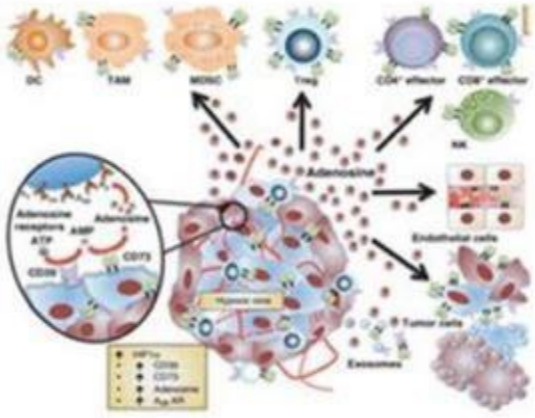
PNQ-201
A Novel Adenosine A_{2B} Receptor Antagonist

PNQ-201 is a novel, targeted therapeutic agent for Colorectal Cancer with potential for therapeutic window superior to current and emerging therapies

Adenosine A_{2B} Receptors: An Attractive Target for Colorectal Carcinoma

A_{2B} receptor antagonism that impacts the Adenosine – hypoxia axis to disrupt signaling leading to increased differentiation and proliferation of tumor cells while down-regulating several anti-inflammatory molecules and immunoregulatory cells



- **Adenosine A_{2B} receptors are consistently up-regulated in colorectal carcinoma tissues and cell lines and promote proliferation of primary tumor cells.**
  - 67% Adenocarcinomas; 17% tubular carcinomas are immuno-positive for A_{2B}
- A_{2B} receptors are ubiquitously expressed on multiple immune cells including T cells, B cells, NK cells , MDSCs and APCs exerts is involved in adenosine mediated effects in tumor microenvironment.
- A_{2B} receptor blockade
  - **directly reduces tumor cell growth**
  - enhances apoptosis,
  - reduces endothelium-derived angiogenesis,
  - Reduces neovascularization and metastasis

PNQ-201: A_{2B} Adenosine Receptor Antagonist
Stage: Ready for IND Filing

- An potent and selective adenosine A_{2B} receptor antagonist - attractive target for Oncology with first in class opportunity
- **Gut-restricted distribution of PNQ-201 offers unique opportunity for "Colorectal cancer"** to attain superior therapeutic window
  - Gut A_{2B} antagonism demonstrated in IBD model
- Patent granted
- IND directed studies completed

PNQ-201: Affinity, Potency & Receptor Subtype Selectivity

Affinity

Receptor Human	Ki (nM)	Selectivity Ratio
A_{2B}	204	1
A_1	1200	50
A_{2A}	~30000	147
A_3	>100,000	490

Right balance of potency and selectivity considering expected high levels of PNQ-201 at the site of action

Functional Potency

Receptor Human	Ki (nM)	Selectivity Ratio
A_{2B}	344	1
A_1	>10,000	>294
A_{2A}	>10,000	>294
A_3	>10,000	>294

Inhibited IL-6 production in **normal human lung fibroblasts** with IC_{50} 1µM

IL-6 is a target & disease relevant biomarker

- PNQ-201 is a gut restricted selective A_{2B} receptor antagonist

The PNQ-201: Key Differentiator

Deliberately selected as a lead candidate due to its low systemic exposure and high colonic/cecal levels for **maximal local benefit while minimizing potential for side effects, if any, to provide a wide therapeutic index**

Rat & Mice

- Bioavailability <1%
- Fecal Recovery ~ 60 % of administered dose

Dog

- Bioavailability ~ 2%
- Fecal Recovery ~ 100 % of administered dose

Pharmacokinetic parameter	PNQ-201		
	Mouse	Rat	Dog
F (%)_PO	< 1 (10 mg/kg)	< 5 (30 mg/kg)	2 (10 mg/kg)
CL_{plasma} (mL/min/kg)_IV	146 (1 mg/kg)	80 (3 mg/kg)	38 (3 mg/kg)
V_{ss} (L/kg)_IV	2.6 (1 mg/kg)	1.2 (3 mg/kg)	0.5 (3 mg/kg)
$t_{1/2}$ (h)_IV	0.3 (1 mg/kg)	0.4 (3 mg/kg)	0.3 (3 mg/kg)
T_{max} (h)_PO	0.5 (10 mg/kg)	0.5 (30 mg/kg)	0.7 (10 mg/kg)
C_{max} (uM)_PO	0.02 (10 mg/kg)	0.9 (30 mg/kg)	0.1 (10 mg/kg)
AUC (uM.h)_PO	0.01 (10 mg/kg)	3.5 (30 mg/kg)	0.2 (10 mg/kg)
% Urinary recovery	2 (IV) < 1 (PO)	<0.5 (PO)	Not Done
% Fecal recovery	65 (IV) 54 (PO)	60 (PO)	100 (IV)

Gut restricted antagonism offers wide therapeutic index

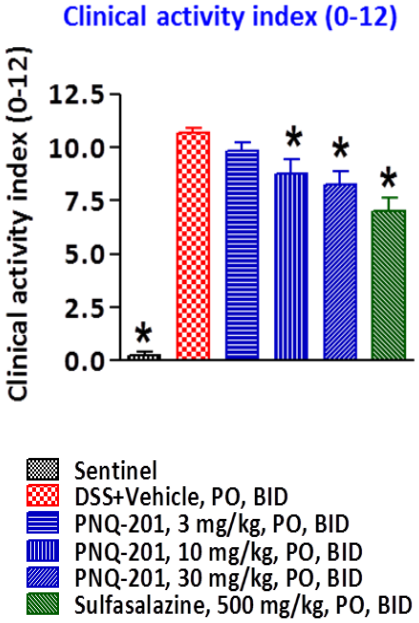
PNQ-201 Exhibits Robust Pharmacology in the Gut Wall

