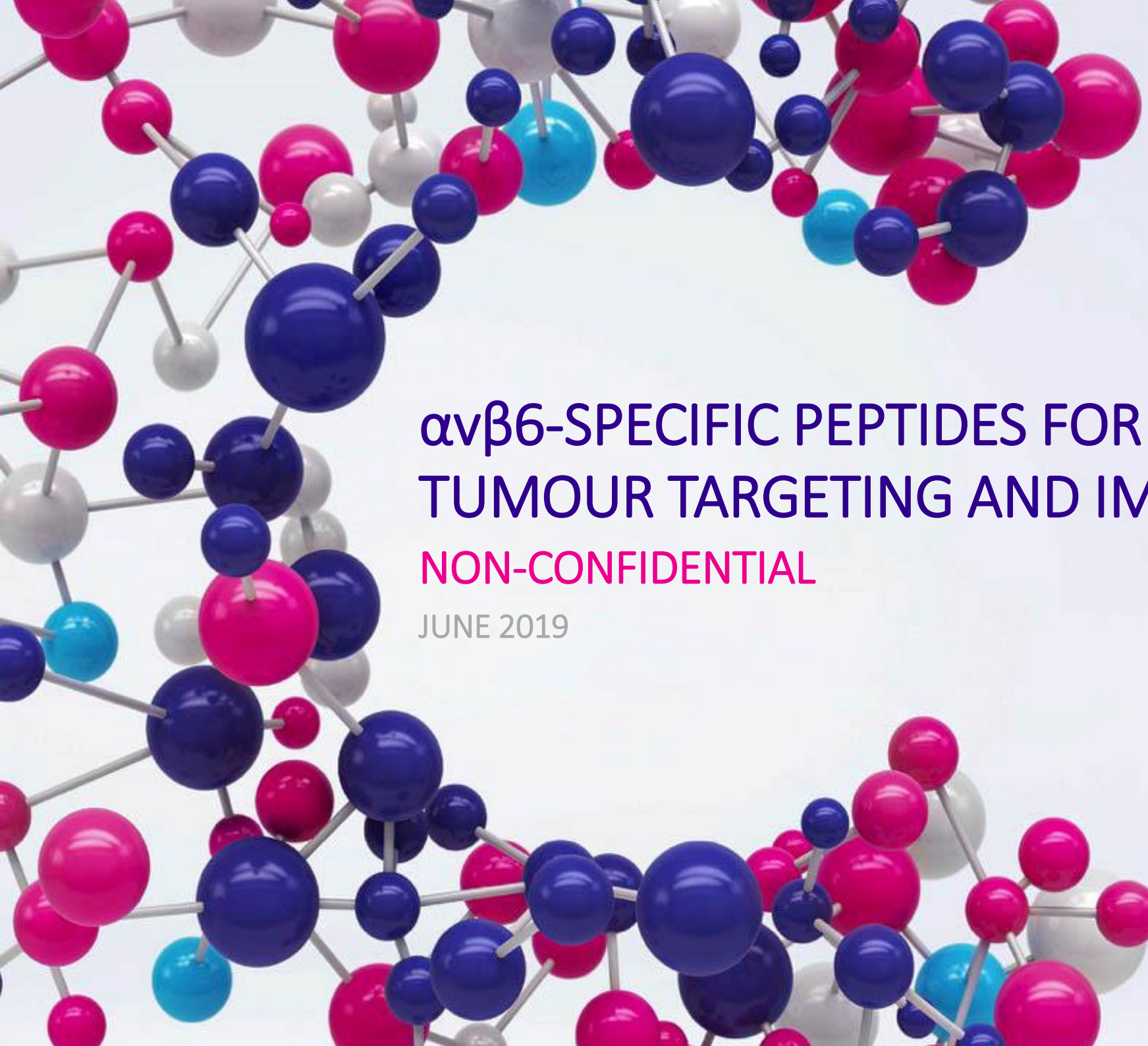


αvβ6-SPECIFIC PEPTIDES FOR TUMOUR TARGETING AND IMAGING

NON-CONFIDENTIAL

JUNE 2019

CANCER RESEARCH UK

SUMMARY

Lead inventor: Prof John Marshall (QMUL)

