesenteric lymph node, the decrease of germinal centers was more prominent as almost all rats at 50 and 75 mg/kg were affected and additionally 2 females at 15 mg/kg. In the spleen, a minimal to moderate depletion of the marginal zone was seen at 50 and 75 mg/kg, and a minimal depletion in few males and females at 15 mg/kg and in 1 male already at 5 mg/kg. At  $\leq$ 75 mg/kg doses of roginolisib in rats, only isolated abnormalities were found in the liver, with multifocal microgranuloma of a minimal severity being the most frequent observed finding. All other organs were congruous with the controls. At the end of the 8-week recovery period no

10^3/µL

╡

week -2

NEU

week 4

ASAT

week 10-12



week -2 week 10-12 week 4 week 4 week 10-12 week -2 week 4 week 10-12 reek -2 GLDH AP TP ٦N Ч ٩L week -2 week 10-12 week -2 week 4 week 10-12 week 4 week 10-12 Neek -2 CHOL AL B 0 mg/kg (m+f) Figure 2. Observed hematology and clinical chemistry changes in 4-week dog toxicity study. Graphs show combined data for male and

PLT

week 4

ALAT

week 10-12

week -2

₹

						4	week oral tc	vxicology stu	dy				
		Day -13/-1	2				Day	16/17		Day 71	Day 82	Day	85
Treatment dose	(mg/kg/day)	0	5	15	45/30 <sup>a</sup>	0	5	15	45/30	45/30	15	0	5
Total n (m/f) CD45RA <sup>+</sup> cells		3/3	5/5	5/5	5/5	3/3	5/5	4/5	5/3	2/1	2/2	3/3	4/5
Counts (/nL)	Mean (m/f) SD (m/f)	.28/.33 .15/.07	.35/.37 .08/.09	.37/.35 .09/.18	.29/.46 .02/.19	.37/.29 .19/.06	.35/.30 .10/.15	.38/.56 .21/.33	.10/.11	.24/.20 .09/-	.46/.35 .30/.17	.33/.36 .14/.11	.33/.32 .13/.13
*%	Mean (m/f) SD (m/f)	16.8/16.5 6.18/5.31	19.4/19.6 4.63/4.56	21.3/18.4 3.11/6.33	17.8/22.3 3.35/4.91	23.6/15.9 10.7/5.12	22.5/17.0 7.21/5.29	27.0/22.3 9.87/6.83	14.1/13.2 2.21/5.37	11.0/15.1 .07/-	27.4/17.9 11.5/5.52	20.5/18.8 7.57/3.80	18.3/18.9 5.78/5.79
CD3 <sup>+</sup> T cells													
Counts (/nL)	Mean (m/f)	1.17/1.34	1.19/1.30	1.05/1.24	1.17/1.20	1.01/1.26	1.07/1.19	.80/1.36	.53/.69	1.64/1.00	.89/1.31	.89/1.22	1.10/.99
	SD (m/f)	.14/.12	.34/.46	.14/.13	.32/.18	.29/.19	.17/.43	.16/.15	.24/.11	.64/-	.12/.18	.13/.14	.14/.26
%	Mean (m/f)	69.3/67.6	64.0/64.8	62.0/69.1	67.1/62.7	63.5/69.2	63.8/70.4	60.4/65.0	77.2/80.0	71.4/70.0	57.9/69.8	58.6/61.9	61.3/60.2
	SD (m/t)	7.01/4.27	3.74/5.78	2.52/8.03	6.50/5.6	10.7/4.11	5.93/5.50	9.20/8.60	4.28/4.91	2.69/-	8.13/3.54	11.5/4.16	7.13/3.77
CD4 <sup>+</sup> T cells													
Counts (/nL)	Mean (m/f)	.87/.95	.84/.91	.75/.92	.86/.82	.75/.91	.78/.87	.57/.93	.34/.46	1.15/.71	00.1/66.	.60/.92	.77/.70
	SD (m/f)	.08/.15	.26/.27	11./60.	.25/.09	.23/.15	.10/.32	.17/.11	.15/.07	.47/-	.06/.17	.13/.24	.11/.18
*%	Mean (m/f)	49.7/67.6	44.7/47.0	45.3/51.7	42.8/44.7	47.5/51.3	44.4/51.5	41.8/45.6	51.2/50.8	48.0/45.8	43.0/53.8	41.3/44.1	42.6/43.0
	SD (m/f)	9.59/4.27	3.41/3.82	5.34/7.13	17.1/5.43	13.7/5.79	3.21/2.16	6.79/7.61	6.42/4.06	4.31/-	7.14/1.20	11.5/3.05	4.77/2.47
CD8 <sup>+</sup> T cells													
Counts (/nL)	Mean (m/f)	.26/.30	.28/.29	.20/.25/	.26/.27	.22/.28	.25/.27	.17/.29	.12/.19	.40/.24	.18/.21	.18/.30	.26/.23
	SD (m/f)	.11/.08	.09/.II	.04/.06	.06/.08	.08/.10	.03/.14	.03/.06	.06/.06	-/20.	.06/.07	.02/.04	.04/.08
*%	Mean (m/f)	13.9/15.4	14.8/14.8	12.1/13.6	14.6/14.2	13.6/15.4	14.3/15.4	13.4/13.8	18.4/20.6	17.2/15.2	11.4/11.0	12.5/14.6	14.5/14.0
	SD (m/f)	3.25/5.31	I.52/2.96	2.05/2.75	2.66/1.53	2.57/4.38	1.31/2.78	3.76/2.54	3.46/3.60	2.47/-	1.06/1.41	1.06/3.84	2.18/1.82
<sup>a</sup> The treatment dc <sup>b</sup> Percentage of to	sse of roginolisib tal lymphocytes.	for this group	was lowered	on Day 15 frc	om the origina	l 45 mg/kg/day	r to 30 mg/kg/r	day;					

