CANCER RESEARCH HORIZONS

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Antibodies and Antibody-Drug-Conjugates targeting Clec14a for solid tumours

Opportunity overview

Problem: Currently angiogenesis targeting agents, as Avastin, block tumour neo-angiogenesis but do not eradicate established tumour vasculature.

Solution: Clec14a is a C-type lectin transmembrane protein which expression is upregulated by reduced shear stress, from reduced blood flow, in endothelial cells. CLEC14A was found to be specifically expressed on the surface of tumour endothelial cells. Antibody-drug-conjugated (ADCs) against CLEC14A disrupt already established tumour vasculature.

Technology: Five antibodies (Abs) selectively targeting Clec14a have been generated. Two have been developed into ADCs.

Findings:

- CLEC14A expression in significantly more abundant in human tumour tissues compare to healthy tissues.
- Clec14a Antibodies mainly localise in tumour tissues in a mouse model of Lewis lung carcinoma.
- Clec14a ADCs showed in vitro cytotoxicity, reduced tumour vascularisation and reduced tumour burden in a murine model of Lewis lung carcinoma.

Clinical impact: renal tumours and other solid tumours.

The Team: Professor Roy Bicknell, Institute of Cardiovascular Sciences, University of Birmingham; Dr Steven Lee, Institute of Immunology and Immunotherapy, University of Birmingham.

Intellectual Property status:

- Patent covering Clec14a inhibitors (WO2011/027132): granted in: Germany, France, UK, US.
- Patent covering antibodies 1-4-5 (WO2016/116760): granted in: Germany, France, UK, Japan, US, Canada, Australia.
- Patent covering antibodies 2-3 (WO2017/158339): granted/pending in: Europe, Japan, US, Canada, Australia, China.

Publications: J. Robinson *et al.* J Pathol Clin Res 2020; Mura, Swain, Zhuang *et al.* Oncogene 2012.

Seeking: We are looking for commercial partners to enable further clinical validation and market access to drive the Clec14a program into the clinic. Collaborative development or straight licensing interests are welcome.

Abs and ADCs targeting Clec14a as therapies for solid tumours

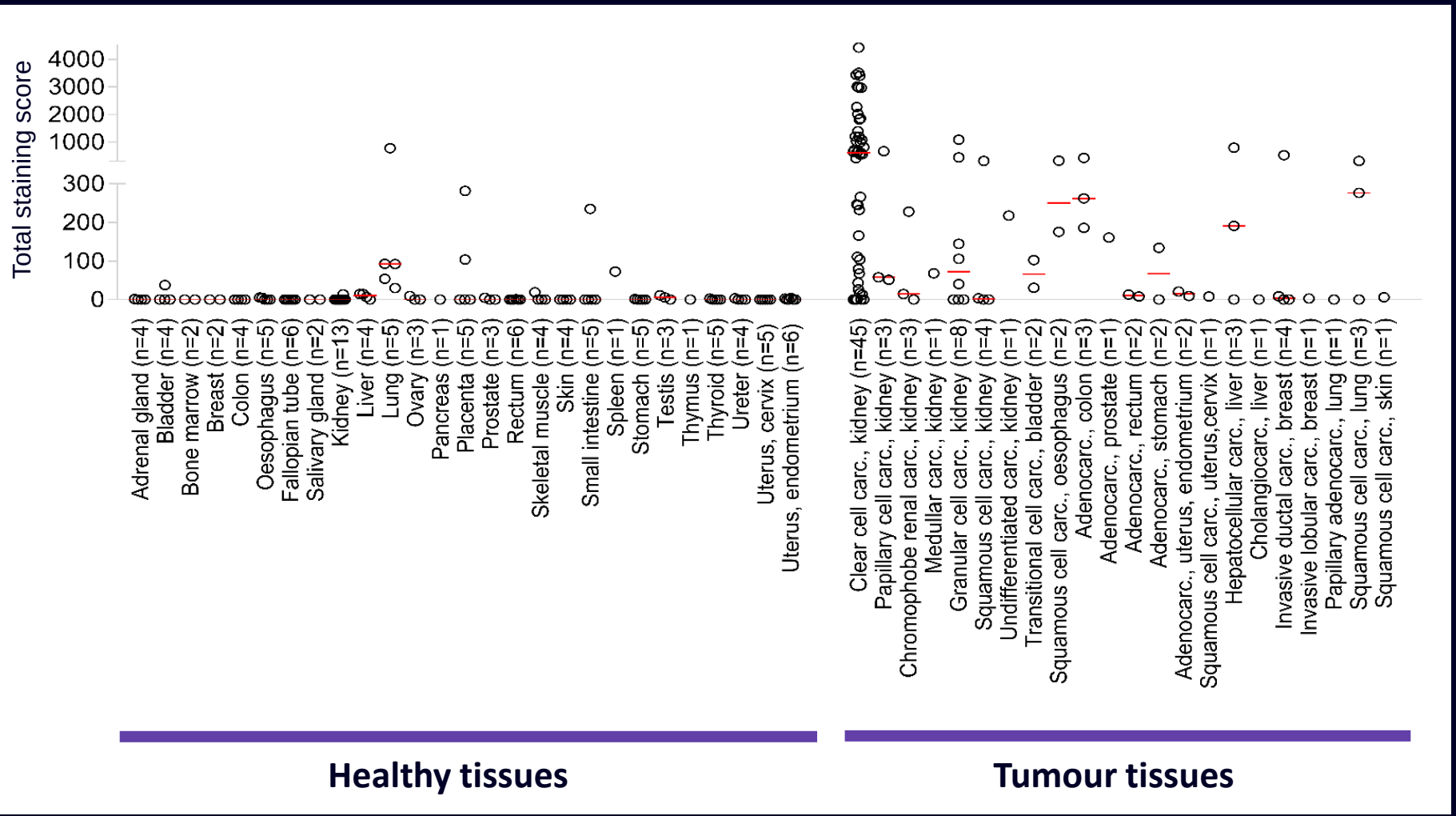
- Clec14a is a C-type lectin transmembrane protein that is specifically expressed on the surface of tumour endothelial cells. It has been demonstrated that CLEC14A expression is upregulated by reduced shear stress from reduced blood flow.

