

Study Title: Effect of novel compounds on IBD induced by chronic DSS treatment

Study Objectives

The goal of the study was to assess the efficacy of novel test compounds on IBD induced by chronic DSS treatment.

INTRODUCTION

Inflammatory bowel disease is a condition that results from a combination of environmental stimuli (the presence of bacterial flora), genetic factors, and a predisposition toward elevated immune response that cannot be replicated in one-dimensional systems such as cell or tissue culture. Because of the genetic complexity of this disease, it is not amenable to computer modeling and requires an *in vivo* system. Mice represent an excellent *in vivo* system as they can be tested in high numbers (increasing the statistical power of any study).

METHODS AND RECORDS

After one week of acclimatization, mice were divided into groups according to body weights. Mice underwent the following procedures:

Beginning on Day 1:

- DSS treatment (Groups 2-7) from Days 1-5. All mice received (2%) DSS in acidified water *ad libitum* for 5 days. Next day, all mice were returned to non-DSS containing water up to Day 12.
- On Day 12, 4 mice from Group 2 were sacrificed to harvest colons and blood samples. Colons were harvested and frozen as specified by the sponsor.

Beginning on Day 13:

- A second DSS cycle was started from Days 13-17.
- Start of dosing in Groups 3-7 with Glatiramer (SC), and test compounds (PO).

Beginning on Day 25:

- A third DSS cycle was started from Days 25-29.

Body weights were recorded daily during DSS cycles and dosing. Water intake was measured daily in all the groups during DSS treatments. Clinical observations including Disease Activity Index (DAI) scoring were conducted (3x a week) during dosing. Cage side observations were conducted daily.

At study termination (4 days after last DSS cycle), mice were sacrificed.

Terminal blood was drawn, processed, and shipped to sponsor. Colons were harvested, flushed, weighed, and lengths were recorded. Each colon was divided into equal three parts; proximal, medial and distal. Each part was placed in a zigzag fashion in a cryomold before freezing. Respective ends were labeled (rectal and small intestine). Samples were shipped to a third party for histology assessment.

Blood Processing Details:

Terminal Blood Collected on: Blood was processed for plasma by collection into an EDTA tube and spun for 10 minutes at 12,500 RPMs in a 4°C centrifuge, and then transferred to 0.5 mL microcentrifuge tubes for storage at -80°C until sent for analysis.

1.1 Treatment Protocol

Gp	n	Treatment	Dose (mg/kg)	Dose Route / Regimen
1	4	Naive	n/a	n/a
2	12 (8+4)	Vehicle	PBS 1X	SC, Days 13-33
3	8	Glatiramer Acetate	100, daily	SC, Days 13-33

Procedural Timeline

	Receive mice	DSS water	Regular water	Dose Groups 3-7 DSS water	Regular water	DSS water	Regular water	Necropsy - Colons Harvested
Date	9/4							
Day	-7	1-5	6-12	13-17	18-24	25-29	30-33	
	Acclimation	Body Weights and DAI Scores						Terminal Blood

Key:
 DAI Disease Activity Index
 DSS Docusate sodium