Targeting CD38 beyond haematological malignancies:
a panel of anti-CD38 antibodies with unique functional properties

Nina Eissler[1],Simone Filosto[1], Pascal Merchiers[1], Beatriz Goyenechea[1], Haw Lu[1], Joseph Mabbitt[1], Rahul Chaitanya Khanolkar[1], Paul Smith[1], Sergio A. Quezada[2], Kevin Moulder[1], Dominic Smethurst[1] and Anne Goubier[1]

1. Tusk Therapeutics Ltd., Stevenage Bioscience Catalyst, Accelerator Building, Gunnels Wood Road, SG1 2FX Stevenage, United Kingdom
2. Cancer Immunology Unit, University College London Cancer Institute, London WC1E 6DD, United Kingdom

Background: Because of its high level of expression on haematological cancers, depleting antibodies targeting CD38, an ectoenzyme with hydrolase and cyclase activity, have been generated and showed clinical benefits in particular against multiple myeloma. Interestingly, CD38 is not restricted to haematological cancer cells but also expressed on different immune subsets including NK and effector T cells, exhausted PD-1+ T cells, suppressive myeloid cells, and regulatory T and B cells. Anti-CD38 antibodies will therefore not only impact CD38-expressing tumour cells but also both effector and suppressive immune cells, as illustrated by the increased interest for CD38 as a target in Immuno-Oncology.

Materials and Methods: An antibody production and screening campaign has been initiated resulting in a panel of fully human CD38-binding antibodies. The antibodies have been screened for their potential to induce ADCC, apoptosis, ADCP, and CDC. The potential to deplete CD38-expressing cells has been verified *in vivo* in lymphoma engrafted mice. Additionally, the antibodies have been evaluated for their ability to stimulate effector T cell and NK cell, in vitro on human cells and in vivo in non-human primates (NHP).

Results: We have produced a portfolio of antibodies targeting distinct epitopes of CD38. These antibodies exert ADCC when directed against CD38-overexpressing targets, while showing differential capacity to induce ADCP and CDC. This translated into differential inhibition of *in vivo* growth of human lymphoma tumours in SCID mice. Most interestingly, some of antibodies augment TCR-induced activation of human T cells *in vitro*, and of NHP CD4 and CD8 T cells in vivo. Regarding CD38 enzymatic function, i.e. hydrolase and cyclase activity, our antibodies display differential profiles in terms of blockade or augmentation of both activities. Effects of anti-CD38 antibodies on immune effector cells, suppressive immune cells as well as tumour cells will be further explored in patient-derived *ex vivo* tumour models.

1. Panel of therapeutic anti-human-CD38 antibodies

1a) KD values and binning groups

Ab ID	KD (M) Avidity	Cross-binning groups
TSK01	1.34E-10	D
TSK02	9.57E-11	E
TSK03	1.81E-11	A
TSK04	1.77E-10	B
TSK05	7.35E-11	H
Darzalex	1.80E-10	F

Fig 1a. hIgG1, CD38 binding antibodies were selected from a discovery campaign at Adimab. KDs and cross-binning groups for selected antibodies are indicated.

1b) Binding to recombinant CD38

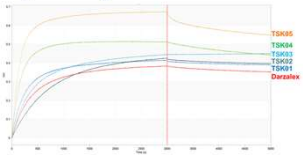
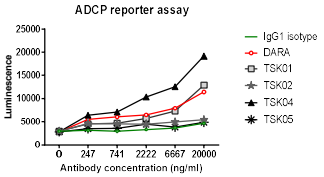


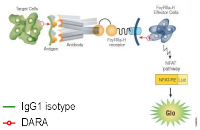
Fig 1b. The kinetics of Tusk Ab interaction with human CD38 were investigated by Octet-based analysis, using CD38-his tag on Ni-NTA biosensors.

2. Tumour-killing activity *in vitro*

2a) Antibody-dependent cellular phagocytosis (ADCP)

© Promega technical manual – G9991, G9995
ADCP reporter assay. NFAT activation in Jurkat cells triggered by FcgRIIa-H engagement is analysed. Target cells: CD38 expressing Raji cell line.





ADCP	activity
TSK01	yes
TSK02	no
TSK03	nd
TSK04	yes
TSK05	no
Darzalex	yes
Isotype hIgG1	no

Fig 2a. Our panel shows differential capacity to induce ADCP ranging from **no** to **high** activity in the reporter assay.

2b) Antibody-dependent cellular cytotoxicity (ADCC)

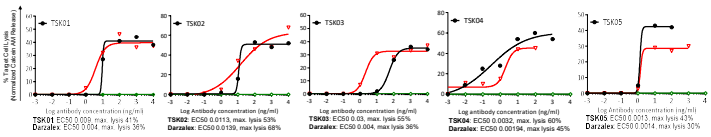


Fig 2b. All antibodies show high potential to induce ADCC against CD38 expressing Daudi cells, comparable to benchmark antibody Darzalex.