- The investigators have established that CLEC14A expression is significantly more abundant in human tumour tissues, compared to healthy tissues.

- Angiogenesis targeting agents, such as the VEGF/VEGF-R inhibitor Avastin, block neo-angiogenesis by inhibiting VEGF-A, but they do not eradicate established tumour vasculature. ADCs against Clec14a can instead specifically disrupt tumour vasculature.

CLEC14A is specifically expressed in tumour tissues in human

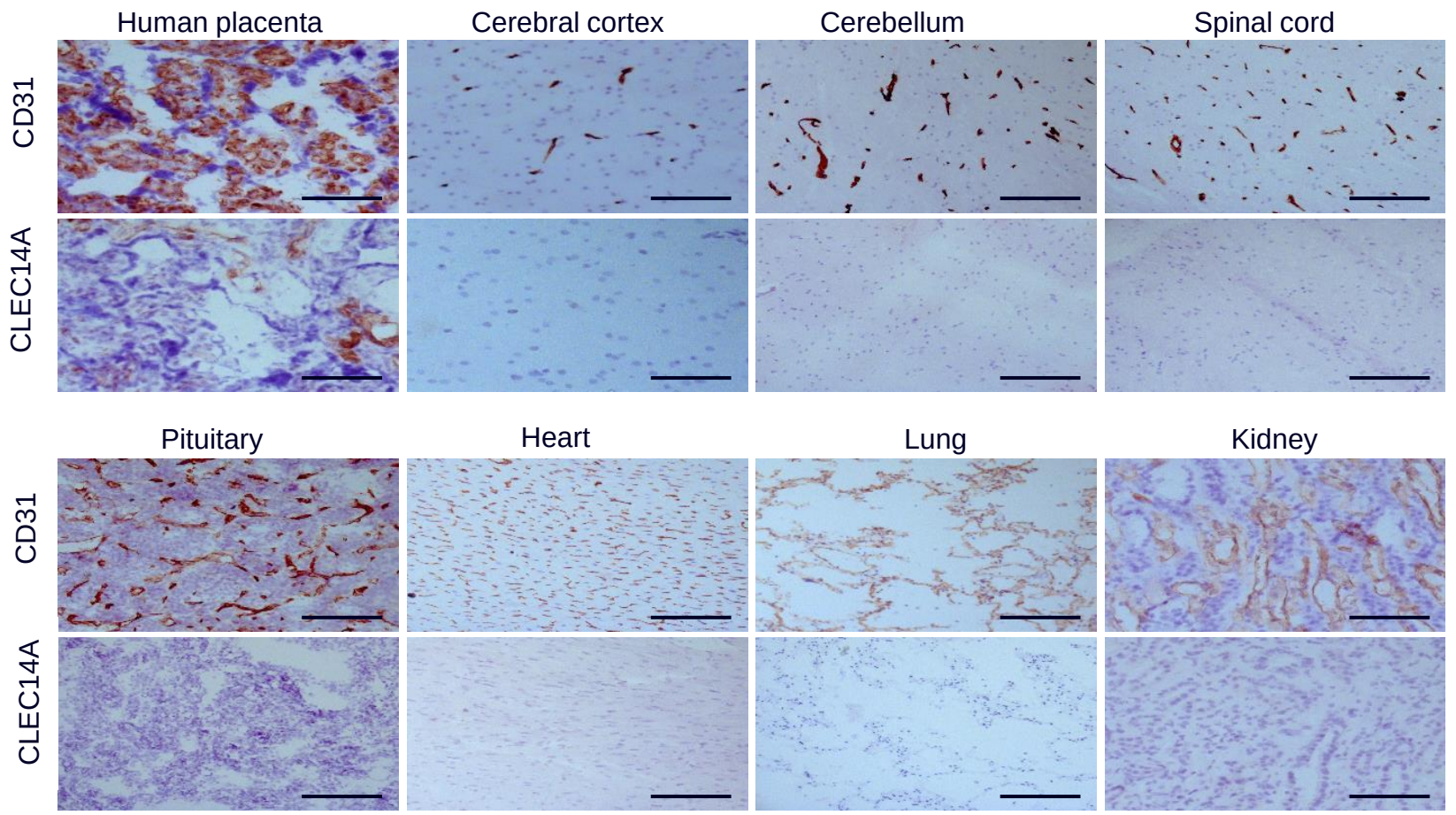
The expression of CLEC14A is regulated by shear stress. Expression of CLEC14A is high on vessels with poor blood flow such as those found in tumours. As blood flow ceases on death but cells do not die immediately, post-mortem tissue cannot be used to analyse CLEC14A expression. Tissue needs to be freshly resected and fixed post collection.



- **<u>Immunohistochemistry:</u>** human healthy tissues and tumour tissues were analysed for CLEC14a and CD31 (vascular marker) expression.
- The graph shows the intensity of Clec14a staining multiplied by the % of vessels stained multiplied by the density of vasculature within the tissue (“total staining score”). (Red line = median score).

Clec14a is highly expressed in kidney tumour tissues.

