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Endoglin Antibody Reduces the NAFLD Activity Score in the STAM™ Model of NASH and Reduces Liver Fibrosis Following Carbon Tetrachloride Treatment

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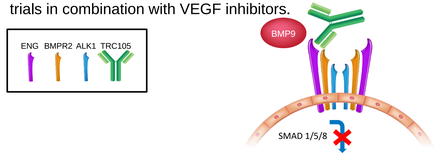
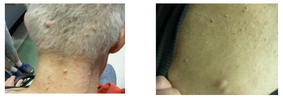
INTRODUCTION

- Endoglin is a membrane receptor required for angiogenesis (Li 1999) that is highly expressed on proliferating endothelial cells in solid tumors (Seon 2011), and is also expressed on activated fibroblasts.
- Endoglin expressed on activated fibroblasts signals through binding transforming growth factor beta (TGF-β) and through binding bone morphogenic protein (BMP).
- Endoglin heterozygosity is associated with the Osler-Weber-Rendu syndrome that results in mucocutaneous telangiectasia.
- TRC105 is a chimeric IgG1 endoglin monoclonal antibody with high avidity (K_D = 5 pM) that inhibits angiogenesis by competitively inhibiting BMP binding (Nolan-Stevaux 2012), potentiates the activity of VEGF inhibitors in preclinical models, and is being studied in multiple oncology trials in combination with VEGF inhibitors.



Inhibition of SMAD 1/5/8 phosphorylation allows unopposed phospho-SMAD 2/3 signaling to restore a quiescent phenotype

- Endoglin antibody reversed cardiac fibrosis and improved survival in preclinical fibrosis models (Kapur 2012; Kapur 2014).
- Marked reduction in cutaneous neurofibromatosis was observed in a sarcoma patient dosed with TRC105 and pazopanib in a Phase 2 clinical trial.



Pictures demonstrate marked reduction of cutaneous neurofibromatosis in a 59 year old man following three months of treatment with TRC105 and pazopanib

Endoglin Antibodies

TRC105

Human chimeric IgG1 antibody to human endoglin that competitively inhibits human BMP binding, and causes endoglin shedding. TRC105 cross reacts with mouse endoglin without inhibiting mouse BMP binding to mouse endoglin.

TRC205

Human IgG4 antibody to human endoglin that competitively inhibits human BMP binding, and causes endoglin shedding. TRC105 cross reacts with mouse endoglin without inhibiting mouse BMP binding to mouse endoglin.

M1043

Rat IgG1 antibody to mouse endoglin that competitively inhibits mouse BMP binding to mouse endoglin.

CCl_4 Fibrosis Study

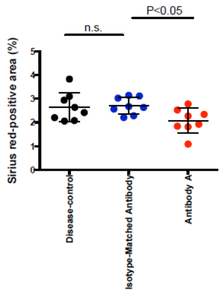
Method

Group	No. mice	Model	Test substance	Dose (mg/kg)	Volume (mL/kg)	Regimen	Sacrifice
1	8	CCl_4	-	-	-	-	Day 28
2	8	CCl_4	Isotype-Matched Antibody	10	5	IV, twice weekly, Day 14, 17, 21 and 24	Day 28
3	8	CCl_4	Antibody A	10	5	IV, twice weekly, Day 14, 17, 21 and 24	Day 28

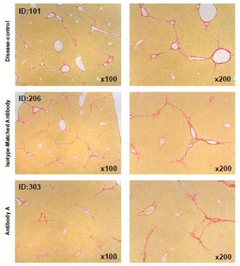
Results

- Antibody A (M1043) significantly decreased the percentage of Sirius red-positive area compared with the Isotype-Matched Antibody control group.
- Extensive collagen deposition and bridging fibrosis were evident in the liver sections from the Isotype-Matched Antibody and disease control groups. The Antibody A (M1043) group demonstrated lower collagen deposition with less frequent formation of bridging fibrosis than the Isotype-Matched Antibody and disease control groups.
- There was no significant difference in mean body weight between the Isotype-Matched Antibody group and the Antibody A (M1043) group.

Fibrosis Area



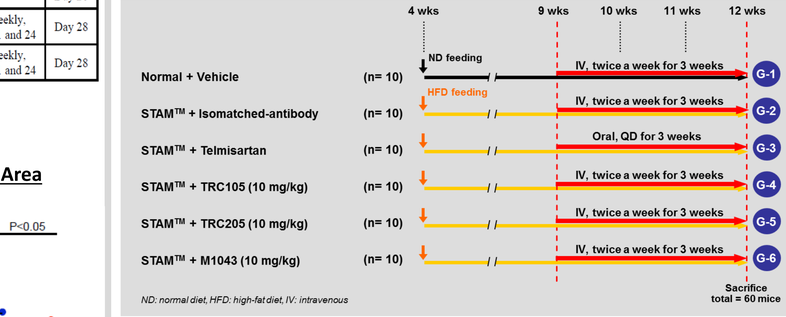
Representative Histopathology



NASH Study

Methods

Study plan for assessing the anti-fibrosis effects of M1043, TRC105 and TRC205 in STAM™ model of NASH



