1. SUMMARY (note: SRK-181 is referred to as "6993-hIgG4" in this report)

The purpose of this study was (1) to evaluate the tolerability of the test articles, LY2109761 (ALK5 kinase inhibitor) or Pan TGFβ-hIgG4 (an antibody, binding to all three isoforms of TGFβ), when administered to female rats via repeat-dose oral gavage (up to 7 days) or single-dose intravenous injection once respectively (in Phase I of the study) and (2) to evaluate the toxicity of the test articles ([redacted], 6993-hIgG4, and [redacted]) when administered to female rats via repeat-dose intravenous injection once weekly for up to 28 days for a total of 4 doses (in Phase II of the study).

Female Crl:CD(SD) rats were assigned to 16 groups, and doses were administered as indicated in the following table. Animals were dosed at a volume of 10 mL/kg. Groups 1 and 4 through 6 were administered vehicle control article or Pan TGFβ-hIgG4 by intravenous (bolus) injection via a tail vein once on Day 1 of Phase I; Groups 2 and 3 were administered LY2109761 via oral gavage once daily for 7 days on Days 1 through 7 of Phase I ([redacted]). Groups 7 through 16 were administered vehicle control article, [redacted], 6993-hIgG4, or [redacted] by intravenous (bolus) injection via a tail vein once weekly for 4 weeks on Days 1, 8, 15, and 22 of Phase II. The vehicle control articles were Phosphate-buffered saline (PBS), pH 7.4 for Pan TGFβ-hIgG4, [redacted], 6993-hIgG4, and [redacted] and 1% (w/v) carboxymethylcellulose, 0.25% (v/v) Polysorbate 80, and 0.05% (v/v) Dow Corning® Antifoam 1510-US in purified water for LY2109761.

Group[a]	No. of Animals Female	Dose Route	Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)
Phase I				
1 (Control)	5	Intravenous	0	0
2 (Low LY2109761)	5	Oral	200	20
3 (High LY2109761)	5	Oral	300	30
4 (Low Pan TGFβ-hIgG4)	5	Intravenous	3	0.3
5 (Mid Pan TGFβ-hIgG4)	5	Intravenous	10	1
6 (High Pan TGFβ-hIgG4)	5	Intravenous	30	3
Phase II				
7 (Control)	5	Intravenous	0	0
8 [redacted]	[redacted]	[redacted]	[redacted]	[redacted]
9 [redacted]	[redacted]	[redacted]	[redacted]	[redacted]
10 [redacted]	[redacted]	[redacted]	[redacted]	[redacted]
11 (Low 6993-hIgG4)	5	Intravenous	10	1.0
12 (Mid 6993-hIgG4)	5	Intravenous	30	3.0
13 (High 6993-hIgG4)	5	Intravenous	100	10.0
14 [redacted]	[redacted]	[redacted]	[redacted]	[redacted]
15 [redacted]	[redacted]	[redacted]	[redacted]	[redacted]
16 [redacted]	[redacted]	[redacted]	[redacted]	[redacted]

a Groups 1 and 7 were administered vehicle control article (Phosphate-buffered saline [PBS], pH 7.4) only.

Assessment of toxicity was based on mortality, clinical observations, body weights, body weight changes, food consumption, and clinical and anatomic pathology. Blood samples were collected for toxicokinetic and anti-drug antibody evaluations.

Phase I

Test article-related mortality occurred on or after Day 3 for all animals administered 200 or 300 mg/kg/dose LY2109761 (Groups 2 and 3 respectively), one animal administered 10 mg/kg/dose Pan TGFβ-hIgG4 (Group 5), and two animals administered 30 mg/kg/dose Pan TGFβ-hIgG4 (Group 6). For some Groups 2 and 3 animals, the cause of the early mortality was attributed to LY2109761-related slight to moderate myocardial degeneration/necrosis which likely resulted in heart dysfunction. One animal administered 200 mg/kg/dose LY2109761 had an additional finding of yellow thoracic cavity fluid, which was likely related to myocardial degeneration/necrosis and may have contributed to the early mortality. Generally, changes in the clinical condition of animals administered 200 or 300 mg/kg/dose LY2109761 included changes in appearance (low body carriage, hunched posture, and/or swollen ventral thorax), behavior (hypoactivity), respiration (irregular or labored), feces, and skin and pelage (pale ears, piloerection, and discolored haircoat). Body weight losses with or without any significant reduction in the mean body weights were noted for animals administered 200 or 300 mg/kg/dose LY2109761. The body weight losses correlated with decreased (-52.9% to -64.7%) food consumption. The cause of the early mortality for Groups 5 and 6 animals was undetermined; however, microscopic heart findings were likely contributory. Changes in the clinical condition of animals administered 10 or 30 mg/kg/dose Pan TGFβ-hIgG4 included changes in appearance (thin body condition), respiration (irregular), and skin and pelage (pale ears and piloerection). A notable decrease (-25%) in food consumption from Days 4 to 7 of Phase I was noted for animals administered 30 mg/kg/dose Pan TGFβ-hIgG4. No test article-related clinical observations or effects on body weights or food consumption were noted for animals administered 3 mg/kg/dose Pan TGFβ-hIgG4.