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
- Toxin-labelled A20FMDV2 controls or clears, *in vivo* murine xenograft pancreatic tumours
- **Clinical efficacy** as PET tracer in solid tumour patients (Ph I trial)
- Intellectual property
- Granted patents in US, EP, CA and JP cover A20FMDV2 lead peptides

αvβ6 - SELECTIVE TARGET IN CANCER

αvβ6 is a RGD-motif binding integrin expressed on the cell surface

- Epithelial specific expression
- **Low/undetectable expression in normal adult tissues**, elevated during tissue remodelling, fibrosis and multiple cancer indications
- Estimated 279,000 new αvβ6+ve tumours diagnosed each year in UK and US combined (excluding melanoma)

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

HIGH αvβ6 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

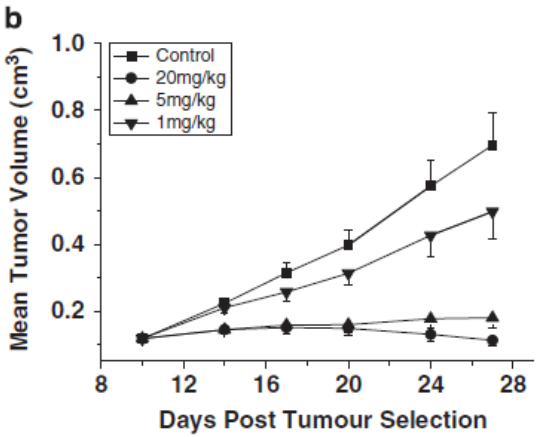
- Correlates with progression to malignant disease (Hamidi et al, 2000)

Increased αvβ6 expression associated with pro-invasive and aggressive phenotype

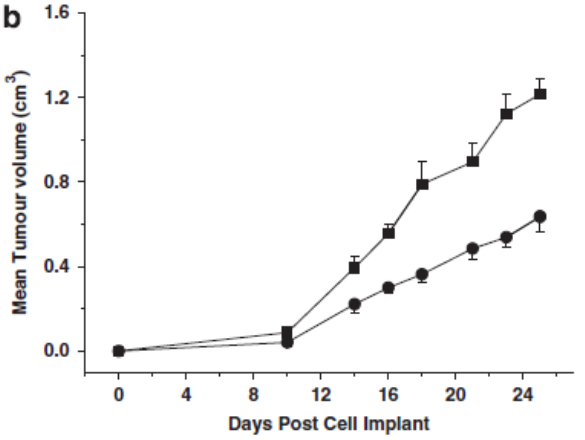
- 77% of metastatic lesions overexpress αvβ6

TARGETING αvβ6 CAN REDUCE TUMOUR GROWTH *in vivo*

Proof of concept established by the αvβ6 (and αvβ8) blocking antibody (264RAD) 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

