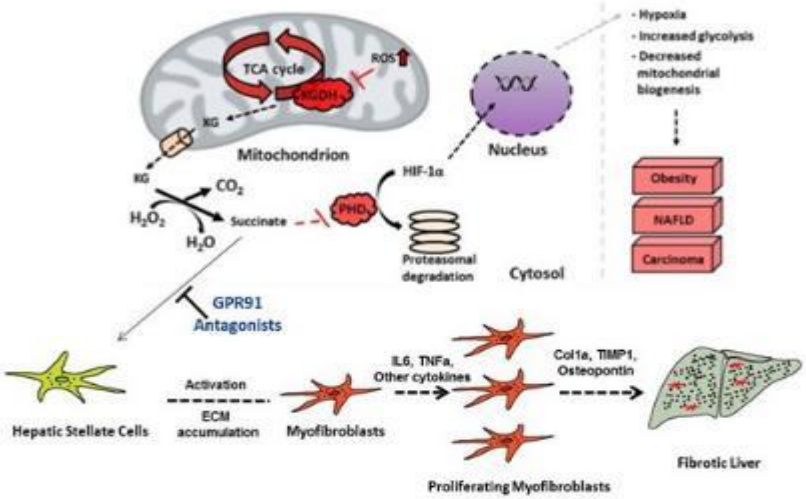
GPR91 Antagonists

Novel target and program for NASH/ NAFLD

GPR 91: A promising target for NASH/ NAFLD

GPR91 is the peripheral oxygen sensor and plays a critical role in multiple pathological conditions involving oxygen deficit



GPR91 is known to be involved in activation of hepatic stellate cells (HSCs) in ischemic conditions
Role of activated HSCs in fibrosis is well established

- Non-Alcoholic Steatohepatitis (NASH), is commonly associated with metabolic syndrome and T2D
- Impaired metabolism and **dysregulation of TCA** intermediates (i.e. isocitrate, succinate, etc.) **and increased oxidative stress** driven by mitochondrial ROS production could drive manifestations of NASH
- NASH can advance to Liver Fibrosis due to **activation of hepatic stellate cells (HSCs)** or trans-differentiation into myofibroblast-like cells, acquiring contractile, proinflammatory, and fibrogenic properties.
- **Activated HSCs** migrate and accumulate at the sites of tissue repair, secreting large amounts of ECM and regulating ECM degradation.
- Hypothesis: **Succinate is paracrine signal by which ischemic hepatocytes trigger stellate cell activation via GPR91**

Potential indications: Diseases based on Retinal Angiogenesis

- Angiogenesis is the physiological process involving the growth of new blood vessels from pre-existing vessels
- Ocular revascularization is a pathological hallmark of some forms of debilitating blindness including diabetic retinopathy, age related macular degeneration and retinopathy of prematurity
- Various evidences suggest that oxygen regulates blood vessel growth in the retina
  - ✓ Pathologic retinal angiogenesis occurs in several diseases that are characterized by retinal ischemia.
  - ✓ The timing and central-to-peripheral direction of retinal vascularization coincide with developmental processes that presumably determine local oxygen tension
  - ✓ The expression of several angiogenic and angio-inhibitory factors is oxygen dependent

AMD

Retinal revascularization

Diabetic Nephropathy

GPR91 has been shown to play an important role in multiple pathways that are implicated in revascularization and angiogenesis

Potential indications:
Diseases based on Hepatic Inflammation and Oxidative Stress

- Non-Alcoholic Steato-Hepatitis (NASH) is characterized by increased oxidative stress which is drive by mitochondrial oxidant production
- Insulin resistance and metabolic syndrome are inherently related to dysregulation of glucose metabolism and the TCA cycle.
- Impaired metabolism and dysregulation of TCA intermediates (i.e. isocitrate, succinate, etc.) may contribute to the manifestation of NASH commonly associated with metabolic syndrome

NASH/ Fibrosis

- NASH can advance to Liver Fibrosis due to activation of HSCs activate or trans-differentiation into myofibroblast-like cells, acquiring contractile, proinflammatory, and fibrogenic properties
- Activated HSCs migrate and accumulate at the sites of tissue repair, secreting large amounts of ECM and regulating ECM degradation
- It is hypothesized that succinate may behave as a paracrine signal by which ischemic hepatocytes trigger stellate cell activation and antagonism of GPR91 receptor may help in blocking succinate induced activation of stellate cells producing anti-hepatic fibrosis effect

Liver Fibrosis

MCD diet induced NASH Model (C57BL/6J mouse)