ND: normal diet, HFD: high-fat diet, IV: intravenous

Analyses

1. General condition
- Body weight
- Liver weight
- Liver-to-body weight ratio

2. Biochemistry
- Liver TG
- Plasma ALT
- Plasma AST
- Plasma ALK phosphatase

3. Histopathological analyses
- HE staining (NAFLD Activity score)
- Sirius red staining (Fibrosis area)
- IHC for F4/80 (Inflammation area)

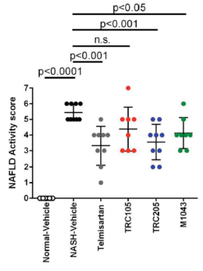
4. Gene expression assay
- TNF-α, MCP-1, α-SMA, IMP-1

5. Sample collection
- Frozen liver samples
- Frozen plasma samples

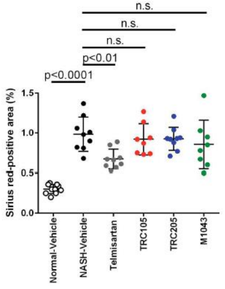
Results

- TRC205 and M1043 significantly reduced the NAFLD Activity Score (NAS). TRC205 and M1043 demonstrated hepatoprotective, anti-inflammatory and anti-NASH effects.
- TRC205 and M1043 reduced inflammatory cell infiltration in the liver, which resulted in decreases in the NAS lobular inflammation score and F4/80 immunostaining. The improvement in serum ALT and AST levels was consistent with TRC205 and M1043 amelioration of hepatocellular injury and inflammation. TRC205 also reduced alkaline phosphatase, liver triglycerides, and α-smooth muscle actin mRNA expression compared to the NASH-Vehicle group.
- M1043, an antibody that competitively inhibits mouse BMP binding to mouse endoglin, more effectively reduced NAS than TRC105, an antibody that does not competitively inhibit mouse BMP binding to mouse endoglin.
- NAS was reduced most significantly with TRC205, an IgG4 antibody that would not be expected to stimulate immune cell infiltration, compared to the IgG1 antibodies TRC105 and M1043.

NAFLD Activity Score



Fibrosis Area



NAFLD Activity Score Table

Group	n	Steatosis 0	1	2	3	Lobular inflammation 0	1	2	3	Hepatocyte ballooning 0	1	2	NAS (mean ± SD)
Normal-Vehicle	10	10	-	-	-	10	-	-	-	10	-	-	0.0 ± 0.0
NASH-Vehicle	9	-	6	3	-	-	1	6	2	-	-	9	5.4 ± 0.5
Telmisartan	9	-	9	-	-	3	4	2	-	1	3	5	3.3 ± 1.2
TRC105	8	1	6	1	-	-	1	5	2	3	-	5	4.4 ± 1.4
TRC205	9	-	6	3	-	5	-	4	-	1	4	4	3.6 ± 1.1
M1043	8	-	6	2	-	1	1	6	-	2	2	4	4.1 ± 1.0

Item	Score	Extent
Steatosis	0	<5%
	1	5-33%
	2	>33-66%
	3	>66%
Lobular Inflammation	0	No foci
	1	<2 foci/200x
	2	2-4 foci/200x
	3	>4 foci/200x
Hepatocyte Ballooning	0	None
	1	Few balloon cells
	2	Many cells/prominent ballooning

Conclusions

CCL_4 FIBROSIS MODEL

- M1043 treatment significantly reduced collagen deposition in the CCl_4 model of liver fibrosis, as evidenced by decreased Sirius red positive area.
- No abnormal findings were observed in the M1043 treated group.

NASH MODEL

- TRC205 and M1043 significantly reduced the NAS.
- TRC205 and M1043 demonstrated anti-inflammatory and anti-NASH effects.
- The increased activity of M1043 versus TRC105 suggests that inhibition of BMP function is an important mechanism of action of endoglin antibodies in models of fibrosis.
- The improved activity of TRC205, an IgG4 antibody versus TRC105 suggests that an endoglin antibody unable to engage immune effector cells will be the preferred clinical candidate.

OVERALL

- Endoglin antibodies have demonstrated activity in cardiac and liver fibrosis models, including NASH.
- Regression of cutaneous neurofibromatosis was observed in a patient with sarcoma treated with the endoglin antibody TRC105 in combination with pazopanib.
- The endoglin antibody TRC205 is being developed to treat patients with fibrosis.

REFERENCES

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- Li DY, Science 284:1534-37, 1999
- Nolan-Stevaux O, PLOS One 7:1-12, 2012
- Seon BK, Current Drug Del 8:135-43, 2011