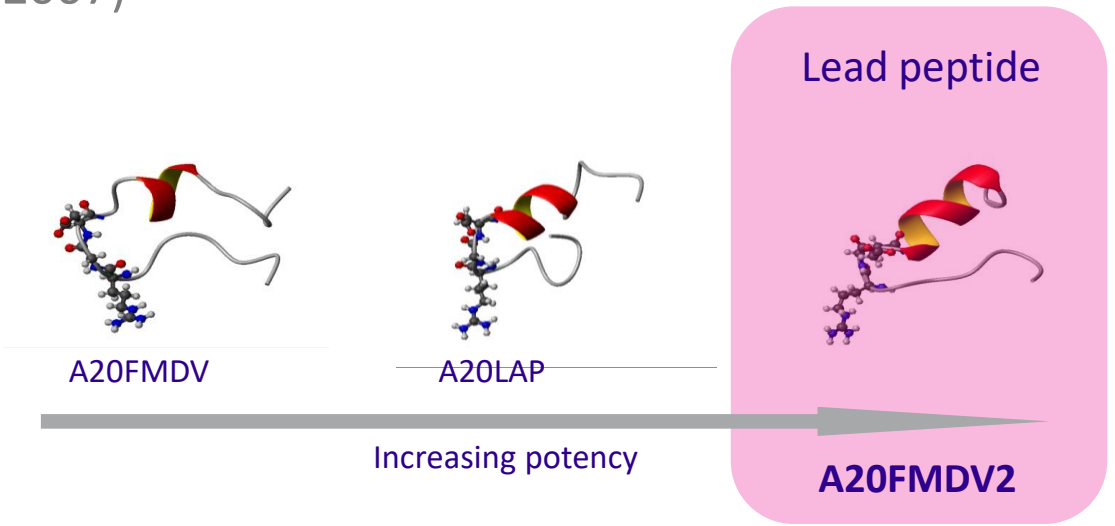


Inhibition of 4T1 tumour growth. Established tumours were treated with 20mg/kg 264RAD twice weekly

GENERATION OF HIGH AFFINITY αvβ6 SPECIFIC PEPTIDES

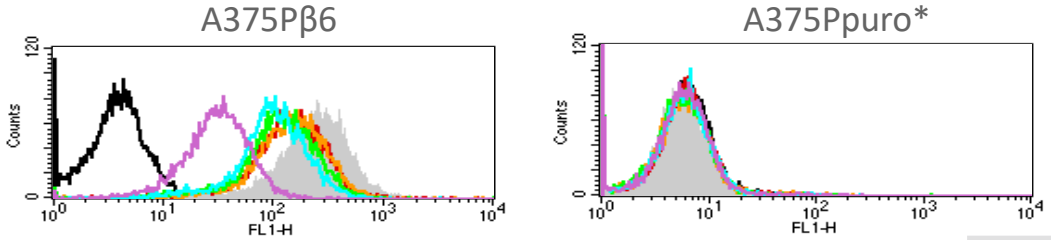
Used peptides derived from natural ligands (TGFβ1-LAP and Foot and Mouth Disease Virus) as a starting point

- Most potent peptides possessed RGDLXXL/I motif
- Discovered that affinity correlated with ability to form alpha-helical structure (DiCara et al., 2007)



In vitro PROPERTIES OF LEAD PEPTIDE A20FMDV2

Specificity



FACS analysis using A20FMDV2

1nM – 10mM

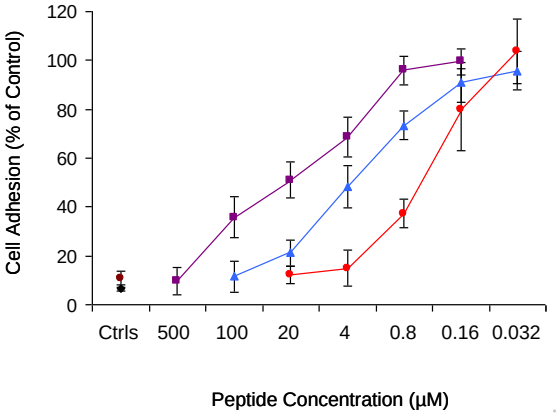
	αvβ6	αvβ3	αvβ5	α5β1
A20FMDV2	3 ± 1 nM	>10 µM	>100 µM	>10 µM

- A20FMDV2 is highly αvβ6-specific (>1000 fold versus other integrins)

*A375Ppuro –ve for αvβ6; **+ve** for αvβ3, αvβ5, α5β1

Inhibition of migration

- A20FMDV2 potently inhibits αvβ6-dependent cell adhesion and migration



CANCER RESEARCH UK

IN VITRO PROPERTIES OF LEAD PEPTIDE A20FMDV2

High affinity binding

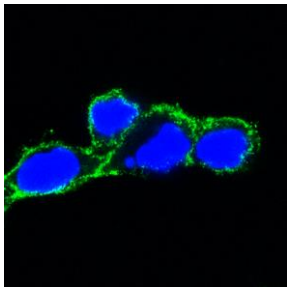
- Binding is highly stable
- A20FMDV2 binds to αvβ6 with affinity constant of 1.73 +/- 0.46 nM

