ANTIBODIES, PEPTIDES, AND PAYLOAD FOR ANTIBODY DRUG CONJUGATES

 LICENSING AND COLLABORATIVE OPPORTUNITIES

March 2016

Non Confidential

ADVANCING DISCOVERIES TO BEAT CANCER
CONTENT

- Introduction to ADCs and CRUK’s / CRT’s biologics capabilities
- Summary of antibody and peptide opportunities
- Non-confidential information on antibodies to the following targets:

<table>
<thead>
<tr>
<th>CEA</th>
<th>Muc1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEACAM5/6</td>
<td>CALLA</td>
</tr>
<tr>
<td>αvβ6 integrin</td>
<td>ICAM3</td>
</tr>
<tr>
<td>TCR mimic antibodies (p53)</td>
<td>CD45RO</td>
</tr>
</tbody>
</table>

- Non-confidential information on peptide to αvβ6 integrin
- Novel toxic payload against CDC2L1/2
ANTIBODIES AND ADCS

• ~50% mAb clinical pipeline comprise antibodies against targets relevant to cancer (Reichert, 2013)
• >30 ADCs in clinical development (Panowski et al., 2014)
• Antibodies in clinical development have an approval success rate of 23%:
  – ADCs success rate is ~35% (Reichert, 2014)
• Conjugation of antibodies to cytotoxic drugs or radionuclides improves their potency & effectiveness
• 2 ADCs approved: brentuximab vedotin & trastuzumab emtansine

Market potential for new generation antibodies 2013-2021 (Evans & Syed, 2014)
Next generation antibodies have the potential to reach sales of $5.7b, ADCs comprise about 1/3 of this
CRT/CRUK ANTIBODY-RELATED DISCOVERY & DEVELOPMENT ACTIVITIES

• Target Discovery & Validation capability:
  – Large network of CRUK funded PIs;
  – CRUK translational funding supports development of drug discovery projects;
  – 2 CRUK 6-year therapeutic antibody programmes (started April ‘09).

• CRUK’s new strategy launched May 2014:
  – Increased investment in biotherapeutic discovery
  – Established dedicated biotherapeutic target and drug discovery funding route
  – Announced establishment of joint biotherapeutic discovery lab with Medimmune helping to catalyse a shift towards biologics (CRUK Medimmune Alliance Laboratory)

• Biotherapeutics Development Unit:
  – GMP manufacturing facility for PhI/II clinical trials: 70L microbial bioreactor & 250L mammalian bioreactor
  – MHRA inspected facility

• Late preclinical/early phase clinical development in CRUK’s Drug Development Office
  – Established in 1982
  – Taken over 120 agents in PhI/II studies
  – 6 products have reached the market
  – 32% of agents in the portfolio are biologics (2014)
ANTIBODY AND PEPTIDE OPPORTUNITIES
<table>
<thead>
<tr>
<th>Target</th>
<th>Molecule Type</th>
<th>Cell surface expressed</th>
<th>Evidence of internalisation</th>
<th>Affinity KD</th>
<th>In vivo data available</th>
<th>Patent: application / granted</th>
<th>Entered clinical trial</th>
<th>Licensing opportunity</th>
<th>Collaborative opportunity</th>
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</thead>
<tbody>
<tr>
<td>CEA</td>
<td>Murine IgG Humanised IgG</td>
<td>√</td>
<td></td>
<td>Low nM-pM</td>
<td>√</td>
<td>√</td>
<td>Clinical imaging trial; Ph1 CAR</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>CEACAM5/6</td>
<td>Mouse IgG</td>
<td>√</td>
<td>Evidence in literature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>√</td>
<td></td>
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<tr>
<td>Bivalent CEA- αvβ6 integrin</td>
<td>scFv Humanised IgG</td>
<td>√</td>
<td>√</td>
<td>Low nM-pM</td>
<td>√</td>
<td></td>
<td></td>
<td>√</td>
<td>√</td>
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<tr>
<td>αvβ6 integrin</td>
<td>scFv scFv-Fc Diabody Humanised IgG</td>
<td>√</td>
<td>√</td>
<td>nM</td>
<td>√</td>
<td>√</td>
<td></td>
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<tr>
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<td>peptide</td>
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<td>√</td>
<td>high</td>
<td>√</td>
<td>√</td>
<td></td>
<td>√</td>
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<tr>
<td>TCR mimics (P53)</td>
<td>Mouse IgG Humanised IgG</td>
<td>√</td>
<td>√</td>
<td>µM</td>
<td>√</td>
<td>√</td>
<td></td>
<td>√</td>
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<td>Muc1</td>
<td>Mouse IgG Humanised IgG</td>
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<td>Evidence in literature</td>
<td>µM</td>
<td>√ as a CAR</td>
<td>√ (HMFG1)</td>
<td></td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>CALLA</td>
<td>Mouse IgG</td>
<td>√</td>
<td>Evidence in literature</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>√</td>
</tr>
<tr>
<td>ICAM3</td>
<td>Mouse IgG</td>
<td>√</td>
<td>Evidence in literature</td>
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<td>√</td>
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<tr>
<td>CD45RO</td>
<td>Mouse IgG</td>
<td>√</td>
<td></td>
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<td></td>
<td></td>
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<td>√</td>
</tr>
</tbody>
</table>
DEVELOPMENT OPPORTUNITIES

• Antibodies, peptide and toxic payload available for licensing or collaborative development

• Opportunity to co-develop novel ADCs with PIs

• Targeting molecules and payload also offer the potential to assist technology development through e.g.
  – Accessing an antibody to enable evaluation of potential proprietary payload
  – Testing of linker chemistry
HIGH AFFINITY ANTI-CEA ANTIBODIES
CEA ANTIBODY SUMMARY

Principal Investigator: Prof Kerry Chester leads the Recombinant Antibody Therapeutics Group at the UCL Cancer Institute.

CRT has a series of high affinity (nM) anti-CEA antibodies for further development, including the following ScFvs:

- murine MFE-23
- humanised MFE-23
- SM3E (high affinity humanised form with 1000 fold improvement in CEA 'off rate' compared to MFE-23) – also available as ScFv-Fc
- Whole IgG humanised antibodies (‘ME1’ and ‘ME2’)
- Dual-specific ScFv-Fc – binds CEA and αvβ6

Potential applications include:

- ADC; Radioimmunotherapy; Systemic cytokine therapy; BiTE; T cell therapy; Gene therapy; Diagnosis and imaging of cancer
Two granted US patent applications:

1. **US 7232888 (Priority Date: 1\textsuperscript{st} July 2003)**
   - Claims to antibody protein sequence SM3E (antibody produced in collaboration with MIT, CRT has commercialisation rights)

2. **US 7626011 (divisional of US 7232888)**
   - also claims nucleic acid sequence and expression vectors
ANTI-CEA ANTIBODY (MFE-23) CLINICAL DATA

Murine versions of MFE-23 antibody have gone into clinical trials (reviewed in Chester et al., Dis Markers. 2000;16(1-2):53-62)
- MFE-23 scFv antibodies tolerated well in clinical imaging studies and as part of an enzyme prodrug therapy (Mayer et al., 2006)
- Feasibility of MFE-23 fused to TCR CD3z chain in 1st generation CAR demonstrated in CRUK Ph1 dose escalation clinical trial (Guest et al., 2014)

Imaging
- Radiolabelled MFE-23 for imaging CEA-expressing tumours
  - SPECT study – 123I-MFE-23
  - Radioimmunoguided surgery (RIGS) – 125I-MFE-23 in primary or metastatic colorectal or pancreatic cancer patients (Mayer et al., 2000. Clin Cancer Res. 6(5):1711)
- Accurate tumour localisation, high tumour-to-blood ratios and lack of toxicity

Production methods
- Proteins produced in E. Coli or P. Pastoris and there were no apparent production issues/limitations
EXPRESSION OF CEA IN HUMAN CANCER TISSUE

Mouse anti-CEA (A5B7) antibody, Celltech (1/200 dilution)

- CEA expression more abundant deeper within the tumour mass than on the surface of the solid tumour mass
GENERATION OF scFv TO CEA

- MFE-23 antibody engineered to increase retention time in tumour relative to normal tissue

- Murine MFE-23 (mMFE-23) resurfaced to present a more human Fv framework surface (hMFE-23).

