DSP502 — A NOVEL APPROACH FOR TARGETING TIGIT AND PD1 PATHWAYS FOR CANCER IMMUNOTHERAPY

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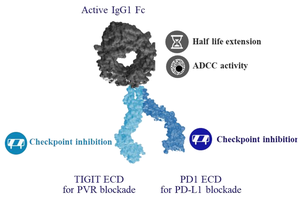
Background

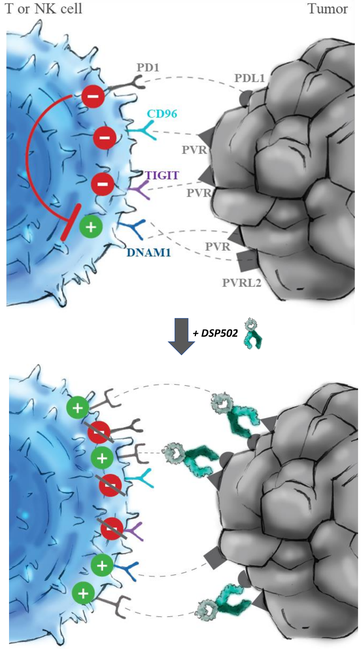
TIGIT, an inhibitory immune checkpoint, is a target of interest for immuno-oncology combination therapies. TIGIT is part of a complex molecular network containing four receptors (DNAM1, TIGIT, PVRIG and CD96) and two ligands (PVR and PVRL2).

Here we describe Dual Signaling Protein 502 (DSP502), a novel, multi-functional IgG1-Fc-fusion protein targeting this molecular pathway in a unique way. DSP502, comprising the extracellular domains of TIGIT and PD1, is designed to simultaneously bind its two respective ligands, PVR and PD-L1, overexpressed on cancer and myeloid cells in the tumor microenvironment.

DSP502 binds PVR preventing inhibitory signaling through TIGIT and CD96 and promoting DNAM1 costimulatory signaling on activated T- and NK-cells. DSP502's PD1 arm binds PD-L1 to unleash effector T-cells through checkpoint inhibition.



In parallel, DSP502's IgG1-Fc delivers an immune-activating signal via Fc receptors. The net effect is enhanced anti-tumor immunity.



PVR overexpression in the TME imbalances inhibitory (TIGIT and CD96) and stimulatory (DNAM1) receptors resulting in immunosuppression. Additionally, PD1 inhibits downstream stimulatory DNAM1

The net effect is immunosuppression

DSP502 blocks PVR to potentiate DNAM1 costimulatory activity while inhibiting TIGIT/CD96 signaling. DSP502 also interferes with PD1/PD-L1 checkpoint and its IgG1-Fc delivers an immune-activating signal

The net effect is enhanced anti-tumor immunity

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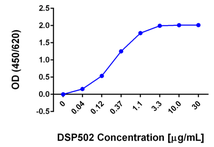
Results

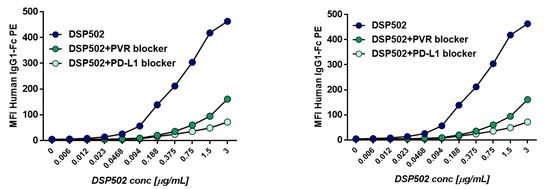
Binding analysis:

DSP502 heterodimer was successfully produced in a mammalian expression system. Both DSP502 arms were shown to bind their cognate ligands in ELISA and on cell surface. DSP502 binding was dependent on the presence of both ligands on cells and was abolished by competing antibodies to the respective targets, demonstrating binding specificity and the 'AND-gate' phenomenon.

DSP502 binds the same ligands on mouse cell-lines.

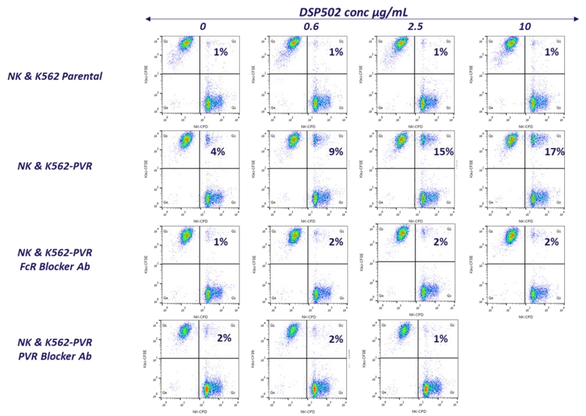


Dual binding ELISA: DSP502 protein efficiently binds plate bound PD-L1 and is detected by PVR human recombinant proteins



DSP502 protein efficiently binds cells expressing both ligands (PVR & PD-L1), and binding is blocked by competing antibodies

Simultaneous binding of DSP502 to fluorescently-labeled tumor and NK-cells was evaluated by FACS, demonstrating increased, dose-dependent, complexation of NK- and tumor cells following DSP502 treatment that was abolished by both PVR and FcR antibodies