Roginolisib.
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6.
Table

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relevant histopathology findings were observed in rats. The observed histopathological findings in rats including the respective severity grade are given in the form of a heatmap in Figure 4.

The pivotal toxicology study in dogs found gross pathological changes in the skin at 5 mg/kg and in the intestinal tract at 15 and 45/30 mg/kg. In addition, no organ weight changes were observed. Histopathology revealed pharmacologically mediated alterations in the lymphatic system at all dose levels. Administration of 5 mg/kg led to a minimal to mild and 15 and 45/30 mg/kg to a moderate to marked depletion of lymphocytes in spleen, thymus, lymph node and lymph follicles of the intestine. At 15 and 45/30 mg/kg, moderate bone marrow depletion, moderate to marked necrosis of the intestinal mucosa, focal necrosis of the gall bladder mucosa, mild to marked liver cell necrosis as well as moderate depletion of the spermatogenic epithelium of the testes were seen. The recovery animals did not exhibit any conspicuous findings except for 1 recovery female at 45/30 mg/kg which developed a dysplastic alteration in the skin of the hind paws within 20 days after treatment. The observed histopathological findings in dogs including the respective severity grade are given in the form of a heatmap in Figure 5.

Overall, in the pivotal oral toxicology study in rats, roginolisib was clinically tolerated and the no observed adverse effect level (NOAEL) was determined at 15 mg/kg. However, in the pivotal toxicology study in dogs, roginolisib was not tolerated due to severe clinical symptoms, that is, mainly epithelial lesions of the skin at 5 mg/kg and necrotizing damage of the intestinal epithelia at 15 and 45/30 mg/kg, which were dose limiting and lethal. A NOAEL could be not established in dogs.

## Discussion

Here, we report the non-clinical DRF and 4-week pivotal toxicology studies of roginolisib, a selective non-competitive

PI3K $\delta$  inhibitor.<sup>19</sup> The level of off-target inhibition, particularly driven by levels of unbound drug within the body, appears to be a key driver of roginolisib's toxicity profile in rats and dogs. Hence, from being a well-tolerated, specific drug, roginolisib may become a poorly tolerated unspecific drug should free drug levels exceed the threshold for selective PI3K $\delta$  inhibition.

The aim of the DRF studies was to evaluate the tolerability and the toxicity of roginolisib and to identify target organs after repeated daily dosing. In addition, the results were used to establish the doses for the 4-week pivotal studies. The aim of the latter studies was to define the starting dose for clinical development based on a no-observed-adverse-effect-level (NOAEL). The oral route was chosen because roginolisib is intended to be given orally in clinical studies, and the rats and dogs were used as species as their metabolic profiles match with that of human (data not reported).