- Sm3E scFv (high affinity anti-CEA antibody) engineered (Graff et al., 2004) with effectively irreversible binding to CEA:

  \[
  K_{off} = 3.0 \times 10^{-7} \text{ s}^{-1} \text{ for a dissociation half-time of 27 days (25°C)}
  \]

  \[
  K_{off} = (1.85 \pm 0.73) \times 10^{-6} \text{ s}^{-1} \text{ averaged from ELISA and cell surface assays, and a dissociation half-time of several days (37°C, ie. physiological conditions)}
  \]
GENERATION OF scFv TO CEA AND/OR AVB6

• **SM3E** was fused to mouse IgG2a Fc

• **SM3EL-Fc** (anti-CEA antibody)
  – compared to SM3E, cysteine residues and a longer linker were used to increase stability (Schumacher FF et al. Sci Rep 2013; 3: 1525)

• **CB5L-Fc** (cross-reacts with CEA and avb6)
  – Created through insertion of a portion of the VP1 loop of foot and mouth disease virus (FMDV) into the anti-CEA parent antibody MFV23)
  – More details available under CDA

• CEA binding retained in dual-specific antibody (CB5L-Fc) but reduced compared to mono-specific SM3EL-Fc
**CHARACTERISATION OF BINDING TO TARGETS**

Anti-CEA Ab (red) bound to CEA-expressing cells only: stably-transfected melanoma cell line (A375CEA) and Capan-1 cells

<table>
<thead>
<tr>
<th></th>
<th>Apparent $K_D$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capan-1</td>
<td>A375-CEA</td>
</tr>
<tr>
<td>SM3L-Fc</td>
<td>7.65 ± 0.8</td>
</tr>
</tbody>
</table>

**Expression of CEA in cells:**
- Binding was specific to CEA
- Nanomolar affinity to CEA in cells
- "Apparent KD" - on immobilised CEA, both mono- and bi-specific antibodies showed a predictive KD in the low picomolar range by SPR
  - Off-rate constants too slow for accurate KD calculation
  - Cell-based assays are more biologically-relevant
BIOLOGICAL EFFECTS OF scFv-Fc IN CELLS

CEA antibodies do not internalise. Only dual-specific and αvβ6 Abs internalise

- There is evidence from a commercial partner that there was good cleavage of their drug/toxin and entry into the target cell following binding of the antibody to the cell surface.

Internalisation of ScFv-Fcs resulted in a reduction of membrane-bound antibody fragments. Presence of ab in cell membrane was analysed by flow cytometry, after incubation at 37°C same levels of SM3EL-Fc were bound to cells. B6L-Fc and CB5L-Fc bound to cells was significantly reduced. CB5L-Fc showed decreased rate of internalisation as it also targeted CEA. Data presented as mean ±SD of 4 independent expts, significance assessed by Student’s t-test.
IN VIVO STUDIES

Antibodies labelled with AlexaFluor-546:
• Injected in mice bearing Capan-1 xenografts (100µg per animal).
• Tumour and blood were taken 3, 6 and 24h after injection.

ScFv-Fc detection in serum

• ScFv-Fc were found in serum up to 24h after injection.
CEACAM5/6 ANTIBODY
BY114: DUAL TARGETING ADC CANDIDATE
CEACAM 5/6 mAb SUMMARY

<table>
<thead>
<tr>
<th>Target</th>
<th>Clone</th>
<th>Isotype</th>
<th>PI Institute</th>
<th>Host</th>
<th>Reactivity</th>
<th>Immunogen</th>
<th>Recommended assays</th>
<th>Ab specific Internalisation data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both CEACAM 5 and CEACAM 6</td>
<td>By114</td>
<td>IgG1 kappa</td>
<td>Karen Pulford University of Oxford</td>
<td>mouse</td>
<td>human</td>
<td>B cell Lymphoma cells</td>
<td>FACS IHC IF IP WB</td>
<td>unknown</td>
</tr>
</tbody>
</table>

CEACAM 5/6 OPPORTUNITY


- Hybridoma available

- Opportunity to licence hybridoma to develop antibody as an ADC
CEACAM5 & 6 TARGET CHARACTERISTICS

CEACAM5 (Aliases: CEA, CD66e); CEACAM6 (NCA, CEAL, CD66c)
• CEACAM 5 & 6 are GPI-anchored cell surface glycoproteins
  – They have an extracellular region containing 3 immunoglobulin-like domains and are linked to the plasma membrane via a glycophosphoinositol-anchor (GPI-A)
• CEACAM 5 & 6 are capable of homo- and hetero-typic interactions with other CEACAM family members on the same cell or on adjacent cells enabling them to function as intercellular signalling molecules (Taheri M. et al., 2000, J. Biol. Chem.)

Rationale for CEACAM5/6 as an ADC
• CEACAM5 & 6 are localised on the cell membrane
• CEACAM5 is capable of being internalised (Schmidt M., 2008, Cancer Immunol. Immunother.)
• CEACAM5 or CEACAM6, or both, are overexpressed in as many as 70% of all human tumours (Chan C.H.F., 2007, Curr. Oncol.)
• CEACAM5 antibodies are in Phase II development as ADCs:
  – Immunomedics (IMMU-130; colorectal cancer)
  – Immunogen in collaboration with Sanofi (SAR-408701; colorectal, lung, NSCLC, cervical, GI, pancreatic, bladder, ovarian, biliary, endometrial and oesophageal)
  – Helix Biopharm developing single domain antibody fragment (L-DOS-47) as an ADC for lung cancer and in pre-clinical development for breast cancer
CEACAM5 ANTIBODIES ARE INTERNALISED

A: anti-CEA abs are trafficked into intracellular pools at 37°C. LS174T cells were labelled with Alexa-488-IgG M8515a on ice for 24h at 4°C or 37°C. Cells were labelled with goat-anti-mouse-PE and imaged. At 37°C a significant fraction of anti-CEA abs are endocytosed into an intracellular pool where they are not labelled by the secondary ab. Scale bar 20µm.

B: Internalised anti-CEA abs partially co-localise with markers of endocytosis. LS174T cells were incubated at 37°C O/N with fluorescently labelled anti-CEA scFvs. Cells were washed and incubated with fluorescently labelled markers of endocytic and lysosomal pathways. The anti-CEA scFvs show partial but incomplete co-localisation with all endocytic pathway markers. Scale bar 10µm.