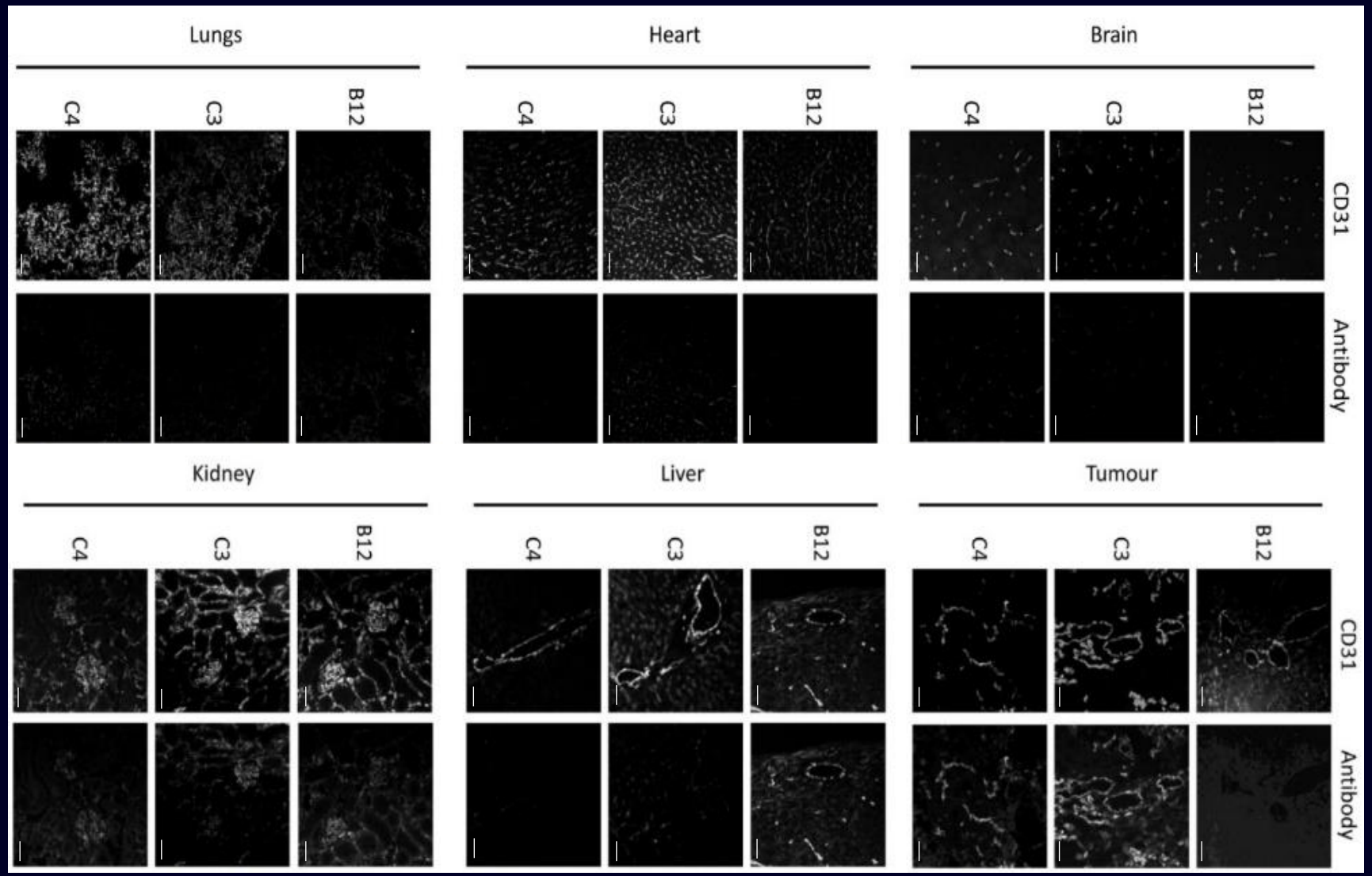
CLEC14A is not expressed in healthy tissues in primates



- **Immunohistochemistry:** CLEC14A protein expression was analysed in cynomolgus macaque tissues.
- Images of CD31 (vascular marker) and CLEC14A staining on frozen sections of human placenta and cynomolgus macaque tissues. Concentration-matched isotype control antibody staining was negative. Scale bars = 100 µm.

Clec14 in not expressed in primate healthy tissues.