Based on the non-clinical toxicity profile of idelalisib<sup>27,28</sup> and the expression of PI3K $\delta$  in human tissue,<sup>29</sup> the target organs for the expected toxicity were the hematopoietic and lymphoid systems, gastrointestinal tract and pancreas, lungs, heart, testes, and liver. The expected toxicities<sup>30</sup> include lymphoid depletion, reduced weight of spleen and thymus, thymic hemorrhage and necrosis, myeloid and granulopoietic hyperplasia (hematopoietic and lymphoid systems), inflammation, infiltration, hemorrhage and ulceration (gastrointestinal tract), increased liver enzymes, increased liver weight, inflammation, and hepatocellular necrosis (liver), myocardium infiltrate, fibrosis (heart; rat hearts were also expected to have increased weight), erythema, dryness, swelling, and redness (skin), and testicular seminiferous tubule degeneration and reduced testicular weight (male reproductive tissues).

Overall, the findings in the DRF studies and the 4-week pivotal studies were similar. Consistent with the expected toxicities, roginolisib, besides depletion of the lymphoid system, predominantly caused hepatotoxicities and gastrointestinal toxicities, especially at the mid and high doses in dogs



Figure 3. Bar graph shows inhibition of pAkt in whole blood rat B cells I hour after treatment with roginolisib.



**Figure 4.** Heat map shows the histopathological findings including the severity grades in the liver and hematopoietic and lymphoid tissues of rats in a 4-week oral toxicology study. No histopathological findings were present in the digestive system or skin of any of the rats in this study. Male findings are represented in the upper colored triangles and female findings in the lower colored triangles. Coloring (i.e., increasing intensity of red) is matched with the severity grade assigned to the finding.

and at the 100 mg/kg dose in female rats. These toxicities were also associated with poor clinical condition of the animals, decreases in body weight gain and food consumption, changes in liver enzymes, and mortality. The mortality observed in female rats at the highest dose level of 100 mg/kg may be explained by a disproportional increase in exposure due to compound accumulation. The gastrointestinal toxicities without a histopathological correlation were likely the result of gluconeogenesis as a result of either poor clinical condition, inhibition of the PI3Ka isoform or both. Contrary to idelalisib, however, no lung and heart toxicities were observed and testicular toxicities were only observed in dogs, not in rats. Importantly, in rats and dogs treated with roginolisib that were assessed at the end of the recovery period, histopathology and clinical pathology investigations were similar to control animals (rats) or animals treated with vehicle alone at Day 85 (dogs). These observations imply that the effects of roginolisib were reversible with no sequelae.

The degree of loss of lymphocytes in all lymphatic tissues observed in all studies, deemed to be related to inhibition of PI3K\delta, was mild at the low dose level and moderate-to-severe at the mid and high dose levels. Lymphoid depletion was most prominent in the B-cell-dependent regions with an almost complete depletion of lymphocytes in the B-cell-dependent marginal zone of the spleen at all dose levels in male dogs. Expectedly, B cells and some classes of T cells were also reduced in blood in response to PI3K8 inhibition in rats and dogs in the 4-week studies. Other hematology parameters were also affected, with a dose-dependent increase observed in platelet and neutrophil counts in dogs. These changes did not progress over time beyond the treatment period, suggesting that roginolisib does not induce thrombocytopenia or neutropenia, 2 common side effects observed in patients receiving treatment with other PI3K inhibitors (e.g., copanlisib).<sup>30,31</sup> Other significant hematological toxicities such as reduction of reticulocytes, monocytes, or eosinophils were also not observed. Contrary to the 4-week studies, the DRF study in dog did not reveal any changes in B and T cells, likely due to the shorter treatment duration in this study. Future animal toxicology studies may be designed to include a comprehensive immunophenotyping panel, including T regulatory cells, memory and exhausted CD8 T cells. Such immunophenotyping will help to inform clinical development on the need of using appropriate monitoring of immune-related adverse events.