CEACAM5 & 6 TUMOUR TISSUE EXPRESSION

IHC of CEACAM5 & 6 localisation using MN-15, MN-3 (binds CEACAM5 and 6); MN-14 (CEACAM5) antibodies (Immunomedics; Blumenthal et al., BMC, 2007).

CEACAM5 is expressed in:
- colon adenocarcinoma
- poorly differentiated pancreatic carcinoma
- large cell lung carcinoma

CEACAM6 is expressed in:
- colon adenocarcinoma
- moderately differentiated to poorly differentiated pancreatic carcinoma
- lung adenocarcinoma, squamous lung carcinoma and large cell lung carcinoma
CEACAM5 & 6 TUMOUR TISSUE EXPRESSION
IHC of CEACAM5 & 6 localisation using MN-15, MN-3 (binds CEACAM5 and 6); MN-14 (CEACAM5) antibodies (Immunomedics; Blumenthal et al., BMC, 2007).

CEACAM6 is expressed in:
- liver metastasis
- endometrioid carcinoma
- prostate carcinoma
- breast cancer
**CEACAM5 & 6 TUMOUR TISSUE EXPRESSION**

Example images of CEACAM5 & 6 localisation (representative using 6 antibodies. **CEACAM5**: HPA011041, HPA019758 (both from Sigma Aldrich), M7072, A0115 (both DakoCytomation); **CEACAM6**: HPA011041 (Sigma-Aldrich), D028-3 (MBL) (Pontén F., 2007, J. Pathol.).

- **CEACAM5** is overexpressed in cancers of the gastrointestinal tract, pancreas, liver, gallbladder, lung, breast, female reproductive system, medullary thyroid, urinary bladder, and prostate (Pontén F., 2007, J. Pathol.)
- **CEACAM6** is overexpressed in cancers of the colon, stomach, pancreas, lung, breast, female reproductive system and acute lymphoblastic leukaemia (Kanderová V. et al., 2010 Exp. Haematol.; Pontén F., 2007, J. Pathol.)
CEACAM 5 & 6 NORMAL TISSUE EXPRESSION

Example images of CEACAM5 & 6 localisation (representative using 6 antibodies. **CEACAM5**: HPA011041, HPA019758 (both from Sigma Aldrich), M7072, A0115 (both DakoCytomation); **CEACAM6**: HPA011041 (Sigma-Aldrich), D028-3 (MBL) (Pontén F., 2007, J. Pathol.).

- **CEACAM 5/6 BY114** antibody can be used to distinguish normal cells from neoplastic cells of granulocyte origin (Mayne et al., 1993, Br. J. Haematol.)
- **CEACAM5**: membrane and cytoplasmic expression in gastrointestinal tract and squamous epithelia. Most abundantly expressed in large intestine (Pontén F., 2007, J. Pathol.)
- **CEACAM6**: expressed in the bone marrow, colon, oesophagus, gallbladder, lung, rectum (Pontén F., 2007, J. Pathol.)
CEACAM 5 & 6 IN DISEASE

• Although CEACAM 5 & 6 are consistently overexpressed in a number of cancers their role in tumour biology is not well understood

• CEACAM 5 & 6 expression is correlated with metastatic disease and poor prognosis (Beauchemin N. et al., 2013, Cancer Metastasis Rev.; Blumenthal R.D. et al., BMC Cancer, 2007; Blumenthal R.D. et al., 2005, Cancer Res.)

• CEACAM 5 & 6 are expressed on epithelial tumour derived microvesicles, which have been implicated in promoting metastasis, tumour-stroma interactions and angiogenesis (Muturi H.T. et al., 2013, PLOS One)

• CEACAM5 & 6 are also markers for Paroxysmal Nocturnal Hemoglobinuria, an acquired hemolytic anemia caused by the complement system (Mayne et al., 1993, Br. J. Haematol.)
AVB6 ANTIBODIES
AVB6 ANTIBODY SUMMARY

Principal Investigator: Prof Kerry Chester (University College London)

- Experienced in generating antibody-based medicines for imaging and therapy for cancer

Series of novel antagonistic antibodies targeting the integrin αvβ6 generated

- Anti-αvβ6 antibodies (sequences and protein) available for licensing or collaboration
- Selective targeting of αvβ6+ve tumours in vitro and in vivo demonstrated
- Antibodies inhibit αvβ6-mediated migration
- Broad utility in oncology and fibrotic disease indications
- Utility as a naked antibody, BiTE, or as an ADC
- Different Ab formats available depending on intended use: scFv, diabodies, IgG
AVB6 ANTIBODY SUMMARY CONT.

Intellectual property
• Patents granted in US (US12/680320) and AU (AU2008303393)
• Patent applications in EP, CA and US (notice of allowance in US)
• Patents granted in US (US12/680320) and AU (AU2008303393)
• Claims under prosecution cover composition of matter and method claims covering insertion of amino acid sequences directing binding specificity (inc. consensus αvβ6 binding seq) into a parent antibody with MFE-23 (anti-CEA) CDR sequences. Allowed claims in US cover antibody, conjugates and methods of cancer treatment.
AVB6 AS A SELECTIVE TARGET IN CANCER

Cell surface-expressed, RGD-directed integrin

- Expression is epithelial specific (Breuss et al, 1995)
- Low/undetectable expression in normal adult tissues, elevated during tissue remodelling, fibrosis and multiple cancer indications (Breuss et al, 1995; Thomas et al, 2006)
- Estimated 279,000 new αvβ6+ve tumours diagnosed each year in UK and US combined (excluding melanoma)

<table>
<thead>
<tr>
<th>Tumour site</th>
<th>% αvβ6 positive</th>
<th>USA + UK incidence</th>
<th># of αvβ6+ve tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix</td>
<td>92%</td>
<td>13,873</td>
<td>12,763</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>64%</td>
<td>22,900</td>
<td>14,656</td>
</tr>
<tr>
<td>Breast</td>
<td>43%</td>
<td>227,960</td>
<td>98,023</td>
</tr>
<tr>
<td>Lung</td>
<td>35%</td>
<td>253,020</td>
<td>88,557</td>
</tr>
</tbody>
</table>

Prof John Marshall & CRUK
HIGH AVB6 EXPRESSION CORRELATES WITH POOR PROGNOSIS

Colon carcinoma
• Reduction in median survival from 16.5 years to 5 years (Bates et al, 2005)

Cervical carcinoma
• Reduction in 5yr survival from 91% to 54% (Hazelbag et al, 2007)

Lung cancer
• Prognostic in early and late-stage cancers, with an independent hazard ratio of 1.9 (Elayadi et al, 2007)

Oral squamous cell carcinoma
• Correlates with progression to malignant disease (Hamidi et al, 2000)

Increased $\alpha_v\beta_6$ expression associated with pro-invasive and aggressive phenotype
• 77% of metastatic lesions overexpress $\alpha_v\beta_6$
RATIONALE: TARGETING AVB6 REDUCES TUMOUR GROWTH IN VIVO

αvβ6 (and αvβ8) blocking antibody (264RAD) generated by Oncology iMED, AstraZeneca, reduces tumour growth and metastasis (Eberlein et al, Oncogene, 2012)

Inhibition of Detroit 562 tumour growth. Established tumours were treated with indicated doses of 264RAD twice weekly.

