DSP107— a first in class, bifunctional, fusion protein targeting both innate and adaptive immunity

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Background

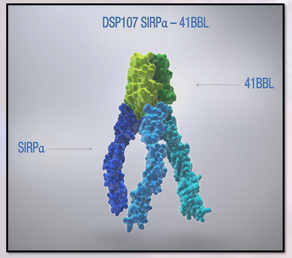
Checkpoint inhibitors have transformed cancer treatment in certain patient subgroups. To improve response rates, considerable effort is now being invested developing novel immune-oncology (IO) drugs and exploring IO target combinations.

Dual signaling proteins (DSPs) are a fusion of extracellular domains of Type-I and Type-II membrane proteins. All TNF superfamily ligands are Type-II proteins, allowing them to be combined with numerous, potential targeting and effector Type-I proteins.

The DSP platform enables production of fusion proteins as trimers, a structure that is essential for TNF receptor family activation.

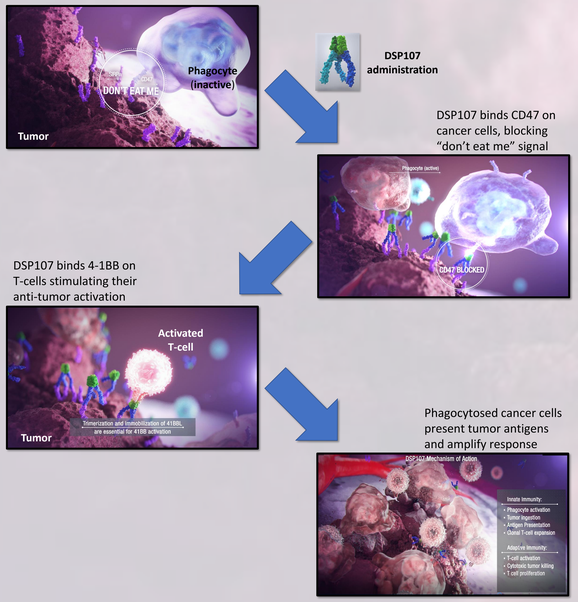
Targeted, dual therapeutic effect enhances efficacy and limits toxicity.

DSPs act as both targeting and effector molecules.



Mechanism of Action

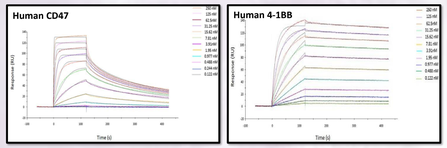
DSP107 produces targeted immune activation leading to macrophage and T-cell mediated tumor destruction



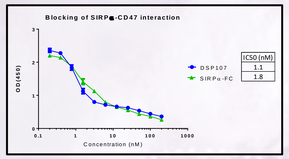
DSP107 targets CD47-overexpressing tumors, simultaneously blocking phagocyte inhibitory signals and delivering an immune costimulatory signal

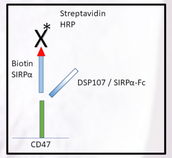
Results

DSP107 interaction with its human, cynomolgus and murine counterpart ligands determined by SPR analysis (Biacore). DSP107 binds with high affinities to both human and cynomolgus CD47 and 4-1BB but not the murine counterparts.



	KD (nM)	
	Monkey	Human
CD47	1.75	1.17
4-1BB	0.34	0.7



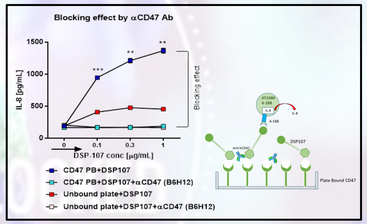


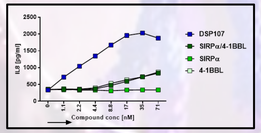
DSP107 and SIRPα-Fc block binding to CD47 at low nanomolar concentrations as measured with biotinylated SIRPα

DSP107 protein efficiently binds CD47 and 4-1BB and blocks SIRPα-CD47 interaction

To measure the stimulatory effect of the 4-1BBL arm of DSP107, two cell-based assay systems were developed. These utilize HT1080 4-1BB OX reporter cells, in a single culture or co-culture system, that secrete IL-8 upon activation of the 4-1BB receptor.