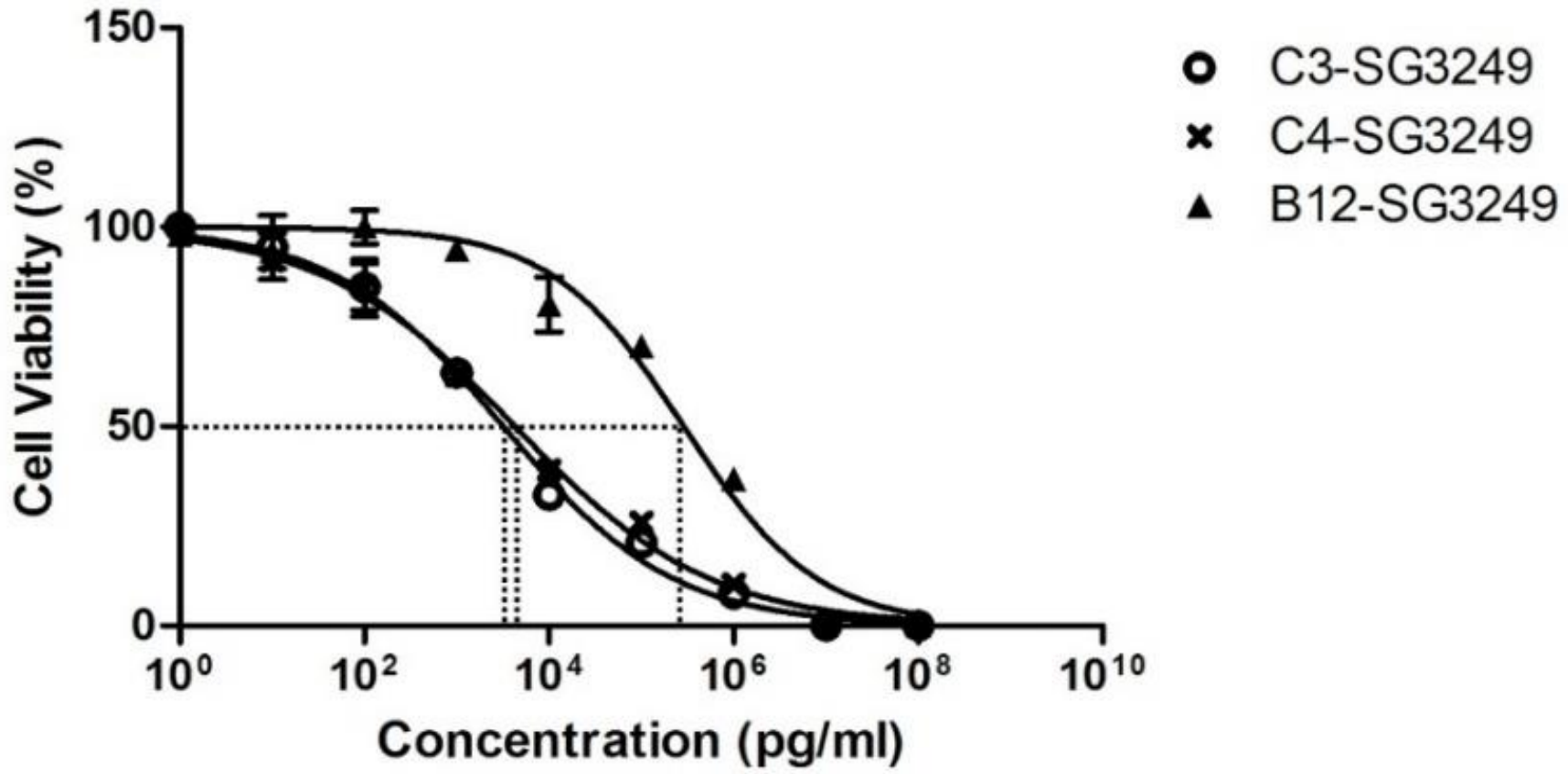
Clec14a Abs localise mainly in tumour tissues in tumour bearing mice



- **Clec14a Antibodies localisation:** the researchers generated five antibodies against Clec14a.
- To characterise antibody distribution *in vivo*, antibodies C3, C4 and B12 (negative control) were administered intravenously to C57BL6 mice with Lewis lung carcinoma. Mice were culled after 90 minutes and tissues were harvested and immune-stained to visualise antibody localisation.