Inhibition of 4T1 tumour growth. Established tumours were treated with 20mg/kg 264RAD twice weekly.
GENERATION OF HIGH AFFINITY AVB6 SPECIFIC PEPTIDES

- Used peptides derived from natural ligands (TGFβ1-LAP and Foot and Mouth Disease Virus) as a starting point
- Most potent peptides possessed RGDLXXL/I motif
- Discovered that affinity correlated with ability to form alpha-helical structure (DiCara et al., 2007)

A20FMDV  A20LAP  A20FMDV2
Increasing potency
Peptide A20FMDV2 can re-direct antibodies to αvβ6  (Kogelberg et al., J Mol Biol, 2008)

• Series of anti-αvβ6 antibodies have been generated through insertion of a 17aa portion of A20FMDV2 into CDR H3 loop of MFE-23 (murine scFv reactive with CEA) resulting in specificity for αvβ6 with no binding to other integrins or CEA
  – Selective – do not bind α5β1, αvβ3, αvβ5, αvβ8 or CEA
  – Block integrin-mediated invasion
  – Internalised
  – Humanised ab retains binding affinity to human αvβ6 of 2.78nM (Biacore)
GENERATION OF SCFV-FC TO AVB6

• SM3E (humanised, high affinity anti-CEA Ab) was fused to mouse IgG2A Fc, then modified as follows:
• Insertion of VP1 loop resulted in gain of binding to integrin αvβ6, while retaining binding to CEA (CB5L-Fc)
• 3 point mutations inhibited binding to CEA, while retaining binding to αvβ6 (B6L-Fc)
### SUMMARY OF AVB6 ANTIBODY SERIES AVAILABLE

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Format</th>
<th>CEA reactive</th>
<th>αvβ6 reactive</th>
<th>Inhibit αvβ6-mediated migration</th>
<th>Inhibit αvβ6-mediated adhesion</th>
<th>Internalised</th>
<th>In vivo targeting of αvβ6-expressing tumours</th>
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<tbody>
<tr>
<td>B6-1</td>
<td>Murine scFv</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Not measured</td>
<td>Not measured</td>
<td>Not measured</td>
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<tr>
<td>B6-2</td>
<td>Murine scFv</td>
<td>No</td>
<td>Yes</td>
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<td>Yes</td>
<td>Yes</td>
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<tr>
<td>B6-3</td>
<td>Humanised scFv</td>
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<td>Yes</td>
<td>Not measured</td>
<td>Yes</td>
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<td>Not measured</td>
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<tr>
<td>B6-3</td>
<td>Humanised Diabody</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>B6L-Fc</td>
<td>scFv-Fc</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>CB5L-Fc (‘Dual Ab’)</td>
<td>scFv-Fc</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>ME3 &amp; ME4 (AvB6-specific)</td>
<td>Humanised whole IgG</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Not measured</td>
</tr>
<tr>
<td>ME5 (‘Dual Ab’)</td>
<td>Humanised whole IgG</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Not measured</td>
</tr>
</tbody>
</table>

scFv, diabody, scFv-Fc, dual-specific CEA and avb6, and humanised whole IgG Abs
ANTI-AVB6 ScFv SELECTIVELY BINDS TO AVB6-EXPRESSING CELLS

Selective binding of B6.3 diabody to αvβ6 on cells
• Antibodies demonstrate specific concentration-dependent binding to αvβ6 transfected cells (versus those expressing other integrins).
• Both cell lines express equivalent levels of several other RGD-binding integrins. (Kogelberg et al., 2013)

Avβ6+ve cell line

Avβ6-ve cell line
ANTI-AVB6 ANTIBODIES INTERNALISED BY AVB6-EXPRESSING CELLS

Antibodies are internalised by $\alpha\nu\beta6$-expressing cells

- Localisation of B6.3 dia body in A375P $\beta6$ (avb6+ve) cells by confocal microscopy.
- Membrane pattern of staining at 4°C
- Internalisation at 37°C (cells incubated for 30mins, 1h and 3h)

Kogelberg et al., 2013
ANTI-AVB6 DIABODY INHIBITS AVB6-MEDIATED MIGRATION

B6.3 diabody (humanised version of αvβ6-specific B6.2 murine scFv) inhibits αvβ6-mediated migration *in vitro*

- B6.3 diabody treatment (and other Ab formats) inhibited migration of A375Pβ6 cells (avb6+ve) to LAP and migration of Capan-1 cells to fibronectin and LAP

Kogelberg et al., 2013
B6.3 SPECIFICALLY TARGETS AVB6-EXPRESSING TUMOURS IN VIVO

B6.3 diabody specifically localises to αvβ6-expressing tumours in vivo

- A375Pβ6 (avb6+ve, left) and A375Ppuro (avb6-ve, right) cells were injected subcutaneously on opposite shoulders and $^{99m}$Tc-labeled B6.3 diabody was injected iv once tumours developed. SPECT/CT cross section imaging of mouse at 5h.

- Highest normal tissue activity in kidneys – typical pattern for radio-metal-labelled compounds due to excretion of $^{99m}$Tc-labeled compound by this organ

Tumour-to-Blood ratios of 40 (avb6+ve tumour) vs 15.5 (avb6-ve tumour) at 24 h
AVB6 PEPTIDES FOR TUMOUR TARGETING AND IMAGING
SUMMARY

Principal Investigator: Prof John Marshall (BCI, Queen Mary University of London)

High affinity αvβ6-specific lead peptide A20FMDV2

• Novel and proprietary peptides with high affinity and selectivity for integrin αvβ6
• Lead peptide selectively targets αvβ6+ve tumours and fibrotic lesions in vivo for imaging and therapy
• Avβ6 is highly expressed in many tumours and clinically correlates with poor prognosis
• In vivo proof of concept for PET & SPECT imaging with radiolabelled A20FMDV2
SUMMARY CONT.

Intellectual property
• Granted patents in US (US 8383593) and JP (JP 5449774) cover A20FMDV2 lead peptide and other peptides in the series
• Patent applications in EP, CA and US (continuation of granted patent)
• Claims under prosecution cover broadest consensus sequence for αvβ6 binding, conjugation of the peptide to therapeutic moieties and methods of diagnosis, prognosis and treatment

Opportunity
• Sequences of peptide available
• Licensing or collaborative opportunity to develop peptide for therapeutic or imaging application
AVB6 AS A SELECTIVE TARGET IN CANCER
αvβ6 expression is up-regulated in cancer

• Correlates with poor prognosis
  – Colon cancer - reduction in median survival from 16.5 years to 5 years (Bates et al, 2005)
  – Cervical carcinoma - reduction in 5yr survival from 91% to 54% (Hazelbag et al, 2007)
  – Lung cancer - prognostic in early and late-stage cancers, with an independent hazard ratio of 1.9 (Elayadi et al, 2007)
  – Oral squamous cell carcinoma - correlates with progression to malignant disease (Hamidi et al, 2000)

• Increased αvβ6 expression associated with pro-invasive and aggressive phenotype
  – 77% of metastatic lesions over express αvβ6

Cell surface-expressed, RGD-directed integrin

• Expression is epithelial specific (Breuss et al, 1995)

• Low/undetectable expression in normal adult tissues, elevated during tissue remodelling, fibrosis and multiple cancer indications (Breuss et al, 1995; Thomas et al, 2006)

• Estimated 279,000 new αvβ6+ve tumours diagnosed each year in UK and US combined (excluding melanoma)

