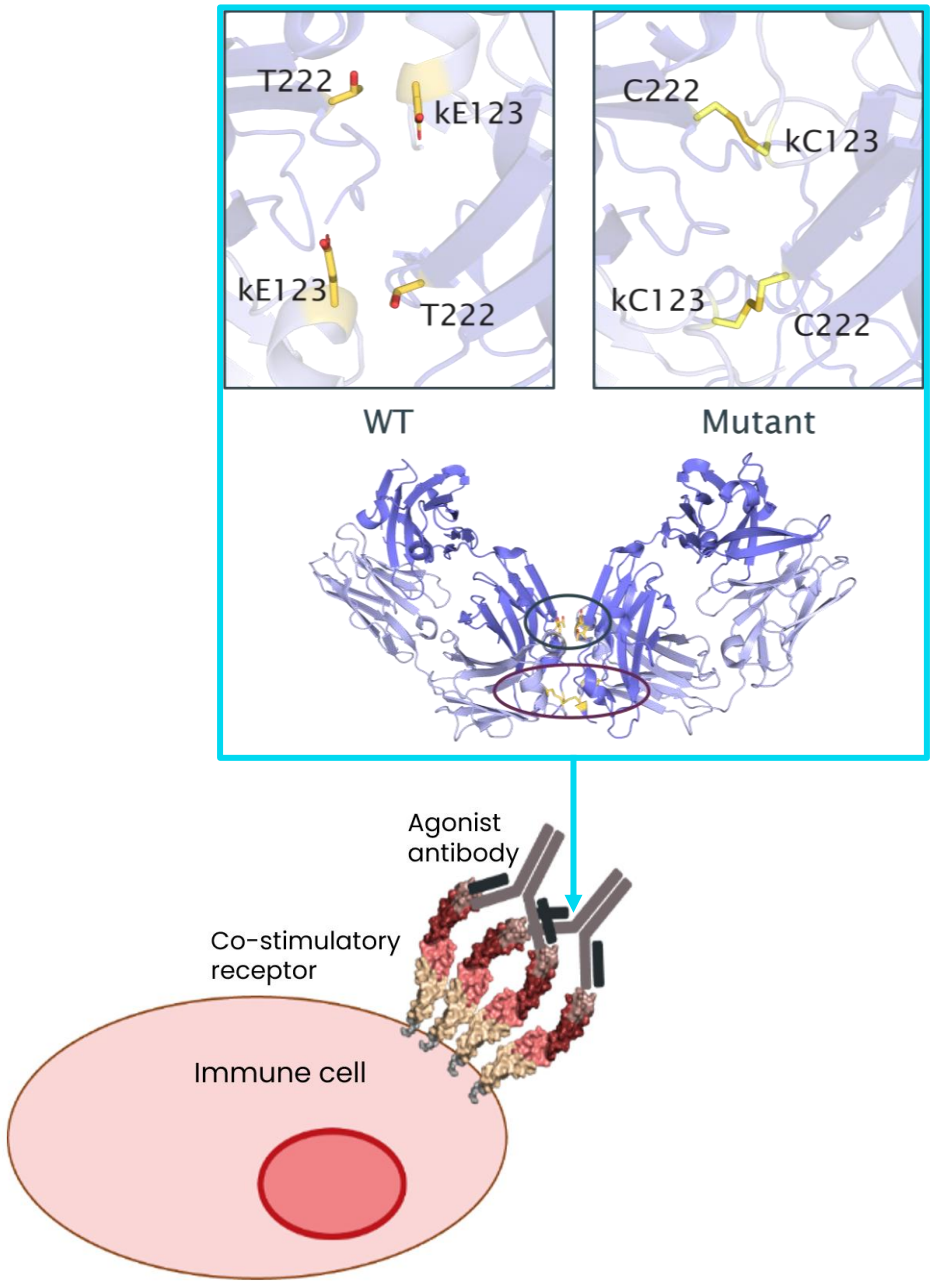


CANCER RESEARCH HORIZONS

# Antibody Clamp for enhanced immunotherapy agonism

Tech ID: CR/2023-005

v1, 2025

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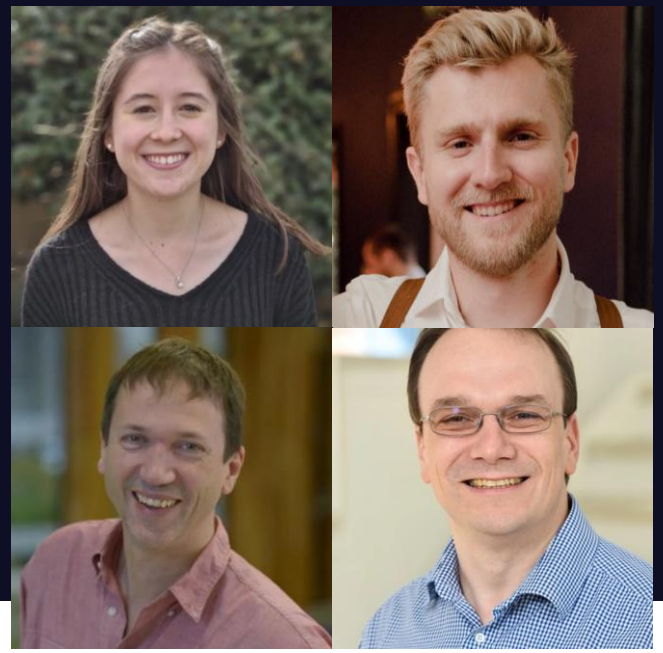
We are beating cancer

# Meet the Technology Team

## Professor Mark Cragg

Professor in Experimental Cancer Biology,
University of Southampton



## Named Inventors

Isabel Elliott, Dr Hayden Fisher
Prof. Ivo Tews, Prof. Jonathan Essex


## Research Group

Centre for Cancer Immunology, world specialists in antibody engineering

CANCER RESEARCH HORIZONS

# Technology Overview

The Ab Clamp is a novel, simple & flexible plug-&-play technology with potential to increase agonism of antibodies for cancer immunotherapy

- Restricts Fab arm mobility via simple mutations
- More compact Abs increase receptor clustering and agonism
- Can be deployed in any isotype and any antibody

# Quick Factsheet

CANCER RESEARCH HORIZONS

## Modality

Antibody engineering platform technology

## USP

Simple plug & play tech applicable to all antibody isotypes

## Lead Op.

TNFR and IgSF family receptor candidates in preclinical validation: CD40, 4-1BB, OX40, CD27 & CD28

## IP

Patents filed 20/12/24 covering method for increasing agonism of monospecific Abs by decreasing Fab arm flexibility

## Indications

Pre-clinical validation using indication-agnostic Abs trialled in solid/blood cancers e.g. NSCLC/ovarian/gastric/breast

