RVX-297, a BET bromodomain inhibitor, has therapeutic effects in preclinical models of acute inflammation and autoimmune disease

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SUPPLEMENTAL:

Plasma exposure

For all animal studies, RVX-297 in plasma was quantified by a validated LC/MS/MS method at Alta Analytical Laboratory (El Dorado Hills, CA) or Climax Labs Inc. (San Jose, CA) to assess maximum plasma exposure post administration.

Multi-analyte profiling in LPS endotoxemic mice

Endotoxemia studies were conducted as described in Methods and Materials. At the study's termination, multi-analyte profiling was performed (RBM, Inc., Austin, TX) to

determine changes in inflammatory factors in the serum of RVX-297 treated animals versus vehicle treated controls.

Histopathology in arthritis models

For all arthritic models, histopathology assessment was performed by Bolder BioPATH Inc. (Boulder, CO). This included microscopic scoring for polyarticular inflammation, cartilage destruction in association with pannus formation and bone resorption. After 1-2 days in formalin and then 4-5 days in a decalcifier, the knee or ankle joint was cut in half longitudinally and in the frontal plane, processed, embedded, sectioned and stained with toluidine blue or H&E. Histopathology scores were generated from 0 to 5 to characterize pharmacodynamic effects as outlined by Bolder BioPATH (www.bolderbiopath.com). Scores were assigned by an experienced observer blinded to the treatment group.

Pharmacokinetic / pharmacodynamic relationship in rat collagen induced arthritis (rCIA)

Pharmacodynamic efficacy based on ankle diameter as well as ankle and knee histopathology were determined in CIA rats at the end of the study, while pharmacokinetics of RVX-297 was determined the day prior. Pharmacokinetic analysis of RVX-297 in plasma was conducted in samples collected at 0, 1, 2, 7 and 14 hours post dose on day 7 of the arthritis phase using six rats per treatment group. All rats had samples drawn pre-AM dose (i.e. 12 hours post dose), and three rats used for 1 and 7 hours post dose, while the other three for 2 and 14 hours post dose. Blood (0.4 mL) was

taken retro-orbitally for determination of RVX-297 in plasma. Maximum plasma exposure was reached between 2 and 7 hours after administration for all doses. PD/PK results were calculated as percent reduction compared to vehicle treated disease controls, and the pharmacokinetic / pharmacodynamic relationship was determined as efficacy versus plasma drug concentration AUC_{0-12}.

Mouse collagen-induced arthritis (mCIA)

mCIA studies were performed at Bolder BioPATH Inc. (Boulder, CO). Bovine type II collagen (Elastin Products, Owensville, MO) was emulsified with Freund's complete adjuvant (Difco, Detroit, MI) supplemented with *M. Tuberculosis*. Male DBA/1OlaHsd mice (Harlan Labs) weighing approximately 20-25 grams were immunized by intradermal injection to elicit an immune response, and a booster was provided 21 days later. Measurable indices of arthritis were present such as ankle joint swelling in the hind paw. Drug treatment was initiated and administered twice per day (b.i.d.) at 100 and 200 mg/kg for RVX-297 or 0.2 mg/kg for dexamethasone for 15 days. Due to body weight loss at 200 mg/kg RVX-297, dosing was reduced from 200 mg/kg to 150 mg/kg and from 100 mg/kg to 75 mg/kg starting on day 6. Dexamethasone treated mice also lost weight, but the treatment protocol was not changed. Efficacy evaluation included body weight and clinical arthritis scores. At the completion of the study, histopathology assessment and scoring was performed on paw joints by an experienced observer blinded to the treatment group. Autoantibody titres against type II collagen in serum were measured by ELISA.

Supplemental Table 1: Circulating Inflammatory Mediators in LPS Stimulated Mice

% Reduction in mouse serum compared to control			
Analyte	**RVX-297**	**Analyte**	**RVX-297**
IL-6	91 ± 3	TNFα	48 ± 5
GM-CSF	88 ± 2	IL-11	44 ± 2
MCP-1	82 ± 3	IL-7	40 ± 6
KC/GRO	79 ± 11	MIP-3β	40 ± 12
MCP-5	79 ± 1	IL-18	39 ± 13
MCP-3	77 ± 4	OSM	39 ± 3
MIP-1β	68 ± 4	RANTES	37 ± 5
MIP-2	67 ± 8	LIF	36 ± 5
IP-10	66 ± 8	SCF	33 ± 8
IL-2	65 ± 0	VEGF	33 ± 5
FGF-9	61 ± 13	Factor VII	31 ± 10
Lymphotactin	59 ± 7	IL-1a	31 ± 10
IL-1β	52 ± 0	MIP-1γ	29 ± 6
IL-17	52 ± 20	MDC	28 ± 8
IFNγ	51 ± 7	SAP	24 ± 8
MIP-1α	50 ± 3	Eotaxin	23 ± 8