During Phase I, test article-related clinical pathology findings observed in one or more of animals sacrificed in a moribund condition between Days 4 and 7 included mildly lower cholesterol and phosphorus concentrations, mildly to moderately lower total protein and albumin concentrations, mildly lower calcium concentration (a fraction of calcium is bound to albumin), and mildly lower alkaline phosphatase activity. These findings were likely secondary to decreased food consumption. Additional clinical pathology findings, likely secondary to a stress response, included minimally to mildly higher neutrophil count; mildly higher glucose concentration; and mildly lower lymphocyte and eosinophil counts, globulin concentration, and red cell mass (red blood cell count, hemoglobin concentration, and/or hematocrit). Few minor Pan TGFβ-hIgG4-related hematology findings were observed on Day 8 in animals administered 30 mg/kg/dose Pan TGFβ-hIgG4, including mildly lower red cell mass (red blood cell count, hemoglobin concentration, and/or hematocrit), with evidence of regeneration (mildly higher red cell distribution width and reticulocyte count) that lacked microscopic correlates.

LY2109761-related microscopic heart findings consistent with TGFβ inhibition occurred in all animals administered 200 or 300 mg/kg/dose LY2109761 and consisted of minimal to slight valvulopathy, slight to moderate myocardial degeneration/necrosis, moderate coronary artery necrosis (with inflammation), and minimal mixed inflammatory cell infiltrates in the atrium. A microscopic finding of slight interstitial edema in the thymus of two animals administered 200 mg/kg/dose LY2109761 correlated with the macroscopic observation of gelatinous thymus and may have been secondary to the heart findings.

Pan TGFβ-hIgG4-related heart findings occurred in all animals administered 3 mg/kg/dose Pan TGFβ-hIgG4 and all animals administered 10 or 30 mg/kg/dose Pan TGFβ-hIgG4 and were similar to those described for LY2109761, except for the lack of myocardial degeneration/necrosis or coronary artery necrosis. The most common finding was minimal to slight valvulopathy, which occurred in all animals administered 3, 10, or 30 mg/kg/dose Pan TGFβ-hIgG4 and was the only Pan TGFβ-hIgG4-related heart finding in animals administered 3 mg/kg/dose Pan TGFβ-hIgG4. In addition to atrium findings, myocardial findings in animals administered 10 or 30 mg/kg/dose Pan TGFβ-hIgG4 consisted of slight mixed cell infiltrates and/or minimal to slight hemorrhage at the heart base, but without degeneration/necrosis. Microscopic heart findings correlated with increased heart weights for animals administered 10 or 30 mg/kg/dose Pan TGFβ-hIgG4. One animal administered 10 mg/kg/dose Pan TGFβ-hIgG4 and three animals administered 30 mg/kg/dose Pan TGFβ-hIgG4 had minimal or moderate diffuse infiltrate of alveolar macrophages in the lung, which was suggestive of a reaction to pulmonary edema and/or congestion secondary to heart dysfunction; one animal administered 10 mg/kg/dose Pan TGFβ-hIgG4 and one animal administered 30 mg/kg/dose Pan TGFβ-hIgG4 had concurrent lung congestion/hemorrhage.

Other LY2109761- or Pan TGFβ-hIgG4-related microscopic findings consistent with TGFβ inhibition occurred in the bones and included minimally to slightly increased thickness of the hypertrophic zone in the endplates of the sternum and minimally increased thickness of the hypertrophic zone in the physis of the femur and/or tibia in animals administered 200 or 300 mg/kg/dose LY2109761 or 10 or 30 mg/kg/dose Pan TGFβ-hIgG4. One animal administered 200 mg/kg/dose LY2109761 had a correlative macroscopic observation of abnormal shape of the caudal sternum.

Additional LY2109761-related microscopic findings occurred in the liver, kidney, and mammary gland of animals administered 200 or 300 mg/kg/dose LY2109761. Liver findings consisted of minimal to slight periportal hepatocyte vacuolation, kidney findings consisted of slight to moderate vacuolation of cortical tubule cells, and mammary gland findings consisted of dilatation of the acini.