<table>
<thead>
<tr>
<th>Tumour site</th>
<th>% αvβ6 positive</th>
<th>USA + UK incidence</th>
<th># of αvβ6+ve tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix</td>
<td>92%</td>
<td>13,873</td>
<td>12,763</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>64%</td>
<td>22,900</td>
<td>14,656</td>
</tr>
<tr>
<td>Breast</td>
<td>43%</td>
<td>227,960</td>
<td>98,023</td>
</tr>
<tr>
<td>Lung</td>
<td>35%</td>
<td>253,020</td>
<td>88,557</td>
</tr>
</tbody>
</table>

Prof John Marshall & CRUK
RATIONALE: TARGETING AVB6 REDUCES TUMOUR GROWTH IN VIVO

αvβ6 (and αvβ8) blocking antibody (264RAD) generated by Oncology iMED, AstraZeneca, reduces tumour growth and metastasis (Eberlein et al, Oncogene, 2012)

Inhibition of Detroit 562 tumour growth. Established tumours were treated with indicated doses of 264RAD twice weekly

Inhibition of 4T1 tumour growth. Established tumours were treated with 20mg/kg 264RAD twice weekly
GENERATION OF HIGH AFFINITY AVB6 SPECIFIC PEPTIDES

- Used peptides derived from natural ligands (TGFβ1-LAP and Foot and Mouth Disease Virus) as a starting point
- Most potent peptides possessed RGDLXXL/I motif
- Discovered that affinity correlated with ability to form alpha-helical structure (DiCara et al., 2007)

A20FMDV2 is a high affinity αvβ6-specific peptide
- Based on structural motif (RGD-helix-hairpin) that confers high potency to αvβ6
- Highly specific for αvβ6 (>1000 fold versus other integrins)
- Selectively binds to αvβ6+ve tumours \textit{in vivo}
**IN VITRO PROPERTIES OF LEAD PEPTIDE A20FMDV2**

**Specificity**
- A20FMDV2 is highly αvβ6-specific (>1000 fold versus other integrins)
- A375 tool paired melanoma cell lines express other integrins α5β1, αvβ3, αvβ5,αvβ8

FACS analysis using increasing concentrations of biotinylated A20FMDV2

![FACS analysis](image)

**Inhibition of migration**
- A20FMDV2 potently inhibits αvβ6-dependent cell adhesion and migration

![Inhibition of migration](image)
A20FMDV2 BINDS WITH HIGH AFFINITY AND INTERNALISES

High affinity binding
• Binding is highly stable (not displaceable with EDTA)
• A20FMDV2 binds to αvβ6 with affinity constant of 1.73 +/- 0.46 nM (saturation binding assays of $^{111}$In-DTPA-A20FMDV2 with A375Pβ6 cells, Leung et al, 2012)
  – DTPA alone has no effect on specificity or affinity of A20FMDV2 (Saha et al, 2010)

A20FMDV2 is rapidly internalised into tumour cells
• 50% radiolabelled A20FMDV2 is rapidly internalized into A375Pβ6 cells within 20 min and reaches 89%–96% by 60 min (Leung et al, 2012)
• Majority of A20FMDV2 internalised into cytoplasm by 30 mins (confocal microscopy) (Saha et al, 2010)
A20FMDV2 CAN BE USED TO IMAGE AVB6 +VE TUMOURS

A20FMDV2 is able to target to tumour cell lines that endogenously express αvβ6

NanoSPECT-CT imaging of $^{111}$Indium-A20FMDV2

Saha et al, J Pathol, 2010

Hausner et al, Cancer Res, 2009

DCIS Breast cancer cell line xenograft  CA1a Breast cancer cell line xenograft

Selective uptake of A20FMDV2 in tumour versus skeletal muscle of > 15:1

BxPC3 pancreatic cancer xenograft

MicroPET imaging of $[^{18}\text{F}]$FBA-(PEG28)$_2$-A20FMDV2
A20FMDV2 CAN TARGET TOXIC PAYLOADS TO AVB6-EXPRESSING CELLS

• Proprietary toxin (third party) conjugated A20FMDV2 has 200-fold selectivity for killing αvβ6+ve versus αvβ6-ve cells in vitro

• A20FMDV2-toxin conjugate shows αvβ6-specific tumour therapy in vivo using avb6-expressing xenografts

• Note: Data cannot be shown due to confidentiality agreement with the company concerned
IN VIVO STABILITY AND CLEARANCE OF A20FMDV2

A20FMDV2 is rapidly degraded ...

• Serum proteases degraded peptide into 3 fragments (Hausner et al., Cancer Res, 2009)
• Half-life of 4 hours in serum ex vivo (Saha et al., J Pathol, 2010)

... and excreted

• 10 mins after iv injection <4% 18F-A20FMDV2 remained in blood, 0.27% at 1 hr (Hausner et al., Cancer Res, 2009)
• 1 hr after iv injection 0.3% 111Indium-A20FMDV2 remained in blood (Saha et al., J Pathol, 2010)
**IN VIVO STABILITY AND CLEARANCE OF A20FMDV2**

In vivo properties can be improved

- PEGylation (Hausner et al., Cancer Res, 2009)
- Tumour : blood ratios of >150:1 have been achieved
- New A20FMDV2 peptide variants generated (in collaboration with University of Auckland) retaining specificity for αvβ6 (confidential information due to new patent filing)
- Lead variants remained 75-80% intact after 24 hour incubation in human plasma at 37°C
- Improved tumour targeting relative to A20FMDV2
- Enhanced binding to αvβ6 on cells (flow cytometry)
TCR MIMIC ANTIBODIES (P53)
OVERVIEW OXFORD THERAPEUTIC ANTIBODY GROUP

CRUK therapeutic antibody programme

• £2.5m over 5 years

• Principal Investigator: Professor Alison Banham
  – Scientist with expertise in production and characterisation of antibodies;
  – Two senior postdoctoral scientists

• Collaborators include:
  – Professor Adrian Harris - academic clinician with extensive experience in angiogenesis and clinical trials
  – Professor Penny Handford - scientist with biochemical studies of molecular mechanisms expertise
  – Professor Susan Lea - scientist with protein:protein structural biology expertise.
T-CELL RECEPTOR MIMIC (TCRm) ANTIBODY APPROACH

• Targeting intracellular proteins exclusively presented on cancer cell surface

• Protein is digested in the proteasome and presented on the cell surface via the HLA system

• HLA is a locus of genes that encode proteins on cell surface responsible for regulating the immune system
  • Human version of MHC

Target
• Antibodies developed against wild type region of p53 as proof of concept

Neefjes et al. 2003 Nat Rev Immunol
P53 TCRm ANTIBODY

P53 target

- Literature based peptide – previously used in vaccines, but not by other TCRms.
- Limited to specific MHC haplotypes e.g. HLA-A*0201 (restricted to caucasian ethnicity)
- Abs are made to the peptide presented by the MHC protein i.e. overlaps

Results

- Antibody only binds cancer cells expressing correct HLA class and p53
  - No expression detected on 16 normal human derived PBMCs (high p53 expression)
  - In vitro data for epitope binding and potential peptide cross reactivity
- FACS analysis suggests antibody is internalised
- Antibody can stimulate in vitro ADCP, CDC and ADCC comparable to Rituximab
- Naked antibody causes significant reduced tumour growth in established xenograft tumours. Patent application filed August 2015
OPPORTUNITIES