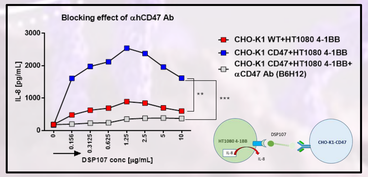
- DSP107 induced IL-8 secretion from reporter cells in the presence of plate-bound CD47
- In the absence of CD47, significantly reduced activation (up to ~3-fold decrease; $p < 0.003$)
- Activity of both plate-bound and "unbound" DSP107 totally abrogated by anti-CD47 Ab





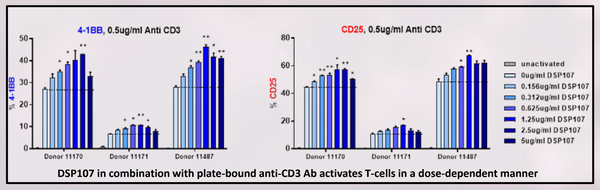
- Plate-bound DSP107 activated 4-1BB signaling more effectively (~3 fold increase) than soluble 4-1BBL or the combination of soluble SIRPα and 4-1BBL, applied at the same molar concentrations
- As expected, soluble SIRPα had no effect

- Addition of DSP107 to a co-culture system with HT1080 4-1BB OX and CHO-K1 CD47 OX cells, significantly increased secretion of IL-8 compared to co-culture of HT1080 4-1BB OX with CHO-K1 WT cells (up to 3.5-fold; $p < 0.0032$)
- IL-8 secretion was significantly blocked by anti-CD47 Ab (up to 7.2-fold; $p < 0.0001$)



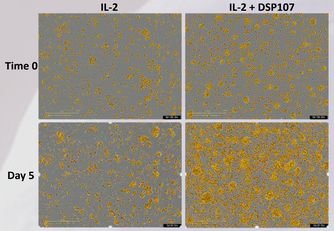
Activation of 4-1BB signaling by DSP107 requires cross presentation of 4-1BBL through SIRPα binding to CD47

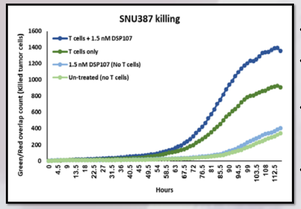
To demonstrate the activation effect of DSP107 on human T-cells, PBMCs were activated using sub-optimal concentrations of anti-CD3, known to induce 4-1BB expression on T-cells. CD25 and 4-1BB activation markers levels were evaluated by FACS.



DSP107 in combination with plate-bound anti-CD3 Ab activates T-cells in a dose-dependent manner

- Effect of DSP107 on T-cell proliferation studied with sub-optimal IL-2 activation
- Proliferation measured using IncuCyte live cell analysis imaging system
- Cell density increased significantly following 5-day incubation of PBMCs with IL-2 and DSP107 (0.33 µg/mL), compared to PBMCs treated with IL-2 alone

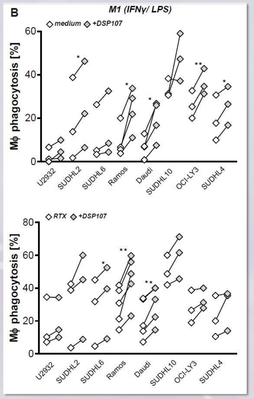


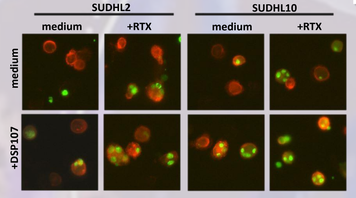


- Effect of DSP107 on T-cell mediated killing of tumor cells evaluated using IncuCyte technology
- No killing effect observed following incubation of SNU387 (hepatocellular cancer) cell line with DSP107 alone (light blue)
- Co-culture in presence of T-cells, activated with sub-optimal concentrations of anti-CD3/CD28 Dynabeads, resulted in killing of cancer cells (dark green)
- Addition of DSP107 (0.33 µg/mL) enhanced T-cell mediated killing of SNU387 cells by 49% (dark blue)

DSP107 augments T-cell activation, proliferation and killing of tumor cells

- Effect of DSP107 on macrophage-mediated phagocytosis of cancer cells tested *in vitro* in co-culture system using macrophages from healthy donors and fluorescently-labeled CD47-expressing cancer cells
- DSP107 added to the co-culture with or without rituximab, and phagocytosis evaluated using IncuCyte fluorescence live cell imaging system
- A significant increase in phagocytosis was demonstrated in five out of eight malignant B-cell lines following administration of DSP107 (2.1 µg/mL) as a single agent
- DSP107 combination therapy with rituximab (RTX) caused a significant increase in phagocytosis in three out of eight cell lines, with a similar trend towards increased phagocytosis for all other cell lines tested



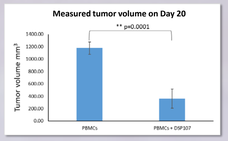


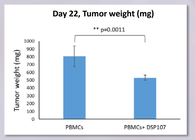
- Phagocytic uptake of SUDHL2 and SUDHL10 cells determined by fluorescent microscopy
- Minimal phagocytosis detected with medium control
- Single agent treatment with either RTX or DSP107 (2.1 µg/mL) increased phagocytosis
- Combined treatment with RTX and DSP107 further augmented number of phagocytosed cancer cells

