Evaluation of the potential for targeted therapy of renal carcinoma and metastatic melanoma with huBC1-huIL12 (AS1409), a potent immunocytokine

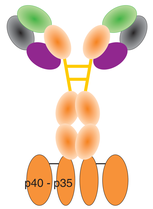
Benjamin Doran, Paul Rogers, David Jones, Colin Green, Nigel Courtenay-Luck • Antisoma Research Ltd., St.George's Hospital Medical School, London, UK

Abstract No.: 922

Introduction

- Angiogenesis is a complex process whereby signals from tumor cells cause existing blood vessel endothelial cells to produce new blood vessels to supply the tumor. Therapies that disrupt this process have the potential to be powerful anti-cancer agents
- BC1 is a monoclonal antibody that recognizes an oncofetal antigen, ED-B fibronectin, expressed predominantly during tumor angiogenesis. ED-B is present in a wide variety of solid tumors
- BC1 recognizes an epitope localized within the type III repeat 7 domain that is is cryptic in fibronectin molecules lacking the ED-B domain but unmasked in molecules containing this sequence
- As BC1 binds to tumor cells, tumor blood vessels and tumor extracellular matrix[1,2], it has clear potential as a tumor-targeting antibody. The accessibility of the extracellular matrix of the tumor neovasculature makes ED-B fibronectin an ideal target for immunotherapy. Murine BC1 has been used successfully for immunoscintigraphy to image brain tumor mass in glioblastoma patients
- IL12 is a heterodimeric cytokine with immunostimulatory and anti-angiogenic properties. IL12 clinical trials have shown signs of anti-cancer activity, including responses, in patients with renal cell carcinoma[3,4] and melanoma[4,5]
- The systemic administration of cytokines is often associated with severe toxic side effects, which prevent the administration of a curative dose. This was the case in IL12 clinical trials. A possible way of increasing the therapeutic index of cytokines is to fuse them to antibodies, which mediate a preferential accumulation of the cytokine at the tumor site: targeted delivery
- huBC1-huIL12 (AS1409) is a fusion protein consisting of the huBC1 antibody and human IL12
- Here we describe the extent of ED-B fibronectin expression in samples taken from human renal carcinoma and melanoma samples, as well as comparing expression in tumor tissue with normal human tissue from two separate studies. The aim was to consider whether these two tumor types are suitable candidates for evaluating huBC1-huIL12 in a phase I clinical trial

Figure 1: huBC1-huIL12 (AS1409) – recombinant huBC1 antibody fused with two heterodimers of human interleukin 12



Methods

All tissue samples obtained from human patients were taken with informed consent and ethics board approval. Tissue sections were evaluated by qualified pathologists. Briefly, sections were prepared as follows:

- **Renal carcinoma:** Samples of renal carcinoma from 20 patients were fixed using the HOPE technique[6], dehydrated with acetone and embedded in paraffin wax. Sections were prepared, mounted on silanized slides and deparaffinized. Sections were incubated with huBC1-huIL12 or huBC1 together with biotinylated anti-human Fab fragments. Detection was by streptavidin/horseradish peroxidise (HRP) and diaminobenzadine (DAB) followed by counter-staining with hematoxylin
- **Metastatic melanoma:** Samples of metastatic melanoma together with draining lymph nodes from 15 patients were obtained and flash frozen. Cryosections were prepared, incubated with huBC1 antibody, then murine anti-human secondary antibodies and then biotinylated anti-mouse antibody. Detection was by streptavidin/HRP with DAB as substrate followed by hematoxylin counter-staining

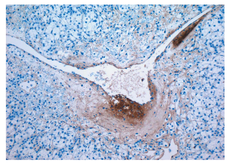
An IgG1 isotype control was used as a comparator for all protocols

Results

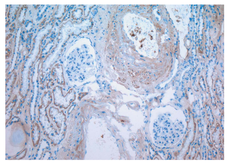
Renal carcinoma

- In 19/20 renal carcinoma samples, blood vessel endothelium stained positively (Figure 2A-G) with huBC1 or huBC1-huIL12. Of the positive results 16 were classed as moderate to strong staining and 3 as weak to moderate staining. Only one sample (Figure 2H) did not stain with huBC1 or huBC1-huIL12. The results of the staining are summarized in Table 1

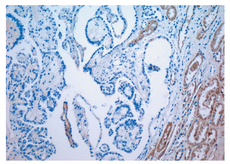
Figure 2: Representative photomicrographs showing ED-B expression in renal carcinoma samples



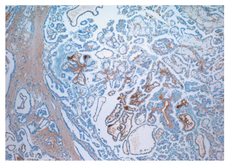
A Clear cell carcinoma (patient sample 3)



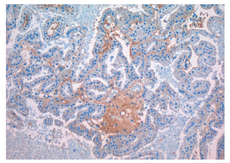
B Onkocytoma (patient sample 4)