Use TCRm approach to develop ADCs against novel targets
  • Utilise a chimeric (mouse/human) HLA receptor as epitope

Develop p53 TCRm antibody via a collaboration or licence
  • Hybridoma available (molG1)
  • Antibody has been humanised by Lonza
  • Tested isotype switched versions (outsourced) molG2a & huG1

  • More information available under CDA
MUC1 ANTIBODIES
# ANTI-MUC1 ANTIBODIES

<table>
<thead>
<tr>
<th>mAb</th>
<th>HMFG1 (hybridoma, mouse IgG1)</th>
<th>HMFG2 (hybridoma, mouse IgG1)</th>
<th>SM3 (hybridoma, mouse IgG1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principle Investigators</strong></td>
<td>Prof. Joyce Taylor-Papadimitriou and Dr. Joy Burchell (King’s College, London)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IP</strong></td>
<td>US Patent (exp 2018)</td>
<td>Material rights (no patent protection)</td>
<td></td>
</tr>
<tr>
<td><strong>Key Characteristics</strong></td>
<td>Recognises under-glycosylated 20-mer repeat, binds epitope “PDTR” (Taylor-Papadimitriou et al, 1981) •Good tumour/normal distinction •Humanised antibody available (Verhoeyen, ME et al, Immunology 1993) •naked humanised antibody was developed to Phase 2 trials (no tox) •IgG internalises rapidly within 15 minutes (Pericleous LM et al., BJC, 2005) •Humanised IgG has $K_d = 1-5 \times 10^{-7}$ M</td>
<td>• Recognises under-glycosylated epitope: “DTR” • Good tumour/normal distinction • Used in multiple radiolabelled imaging trials in ovarian, breast, gastrointestinal, and bladder cancers • Greater tumour/blood distribution compared to brentuximab vedotin (Fromm et al., Clin Lymph, Myeloma &amp; Leuk 2012) • Efficacy demonstrated as a CAR in vivo (Willkie et al, I Immunol 2008)</td>
<td>• Recognises under-glycosylated epitope: “APDTRP” • Good tumour/normal distinction</td>
</tr>
<tr>
<td><strong>Internalisation</strong></td>
<td>Internalisation demonstrated with other anti-MUC1 antibodies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### BINDING CHARACTERISTICS OF MUC1 ANTIBODIES

#### Binding to recombinant MUC1 glycoforms

- HMFG2 has broadest capacity for strong binding to tumour associated MUC1 glycoforms

<table>
<thead>
<tr>
<th>Recombinant MUC1 Glycoform&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Unglyc</th>
<th>Tn</th>
<th>STn</th>
<th>T</th>
<th>ST</th>
<th>Affinity (M × 10&lt;sup&gt;-8&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMFG2</td>
<td>0.23</td>
<td>0.07</td>
<td>0.09</td>
<td>0.07</td>
<td>0.08</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>± 0.04</td>
<td>± 0.06</td>
<td>± 0.02</td>
<td>± 0.04</td>
<td>± 0.05</td>
<td></td>
</tr>
<tr>
<td>SM3</td>
<td>0.71</td>
<td>0.19</td>
<td>0.33</td>
<td>0.16</td>
<td>1.06</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>± 0.29</td>
<td>± 0.11</td>
<td>± 0.02</td>
<td>± 0.18</td>
<td>± 0.58</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Antibody concentration (ng/ml) at which 50% of maximal binding was determined. Glycosylation of Muc1 is dysregulated in cancer. Tumour-associated Muc1 is mainly shorter glycans including Tn, sialyl Tn (STn), T (Thosen-Friedenreich), and ST.
**IMMUNOHISTOCHEMISTRY ANALYSIS**

Percent of positive cells in prostate tissue biopsies stained by anti-Muc1 antibodies

<table>
<thead>
<tr>
<th>MAb</th>
<th>B/N</th>
<th>PIN 1, 2</th>
<th>3-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BrE-3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.8±30.7</td>
<td>26.0±33.6 (2)</td>
<td>60.9±31.9 (4)</td>
</tr>
<tr>
<td>SM3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.5±43.2</td>
<td>70.5±49.0 (2)</td>
<td>70.4±38.8 (2)</td>
</tr>
<tr>
<td>B27.29</td>
<td>33.0±38.2</td>
<td>43.8±36.1 (1)</td>
<td>39.4±39.0 (1)</td>
</tr>
<tr>
<td>BC2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.4±35.6</td>
<td>39.1±34.5 (2)</td>
<td>55.3±35.6 (3)</td>
</tr>
<tr>
<td>EMA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.7±38.4</td>
<td>42.3±40.1 (1)</td>
<td>51.1±34.3 (2)</td>
</tr>
<tr>
<td>HMFG-1</td>
<td>13.1±26.2</td>
<td>9.8±17.5 (1)</td>
<td>13.6±25.0 (1)</td>
</tr>
<tr>
<td>NCL MUC1 core</td>
<td>13.8±29.6</td>
<td>9.8±17.2 (1)</td>
<td>13.5±25.0 (1)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean percent (± SD) obtained by averaging percents of stained (apical or cytoplasmic) cells. <sup>b</sup>MAbs demonstrating a significant correlation of staining with Gleason grade. Numbers in parentheses indicate the ratio of percent positive cancer cells to percent positive benign/normal cells.

Burke et al., Int J Oncol 2006

Staining of breast tissue with HMFG2

Wilkie et al., J Immunol 2008
HMFG1 INTERNALISES RAPIDLY IN BREAST CANCER CELLS

HMFG1 IgG antibody internalisation

0 mins

15 mins

MCF-7 breast cancer cells (Pericleous LM et al., BJC, 2005)
IN VIVO TESTING OF HMFG2 CONTAINING CAR

Mice (SCID Beige mice) bearing established tumour (MDA-MB-435 cells engineered to express Muc1) treated i.p. with single dose of HMFG2 containing CAR (HOX) or control CARs (DOX, HDFTr)

Administration of HOX+ T cells resulted in significant delay in tumour growth

Wilkie et al., J Immunol 2008
OPPORTUNITY

• Collaboration or licensing opportunity to develop Muc1 antibodies

• Some protein available for each antibody; bulk HMFG1 available (>2kg) and GMP grade cell lines for antibody production

• DNA sequences available for HMFG1 and SM3 in public domain, HMFG2 sequence is confidential

• Tools and reagents available for antibody testing; soluble, glycosylated MUC1 protein and transgenic mouse model of Muc1 available for in vitro and in vivo proof of concept studies
ANTI-CALLA (CD10) ANTIBODY
CLONE SS2/36
## ANTI-CALLA CLONE SS2/36

<table>
<thead>
<tr>
<th>(Clone)</th>
<th>SS2/36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principal Investigator</td>
<td>Jackie Cordell, University of Oxford</td>
</tr>
<tr>
<td>Intellectual Property</td>
<td>Material rights (no patent protection)</td>
</tr>
</tbody>
</table>
|Characteristics | • Generated via mouse hybridoma technology  
• Immunogen: Common acute lymphoblastic leukaemia cells  
• Mouse IgG1  
• Binds human CALLA |
|Internalisation | No direct data on clone SS2/36 available. However, studies in the literature have shown that other mAbs cross-linked to CALLA are internalised (Pesando et al, 1981; Pesando et al, 1983) |
|Opportunity | Licence to the hybridoma |
TARGET VALIDATION AND EXPRESSION