## Partnering

Opportunity for co-development and licensing, including those with stalled or in-development immunostimulatory antibodies

# Technology Overview

**Problem:** There is a clinical need for new cancer immunotherapy approaches, as progress has stalled and efficacy is limited:

- 70-80% of cancer patients are ICI unresponsive

**Solution:** Immunostimulatory antibodies (ISAs) target & bind co-stimulatory receptors expressed on immune cells to elicit agonistic effects, including (i) boosted anti-tumour immune responses & (ii) synergy with ICIs.

- BUT many ISAs are stalling in clinical trials, highlighting an unmet need for more agonistic & less toxic ISAs

**Technology:** **Antibody Clamp is positioned to unlock powerful & safe agonists for broad clinical impact**.

- Simple mutations of two residues (conserved across isotypes) to cysteine introduces two disulfide bonds
- This limits relative Fab arm flexibility, resulting in compact Abs that increase receptor clustering/agonism
- Clamped ISAs are potentially more effective and less toxic
- Wide range of targets - can be applied to any isotype and any antibody
- Has potential to synergise with other Ab engineering methods (e.g. bispecificity/multi-valency)

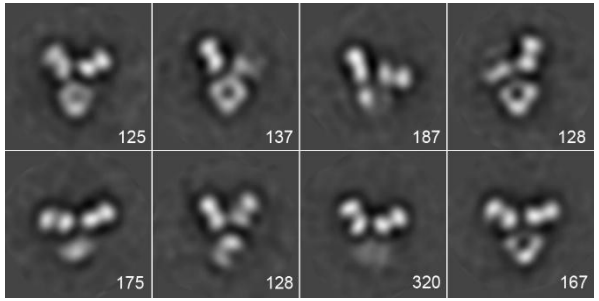
**Findings:**

- Ab Clamp has been successfully applied to reduce flexibility of multiple ISAs to receptors in the TNFR & IgSF families
- POC data validates that Clamped ISAs elicit increased agonism
- Compelling in vivo data demonstrating improved agonism of Clamped v WT Chi Lob 7/4 (CD40)