The dermatological manifestations in dogs (e.g., hyperkeratosis, epidermal-hyperplasia, erythema, and maturityonset alopecia), appearing a month after roginolisib treatment at 5 mg/kg, were considered PI3K $\delta$  related. Dysregulation of PI3K $\delta$  may have affected the maintenance of keratinocyte proliferation and migration as the PI3K/AKT signaling plays a role in maintaining skin-derived precursors in vitro<sup>32</sup>; moreover, PI3K $\delta$  specifically has been shown to be expressed by hyperproliferating keratinocytes in psoriatic plaques and implicated in the psoriatic inflammatory infiltrate.<sup>33</sup>

Strong inhibition of the pharmacodynamic biomarker pAkt occurred in rats treated with 5 to 75 mg/kg l hour after dosing. There was a trend towards higher inhibition at higher dose levels and longer treatment duration. At Day 28, almost complete inhibition of pAkt was observed for all dose levels, indicating that the rat is a relevant species for the toxicological characterization of roginolisib. The therapeutically active dose level is  $\geq$  5 mg/kg, similar to the results observed in a rat collagen-induced arthritis (CIA) model (unpublished data). The efficacy data from the CIA model were subsequently confirmed in mouse models of cancer.<sup>19</sup>

Toxicokinetics analysis showed that at 5 mg/kg, roginolisib free plasma levels exceeded the cellular target engagement IC<sub>90</sub> for PI3K $\delta$  (10 ×IC<sub>50</sub> value of 10 ng/mL<sup>19</sup>) for  $\geq$  12 hours (Figure 6). In rats the dose of 5 mg/kg was well tolerated. In dogs, no NOAEL was established; however, the observed adverse effects at the 5 mg/kg dose level were considered monitorable and reversible. At the dose levels of  $\geq$  15 mg/kg free plasma levels exceeded the IC<sub>90</sub> for PI3K $\delta$  up to the complete dosing period and the IC<sub>50</sub> for PI3K $\beta$  for  $\geq$  12 hours (IC<sub>50</sub> value of 226 ng/mL<sup>19</sup>). Additionally, the free C<sub>max</sub>

	6			_			l.	-					-	4-	Inc		- 11	
Dose	101	mg/	m	m	51	mg/	m	m	m/1	m/	mg/	rg m/	m	45 m/	/30 m/	m	m/	m
Sex	4	2	2	1	1	2	1	5	11	20	1	1	20	1	20	1	20	25
Stomach	1	2	3	1	2	3	4	5	1-	25	1 4	27	5- 1	1-	2"	1,	2	2.
Hypersecretion, gastric, foveolar																		
Nos, bacteria, pylorus, foveola									1			1	1	J				
Nos, gross lesion, description only	$\vdash$	_	-	_	-		_	-	+	-			-	4	+			_
Nos, necrosis, roveolar epitnelium, rocal	$\vdash$		-	-	-	H	-	-	+	+	+	7	+	+	+	4		-
Intestine, small duodenum													+	+		1		_
Autolysis							-											
Hyperemia, mucosa				_				_					_		_			
Necrosis, mucosa, villi, diffuse	$\square$		_	_				-	-		4	4	+	+	-	_		_
Calcification serosa dystrophic focal		-	-	-	-		_	-	4	4		-	-	+	-			_
Hemorrhage										+	+	+	+	+	ť	7		-
Hyperemia																		
Necrosis, mucosa, villi, diffuse								_			4	4			_			
Intestine, small, ileum								4	4	4				4	_			4
Depletion, lymphatic follicle(s)	$\vdash$		-		-	H	4	4	+	-		-	+	4	-	4		-
Infiltrates, granulocytic				-	-			-	+	-	+	4	+	+	+	-		_
Necrosis, lymphocyte	H								+	+				+				_
Necrosis, mucosa, villi, diffuse																		
Intestine, large, cecum																4	1	
Depletion, lymphatic follicle(s)									1			1		4				
Necrosis, mucosa, crypt, diffuse				_					_			4	-	-	_			_
Intestine, large, colon	H					-			-	-			+		-	1		
Depietion, lymphatic follicle(s)	H		-	-	-			+	+	-		+	+	4	+			-
Infiltrates, ganulocytic, lymphatic follicle	H			-	-			-	+	-	-	+	+		+			-
Necrosis, mucosa, crypt, diffuse	H							+	+				1	1				-
Intestine, large, rectum																		
Depletion, lymphatic follicle(s)																		
Hyperemia, mucosa				_				_	4	_	4	4	+	4	-	_		_
Hyperplasia, epithelial, crypt, focal			_	_	-		_	-	+	+	4	+	+	+	+	_		_
Necrosis, lymphocyte	$\vdash$		-	-	-	$\vdash$	-	+	+	-			+	+	+			-
Liver	H								+	1		-	+	+	1			-
Activation, kupffer cell(s)									1					T				_
Congestion																		
Deposition, fatty, centrilobular																		
Inflammation, granulomatous, focal				_				_	4	4	-	4	_	4	_	_		_
Infiltrates, mixed, perivascular		4		_	4			-			-	-	-	-			4	
Nicrogranuloma	$\vdash$				-	Η		-	4	-			-	-	4			-
Necrosis, large, focal					$\vdash$				+	-	-			+	1	-		_
Necrosis.small, multifocal									1	+	+	ť	1	1				_
Stasis of fliuid, bile duct, focal																		
Skin				Z	/	7		4		Ζ.	4		/					
Alopecia, axillary, bilateral								_	_		-	_		_		_		
Cyst(s), keratin, focal	$\square$		_	_	-		_	_	+	+	4	4	+	4	4	_		_
Dysplasia, epidermis, paw, focal				_						+		+		┥	4	$\neg$		_
Hyperkeratosis, epidermis, local									-	-	4	+	4	+		-		-
Hyperkeratosis, scrotum, multifocal	H			-							+	+	+	┥				-
Hyperplasia, epidermis, focal		/																_
Inflammation, granulomatous, hair follicle, focal																		
Inflammation, ulcerative, scrotum, multifocal																		
Maturation defect, hair follicle, focal								_	_		_							
Parakeratosis, hind paw, pad, focal				_				_	_	$\rightarrow$	+	_	4	4	4	_		
Pustules, intraepidermal, focal			_	-				4	-	-	+	+	+	-	-	_		_
Peyer's patches			-	-					+			+		+				-
Lymph node, mandibular	H							-		-	+	1	+	d	-			
Depletion, lymphoid paracortex																		
Depletion																		
Lymph node, mesenteric											4		4					
Sinus histiocytosis														1	1			
Depletion, lymphoid paracortex	$\vdash$			_	-			_				4		$\downarrow$	4			_
Depletion Splean	$\vdash$		-	-	-	$\vdash$		-	-	1		-	4	+	-			4
Depletion marginal zone	H			-														-
Depletion	H			-					+			1		-	-			*
Thymus																		
Depletion																		
Cyst																		
Necrosis, lymphocyte									_	1	1		1		1			
Involution				_								4						_
Depletion, lymphoid cortex		_																

