CB699: A novel mesothelin-binding Humabody® CD40 and CD137 dual-agonist for enhancing immune cell responses against MSLN⁺ tumors

Poster #5302

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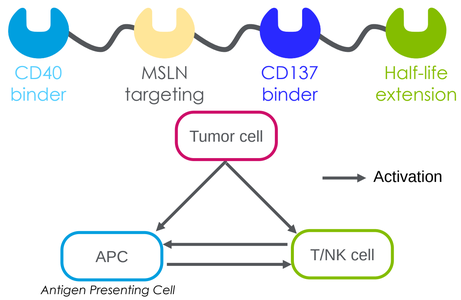
CB699 Mechanism of Action

Figure 1. CB699: a half-life extended MSLN x CD137 x CD40 molecule that enhances immune – immune and immune – tumor crosstalk



CB699 is a half-life extended tri-specific Humabody® V_H immune cell enhancer.

- CD40 (TNFRSF5) and CD137 (TNFRSF9) are costimulatory molecules expressed on B cells, DCs and macrophages, and T and NK cells, respectively
- MSLN is widely expressed on multiple tumor types, including ovarian, pancreatic and mesothelioma

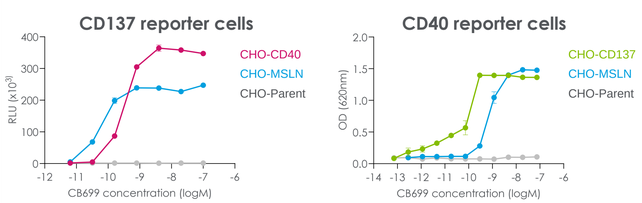
CB699 binds MSLN, CD137, CD40, and HSA

Figure 2. CB699 binds immobilised target and clusters cell expressed target

CB699 affinity was assessed by SPR (Biacore). Clustering of CD137 and CD40 was assessed using CD137 (Promega), and CD40 (InvivoGen) reporter cells.

	ka (1/Ms)	kd (1/s)	KD (nM)	Rmax (RU)
CD40-hFc	3.31E+05	9.61E-03	29.0	117.4
MSLN-hFc	1.90E+05	4.69E-04	2.47	131.4
CD137-hFc	5.51E+04	4.22E-05	0.766	182.6
HSA	2.17E+05	1.13E-01	522	36.1

✓ CB699 has low nM affinity for CD40, MSLN, and CD137

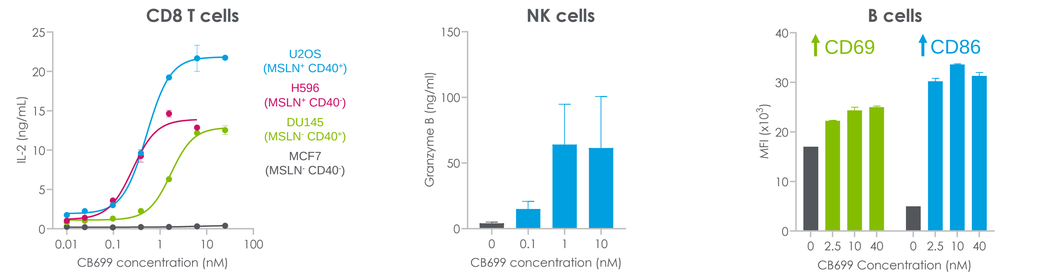


✓ CB699 clusters CD137 in the presence of CHO-CD40 or CHO-MSLN cells
✓ CB699 clusters CD40 in the presence of CHO-CD137 or CHO-MSLN cells

CB699: Potent immune cell activation

Figure 3. CB699 conditionally activates T cells, NK cells, and B cells

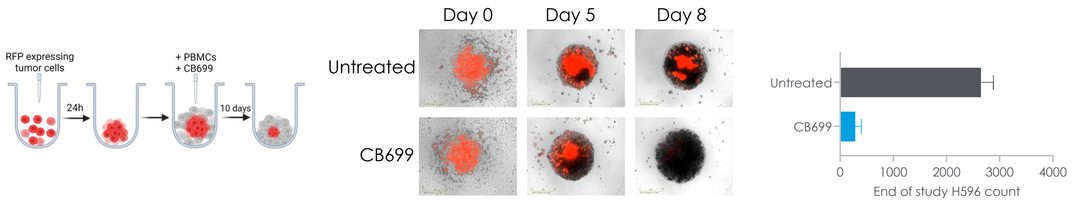
CD8 T cells were cocultured with indicated cell lines in the presence of αCD3 and IL-2 secretion assessed by MSD. NK cells were activated with IL-2, cocultured with MSLN⁺ H226 cells, and activation granzyme B secretion assessed by MSD. PBMCs were cocultured with CD137⁺ Jurkat cells and B cell activation assessed by flow cytometry.