**Development:** Awarded a CRH translational grant to benchmark increased Ab agonism vs other Ab engineering methods, and to perform head-to-head comparison experiments for Clamped v WT ISAs in:

i. CRC tumour model (PK & efficacy)
ii. PBMC humanized mouse model (toxicity)

# Key Technology Data:
## Negative stain electron microscopy (nsEM) to investigate conformation of Clamped Abs

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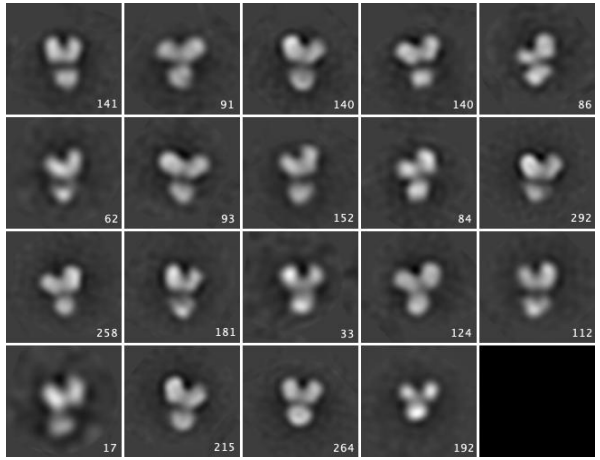



