αvβ6 ANTAGONISTIC ANTIBODIES

- Novel antagonistic antibodies targeting the integrin αvβ6
- Selective targeting of αvβ6-positive tumours in vivo demonstrated
- Broad utility on oncology and fibrotic disease indications
- Utility as naked antibody and ADC approaches

COMMERCIAL OPPORTUNITY

The anti-αvβ6 antibodies come with a package of data demonstrating inhibitory action against αvβ6 in vitro and selective targeting of αvβ6 positive tumours in vivo. The availability of humanised antibodies (both IgG and diabody) provides an opportunity for rapid development of an antibody towards and into the clinic, as a naked antibody or as part of an ADC approach. These antibodies have considerable therapeutic potential with utility in a range of common tumour types (including pancreatic, breast, oesophagus, head and neck, skin, lung and ovarian), each year an estimated 250,000 αvβ6 positive tumours are diagnosed in the US and UK alone. In addition there is therapeutic potential in fibrotic diseases. CRT is seeking a collaborative or licensing partner for the further development of anti-αvβ6 antibodies.

THE TECHNOLOGY

A series of anti-αvβ6 antibodies has been generated through a loop grafting approach [1], which involved taking advantage of the A20FMDV2 peptide, which has high affinity and selectivity for αvβ6 [2], by inserting it into the CDR H3 loop of the murine anti-CEA MFE-23 antibody. Therefore, the series of anti-αvβ6 antibodies includes antibodies engineered to be highly αvβ6-specific (B6.2 and humanised B6.3), and an antibody which also cross-reacts with the tumour-associated antigen, CEA (B6.1). Importantly, B6.2 was determined to be as structurally stable as the parent scFv, indicating that insertion of the A20FMDV2 peptide and the Y100bP mutation (removes CEA cross-reactivity) was not detrimental to the protein structure [1]. The humanised variant B6.3 has been generated in diabody [5] and full IgG formats, the diabody variant of which has a Kd of 2.78 nM (as measured by Biacore).

The antibodies have demonstrated specific concentration-dependent binding to αvβ6 transfected cells (versus those expressing other integrins, eg B6.1 scFv - Figure 1), and inhibition of αvβ6-mediated migration and adhesion (eg B6.3 diabody - Figure 2).

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CRT OPPORTUNITY
Biological Therapeutics – In Vivo Proof of Principle
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**B6.3 inhibits αvβ6-mediated migration in vitro**

Figure 2: B6.3 diabody treatment inhibited migration of A375Pβ6 cells to LAP and migration of Capan-1 cells to fibronectin and LAP.

Imaging and biodistribution studies of radiolabelled B6-3 diabody demonstrated that it specifically targets αvβ6-expressing tumours in vivo (Figure 3), with Tumour-to-Blood ratios of 40 for αvβ6 positive tumours and 15.5 for αvβ6 negative tumours. The bivalent nature of diabodies holds the advantage of higher tumour uptake. B6.2 (Figure 4) and B6.3 diabody are both rapidly internalised (starting after 30 mins) into αvβ6-expressing cells, demonstrating the potential for these antibodies to be used for the delivery of toxic payloads in an ADC approach.

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

Figure 3: A375Pβ6 (αvβ6 positive, left-hand side) and A375Ppuro (αvβ6 negative, right-hand side) cells were injected subcutaneously on opposite shoulders and 99mTc-labeled B6.3 diabody was injected intravenously once tumours had developed. SPECT/CT cross section imaging of mouse at 5h time point.

**Internalisation of B6.2 into αvβ6-expressing cells**

Figure 4: B6.2 is internalized by αvβ6-expressing cells. αvβ6-expressing (A375Pβ6, a–h) and non-expressing (A375Ppuro, i–p) cells were incubated with B6.2 for 1 h at 4 °C, free scFv was subsequently removed and the cells were incubated at 37 °C for the times indicated. Zoom boxes show the predominant plasma membrane pattern of staining at 10 min (d) and internalized vesicular staining at 3 h (h). Similar results were observed at 30 min and 1 h (data not shown). Scale bars represent 20 mm.

**SUPPORTING RATIONALE**

The expression of αvβ6 is restricted primarily to epithelial cells where it is expressed at low levels in healthy tissue and significantly up-regulated during wound healing, fibrosis and in tumourigenesis. αvβ6 has multiple regulatory functions in tumours including TGF-β activation, cell proliferation, MMP production, cell invasion and survival. The αvβ6 antibody STX-100 is in Phase II clinical trials for Idiopathic Pulmonary Fibrosis and antibody-mediated blockade of αvβ6 has been demonstrated to inhibit tumour growth in vivo [6, 7], which support the use of αvβ6-targeted agents in therapy for both cancer and fibrosis. In cancer patients, elevated αvβ6 expression has been correlated with poor prognosis in tumours including colorectal, ovarian and lung. Numerous publications have identified αvβ6 as a key regulator of the epithelial to mesenchymal transition. αvβ6 has also been linked with maintenance of a pluripotent cancer stem cell phenotype in oral cancer, and as a key member of a “K-Ras dependency signature” in lung and pancreatic tumours [8].

**INTELLECTUAL PROPERTY**


**REFERENCES**

5. Kogelberg H. et al Plos ONE (2013) 8(9) e73260