Preclinical characterization of bi-functional NK-aHSC engager for liver fibrosis

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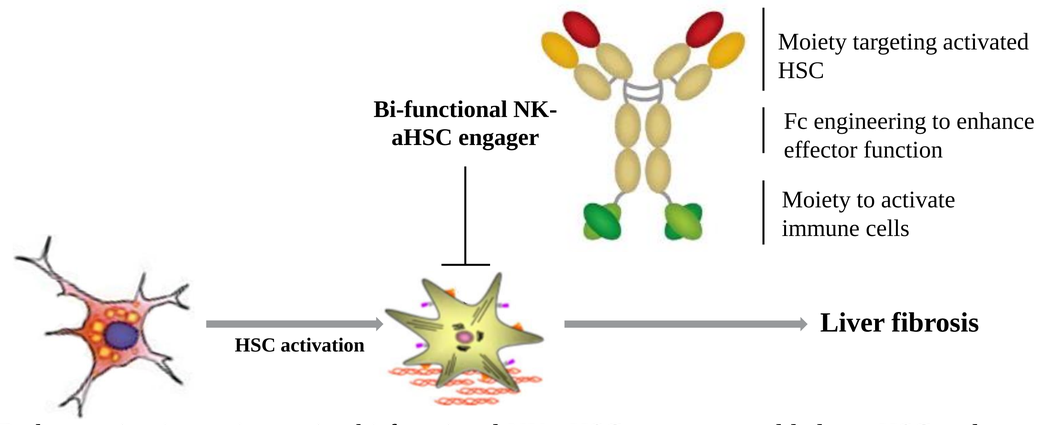
Background and Objective

Activated Hepatic Stellate Cells (aHSC) are the primary source of hepatic myofibroblasts, which are the main contributors for liver fibrogenesis. Depletion of aHSC using either genetic manipulations or pharmacological approaches (e.g. ADC, CART) has demonstrated anti-fibrosis effect in various animal models. However, these approaches have not been applied to clinical setting. To translate these findings, Laekna has developed a proprietary antibody-based discovery platform for aHSC depletion and designed bi-functional NK-aHSC engager lead molecules, with the goal to discover and develop novel therapeutics for liver fibrosis.

Disease model	Target	Modality to Deplete myofibroblast
Liver fibrosis	GFAP+ HSC	Transgenic [1]
Liver fibrosis	uPAR	CART [2]
Cardiac fibrosis	FAP	CART [3]
Liver fibrosis	CD248	ADC [4]
Liver fibrosis	MSLN	ADC [5]
Liver / Lung fibrosis	ADAM12 / GLI1	Vaccination [6]

ImmunoHEP: Laekna Proprietary aHSC Depletion Platform



By harnessing innate immunity, bi-functional NK-aHSC engagers could clear aHSC and ameliorate fibrosis in the liver. This approach could also be potentially applied to other type of fibrotic diseases and autoimmune diseases, where activated fibroblasts play a pathological role in disease progression.

Antibodies/Biologics Targeting aHSC Surface Antigens Exert ADCC and ADCP Activity

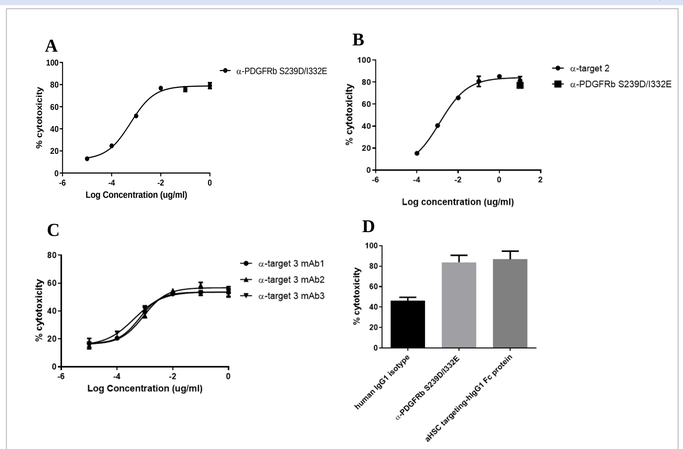


Figure 1. Antibodies or proteins targeting aHSC increased cytotoxic activity of primary NK cells or PBMC cells against TGFβ1-activated primary human HSC cells.

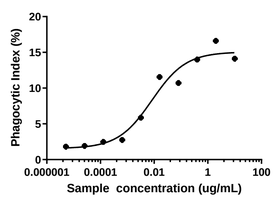


Figure 2. Antibody targeting human PDGFRβ increased phagocytosis activity of human primary macrophage against TGFβ1-activated primary human HSC cells.