- Images show the antibodies' staining and CD31 (vascular marker) staining. Scale bar = 10 µm

The antibodies C3 and C4 showed strong staining within tumour sections and showed no staining in the key organs (lungs, heart, brain, liver) and low staining in the kidney, a known major route for antibody clearance.

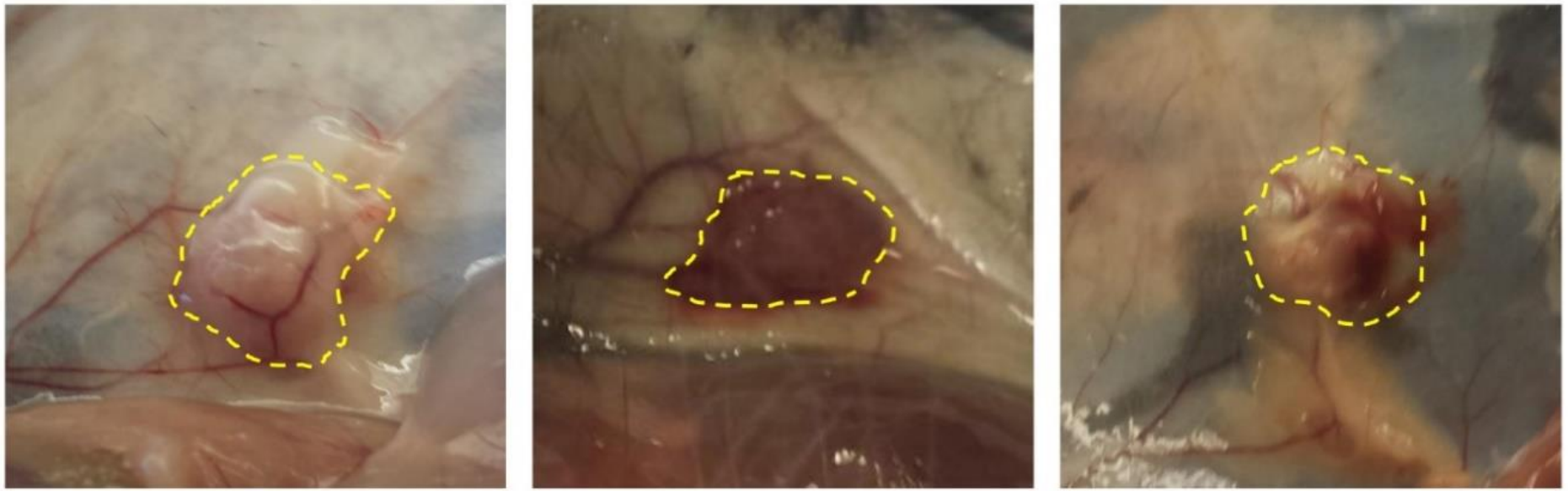
ADCs against Clec14a cause *in vitro* cytotoxicity