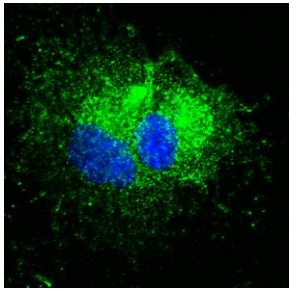
Ligand internalisation

- A20FMDV2 is rapidly internalised

into tumour cells

0 min





45 min

Saha et al, J Pathol, 2010

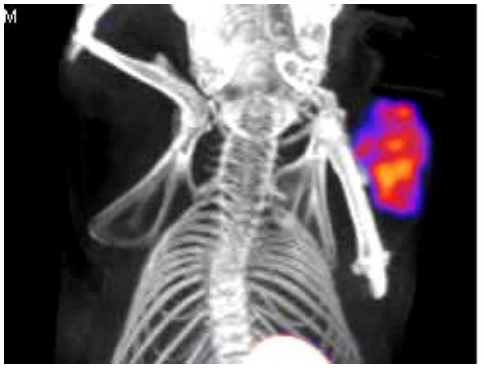
In vivo EFFICACY AS TOXIN-TARGETING THERAPEUTIC

High affinity binding

- Toxin-conjugated A20FMDV2 demonstrated control or clearance of distinct, *in vivo*, murine human xenograft pancreatic cancer models
- no signs of toxicity
- Toxin-conjugated A20FMDV2 demonstrated selective killing of numerous αvβ6+ (vs αvβ6-) tumour cell lines – with nM IC50s (*in vivo* and *in vitro* **data available under CDA**)

A20FMDV2 LABELS αvβ6+ TUMOURS

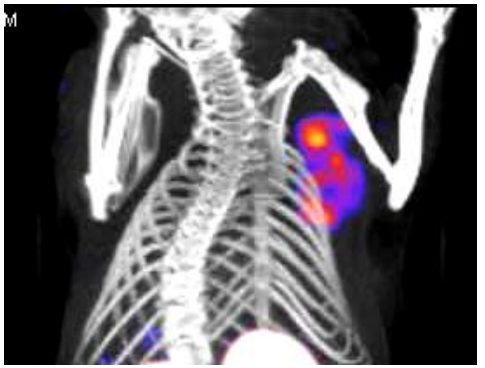
A20FMDV2 is able to target tumour cell lines that endogenously express αvβ6 in murine models