✓ CB699 enhances IL-2 secretion from CD8 T cells in the presence of MSLN or CD40 expressing tumor cells
✓ CB699 enhances granzyme B secretion from NK cells in the presence of MSLN expressing tumor cells
✓ CB699 enhances B cell activation and maturation in the presence of a CD137 expressing T cell line

Figure 4. CB699 enhances immune cell killing of tumor spheroids

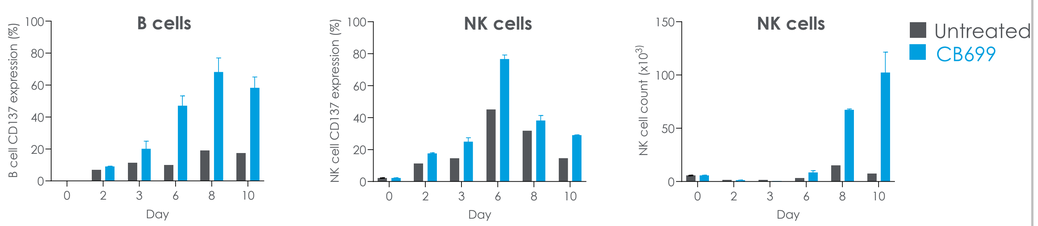
H596-RFP spheroids were plated in ULA plates for 24 hours prior to addition of PBMCs and CB699. Images were acquired by IncuCyte and viable tumor cells at the end of the study were quantified by flow cytometry.



✓ CB699 enhanced immune cell killing of H596-RFP spheroids does not require exogenous TCR co-stimulation

Figure 5. CB699 activates B and NK cells in the tumor spheroid assay

H226-RFP spheroids were plated in ULA plates for 24 hours prior to addition of PBMCs and CB699. CD137 expression on B and NK cells as well as NK cell counts were assessed at indicated timepoints by flow cytometry.

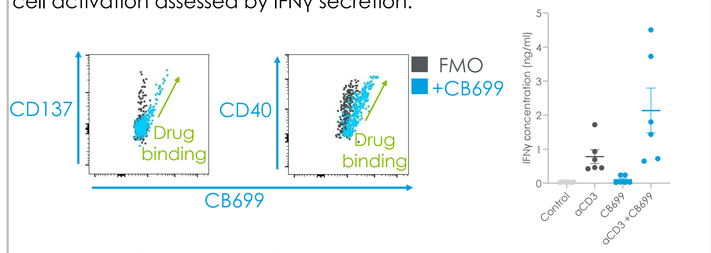


✓ CB699 enhances CD137 expression on B and NK cells, and enhances NK cell proliferation

CB699 is active *ex vivo* and *in vivo*

Figure 6. CB699 enhances TIL activity *ex vivo*

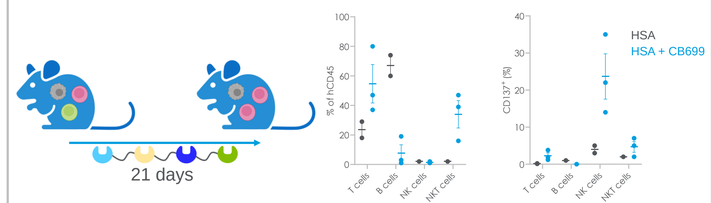
CB699 binding to TILs in a primary NSCLC tumor was assessed by flow cytometry. 1-2mm tumor chunks were cultured with CB699 and immune cell activation assessed by IFNγ secretion.



✓ CB699 binds to TILs and enhances cytokine secretion

Figure 7. CB699 expands and activates immune cells *in vivo*

huNOG-EXL (humanised) mice were treated with HSA or HSA+CB699 for 21 days and peripheral immune cells were analysed by flow cytometry



✓ CB699 reduced B cells but increased T and NKT cell counts in PBMCs
✓ CB699 enhanced CD137 expression on NK cells

Figure 8. NHP assessment enables GLP toxicology studies

CB699 PK was assessed in a non-GLP DRF toxicology study in cynomolgus monkeys as well as in GenOway (HSA / hFcRN) mice.

Species	Dose (mg/kg)	$T_{1/2}$ (hours)
Cynomolgus monkey	4.5	71.8
Cynomolgus monkey	44.1	86.9
GenOway mouse	2	20.4

✓ Half-life suitable for clinical development

Conclusions

✓ CB699 is a tri-specific half-life extended Humabody® designed to simultaneously enhance myeloid cells and lymphocytes
✓ CB699 has demonstrated potent activity *in vitro* and *ex vivo*
✓ Encouraging *in vivo* data supports further development

See our sister poster #5313: Section 3, Board 22, *"CB307: A dual targeting costimulatory Humabody® VH therapeutic for treating PSMA-positive tumors"*