- **Clec14a ADCs *in vitro* cytotoxicity:** ADCs were generated with two anti-Clec14a antibodies conjugated to SG3249, a pyrrolobenzodiazepine dimer-based DNA crosslinking agent which induces cell death by causing DNA damage.
- The graph shows the viability of HUVECs (Human umbilical vein endothelial cells, primary endothelial cells that express Clec14a), treated with increasing concentrations of Clec14a targeting ADCs, C3-SG3249, C4-SG3249, or B12-SG3249 (control) for 96 hours.

ADCs against CLEC14A reduce vascularisation and tumour burden in tumour bearing mice

A



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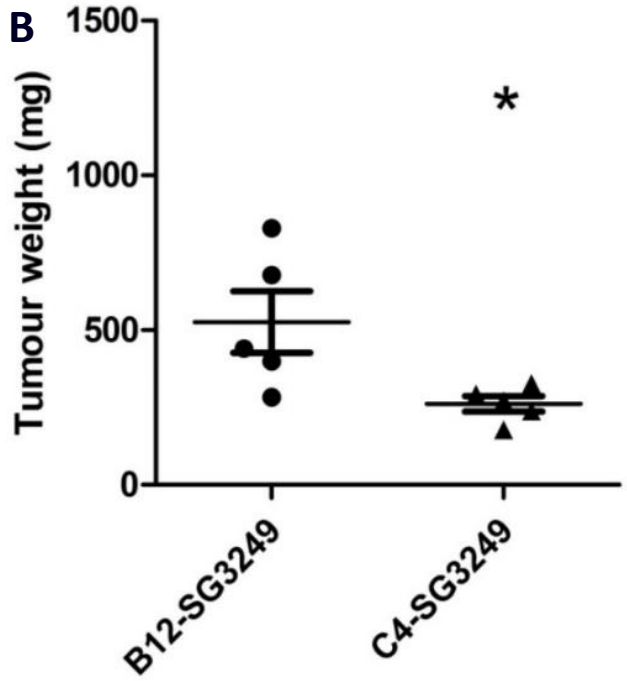

A) C57BL6 mice with Lewis lung carcinoma were treated with 1mg/kg of C3-SG3249, C4-SG3249 or B12-SG3249 (control) and culled after 24 hours. Leakage of blood into surrounding tissues was observed with C3-SG3249 and C4-SG3249 treatment, indicating vascular damage. Yellow dashes delineate tumour boundaries.

B) Endpoint weights of Lewis lung carcinoma tumours in C57BL6 mice after two weekly treatments with C4-SG3249 or B12-SG3249. n=5.

The researchers hypothesise that the CLEC14A ADC kills the CLEC14A expressing tumour endothelial cells. This leads to haemorrhagic necrosis of the tumour.

Clec14a technologies patent portfolio

Patent family and application number	Publication Number	Filing Date	Status
Inhibitors Patent PCT/GB2010/001689	WO2011/027132	03/09/2010	Granted in UK, Germany, France, and US
Antibodies C1-4-5 Patent PCT/GB2016/050134	WO2016/116760	21/01/2016	Granted in JP, AU, CA, UK, Germany, France, US
Antibodies C2-3 patent PCT/GB2017/050689	WO2017/158339	14/03/2017	Granted/pending in AU, CA, EP, JP, CN, US

Seeking

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