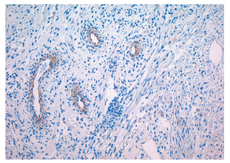
C Chromophilic / tubulo-papillary renal carcinoma (patient sample 8)



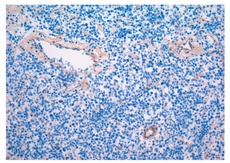
D Tubulo-papillary renal carcinoma (patient sample 5)



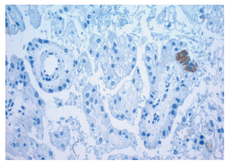
E Clear cell / papillary carcinoma (patient sample 11)



F Angioleiomyolipoma (patient sample 12)



G Lymphoma (patient sample 13)



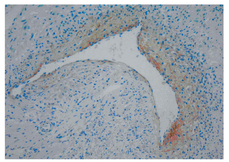
H Clear cell / chromophilic renal carcinoma (patient sample 2)

Table 1: Staining of renal carcinoma samples for ED-B expression using huBC1-huIL12 fusion protein or huBC1 antibody

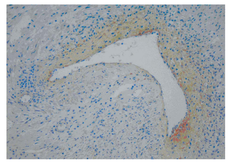
Case	Tumor type	Staining result
1	Clear cell	✓
2	Clear cell/ chromophilic	✗
3	Clear cell	✓
4	Onkocytoma	✓
5	Tubulo-papillary	✓
6	Clear cell/ chromophilic	✓
7	Chromophilic	✓
8	Chromophilic/ tubulo-papillary	✓
9	Clear cell, heteromorph	✓
10	Clear cell	✓
11	Clear cell + papillary	✓
12	Angioleio-myolipoma	✓
13	Lymphoma	✓
14	Chromophilic/ papillary	✓
15	Regressive clear cell	✓
16	Clear cell, regressive cystic	✓
17	Clear cell/papillar	✓
18	Clear cell + oxyphil	✓
19	Chromophilic	✓
20	Chromophilic + partly oncocytoma	✓

- Cytoplasmic staining of tumor cells was seen in 7/20 samples, mainly in clear cell carcinoma or tubulo-papillary tumors
- Occasional positive staining of tubules, stroma and papillae was seen. Glomeruli were not stained
- Little or no staining of blood vessels in non-malignant tissue was seen
- The extent and intensity of ED-B fibronectin expression was similar whether evaluated using the antibody alone (huBC1) or AS1409 (Figure 3A-D)

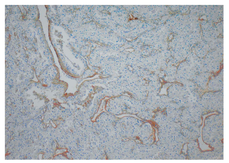
Figure 3: Comparison of ED-B staining with huBC1 and huBC1-huIL12 in two renal carcinoma samples



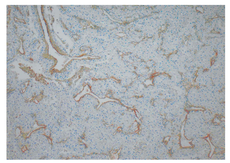
A Renal carcinoma (patient sample 12) stained with huBC1 antibody



B Renal carcinoma (patient sample 12) stained with huBC1-huIL12 fusion protein



C Renal carcinoma (patient sample 20) stained with huBC1 antibody



D Renal carcinoma (patient sample 20) stained with huBC1-huIL12 fusion protein

Metastatic melanoma

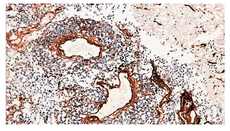
- huBC1 exhibited very specific staining of blood vessel walls and/or endothelial cells in all samples (Figure 4A-F), with very little or no staining of tumor cells. The results of the staining are summarized below (Table 2):

Table 2: Staining of metastatic melanoma samples for ED-B expression using huBC1

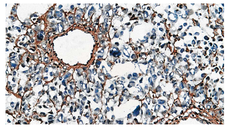
Case	Tissue	Tumor stroma		Blood vessel endothelium	
		Frequency	Intensity	Frequency	Intensity
1	Mediastinum	4	+	4	+++
2	Lung	4	++	4	+++
3	Skin	4	++	4	+++
4	Mesentery	2	+	4	+++
5	Brain	–	–	4	+++
6	Skin	4	+++	3	+++
7	Lung	Negative	Negative	Negative	Negative
8	Skin	2	++	4	+++
9	Skin	4	++	4	+++
10	Small intestine	4	++	3	+++
11	Stomach	4	++	1	++
12	Brain	–	–	4	+++
13	Small intestine	4	++	3	+++
14	Breast	4	++	3	+++
15	Small intestine	1	++	2	++

Frequency: 1 few, 2 several, 3 many, 4 most
Intensity: 0 no staining, + weak staining, ++ moderate staining, +++ strong staining

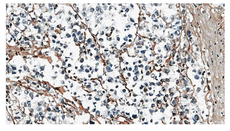
Figure 4: Representative photomicrographs showing staining for ED-B expression in metastatic melanoma samples



