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NOVEL IMAGING AGENTS FOR DETECTING CELL DEATH

A team led by Professor Kevin Brindle has developed a novel agent for use in molecular imaging:

- A 14kDa protein that binds tightly to dying cells
- Can be cross-linked to a variety of contrast agents
- Is suitable for use with *in vivo* imaging technologies such as MRI, PET and SPECT
- Is suitable for *in vitro* imaging technologies, such as fluorescence microscopy and flow cytometry

May be used as a prognostic indicator of treatment outcome for:

- Cancer
- Cardiac infarction
- Cardiac plaque formation
- Age-related macular degeneration

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Background

Targeted imaging agents that are able to detect the onset and extent of cell death *in vivo* following therapy are considered to be good predictive indicators of treatment outcome.

Currently, apoptosis (a form of cell death) can be detected *in vivo* by radionuclide imaging, using radiolabelled Annexin V. Annexin V is a large protein that is cleared slowly in humans by the liver and kidneys and which is complex and expensive to manufacture.

Technology

Prof Brindle and colleagues at the University of Cambridge have developed a series of imaging agents built around a modified 14kDa C2A domain of the protein Synaptotagmin I, which incorporates a specific Ser-Cys mutation. This modified C2A domain retains its ability to bind tightly to phosphatidylserine, which is externalised on the surface of dying (apoptotic) cells.

The Ser-Cys mutation allows the introduction of a chemical linker and incorporation of different contrast agent labels onto the C2A domain, allowing real time *in vivo* imaging of cell death using a range of methods. The choice of label is determined by the molecular imaging modality to be used, such as MRI, PET, SPECT, radiography, fluoroscopy or fluorescence imaging. Attachment of a fluorescent label also allows apoptosis detection *in vitro*, using fluorescence microscopy or flow cytometry.

The technology can be used to assess the level of cell death at a particular site and may be used to assess the effectiveness of therapy in treating disease (for example cell death in tumours) or to assess the level of cell damage following a traumatic event such as cardiac infarction.

Reference

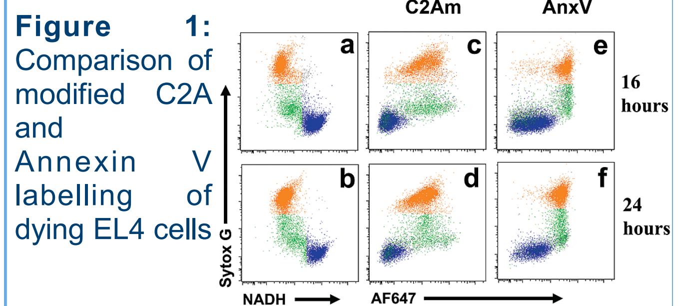
Alam, I *et al*. Comparison of the C2A domain of Synaptotagmin I and Annexin V as probes for detecting cell death. *Bioconjug Chem.* 2010 Apr 19 [epub ahead of print].

Commercialisation

The C2A technology allows the development of a suite of reagents, with enhanced specificity for dying cells, for clinical and pre-clinical high resolution imaging. These products are likely to be easier and cheaper to produce than existing markers and have the added benefit of patent protection.

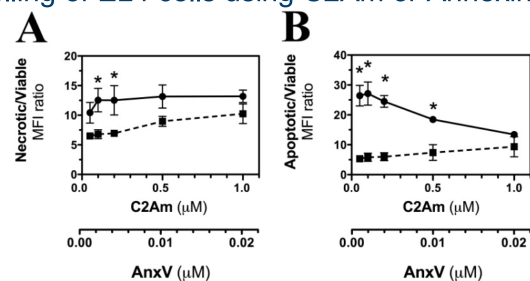
We are seeking commercial partners for licensing, collaboration and development of this technology, which is protected by patent application, published as WO2009/133362.

Figure 1: Comparison of modified C2A and Annexin V labelling of dying EL4 cells



Flow cytometic measurements of (a.b) NADH autofluorescence and Syntox green nuclear staining or AlexaFlor-647 C2A (c,d) or Annexin V (e,f) showing apoptotic (green), necrotic (orange), and viable (blue) cells populations.

Figure 2: The effect of probe concentration on labelling of EL4 cells using C2Am or Annexin V



Data are represented as ratios of mean fluorescence intensity (MFI): necrotic/viable (A) and apoptotic/viable (B). C2Am (closed circles) or AnxV (closed squares). C2Am labelled more specifically both necrotic (A) and apoptotic (B) EL4 cells, compared with Annexin V. Data shown as means ± SEM. * $P<0.05$, significant difference (N=3, two-way ANOVA).

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