Giving T cells a boost

Defining the molecular characteristics of immune-stimulatory antibodies for cancer immunotherapy

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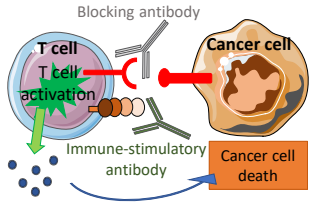
Cancer in the UK

- One diagnosis every two minutes
- 375,000 new cancer cases every year
- 166,000 cancer deaths every year
- Equivalent to 450 deaths every day
- Cancer survival has doubled in the last 40 years due to novel treatments[1]

The promise of antibody immunotherapy

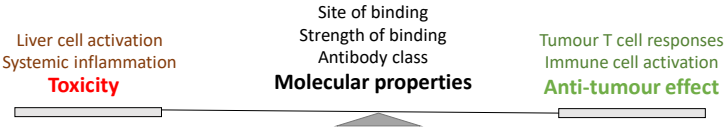


- **T cells** are cells which can **kill** pathogen-infected cells and **cancer cells**.
- T cells have activating and inhibitory receptors on their surface which control their function.
- **Monoclonal antibodies** can bind these receptors to **activate T cells** and unleash their killing activity[2].

The challenge of targeting the activating receptor 4-1BB

- **4-1BB is an activating receptor** on T cells, and antibodies binding 4-1BB can be used to activate these cells.
- Two 4-1BB antibodies have been in clinical trials[3]:
  - **Urelumab** was a potent anti-tumour agent but trials were halted due to **toxicity.**
  - **Utomilumab** was safe but exerted very **limited anti-tumour activity.**

The antibody properties which determine efficacy and toxicity remain unclear

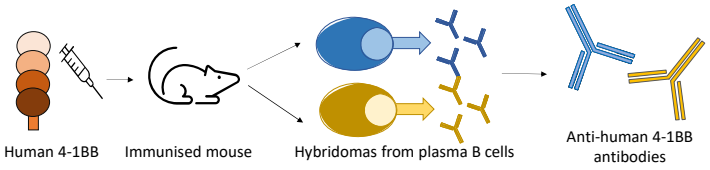


We aim to generate a novel panel of 4-1BB antibodies, characterise their molecular properties and establish which antibody characteristics are responsible for efficacy and toxicity.

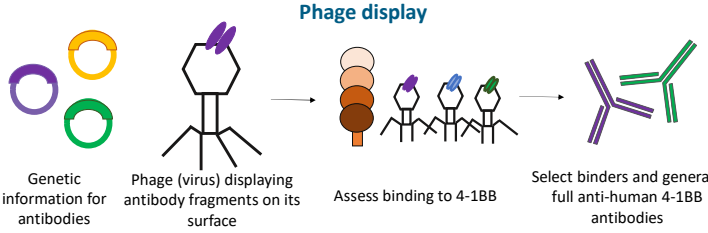
The Project

1. Generating antibodies against human 4-1BB

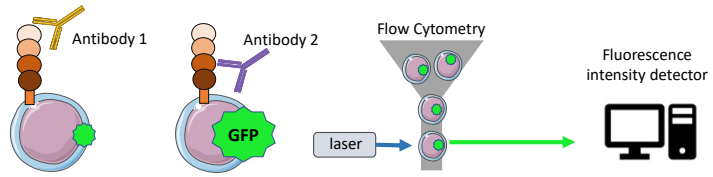
Hybridoma technology



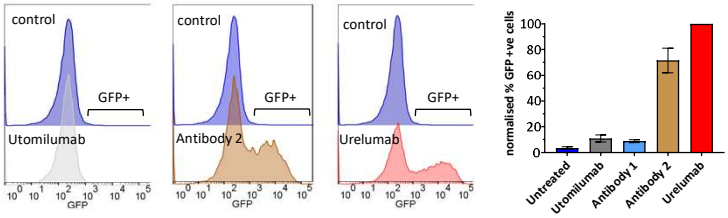
Phage display



2. How effective are anti 4-1BB antibodies at activating T cells?

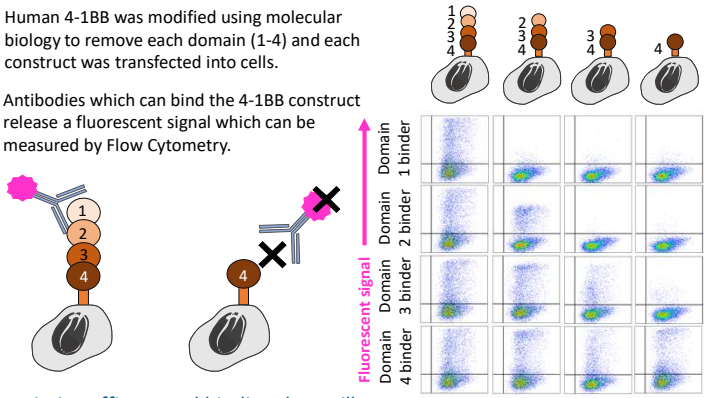


4-1BB signalling induced by the antibodies results in a green fluorescent protein (GFP) which can be detected by Flow cytometry. **Potent stimulatory antibodies induce a high GFP signal.**



3. Molecular characterisation of anti 4-1BB antibodies

- Human 4-1BB was modified using molecular biology to remove each domain (1-4) and each construct was transfected into cells.
- Antibodies which can bind the 4-1BB construct release a fluorescent signal which can be measured by Flow Cytometry.

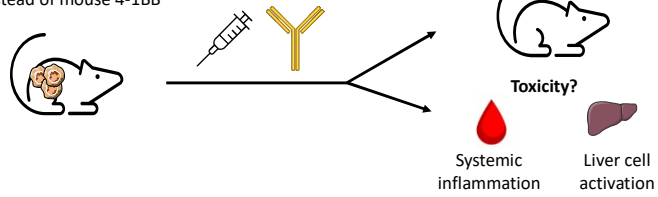


Associating efficacy and binding data will tell us whether targeting a specific site is preferential for immune stimulation

We have mapped the binding site of 30 unique antibodies in total

4. Next: Can our antibodies cure tumours without toxicity in transgenic mice expressing human 4-1BB?

Transgenic mouse
Its cells express human 4-1BB instead of mouse 4-1BB



In summary

- We have a panel of 30 **novel anti human 4-1BB monoclonal antibodies.**
- We have identified poor (antibody 1) and potent (antibody 2) activators.
- We have begun the **molecular characterisation** of these antibodies to establish **associations between effectiveness and antibody properties.**

Potential Impact

Determining the molecular characteristics of potent and safe agonists will contribute towards:

- Rational monoclonal antibody engineering
- Selection of optimal therapeutic agents

Clinical success of immune-stimulatory antibodies

Therapeutic success and improved patient survival

Acknowledgements

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References

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2. Mayes, Hance and Hoos. The promises and challenges of immune agonist antibody development in cancer (2018). Nat. Rev. Drug Discov. 177 *17*, 509–527.
3. Makkouk, Chester and Kohrt (2016). Rationale for anti-CD137 cancer immunotherapy. Eur. J. Cancer *54*, 112-119.

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