Prophylactic treatment mode

MCD diet :A02082002B
Control Diet: A02082003B, Research Diet® USA

Study protocol

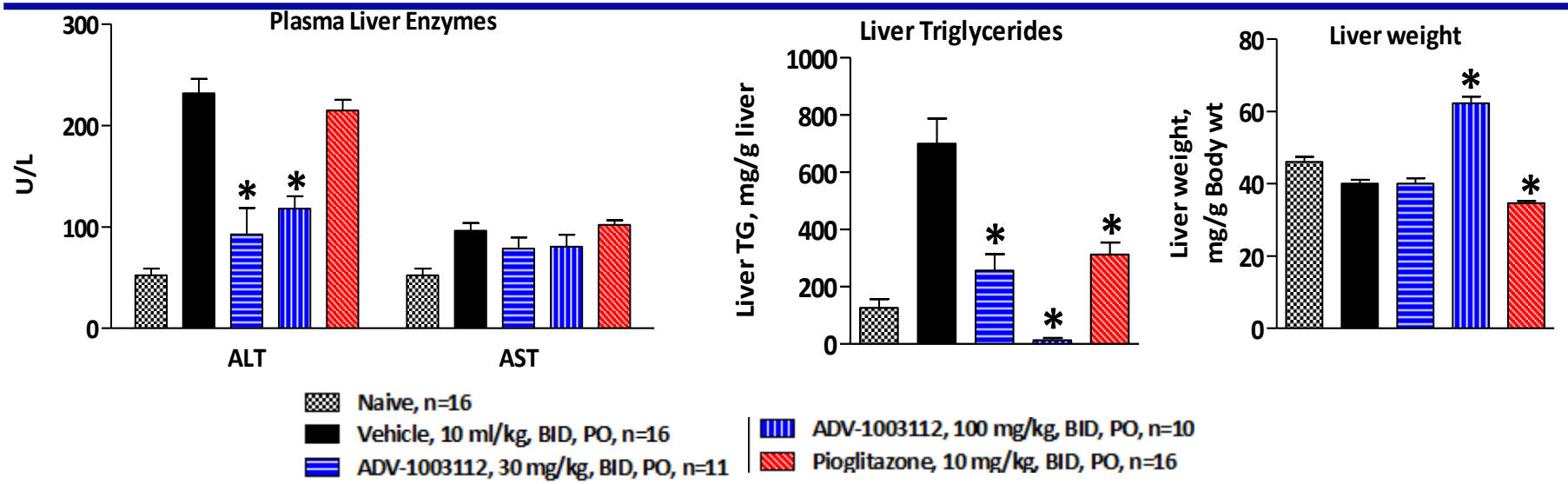
Animals: Male C57BL/6J mice
Age: 8-10 weeks
Diet :MCD diet, Research Diet®, Control Diet Research Diet®

Termination (n=12)
Serum chemistry and mRNA biomarkers

* Part of liver was used for mRNA estimation and remaining liver for histopathology

Treatment Groups (n=16/Group)
Naïve (Control Diet)
Vehicle (MC/tween 80), PO, BID
ADV-1003112, 30 mg/kg, BID
ADV-1003112, 100 mg/kg, BID
Pioglitazone, 10 mg/kg, PO, BID

Preclinical Data Highlights : NASH/ Fibrosis



Gene	Expression decrease in % Compared to Vehicle		
	ADV-1003112, 30 mpk	ADV-1003112 100 mpk	Pioglitazone
Collagen 1a	>90% (**)	90% (**)	>80%(*)
TIMP-1	75%	>85% (*)	80% (*)
Osteopontin	50% (n.s.)	>60% (*)	>70% (*)
TNF-α	60% (n.s.)	75% (**)	70% (*)
TGF-β	25% (n.s.)	30% (n.s.)	30% (n.s.)
MCP-1	40% (n.s.)	>80% (*)	60% (n.s.)

Treatment with GPR-91 antagonists caused significant lowering in Liver Function as well as in genetic markers of liver health

GPR91 Antagonists: Summary

- **Program in Lead Optimization Over**
  - 1000 compounds synthesized towards optimized leads – SAR well understood

- Potential first-in-class compound for multiple indications:
  - **Treatment of NASH/liver fibrosis**
  - Diabetic Retinopathy
  - Age Related Macular Degeneration
  - Potential for kidney Fibrosis

- Convincing Preclinical PoC data with lead compound for NASH with Liver Fibrosis, validating the hypothesis about MoA

- All *in vitro* assays in place for rapid advancement

Impetis GPR91 Program : Summary

- Extensive experience with target

- Potent and selective **proprietary series** of GPR91 antagonists have been identified
  - 1000 compounds synthesized towards optimized leads – SAR well understood
  - High nanomolar to submicromolar potency hGPR91
  - Selective over GPR99
  - Maintain cross reactivity between human & mouse GPR 91
    - Refined and validated homology model for human, rat and mouse GPR91
  - Excellent PK profile in mouse & rat

- First to demonstrate pharmacological validation
  - **Robust efficacy in NASH model of liver fibrosis**

GPR91 in Liver Diseases: Literature Summary

Indication	Literature summary	References
Liver fibrosis	• Hepatic stellate cells (HSCs) are the main extracellular matrix (ECM) producing cells in the injured liver. • Following liver injury, HSCs activate or trans-differentiate into myofibroblast-like cells, acquiring contractile, proinflammatory, and fibrogenic properties. • GPR91 was found to be expressed in quiescent hepatic stellate cells, and its expression was decreased with activation. • Succinate levels are increased ~ 14 fold higher during hepatic ischemia and exposure to succinate leads to activation of HSCs. • Antagonism of GPR91 receptor may help in blocking succinate induced activation of stellate cells producing anti-hepatic fibrosis effect.	• Correa PR et al, Journal of Hepatology 47: 262-269 (2007) • Gabele et al, 2003 • Milani et al, 1990 • Marra, 1999
Nonalcoholic steatohepatitis (NASH)	Succinate receptor GPR91 potentially mediates hepatic oxidative damage by succinate.	Montez et al, Endocrinology. 2012 Dec; 153(12): 5746–5759

Thank You