A best in class anti-CD38 antibody with anti-tumour and immune modulatory properties.

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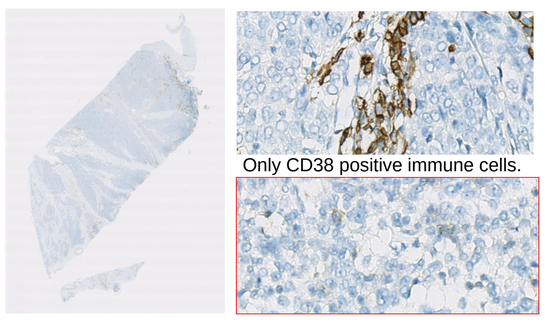
Targeting CD38 in multiple myeloma has resulted in outstanding responses. CD38 is widely expressed on myeloma cells and other haematological malignancies, however, not much is known about its expression in solid tumours and its role in the immune system. We have analysed a range of solid tumours for CD38 expression and distribution. To optimally target CD38, we have generated a novel antibody that depletes CD38-high expressing cells, and additionally exhibits immune modulatory properties.

CD38 expression in solid tumours: various methods have been applied (analysis of mRNA expression libraries, IHC on tumour sections, flow cytometry on patient tumour material), and revealed varying CD38 expression among all cancers analysed. IHC and flow cytometry confirmed CD38 expression across common cancer types, mostly confined to infiltrating lymphocyte and myeloid subsets, while expression on tumour cells was patient dependent. Of note, high expression of CD38 was found on a population of CD3-CD57- immune cells, which could represent myeloid cells (MDSCs or macrophages). Finally, we observed that co-culture with activated NK cells or treatment with IFNγ increases CD38 expression on some tumour types, indicating that some tumours could respond to immune activation by upregulating CD38.

Antibody development to target CD38 in solid tumours: among a panel of antibodies binding to distinct epitopes of CD38 and exerting unique functional properties, we have identified a fully human antibody, with strong capacity to deplete CD38-high cells *in vitro* and *in vivo* by varying killing mechanisms. This antibody was also found to increase TCR-mediated signalling and proinflammatory cytokine secretion by human T cells, and further to enhance NK cell activation *in vitro*. Low dose injection to non-human primates resulted in increased expression of activation markers on both CD4 and CD8 T cells, while no T cell depletion was observed.

1. CD38 expression in solid tumours is mostly confined to immune cells and increases upon immune activation

a) CD38 expression mostly restricted to immune cells



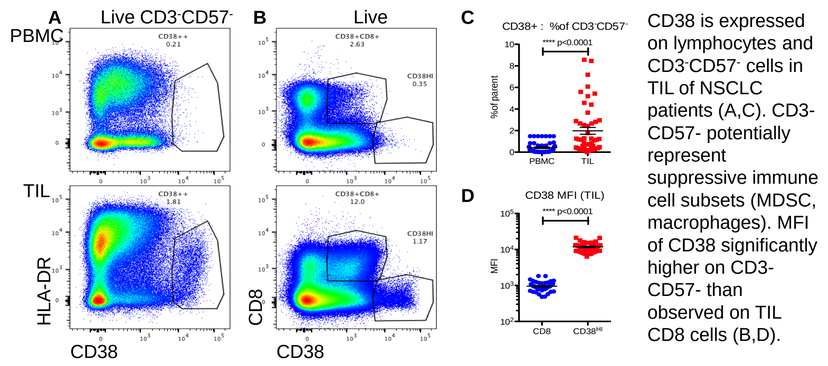
Only CD38 positive immune cells.

Lung sample, no checkpoint inhibitor pre-treatment: CD38 positive tumour cells →**0,5%** of the tumour area.
CD38 pos. tumour cell only found in 1/18 samples:

Faint CD38 positive tumour cells observed.

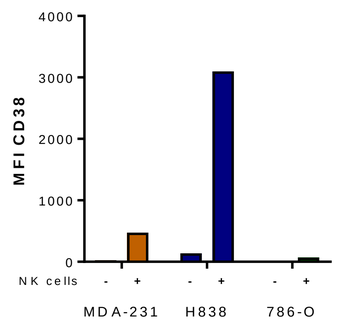
Stainings by HalioDX.

b) CD38^{high} immune cells in tumours



CD38 is expressed on lymphocytes and CD3-CD57- cells in TIL of NSCLC patients (A,C). CD3-CD57- potentially represent suppressive immune cell subsets (MDSC, macrophages). MFI of CD38 significantly higher on CD3-CD57- than observed on TIL CD8 cells (B,D).