Specific aHSC Killing Activity of Bi-Functional Engagers

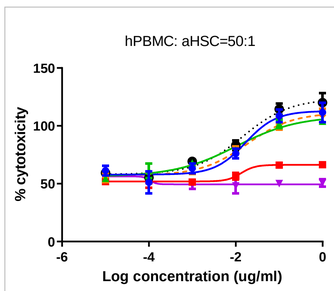


Figure 3. Monoclonal antibodies with different Fc mutations (mutations to enhance ADCC activity and mutation to disable ADCC) had different ADCC cytotoxicity against TGFβ1-activated human primary HSC cells

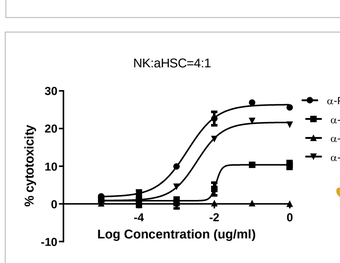


Figure 4. Bi-functional engager with a NK activation moiety further increased cytotoxic activity of primary NK cells against TGFβ1-activated human primary HSC cells

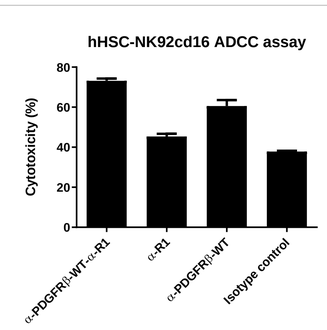


Figure 5. Bi-functional engager with a moiety to block immune inhibitory receptor further increased cytotoxic activity against TGFβ1-activated human primary HSC cells

Reduction of PDGFRβ and Activation of NK cell Activity After a Single Dose of Anti-mouse PDGFRβ Antibody (mIgG2a S239D/I332E) in Liver Fibrosis Models

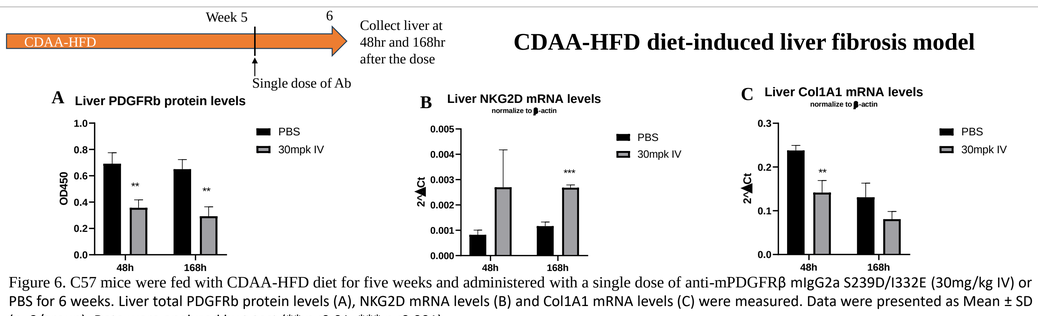


Figure 6. C57 mice were fed with CDAA-HFD diet for five weeks and administered with a single dose of anti-mPDGFRβ mIgG2a S239D/I332E (30mg/kg IV) or PBS for 6 weeks. Liver total PDGFRb protein levels (A), NKG2D mRNA levels (B) and Col1A1 mRNA levels (C) were measured. Data were presented as Mean ± SD (n=3/group). Data were analyzed by t-test (** p<0.01; *** p<0.001)

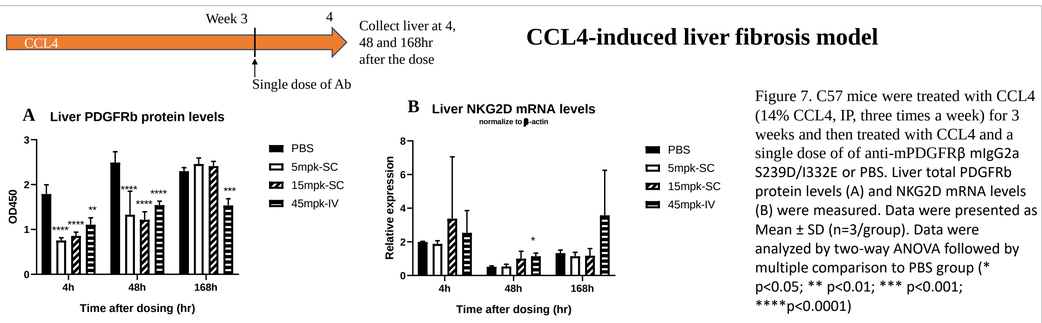


Figure 7. C57 mice were treated with CCL4 (14% CCL4, IP, three times a week) for 3 weeks and then treated with CCL4 and a single dose of of anti-mPDGFRβ mIgG2a S239D/I332E or PBS. Liver total PDGFRb protein levels (A) and NKG2D mRNA levels (B) were measured. Data were presented as Mean ± SD (n=3/group). Data were analyzed by two-way ANOVA followed by multiple comparison to PBS group (* p<0.05; ** p<0.01; *** p<0.001; ****p<0.0001)

aHSC-Depletion Reduced Liver Fibrosis in a Fc Effector Function Dependent Manner *in vivo*