Model	Doses	Outcomes
DSS-Induced Colitis in B6 Mice	Single Dose 30 mg/kg, BID Prophylactic; 3,10 & 30 mg/kg, BID; Therapeutic 10,30 &100 mg/kg	▪ Significant improvement in clinical activity index with improvement in histo-pathological score when treated in prophylactic as well as therapeutic mode ▪ Prophylactic treatment -decrease in IL-6 & MPO
TNBS-Induced Colitis in SD Rats	Prophylactic -10 ,30 and 100 mg/kg, BID	▪ Significant improvement in disease activity index (macroscopic evaluation) in TNBS-induced colitis rat model when treated in prophylactic mode ▪ These changes were accompanied by decrease in IL-6

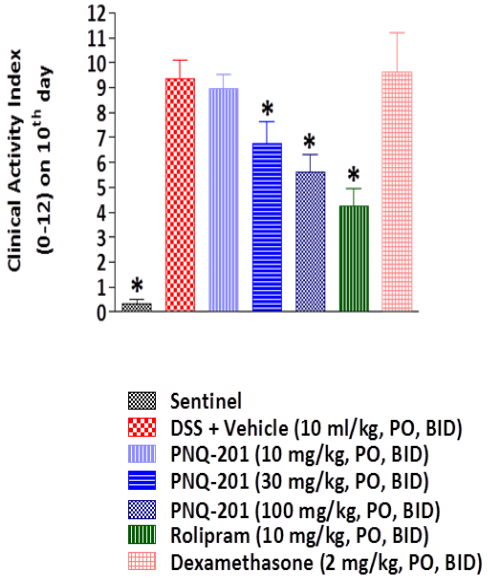
These models were selected to demonstrate gut A_{2B} antagonism in absence of suitable preclinical model for such compound

PNQ-201: Gut A_{2B} Antagonism in DSS Induced Colitis

Prophylactic Treatment

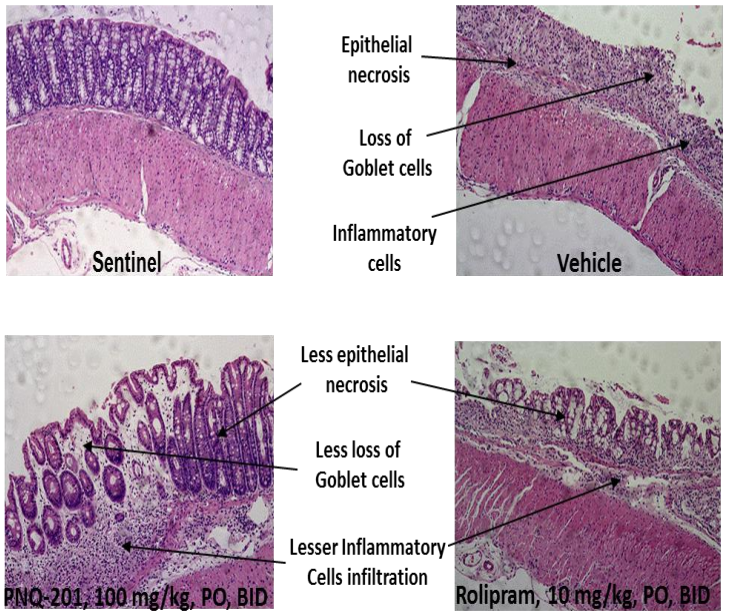


Therapeutic Treatment



Therapeutic Treatment

Histopathology Images



- Gut A_{2B} antagonism resulted in improvement in clinical activity index
- Histo-pathological evidence for beneficial effect

PNQ-201 has the potential for an excellent Therapeutic Window

Efficacy

- Adenosine A_{2B} receptors are consistently up-regulated in colorectal carcinoma tissues and cell lines compared to normal colorectal mucosa
  - 67% Adenocarcinomas ; 17% tubular carcinomas are immuno-positive for A_{2B}
- These Adenosine A_{2B} receptors are known to promote cancer cell growth in addition to it's other effects on tumor cell migration, apoptosis, angiogenesis and neovascularization.
- Effect on immune cells in tumor microenvironment

Safety

- Gut restricted distribution

These might offer wide unprecedented therapeutic window

PNQ-201: Summary

- IND enabling studies, including safety pharmacology studies, have been completed : Clean preclinical safety profile
  - Regulatory toxicology studies up to 1000 mg/kg *po* (rats) and 750 mg/kg *po* (dogs)
  - Subcutaneous administration to give exposure > 50 X that at 1000 mg/kg *po* was attempted to identify target organ – No toxicity observed
- Long patent life till 2031
  - US, EP and Japanese patents granted
- CMC
  - A robust, reproducible and scalable process to manufacture drug substance has been developed
  - Drug substance is stable for 6 months under storage conditions
- Manufacturing of human dosage form and FIH studies planned

Thank You