DCIS Breast cancer cell line xenograft

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

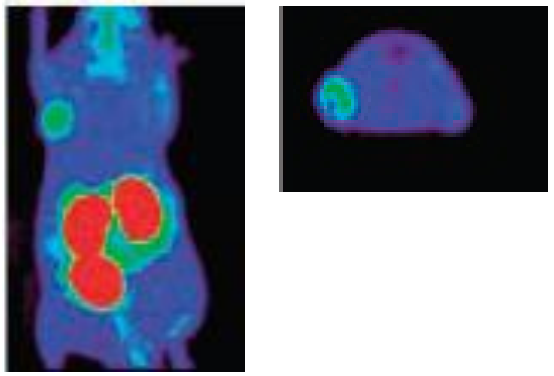
Saha et al, J Pathol, 2010



CA1a Breast cancer cell line xenograft

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

Saha et al, J Pathol, 2010



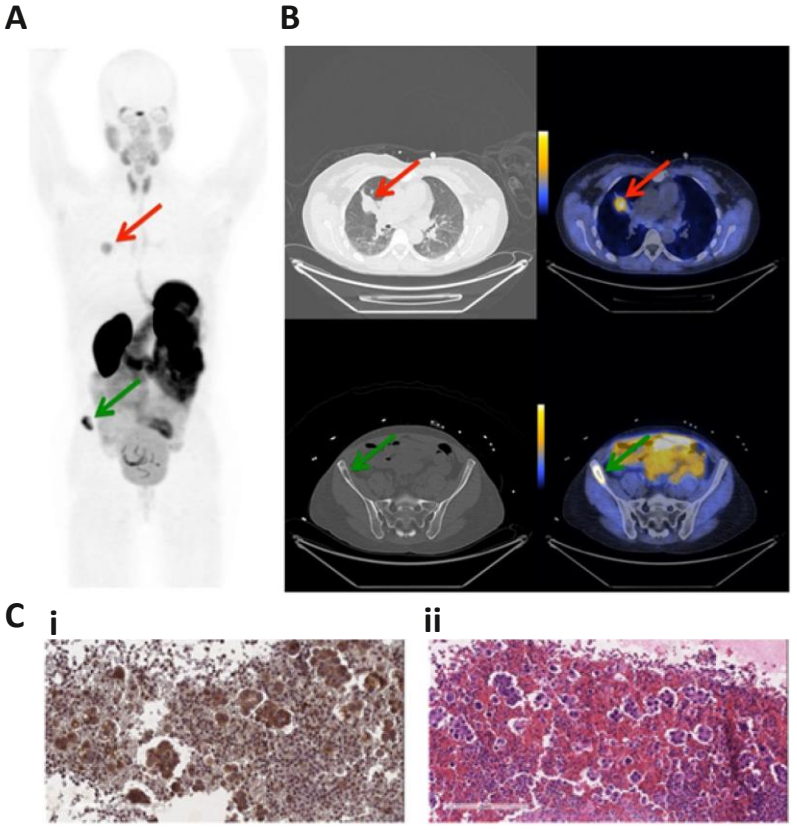
BxPC3 pancreatic cancer xenograft

microPET imaging of [^{18}F]FBA-$(PEG28)_2$-A20FMDV2

Hausner et al, Cancer Res, 2009

EFFICACIOUS PET-IMAGING IN Ph I TRIAL IN SOLID TUMOUR PATIENTS

Academic lead, PET-imaging Ph I clinical trial in solid tumour patients using a PEG-modified, [^{18}F]-labelled, A20FMDV2 ([^{18}F]αvβ6-BP)[1]