CALLA (CD10/NEP/MME) is a common zinc-dependent metalloendoprotease that inactivates a number of signalling peptide hormones including glucagon, enkephalins, substance P, neurotensin, oxytocin and bradykinin (http://www.ncbi.nlm.nih.gov/gene/4311)

CALLA expression patterns
• Cell surface marker for human acute lymphocytic leukaemia (ALL)
  – Present on leukemic cells of pre-B phenotype, which represent 85% of cases of ALL and is absent from normal peripheral blood mononuclear cells (PMBC) (Letarte et al. 1998 J Exp Med; Al Gwaiz et al. 2008 Histol Histopathol)
• Expressed in B-lymphoblastic leukaemia/lymphoma and in certain mature B-cell lymphomas (plasma cell myeloma, follicular lymphoma, diffuse large B-cell lymphoma and Burkitt lymphoma)
• It’s presence has been observed in multiple haematological and solid cancers including:
  – DLBCL (Holler et al. 2009 J Hematop)
  – Melanoma cell lines (Velazquez et al. 2007 J Transl Med)
  – Glioma cell lines (Monod et al. 1989 Int J Cancer)
  – Breast cancer (Jana et al. 2014 Indian J Pathol Microbiol)
  – Hepatocellular carcinoma (Xiao et al. 2001 Am J Pathol)
  – Kidney (Chu et al. 2000 Am J Clin Pathol)
  – Lung (Cohen et al. 1996 Cancer Res)
  – Liver (Borscheri et al. 2001 Am J Surg Pathol)

(Al Gwais et al. 2008 Histo Histopathol)
IMMUNOHISTOCHEMISTRY: SOLID CANCERS

**Prostate**
(http://www.proteinatlas.org)

**Liver**
(http://www.proteinatlas.org)

**Metastatic melanoma**
(Velazquez et al. 2007 J Transl Med)
Diffuse expression (red stain) (20×)

Focal expression of NEP (red stain) (20×).

**HCC**
(Xiao et al. 2001 Am J Pathol)

A: Strong membranous-positive reaction with anti-CD10.
B: Weaker reactivity in normal area of the same liver.
A and B were from the same section. (125x).

**Breast**
(Jana et al. 2014 Indian J Pathol Microbiol)
Stromal staining of breast carcinoma grade 3 (400×)
IMMUNOHISTOCHEMISTRY: NORMAL TISSUES

No significant staining of CALLA detected on the majority of normal tissues with the exception of the kidneys.

http://www.proteinatlas.org
INTERNALISATION OF CALLA

Laz 221 cells, incubated with (A) or without (B) J5 anti-CALLA antibody 24hr at 37°C, were surface labelled with $^{125}$I to determine if gp 100-bearing CALLA remains exposed on surface of cells after modulation. Modulated cells were harvested for labelling (G/M FITC) at 10 and 40hr (solid lines). Dashed lines represent control antiserum. Cells were analysed by FACS. Modulation or loss of antigenic determinant on cell surface is >90% complete in Panel B.

Pesando JM et al. 1981 J Immunol
This model illustrates the role of CD10 in normal (left panel) and tumoral (right panel) context with a CD10-enzymatic deregulation (upper [A] and middle [B] panel) or an alteration in the CD10 signalling (lower [C] panel). (A): Transformation of ECP and/or P induces the decrease of the CD10-enzymatic activity and/or the decrease of the number of CD10-expressing cells that induce the accumulation of peptides, normally cleaved by the CD10, which mediate the proliferation of progenitor cells. (B): Transformation of ECP induces their proliferation and an increase in CD10-expressing cells, which cleaved differentiating signalling peptides. (C): CD10 signalling deregulation in transformed ECP cells could block PTEN activity and induce cell growth by the activation of Akt pathway. (Maguer-satta et al. 2011 Stem cells.)
ICAM3: INTERCELLULAR ADHESION MOLECULE 3 ANTIBODIES
ICAM3.1, ICAM3.2
ICAM3 MONOCLONAL ANTIBODIES

<table>
<thead>
<tr>
<th>Target</th>
<th>Clone</th>
<th>Isotype</th>
<th>PI, Institute</th>
<th>Host</th>
<th>Reactivity</th>
<th>Immunogen</th>
<th>Recommended assays</th>
<th>Ab specific Internalisation data</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM3</td>
<td>ICAM3.1</td>
<td>IgG1</td>
<td>University of Oxford</td>
<td>mouse</td>
<td>human</td>
<td>ICAM-3/Fc chimeric fusion protein</td>
<td>ELISA, FACS, IHC, IF, IP, RIA, WB</td>
<td>unknown</td>
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<tr>
<td>ICAM3</td>
<td>ICAM3.2</td>
<td>IgG1</td>
<td>University of Oxford</td>
<td>mouse</td>
<td>human</td>
<td>ICAM-3/Fc chimeric fusion protein</td>
<td>ELISA, FACS, IHC, IF, IP, RIA, WB</td>
<td>unknown</td>
</tr>
</tbody>
</table>

ICAM3 OPPORTUNITY

- Hybridoma available for both ICAM3.1 and ICAM3.2 (Bossy D., 1995, Eur. J. Immunol.)

- Antibody stock available for ICAM3.1

- CRT are looking for licensing partners to develop this antibody as an ADC
ICAM3 TARGET CHARACTERISTICS

Cancer target ICAM3 (Aliases: CD50, CDW50, ICAM-R)

• Human ICAM3 is a 130kDa type I transmembrane glycoprotein protein
• Binds LFA-1 (integrin alpha-L/beta2) and integrin alpha-D/beta-2 on APC cells
• ICAM3 is closely related to ICAM1, it consists of 5 immunoglobulin domains and binds LFA1 through its two N-terminal domains
• ICAM3 has a role in regulating leukocyte adhesion, recruitment and transendothelial migration (del Pozo M., 1997, J. Cell Biol.)
ICAM3 TARGET CHARACTERISTICS CONT

Rationale for ICAM3 as an ADC

• ICAM3 is localised intracellularly and on the cell membrane (Serrador J.M., 2002, J. Biol. Chem.)
• ICAM3 expression in normal tissue is highly restricted to the haematological system (de Fougerolles A., 1992, J. Exp. Med.; Cordell J. et al., 1994 J. Clin. Pathol.)
• Although there is no data showing internalisation for ICAM-3, there is data on its close relative ICAM-1 showing that ICAM-1 is internalised (Muro S., 2003, J. Cell Sci.)
• The ICAM3-ADC could be used to target ICAM3(high) cells, while leaving the activated ICAM3(low) T-cells
ICAM-1 INTERNALISATION

HUVEC were treated with 250U TNF-α for 24h. Confluent monolayers were incubated at either 4°C (c) or 37°C (A,C-F) in the presence of either large (A; >1000 nm diameter), small (b; <500 nm diameter) biotin-anti-ICAM-1/streptavidin conjugates, anti-ICAM-1 immunobeads (C,D) or beads previously coated with control murine IgG(E,F). Merged images were pseudo-coloured to show single-labelled, internalised immunoconjugates/immunobeads as green (arrows) and double-labelled immunoconjugates/immunobeads on the cell surface as yellow (arrowheads). The phase-contrast image shown in E corresponds to the fluorescence image shown in F. Bar, 10 μm.
ICAM3 NORMAL & TUMOUR TISSUE EXPRESSION

Example images of ICAM3 localisation ((NCL-CD50-366 antibody (Novocastra); Pontén F., 2007, J. Pathol.).