LPS stimulated C57Bl/6 mice were treated orally with RVX-297 at 75 mg/kg b.i.d. Levels of inflammatory mediators in serum were determined by multi-analyte profiling. Data were generated from 4 mice per group, and presented as mean percent reduction in RVX-297 treated animals versus vehicle treated controls. Variability is shown as standard

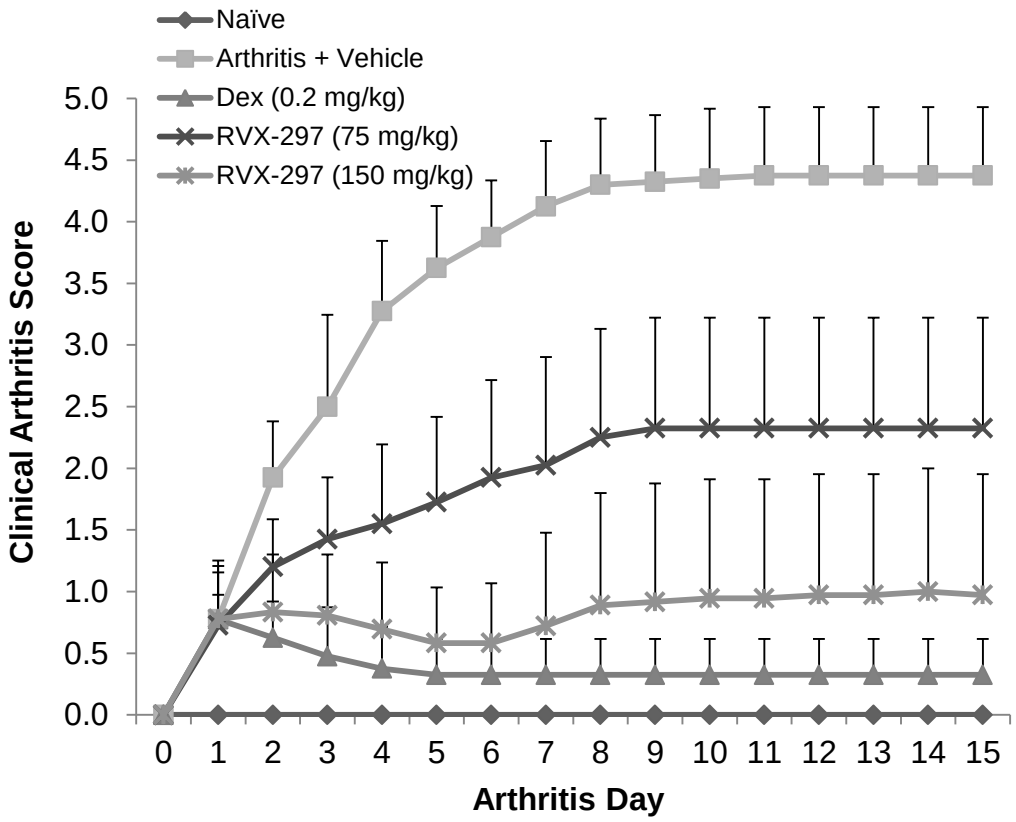
deviation. All data from RVX-297 treated animals vary significantly from vehicle treated controls (Student's t-test, $p<0.05$).

Supplemental Figure 1: Mouse Collagen Induced Arthritis (mCIA)

A: Clinical arthritis scores (scored 0-5 outlined at www.bolderbiopath.com) are presented as the mean ± standard deviation (10 mice per group except naïve n=4). Differences between vehicle treated controls and RVX-297 or dexamethasone treated mice were statistically significant starting at day 2 using a one-way ANOVA followed by Tukey's Multiple Comparison Test ($p<0.05$).

B: Anti-collagen II IgG levels in serum of vehicle or RVX-297 treated CIA mice ± standard deviation. Statistical significance was determined with a one-way ANOVA followed by Tukey's Multiple Comparison Test (**, $p<0.01$; NS=not significant).

Supplemental Figure 1A:


Naïve
Arthritis + Vehicle
Dex (0.2 mg/kg)
RVX-297 (75 mg/kg)
RVX-297 (150 mg/kg)
Clinical Arthritis Score
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Arthritis Day

Supplemental Figure 1B