Other findings were considered secondary to stress and/or decreased food consumption. Animals administered 200 or 300 mg/kg/dose LY2109761 had minimal to slight adrenal cortical hypertrophy and/or slight individual cell necrosis, decreased lymphocytes in the spleen marginal zone (slight to marked) or thymus (minimal to slight), moderate to marked lymphocyte necrosis in the thymus, and slightly to moderately decreased zymogen granules in the pancreas. Correlative macroscopic findings included bilateral large and/or bilateral discolored tan adrenal glands and/or small spleen in animals administered 200 or 300 mg/kg/dose LY2109761. Animals administered 3, 10 or 30 mg/kg/dose Pan TGFβ-hIgG4 had minimal adrenal cortical hypertrophy, decrease lymphocytes in the spleen marginal zone (slight to moderate) or thymus (minimal), minimal lymphocyte necrosis in the thymus, and slightly decreased zymogen granules in the pancreas.

Phase II

One animal administered 30 mg/kg/dose [redacted] was found dead immediately postdose on Day 8 of Phase II. This death was related to dosing procedure and not test article related. No other mortality occurred during the duration of Phase II.

Phase II non-adverse, test article-related decreases in body weight were observed for animals administered ≥10 mg/kg/dose 6993-hIgG4 or ≥10 mg/kg/dose [redacted].

During Phase II, test article-related clinical chemistry findings on Day 29 were limited to mildly higher total protein and globulin concentrations in animals administered 100 mg/kg/dose [redacted] or ≥30 mg/kg/dose 6993–hIgG4. These findings were suggestive of an inflammatory response to test article or the presence of the test article (an IgG4 immunoglobulin) and lacked microscopic correlates.

No test article-related mortality or effects on food consumption, organ weights, macroscopic or microscopic findings were noted.

In conclusion, administration of 200 or 300 mg/kg/dose LY2109761 to female rats via oral gavage daily for up to 5 days was not tolerated and resulted in mortality and adverse clinical observations, reductions in body weights and food consumption. Microscopic changes were noted in heart (valvulopathy, myocardial degeneration/necrosis, coronary artery necrosis and inflammation, and mixed inflammatory cell infiltrates in the atrium), bones (increased thickness of the hypertrophic zone in the physis), liver (periportal hepatocyte vacuolation), kidney (vacuolation of cortical tubule cells), mammary gland (dilatation of the acini), adrenal (cortical hypertrophy and/or slight individual cell necrosis), spleen (decreased lymphocytes), thymus (lymphocyte necrosis), and pancreas (decreased zymogen granules). Clinical pathology changes secondary to decreased food consumption or stress were also observed in these animals. Based on these results, 200 or 300 mg/kg/dose of LY2109761 was not tolerated and considered adverse.

Administration of vehicle control article or 3, 10, or 30 mg/kg/dose Pan TGFβ-hIgG4 via a single intravenous (bolus) injection resulted in mortality in animals administered 10 or 30 mg/kg/dose and adverse clinical observations. Reductions in food consumption was observed for animals administered 30 mg/kg/dose. Microscopic changes were noted in heart (valvulopathy) of animals administered ≥3 mg/kg/dose and in the heart (mixed inflammatory cell infiltrates and/or hemorrhage), lung (diffuse infiltrate of alveolar macrophages and congestion/hemorrhage), and bones (increased thickness of the hypertrophic zone in the endplates) of animals administered ≥10 mg/kg/dose. Clinical pathology changes for animals administered 30 mg/kg/dose Pan TGFβ-hIgG4 included mildly lower red cell mass and creatine kinase (CK) activity and; minimally to mildly lower phosphorus, potassium, and cholesterol concentrations. Due to mortality, dose levels of 10 or 30 mg/kg/dose were above the maximum tolerated dose. Although all animals administered 3 mg/kg/dose survived to scheduled sacrifice, due to the nature of microscopic findings (valvulopathy in the heart), this dose level was also considered adverse.

During Phase II, female rats were administered vehicle control article or 10, 30, or 100 mg/kg/dose [redacted], 6993-hIgG4, or [redacted] via repeat-dose intravenous injection once weekly for up to 28 days for a total of four doses. Non-adverse minimal to slight decreases in body weight were observed for animals administered ≥10 mg/kg/dose 6993-hIgG4 or ≥10 mg/kg/dose [redacted] with a lack of associated clinical signs and microscopic findings. No test article-related alterations in food consumption were observed. Clinical pathology changes in animals administered 100 mg/kg/dose [redacted] or ≥30 mg/kg/dose 6993-hIgG4 included mildly higher total protein and globulin concentrations. Due to the minimal severity of findings and the lack of any impact on the health and well-being of animals administered 10, 30, or 100 mg/kg/dose of [redacted], 6993-hIgG4 or [redacted], these dose levels were considered non-adverse and well-tolerated. Thus, the no observed adverse effect level (NOAEL) for [redacted], 6993-hIgG4 and [redacted] is 100 mg/kg/dose.