Clamp

**Figure 1)** nsEM showing hIgG1 conformation restricted by Clamp

Defined binding geometry, with the two Fab arms closer together

# Key Technology Data:
## Restriction and agonism of clamped hIgG1

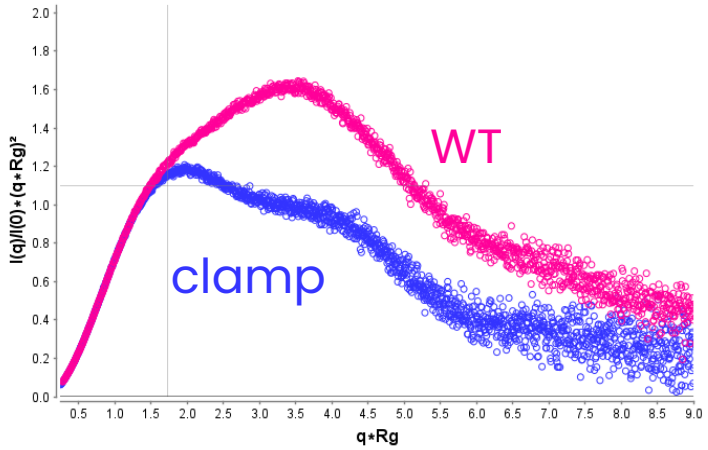



**Figure 2a)** SAXS showing hIgG1 conformational restriction

Achieved in 7/7 mAbs for TNFR and IgSF antibodies

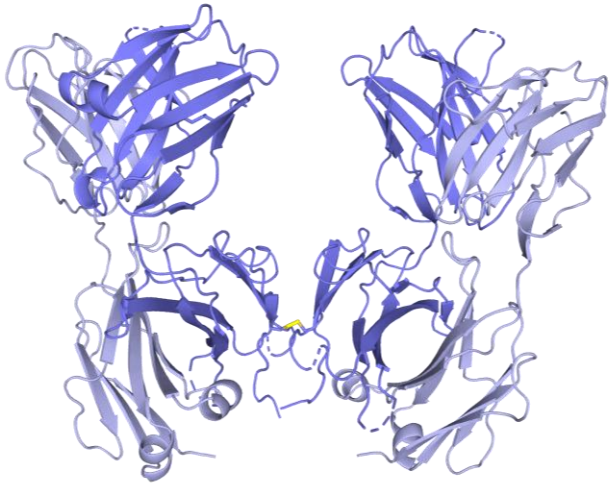


**Figure 3)** Simple mutations

Plug & play
Low risk of liability
Typical IgG yields

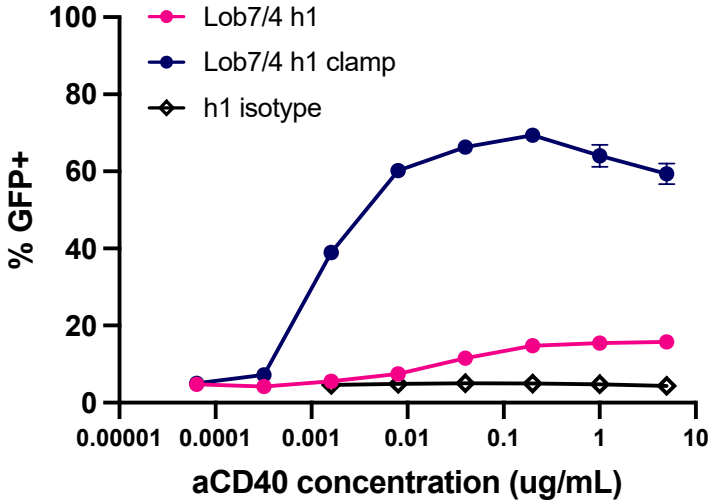



**Figure 4a)** Clamp evoking increased hIgG1 Ab agonism, applicable across isotypes

Exploit new MoAs

# Key Technology Data:
## Restriction and agonism of clamped hIgG4

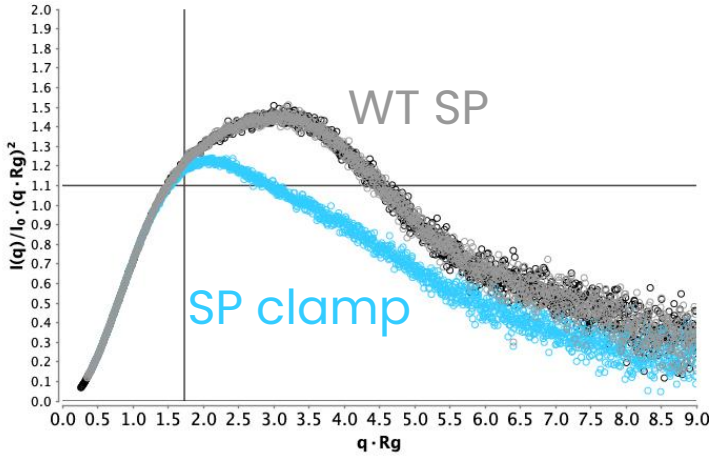
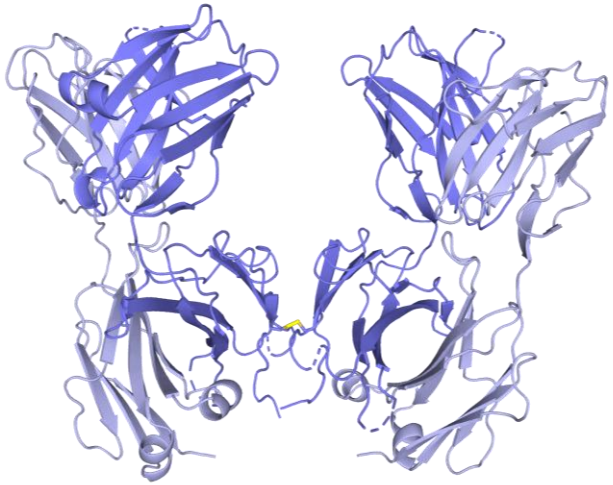



**Figure 2b)** SAXS showing hIgG4 conformational restriction

Achieved in 7/7 mAbs for TNFR and IgSF antibodies



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Plug & play
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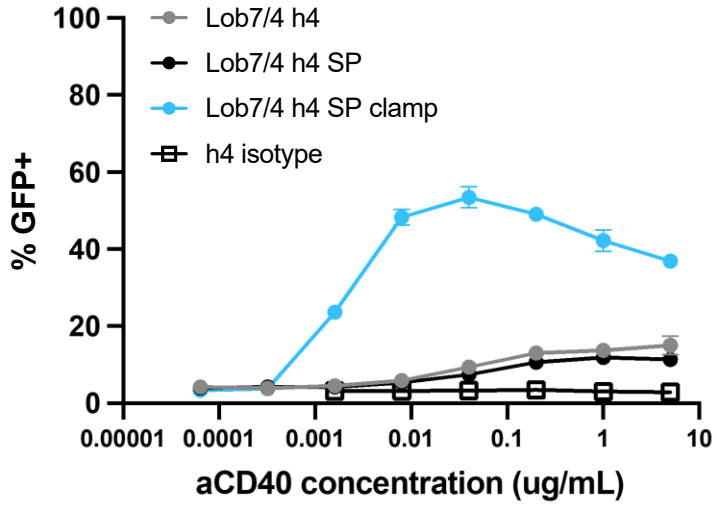