2c) Complement-dependent cytotoxicity (CDC)

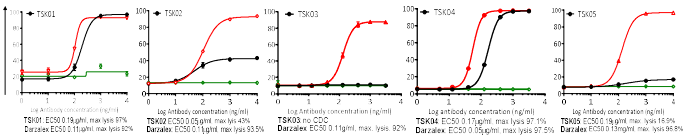
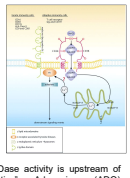


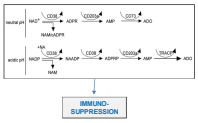
Fig 2c. The potential of our antibodies to kill CD38 expressing Daudi cells via complement dependent cytotoxicity (CDC) ranges from antibodies inducing strong CDC, comparable to benchmark antibody Darzalex, to medium or no CDC induction.

3. Modulation of CD38 enzymatic activity

Cyclase activity: CD38 products (cADPR and NAADP) can be internalised and initiate Ca2+ signalling. *Malavasi et al. Blood 2011*



Hydrolase/NADase activity is upstream of the "alternative" Adenosine (ADO) generation pathway. *Horenstein et al. Mol Med. 2016*



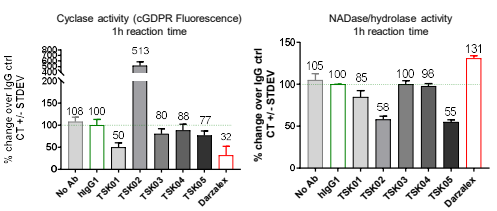
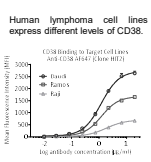
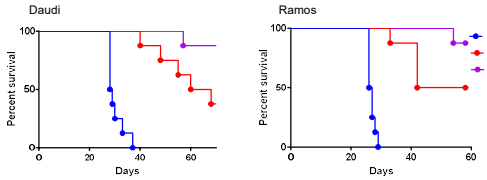


Fig 3. Effect on cyclase and NADase activity in Jurkat. Conversion of surrogate substrates (NGD+ and E-NAD) into fluorescent products. *Cyclase inhibition*: TSK01 and Darazalex strong; TSK03, TSK04 and TSK05 moderate. *Cyclase activation*: TSK02 strong. *NADase inhibition*: TSK01, TSK02, TSK05. *NADase activation*: Darzalex. *No effect*: TSK03 and TSK04.

4. Anti-tumour activity *in vivo*

4a) *In vivo* activity in disseminated tumour models

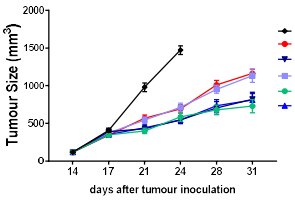




Experimental details: CB17 SCID mice, n=8 /group
- d0 i.v. injection Daudi or Ramos
- 10mg/kg for each Ab i.p., start d5, twice a week for 3 weeks.

Fig 4a. TSK04 shows strong therapeutic activity against B cell lymphomas (CD38 high expressing Daudi cells, CD38 lower expressing Ramos cells). *In vivo* activity of TSK04 in both models is enhanced compared to Darzalex.

4b) *In vivo* activity 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

Fig 4b. All antibodies tested show strong anti-tumour activity against s.c. injected Ramos cells. Activities are either equivalent (TSK02) or enhanced (TSK01, TSK04 and TSK05) when compared to Darzalex.

5. Agonist activity of anti-CD38 antibodies

5a) Anti-CD38 antibodies increase T-cell receptor signalling *in vitro*

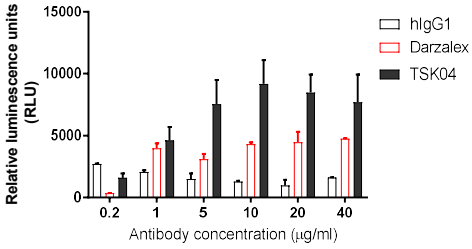


Fig.5a. TSK04 and, to a lower extent, Darzalex, increase T-cell receptor signalling as evaluated by measuring NFAT activation in luc_reporter Jurkat cells stimulated with anti-CD38 antibodies cross-linked on the cell surface and in the presence of 1µg/ml soluble anti-CD3. Cells are stimulated with antibody concentrations between 0.2 and 40µg/ml.

5b) Low dose anti-CD38 antibody increases T cell activation in non human primates

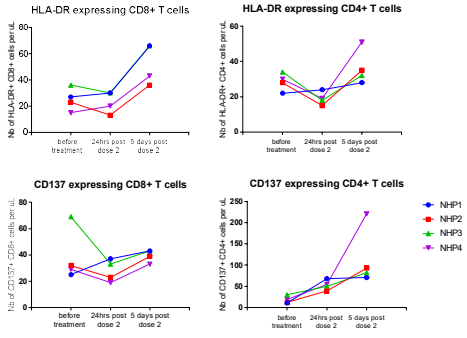


Fig. 5b. Non-human primates (cynomolgus monkeys) were treated with 0.03mg/kg TSK04 i.v. on days 1 and 8. We analysed peripheral T cell frequencies and activation markers (CD69, CD137 and HLA-DR) before the first dose, as well as 24hrs and 5 days after the second dose. T cells showed signs of increased 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.

Conclusions: We present a portfolio of CD38-targeting antibodies with distinct activity profile. The broad expression and multiple functions described for CD38 underline the importance of being able to choose from a range of antibodies that can address the different functionalities depending on the most prominent role of CD38 in each disease setting. The new class of anti-CD38 antibodies presented here will be further explored for their potential to improve response rates, especially in solid tumours.