**Figure 5.** Heat map shows the nature and severity of histopathological findings in the digestive system, liver, skin, and hematopoietic and lymphoid tissues of dogs during a 12-week oral toxicology study. Male findings are represented in the upper colored triangles and female findings in the lower colored triangles. Coloring (i.e., increasing intensity of red) is matched with the severity grade assigned to the finding. <sup>a</sup>The treatment dose for this group was lowered on Day 15 from the original 45 mg/kg to 30 mg/kg; <sup>b</sup>These animals were "recovery" animals and were investigated after an 8-week, treatment-free recovery period; <sup>c</sup>This animal died/was prematurely euthanized.



**Figure 6.** Graphs show the free concentration of roginolisib (ng/mL) in plasma at each measured timepoint following daily administration, as calculated for rat A and dog B at escalating doses (5, 15, 50, and 75 mg/kg for rat and 5, 15, and 45/30 mg/kg for dog) based on plasma protein binding data (24% for rat and 56% for dog).  $IC_{50}$  thresholds for PI3K $\alpha$ , PI3K $\beta$ , and PI3K $\delta$  and  $IC_{90}$  threshold PI3K $\delta$ , based on intracellular target engagement measured in Jurkat cell lysate (Kinativ®), are depicted with dotted lines. For comparison the free concentration of roginolisib as measured in patients at the 80 mg dose is depicted in Figure 6B as well.

Table 7. Plass	na Protein Binding	, pAkt Whole	Blood Potency, and Pre	edicted Clearance of Rogi	nolisib in Mouse, Rat, Dog, and Human.
		2/ 1	<b>1</b> '		, , D,

Study	Mouse	Rat	Dog	Human
Plasma protein binding (percentage bound at 1 $\mu$ g/mL)	49%	24%	56%	82%
Whole blood pAkt IC <sub>50</sub> (ng/mL) <sup>a</sup>	244	270	—	815
Predicted CL (L/h/kg) <sup>b</sup>	1.57	.65	.34	<.15

<sup>a</sup>B-cell intracellular pAkt after stimulation with anti-IgD/M as measured by flow cytometry; <sup>b</sup>Based on stability in hepatocyte incubations.