- Constitutively expressed by all normal leukocytes (Fawcett J., 1992, Nature)
- Strong cytoplasmic and membrane staining found in malignant lymphomas (Berglund L., 2007, Mol. Cell Proteomics)
- Expressed in epidermal langerhan’s cells (Montazeri A., 2006, Br. J. Dermatol.)
ICAM3 IN DISEASE


• ICAM3 promotes NSCLC cell migration and invasion (Park J.K., 2010, Int. J. Oncol.)

• A genetic variant of ICAM3 is thought to have a role in susceptibility to SARS infection (Chan K.Y., 2007, J. Infect.Dis.)
CD45RO ANTIBODY
UCHL-1
## CD45RO ANTIBODY

<table>
<thead>
<tr>
<th>Target</th>
<th>Clone</th>
<th>Isotype</th>
<th>PI, Institute</th>
<th>Host</th>
<th>Reactivity</th>
<th>Immunogen</th>
<th>Recommended assays</th>
<th>Ab specific Internalisation data</th>
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</thead>
<tbody>
<tr>
<td>CD45RO</td>
<td>UCH-L1</td>
<td>IgG1</td>
<td>Peter Beverley, CRUK LRI</td>
<td>mouse</td>
<td>human</td>
<td>Cultured T cells from an IL-2-dependent T-cell line (CA1) prepared from human peripheral blood activated with influenza virus</td>
<td>FACS, IHC, IP, WB</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

### CD45RO OPPORTUNITY

- Hybridoma and antibody stock available (Smith S. et al., 1986, Immunology)
- CRT are looking for licensing partners to develop this antibody as an ADC
CD45RO TARGET CHARACTERISTICS

Cancer target CD45 RO (Aliases: PTPRC; LCA; GP180; LYS; T200)
• CD45RO is 180kDa splice variant of the transmembrane Tyr phosphatase CD45. It is the shortest CD45 isoform and lacks the RA, RB, and RC exons.
• CD45RO enhances both T cell receptor and B cell receptor signalling mediated activation
• Most regulatory T cells in adults are CD45RO+ and the percentage increases with age (Booth N.J., 2010, J. Immunol.)
• CD45RO ligand is CD22

Rationale for CD45RO as an ADC
• CD45RO antibodies have been used for targeted nanoparticle drug delivery (Tang X., 2015, Nanoscale Res. Lett.)
• A CD45RO ADC could be used to pre-treat patients to eradicate immune suppressive T-cells enabling improved response to the subsequent primary treatment.
CD45RO NORMAL & TUMOUR TISSUE EXPRESSION

CD45RO NORMAL PROTEIN EXPRESSION


CD45RO TUMOUR EXPRESSION


CD45RO IN DISEASE


• CD45RO+ T cells are more common than naïve T cells (CD45RA+) in inflammatory myopathies (De Bleecker J.L., 1995, Am. J. Pathol.)

• CD4+ CD45RO+ T cells are a major latent viral reservoir in HIV+ individuals (Spiegel H., 1992, Am. J. Pathol.)
CDC2L1/2 INHIBITORS AS A NOVEL TOXIC PAYLOAD FOR AN ADC
CDC2L1/2 INHIBITORS

• CRT-DL kinase project identified a sub-series with nM cellular potency
  – Not associated with on-target inhibition
  – CRT initiated target de-convolution
  – Target identified as a pro-apoptotic kinase CDC2L1/2
    » Strong inducer of apoptosis

• siRNA knockdown phenocopies pharmacological target inhibition

• Inhibitors demonstrate broad and highly potent activity across a range of cancer types

• Novel and synthetically tractable series of compounds available

• Licensing opportunity to develop inhibitors as toxic payload for ADC
NOVEL KINASE TARGET IDENTIFIED BY FORWARD CHEMICAL GENETICS

Congeneric series with unusually potent anti-proliferative activity

- Exquisite correlation between kinase binding affinity and cellular phenotypic potency
- Strong induction of apoptosis

$r^2 = 0.92$

Apoptosis induction in H1703 NSCLC
CRT0160148 (CDC2L1/2 INHIBITOR): IN VIVO PROOF OF CONCEPT

• Efficacy in H1703 NSCLC xenograft model
  – 69% tumour growth inhibition @ 1 mg/kg (1x/day, ip)
CDC2L1/2 BIOLOGY

- CDC2L1 (CDK11B) and CDC2L2 (CDK11A) lie in close proximity on chromosome 1p36 and are nearly identical
  - 100% sequence homology in the ATP binding site

- Serine/threonine protein kinases and members of the p34Cdc2 protein kinase family

- Both genes encode multiple isoforms for which the literature suggests diverse functions including
  - Apoptosis, autophagy, transcription, G2/M phase of the cell cycle, cytokinesis and maintenance of sister chromatid cohesion
TARGET VALIDATION

siRNA-mediated knockdown studies

- Knockdown experiments in multiple cancer cell lines with multiple siRNAs have shown decreased viability and induction of apoptosis

**Cell viability**

U2OS cells

<table>
<thead>
<tr>
<th>UN</th>
<th>Mock</th>
<th>NTC</th>
<th>SiTox</th>
<th>ABI-32</th>
<th>ABI-34</th>
<th>ABI-92</th>
<th>Q-7700</th>
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NCI-H1703 cells

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<th>ABI-32</th>
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SKOV3 cells

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**cdc2L1/2 siRNA**

- U2OS cells
- SKOV3/NCI-H1703 cells

**Q-7700**

- NTC: non-targeting control
- UN: untransfected cells
- SiTox: +ve control toxin
**TOXIC PAYLOAD PROFILES**

<table>
<thead>
<tr>
<th>Compound</th>
<th>MW</th>
<th>CDC2L1 (Kd nM)</th>
<th>CDC2L2 (Kd nM)</th>
<th>S(90)_{55} (@ 1 μM)</th>
<th>U2OS (IC(_{50}) nM)</th>
<th>H1703 (IC(_{50}) nM)</th>
<th>SKOV3 (IC(_{50}) nM)</th>
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<td>CRT0160148</td>
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<td>0.018</td>
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<td>10</td>
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- Low MW synthetically tractable series
- High potency dual CDC2L1/2 inhibitors
- Selective against other kinase targets
- Highly potent antiproliferative activity (BrdU incorporation @ 24 hours)
COMPOUND ACTIVITY BY CELL TYPE

- In a panel of 90 cancer cell lines (Eurofins Panlabs) CRT0160148 showed broad and highly potent activity across a range of cancer types.

5x apoptosis induction by CRT0160148

![Graph showing apoptosis induction by CRT0160148 at different concentrations for various cell lines. The graph indicates a 5x fold increase at 100 nM and 10 nM compared to the control (U2OS).]
SUMMARY

• High potency inhibitors identified against a novel cancer target

• Strong induction of apoptosis across a diverse panel of cancer cell lines and in tumour explants (information available under CDA)

• Efficacy in two models (SKOV3 and HI703)

• Novel, chemically tractable series with a primary amine moiety for linker attachment

• Compounds have not been optimised against CDC2L1/2 target

• Additional information available under CDA