DSP107 promotes phagocytosis of lymphoma and cancer cell lines and augments phagocytosis in combination with therapeutic Abs

In vivo effect of DSP107 on human DLBCL tumors evaluated in humanized NOD Scid Gamma (NSG) mice
1 x 10^6 SUDHL6 cells inoculated sc on Day 0. On Day 7, mice randomly assigned according to scheme below
Freshly isolated PBMCs, drawn from same donor, administered on Days 7 and 13

Group	N	Inoculated cell line	Human PBMCs (IV)	Treatment	Dose level (µg/mouse)	Route	Dosing Frequency & Duration
1	5	DLBCL SUDHL6	+	Vehicle (PBS)	-	IP	3 x / week (total of 6 doses)
2	6	DLBCL SUDHL6	+	DSP107	250	IP	3 x / week (total of 6 doses)



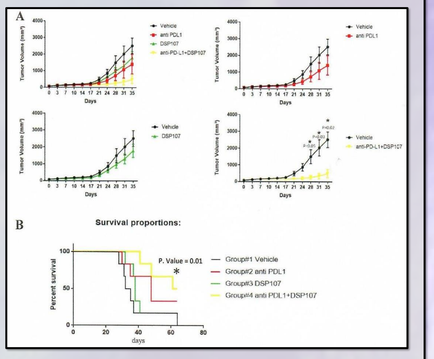


Treatment of SUDHL6 DLBCL tumors in humanized NSG mice with DSP107 resulted in a significant reduction in tumor volume (~70%) and tumor weight (34%)

DSP107 in vivo efficacy also evaluated in h4-1BB knock-in mice carrying hCD47-expressing MC38 tumors.
Six mice / group randomized according to scheme below:

Group	N	Inoculated cell line	Treatment	Dose level (µg/mouse)	Route	Dosing Frequency & Duration
1	6	hCD47-MC38	Vehicle (PBS)	-	IP	Day 0,1,2,3,4
2	6	hCD47-MC38	anti PD-L1	60	IP	BIW x 3 weeks
3	6	hCD47-MC38	DSP107	250	IP	Day 0,1,2,3,4
4	6	hCD47-MC38	DSP107	250	IP	Day 0,1,2,3,4
			anti PD-L1	60	IP	BIW x 3 weeks

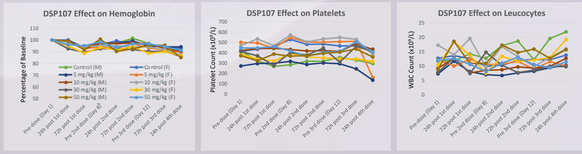
- DSP107 treatment well tolerated as monotherapy and in combination with anti PD-L1
- Treatment with anti PD-L1 and DSP107 monotherapy trended towards inhibition in tumor growth
- DSP107 + anti PD-L1 combination resulted in statistically significant TGI of 83% ($p = 0.02$), compared to control mice
- Using Mantel-Cox analysis, combined treatment with DSP107 and anti PD-L1 resulted in a statistically significant improvement in survival compared to control mice ($p = 0.01$)



DSP107 demonstrates efficacy as monotherapy and in combination with anti PD-L1 in murine tumor models

16-day intravenous (infusion) administration range-finding toxicity study in cynomolgus monkeys (Study #8402826, Covance, UK) – Non-GLP
4 administrations of either 0, 5, 10, 30 or 50 mg/kg DSP107 on Days 1, 8, 12, 16 to one male + one female monkey / dose group

- DSP107 well tolerated during repeated administration of doses up to 50 mg/kg
- No clinically significant hematological or hepato-toxicity observed
- No evidence of cytokine release
- No macroscopic or microscopic findings



Summary and Conclusions

- DSP107 is a bifunctional, trimeric fusion protein with a unique MoA targeting both innate and adaptive immunity
- DSP107 blocks interaction of SIRPα with CD47, induces phagocytosis of cancer cells as a single agent and when combined with targeted therapies
- DSP107 promotes activation and proliferation of T-cells and enhances T-cell mediated killing of tumor cells
- DSP107 is efficacious as monotherapy and in combination with anti PD-L1 in murine cancer models
- Repeated doses up to 50 mg/kg were safe and well-tolerated in cynomolgus monkeys with absence of hematological or hepato-toxicity
- GLP tox and IND enabling studies ongoing
- Clinical studies scheduled to commence in Q2 2020

For further information: info@kahr-medical.com