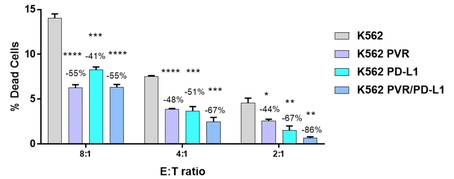


DSP502 increases doublet formation when co-cultured with K562-PVR and NK cells. No effect seen when co-cultured with K562 (PVR negative); Doublet formation is inhibited by PVR or FcγRIII blocking

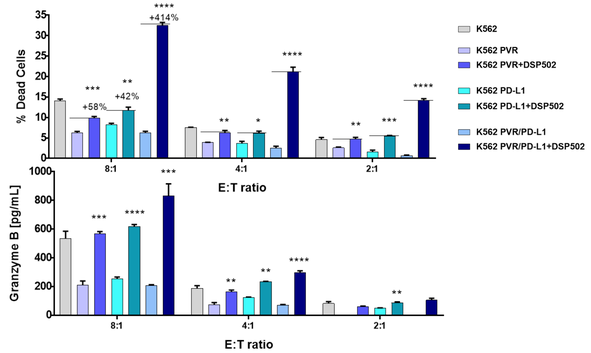
NK killing effect:

The effect of DSP502 on human NK-cell mediated cytotoxicity was studied in co-culture assay with human K562 CML cells overexpressing PVR, PD-L1 or overexpressing both PVR and PD-L1.

K562 cells were incubated for 24h with purified NK cells, in different E:T ratios and with and without treatment with DSP502. Cell viability was analyzed by flow cytometry and Granzyme B by ELISA.



Overexpression of DSP502 ligands protects tumor cells from NK-cell mediated cytotoxicity

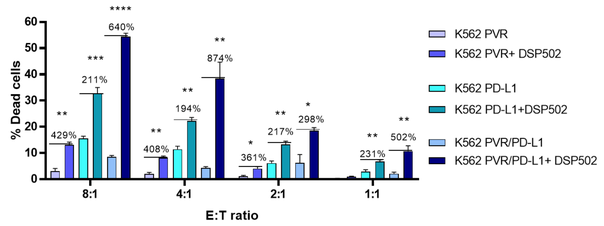


DSP502 significantly increases NK-mediated cytotoxicity; Toxicity level is dramatically higher when both ligands are expressed

PBMC killing effect:

The effect of DSP502 on human PBMC mediated cytotoxicity was studied in co-culture assay with human K562 CML cells overexpressing PVR, PD-L1 or overexpressing both PVR and PD-L1.

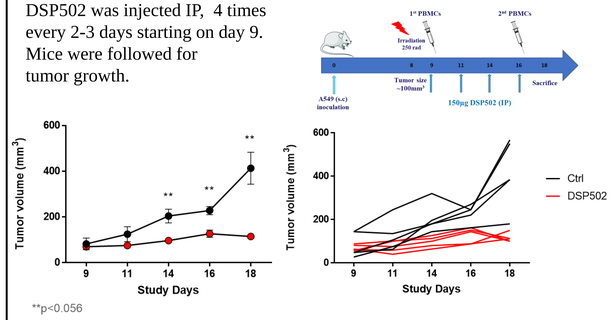
K562 cells were incubated for 48h with purified PBMCs, in different E:T ratios and with increasing concentrations of DSP502. Cell viability was analyzed by flow cytometry



DSP502 augmentation of PBMC mediated tumor cell killing correlates with its ligands' expression pattern

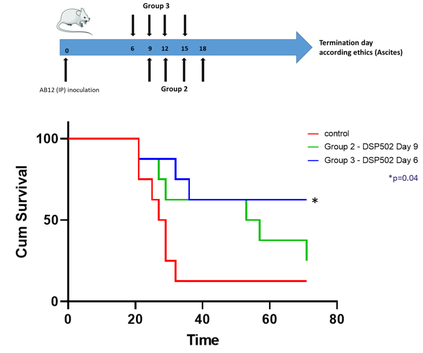
In-vivo studies:

DSP502 in vivo efficacy was evaluated in humanized and syngeneic mice tumor models. For the humanized model, A549 cells were injected SC to NSG mice. On day 9, mice were irradiated and human PBMCS were injected IV followed by a second injection of human PBMCs on day 16.
DSP502 was injected IP, 4 times every 2-3 days starting on day 9. Mice were followed for tumor growth.



DSP502 treatment is well tolerated; DSP502 inhibits tumor growth of A549-NSCLC xenograft in a humanized NSG mouse model, with all mice being tumor-free at the end of the study

For the syngeneic model, AB12, a mouse mesothelioma cell-line was studied. Cells were injected IP to immunocompetent mice. Treatment with DSP502 started on Day 6 or 9 after cell inoculation. A total of 4 doses were administered, once every three days. Survival and clinical signs were monitored daily.



DSP502 significantly prolongs the survival of mice bearing AB12 mesothelioma tumors

Summary and conclusions:

Here we report the design and function of DSP502 a novel immunotherapeutic fusion protein

- DSP502 offers multiple functionalities that can synchronously and synergistically drive anti-tumor immunity
- Beyond targeting PVR and PDL1, DSP502 has the potential to additionally impact the TIGIT pathway through its effects on CD96 and DNAM1
- DSP502 is currently in IND-enabling studies and CMC development