approached the IC<sub>50</sub> for PI3K $\alpha$  (IC<sub>50</sub> value of 5.3 µg/mL<sup>19</sup>). The dose level of 15 mg/kg was well tolerated in rats and defined as the NOAEL. By contrast, in dogs this dose level was associated with high mortality and severe side effects. Dose levels  $\geq$  45 mg/kg resulted in free plasma levels exceeding the IC<sub>50</sub> for PI3K $\alpha$  (>3 fold in both rat and dog at

100 mg/kg) and were associated with increasing toxicity in both species. These results indicate that roginolisib's toxicities in rats and dogs are not driven exclusively by the level of PI3K $\delta$  inhibition. Aggravated toxicity is observed, especially in dogs, at dose levels projected to also inhibit PI3K $\beta$  and especially PI3K $\alpha$ . Data from these and other studies indicates

a complex association between isoform selectivity and the safety profiles of PI3K inhibitors. For example, a quantitative systems pharmacology model-based study concluded that co-inhibition of the PI3K $\alpha$  isoform as a consequence of prolonged PI3K $\delta$  inhibition exacerbates colitis in humans.<sup>34</sup> Similar to this observation in humans, unbound plasma levels of roginolisib at or in excess of the IC<sub>50</sub> for PI3K $\alpha$  resulted in gastrointestinal toxicities in dogs.

Toxicokinetic exposure observed at the 50 mg/kg dose in the 4-week oral rat study was comparable with the exposure delivering a pharmacological benefit in a rat CIA model at a dose of 30 mg/kg (unpublished data). The observed C<sub>min</sub> exposure levels of 372 ng/mL and 467 ng/ml in males and females, respectively, were above the IC<sub>50</sub> of 270 ng/mL for pAkt inhibition as determined in rat whole blood B cells (see Table 7). These results are consistent with the PK/PD model developed based on the data from murine lupus models predicting the full therapeutic efficacy that can be achieved by 50% target coverage over time.<sup>20</sup> Interestingly, roginolisib demonstrates a 3-fold higher plasma protein binding in human compared to rat (82% vs 24%) resulting in a concomitant 3-fold higher IC<sub>50</sub> in the whole blood pAkt assay of 815 ng/mL (see Table 7). These data suggests that roginolisib potency and possibly toxicity is driven by the nonprotein bound exposure of roginolisib rather than total exposure. Higher protein binding in humans likely also contributes to roginolisib's high metabolic stability in human hepatocytes and its concomitant low predicted clearance, resulting in a human PK profile with a low peak-to-trough ratio.<sup>35</sup> This is confirmed by preliminary Day 1 PK data from the ongoing clinical study wherein after a single dose of 80 mg an average free C<sub>max</sub> of 224 ng/mL and an average C24h of 121 ng/mL are observed (see Figure 6B). Such PK profile is highly beneficial for continuous selective inhibition of PI3K\delta over time hereby preventing any off-target, for example, PI3K $\alpha$ , mediated toxicities. Under these conditions, roginolisib appears to be well tolerated, and hence, future non-clinical toxicology studies will have to be designed to better simulate the PK profile observed in human and separate the toxicities related to selective and unselective PI3K $\delta$  inhibition.

## Conclusion

Roginolisib has a toxicology profile similar to other PI3K $\delta$ inhibitors: the compound is well-tolerated in rats, but is associated with skin and gastrointestinal toxicities in dogs. Though dose-dependent toxicities were observed, especially at doses resulting in concomitant inhibition of PI3K $\alpha$ and PI3K $\beta$ , these effects were mostly reversible. Our data indicate that doses that do not selectively inhibit PI3K $\delta$  and result in inhibition of additional PI3K isoforms are associated with adverse effects. Based on this concept, doses for human investigation were chosen not to exceed a daily oral dose of 200 mg to prevent any inhibition of PI3K $\alpha$  at  $C_{max}$  and the optimal dose range for selective PI3K $\delta$  inhibition was deemed to be in the range of 20 mg to 100 mg. In addition, roginolisib shows no apparent risk for metabolite generation (unlike idelalisib<sup>36</sup>) and a predictable PK profile in human. All these properties make it a candidate for future clinical investigation, and current clinical data<sup>35</sup> confirm this anticipated profile of a welltolerated PI3K $\delta$  inhibitor.

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#### **Author Contributions**

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#### **Declaration of conflicting interests**

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