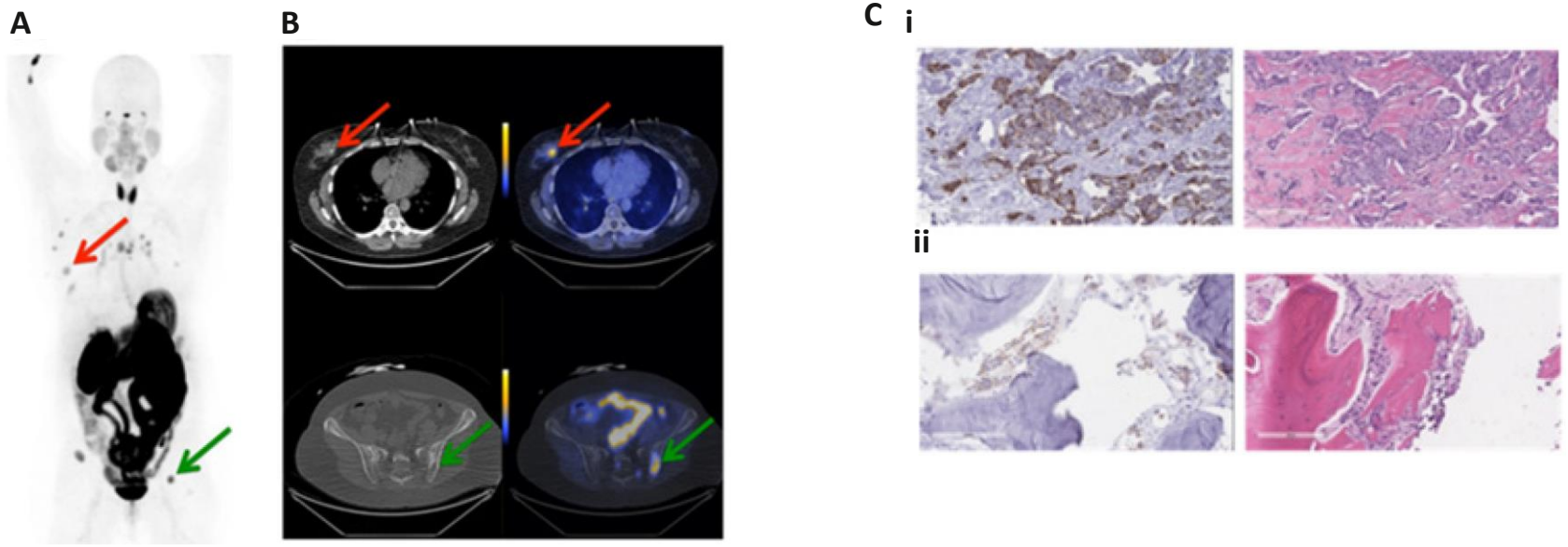


Representative PET, CT, and PET/CT images and IHC staining for subject 1. Subject 1 was a 53-year-old female never-smoker with no significant past medical history diagnosed 20 months prior to study enrollment with stage IV adenocarcinoma of the lung. (A) Coronal maximum intensity projection PET image (scaled to SUVmax 15.0) shows distribution of [^{18}F]αvβ6-BP 1 hour after intravenous administration. Red arrow indicates uptake of [^{18}F]αvβ6-BP in primary lung lesion (SUVmax 5.2) and green arrow in the right iliac wing metastasis (SUVmax 13.5). (B) Corresponding axial CT (left) and PET/CT (right) images (scaled to SUVmax 7.0) show distribution of [^{18}F]αvβ6-BP in lung mass (top) and right Iliac bone metastasis (bottom). (C) IHC section of sample obtained from pleural fluid (i); no tissue available for the primary tumor or the right iliac wing metastasis) stained for integrin αvβ6-expression and corresponding H&E staining (ii).