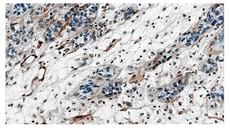
A Metastatic melanoma – brain (case 12)



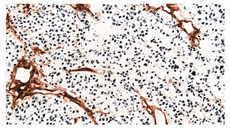
B Metastatic melanoma - representative draining lymph node



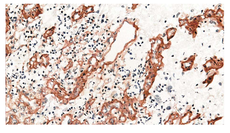
C Metastatic melanoma – stomach (case 11)



D Metastatic melanoma – skin (case 8)



E Metastatic melanoma – mesentery (case 4)



F Metastatic melanoma – brain cerebellum (case 5)

- Staining of endothelium was more intense or frequent within areas of tumor than within normal tissue
- A network of moderate to intensely stained fibres was identified in several of the metastatic tumor samples. These appeared to be physically anchored to the blood vessels and are likely to be related to new capillary networks
- Positive staining of stroma was present in all samples. This was mostly found to be non-specific, although on occasions there appeared to be some difference in staining intensity and/or frequency between parenchymal stroma and the stroma found within a tumor. Where necrotic tissue was present, it exhibited varying levels of non-specific staining

Conclusions

- 19/20 renal carcinoma samples and 15/15 metastatic melanoma samples (and draining lymph nodes) stained positively with huBC1 antibody or huBC1-huIL12 fusion protein (AS1409) targeted against ED-B fibronectin
- Staining was characterized as widespread and intense and was mostly associated with blood vessel endothelium and tumor stroma. Some staining of tumor cell cytoplasm was seen in renal carcinoma samples; no staining of melanoma tumor cells was reported
- Little or no staining of normal blood vessels was seen. Rapidly vascularizing tissue such as placenta stained positively as expected
- The widespread expression of ED-B containing fibronectin in both melanoma and renal carcinoma together with the apparent sensitivity of these tumor types to cytokine therapy suggests that renal cancer and melanoma are suitable indications in which to start the clinical evaluation of huBC1-huIL12
- A phase I trial in renal cancer and melanoma is expected to commence in H2 2007

References

1 P Pujuguet, A Hammann, M Moutet, JL Samuel, F Martin and M Martin. Expression of fibronectin ED-A+ and ED-B+ isoforms by human and experimental colorectal cancer. Contribution of cancer cells and tumor-associated myofibroblasts. Am J Pathol. 1996;**148**:579–592.

2 Marta Midulla, Rakesh Verma, Massimo Pignatelli, Mary A. Ritter, Nigel S. Courtenay-Luck and Andrew J. T. George. Source of oncofetal ED-B-containing fibronectin: implications of production by both tumor and endothelial cells. Cancer Res. 2000;**60**:164–169.

3 Giuliano Mariani, M.D., Arben Lasku, M.D., Antonio Pau, M.D., Giuseppe Villa, M.D., Cinzia Motta, M.D., Giuseppina Calcagno, M.D., Gioconda Z. Taddei, M.D., Patrizia Castellani, Ph.D., Kostas Syrigos, M.D., Alessandra Dorcaratto, Ph.D., Agamennon A. Epenetos, Ph.D., Luciano Zardi, Ph.D., Giuseppe A. Viale, M.D. A pilot pharmacokinetic and immunoscintigraphic study with the technetium-99m-labeled monoclonal antibody BC-1 directed against oncofetal fibronectin in patients with brain tumors. Cancer. 1997;**80**:2484–9

4 Jared A. Gollob, James W. Mier, Korina Veenstra, David F. McDermott, Daniel Clancy, Marguerite Clancy, and Michael B. Atkins. Phase I Trial of Twice-Weekly Intravenous Interleukin 12 in Patients with Metastatic Renal Cell Cancer or Malignant Melanoma: Ability to Maintain IFN-γ Induction Is Associated with Clinical Response. Clin Cancer Res, 2000;**6**:1678–1692

5 Gheath Alatrash, Thomas E. Hutson, Luis Molto, Amy Richmond, Cheryl Nemec, Tarek Mekhail, Paul Elson, Charles Tannenbaum, Thomas Olencki, James Finke, Ronald M. Bukowski. Clinical and immunologic effects of subcutaneously administered interleukin-12 and interferon alfa-2b: phase I trial of patients with metastatic renal cell carcinoma or malignant melanoma. Am J Clin Oncol. 2004 Jul 15;**22**:2891–900.

6 Wiedorn K.H.; Olert J.; Stacy R.P.; Goldmann T.; Kühl H.; Matthus J.; Vollmer E.; Bosse A. HOPE – a new fixing technique enables preservation and extraction of high molecular weight DNA and RNA of > 20 kb from paraffin-embedded tissues. Hepes-Glutamic acid buffer mediated Organic solvent Protection Effect. Pathol. Res. Pract. 2002;**198**:735–740

2007 AACR Annual Meeting