c) CD38 expression increases on tumours after immune activation

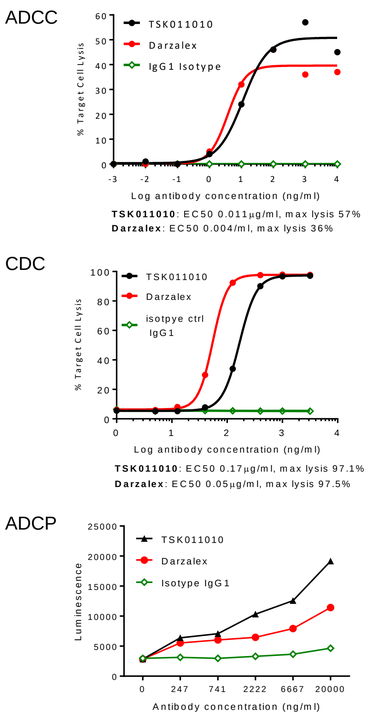


Co-culture with activated NK cells (or IFNγ, not shown) leads to upregulation of surface expression of CD38 on CD38 negative tumour cell lines.
MDA-MB-231 (breast), H838 (lung), and 786-O (ovarian) cancer cell lines co-cultured for 24hrs with NK cells pre-activated with IL-2.

Conclusion I: We found heterogenous expression of CD38 in solid tumours, mostly confined to immune subsets (shown here for NSCLC). Within TILs, a non-lymphocyte fraction of cells, potentially MDSC/macrophages, expressed high levels of CD38. Factors released by activated NK cells, e.g. IFNγ, increased CD38 expression on tumour cell lines to different extents, indicating that checkpoint inhibitor treated patients might show upregulated CD38 expression on tumour cells. The lead anti-CD38 Ab candidate has thus been selected based on its ability to induce killing of CD38high cells and on its immune modulatory properties.

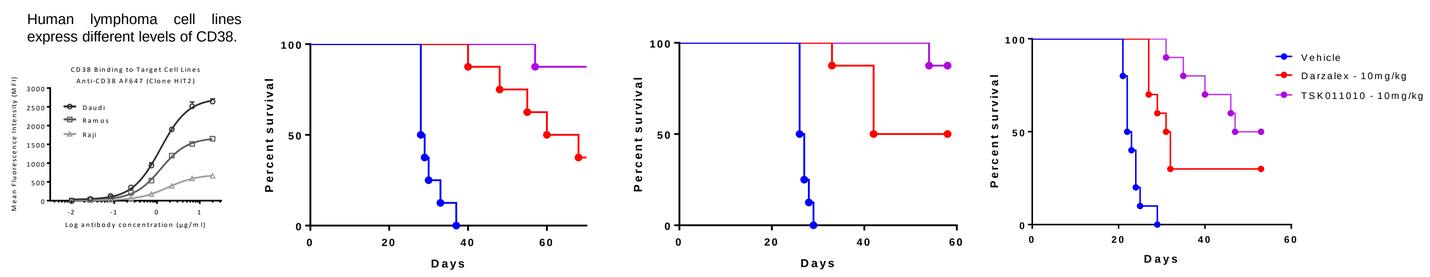
2. Lead candidate antibody TSK011010 – killing of CD38^{high} cells *in vitro* and *in vivo*

a) *In vitro* killing activity of TSK011010



In vitro killing capacity of TSK011010 against CD38-expressing target cells was assessed by ADCC and CDC assays against Daudi cells, and by a reporter ADCP assay (Promega FcgRIIa-H). TSK011010 performed similarly or better for ADCC and ADCP when compared to Darzalex, with slightly less activity in CDC.

b) *In vivo* killing activity of TSK011010 – in disseminated tumour models

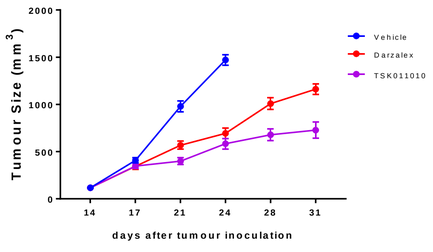


Experimental details:
CB17 SCID mice, n=8 /group
- d0 i.v. injection Daudi, Ramos or Raji cells
- 10mg/kg for each Ab i.p., start d5, twice a week for 3 weeks.

TSK011010 shows strong therapeutic activity against B cell lymphomas (CD38 high expressing Daudi cells, CD38 lower expressing Ramos and Raji cells). *In vivo* activity of TSK011010 in all three models is enhanced compared to Darzalex.

- and in solid tumour model

Experimental details: CB17 SCID n=10/group, 1x10e7 Ramos cells inj. s.c. d0, 10mg/kg started at 100-130mm3 tumours, twice a week for 3 weeks.

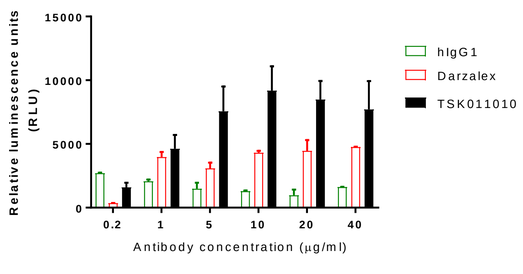


TSK011010: enhanced anti-tumour activity against s.c. injected Ramos cells compared to Darzalex.