1. Hausner, S et al., Clin Can Res. 2019

EFFICACIOUS PET-IMAGING IN Ph I TRIAL IN SOLID TUMOUR PATIENTS

(…continued) Subject 2 diagnosed with stage IV invasive mammary carcinoma

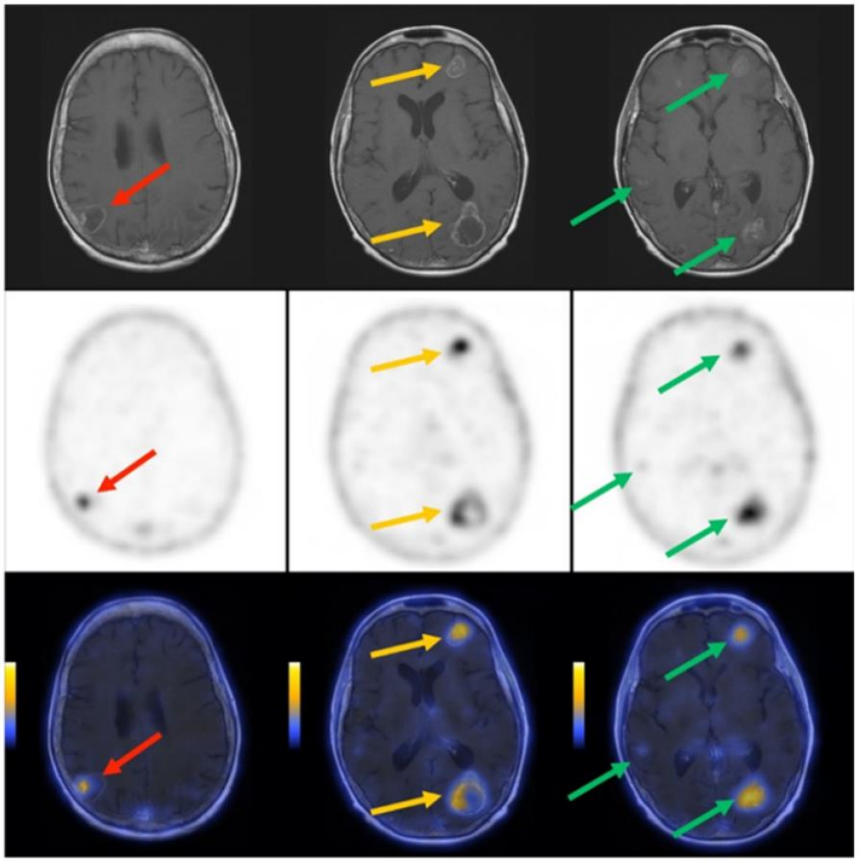


Representative PET and PET/CT images, and IHC staining for subject 2. Subject 2 was a 59-year-old female diagnosed with stage IV invasive mammary carcinoma. (A) Coronal maximum intensity projection PET image (scaled to SUVmax 15) shows distribution of [^{18}F]αvβ6-BP 1 hour after intravenous administration. Red arrow indicates uptake of [^{18}F]αvβ6-BP in primary breast lesion and green arrow in the left iliac metastasis. (B) Corresponding axial CT (left) and PET/CT (right) images (scaled to SUVmax 7) show distribution of [^{18}F]αvβ6-BP in breast mass (SUVmax 3.9) and left Iliac bone metastasis (SUVmax 13.1). (C) IHC section stained for integrin avb6-expression and corresponding H&E staining of primary breast tumor (i) and (ii), respectively, and left Iliac metastasis (c and d), respectively.

1. Hausner, S et al., Clin Can Res. 2019

EFFICACIOUS PET-IMAGING IN Ph I TRIAL IN SOLID TUMOUR PATIENTS

Subject 3 diagnosed with moderately differentiated adenocarcinoma of the lung



Representative MRI, PET, and PET/MRI images of brain for subject 3. Subject 3 was a 56-year-old female diagnosed with moderately differentiated adenocarcinoma of the lung. Contrast enhanced T1-weighted MRI demonstrating multiple bilateral metastases (top row). [^{18}F]αvβ6-BP PET images of the brain 1 hour after intravenous administration [^{18}F]αvβ6-BP demonstrating multifocal elevated activity (scaled to SUVmax 2.0; middle row). Coregistered fusion images of [^{18}F]αvβ6-BP PET and MRI demonstrating PET activity matched to enhancing lesions (scaled to SUVmax 2.0; bottom row).

1. Hausner, S et al., Clin Can Res. 2019

EFFICACIOUS PET-IMAGING IN Ph I TRIAL IN SOLID TUMOUR PATIENTS

Summary of trial to date (trial ongoing - NCT03164486):

- Low uptake in normal bone, lung, liver and brain
- clear uptake and differential labelling of both 1° lesions and metatstases within those same tissues
- Able to image subcentimetre metastases
- Safe, with no evidence of toxicity in patients

1. Hausner, S et al., Clin Can Res. 2019

PATENT POSITION

Claims under prosecution cover peptide consensus sequence, novel variants, including peptide, conjugate and method of treatment claims (including conjugation to detectable moieties for imaging and therapeutic moieties)

Granted claims to lead peptide and pharmaceutical composition

Application/Patent number	Filing date	Status
US8383593B2	02/04/2008	Granted
US8927501B2 (continuation)	17/01/2013	Granted
US9650416B2	11/12/2014	Granted
EP1957522B1	02/04/2008	Granted - Validated in CH, DE, ES, FR, GB, IT
JP5449774B2	02/04/2008	Granted
CA2624618	02/04/2008	Allowed
CA2854550 (Divisional)	01/06/2014	Pending
GB1706472.6 New Filing Covering Peptide Variants	24/04/17	(WO2018197490A1) Pending

A20FMDV2 – SUMMARY

αvβ6 expression is upregulated in cancer

- Correlate with poor prognosis

High affinity αvβ6-specific lead peptide A20FMDV2

- Highly specific for αvβ6 (>1000 fold versus other integrins)
- Selectively binds αvβ6+ve tumours *in vivo*
- Selective killing *in vitro* and *in vivo* with A20FMDV2-toxin conjugate
- Clinical safety and efficacy of radiolabelled A20FMDV2 as PET-tracer in solid tumour pts
- Cancer-related PET and SPECT imaging in αvβ6+ve tumours

Comprehensive, granted patent territory

THANK YOU

Contact: jesse.toe@cancer.org.uk

CANCER RESEARCH UK