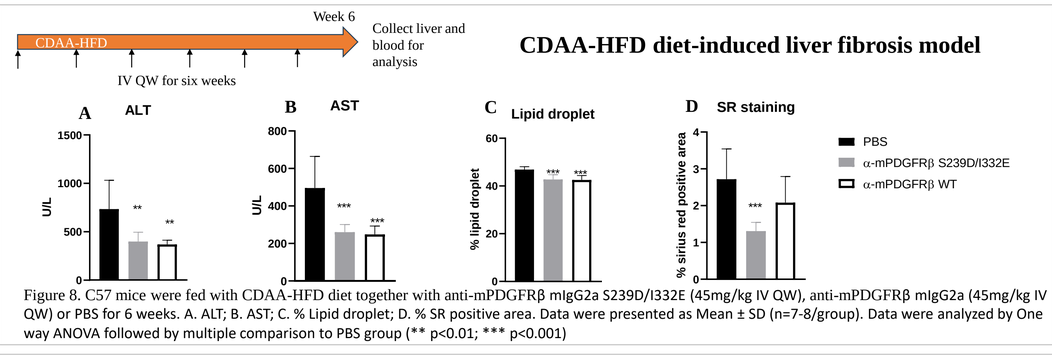


Figure 8. C57 mice were fed with CDAA-HFD diet together with anti-mPDGFRβ mIgG2a S239D/I332E (45mg/kg IV QW) or PBS for 6 weeks. A. ALT; B. AST; C. % Lipid droplet; D. % SR positive area. Data were presented as Mean ± SD (n=7-8/group). Data were analyzed by One way ANOVA followed by multiple comparison to PBS group (** p<0.01; *** p<0.001)

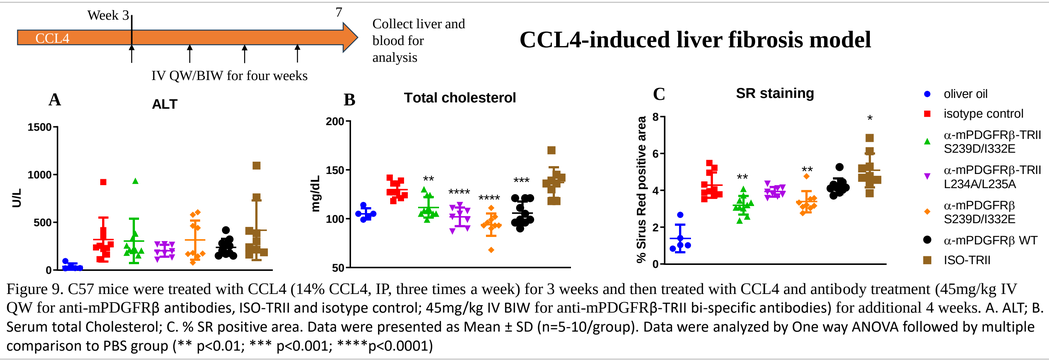


Figure 9. C57 mice were treated with CCL4 (14% CCL4, IP, three times a week) for 3 weeks and then treated with CCL4 and antibody treatment (45mg/kg IV QW for anti-mPDGFRβ antibodies, ISO-TRII and isotype control; 45mg/kg IV BIW for anti-mPDGFRβ-TRII bi-specific antibodies) for additional 4 weeks. A. ALT; B. Serum total Cholesterol; C. % SR positive area. Data were presented as Mean ± SD (n=5-10/group). Data were analyzed by One way ANOVA followed by multiple comparison to PBS group (** p<0.01; *** p<0.001; ****p<0.0001)

Conclusion

We have established a proprietary antibody-based discovery platform for aHSC depletion. Preclinical characterization of lead molecules has demonstrated effective aHSC killing and anti-fibrosis activity. These results support the further development of the lead molecules and continue to generate the next generation molecules using the platform-for liver fibrosis, with potentials to expand further to other fibrotic disease indications.

Reference:

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