Conclusion II: To target CD38 in cancer, we present an anti-CD38 antibody with potent killing activity *in vitro* and *in vivo*, outperforming anti-CD38 reference antibody Darzalex. This antibody is suitable to deplete CD38^{high} expressing target cells, such as tumour cells or suppressive myeloid cells.

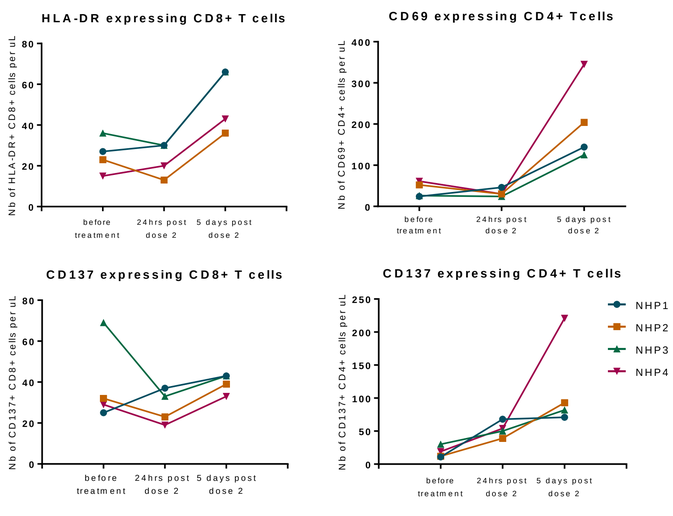
3. Immune-modulatory activity of TSK011010

a) TSK011010 increases T-cell receptor signalling



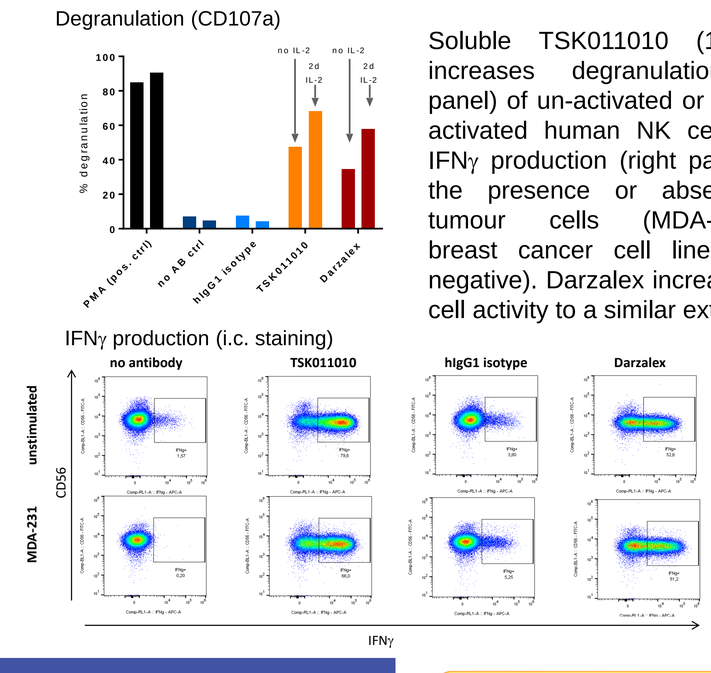
TSK011010, and to a lesser extent Darzalex, increases T-cell receptor signalling, evaluated by NFAT activation in luc_reporter Jurkat cells stimulated with anti-CD38 antibodies cross-linked on the cell surface in the presence of 1µg/ml soluble anti-CD3 AB. Antibody concentration tested from 0.2 - 40µg/ml.

b) Low dose TSK011010 increases T cell activation in non-human primates



Non-human primates (cyno) were treated with 0.03mg/kg TSK011010 i.v. on days 1 and 8. Peripheral T cell frequencies and activation markers were analysed before the first dose, 24hrs and 5 days after the second dose. T cells showed increased activation after dosing, most prominently by upregulation of CD69 and CD137 on CD4 T cells, and HLA-DR on CD8 T cells. No immune activation-related adverse reactions were observed.

c) TSK011010 increases NK cell IFNγ production and degranulation



Soluble TSK011010 (10µg/ml) increases degranulation (left panel) of un-activated or IL2 pre-activated human NK cells, and IFNγ production (right panel) in the presence or absence of tumour cells (MDA-MB-231 breast cancer cell line, CD38 negative). Darzalex increases NK cell activity to a similar extent.

Conclusion III: In addition to depleting CD38^{high} expressing target cells, TSK011010 has the capacity to increase the function of immune effector cells. This dual activity will allow Tusk to fully exploit the therapeutic potential of targeting CD38, not only in haematological malignancies but also in solid tumours.

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