**Figure 4b)** Clamp evoking increased hIgG4 Ab agonism, applicable across isotypes

Exploit new MoAs

**Tech ID**: CR/2023-005, non-confidential deck v1 2025

# Upcoming pre-clinical work

## Molecule production & in-vitro studies

CANCER RESEARCH HORIZONS

**Work Package 1**

- Produce a series of engineered Clamped antibodies using different technologies, and combinations thereof.
- Assess yield, aggregation, receptor binding, affinity and FcgR binding.

**Aims**

- Demonstrate whether these various molecules can be produced in good yield, without liabilities.

**Work Package 2**

- Evaluate efficacy in vitro using a series of well-established assays, including in primary cell activity assays.

**Aims**

- Determine which technologies display the highest agonism *in vitro* and whether this requires FcgR expressing cells.

# Upcoming pre-clinical work

## In-vivo efficacy & toxicity studies

CANCER RESEARCH HORIZONS

**Work Package 3**

- Evaluate the most promising antibodies *in vivo* for both efficacy and toxicity.
- Use of MC38 CRC mouse model (for efficacy).
- Use of PBMC humanized mouse model (for toxicity).

**Aims**

- Define if the Clamp is more agonistic (and therefore therapeutic) *in vivo*.
- Evaluate if Clamped antibodies display toxicity compared to wild type antibodies.
- Benchmark the clamp against other agonising technologies, revealing in what scenarios it would be best employed.

# Opportunity Overview

CANCER RESEARCH HORIZONS

**Intellectual Property status:**

- Protected by 20/12/2024 PCT filing (*PCT/GB2024/053188*).
- Claiming a method of increasing activity of an antigen-binding protein by modifying amino acids in specific locations on heavy & light chains.
- Covers all antibody isotypes and a broad range of receptors:
    - **TNFR**: CD40, 4-1BB, OX40, CD27, DR4, DR5
    - **IgSF**: CD28, ICOS, PD1
- Covers the engineered antigen-binding proteins, and associated nucleic acids, expression vectors, and host cells.
- Covers pharmaceutical compositions comprising the engineered antigen-binding proteins.

**Key publication:**

- Elliott, I.G., Fisher, H., Chan, H.T.C. *et al.* Structure-guided disulfide engineering restricts antibody conformation to elicit TNFR agonism. *Nat Commun* **16**, 3495 (2025).

**Seeking:**

- We are looking for commercial partners to enable further pre-clinical validation and market access to drive the Antibody Clamp into the clinic, including those with stalled or in-development ISAs.
- Co-development collaborations or straight licensing interests are welcome.

# CR/2023-005: Antibody Clamp for enhanced immunotherapy agonism

CANCER RESEARCH HORIZONS

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We are beating cancer

## Contact


Gregor Lawrence

Business Development Associate

Therapeutics

[Click here] to email Gregor

# Academic background

- Cragg lab researching receptor agonism
  - IgG2 is optimal for agonism
    - Has the unique property amongst human isotypes in driving stimulation of CD40 in an FcgR independent manner
  - Driven by receptor clustering
  - Hinge is central and unique – disulphide shuffling

- Cragg lab designed a series of hinge variants with restricted diS (Cys to Ser (C-S) mutations)
  - C-S variants span a range of agonistic activities
    - Epitope identical – only difference is in the hinge
  - Structures of C-S variants elucidated
    - Anomalous scattering shows disulfide positions
    - Reveals a clear disulphide cross-over in agonistic forms

- Agonistic variants are more compact
  - To better appreciate dynamics of this, used the structural data to run atomistic Molecular Dynamics simulations
  - Took these structures and incorporated SAXS data to represent the solution phase conformations
  - Found that hIgG2 hinge restriction drives activity

# Academic background

- Cragg lab linked antibody flexibility to activity when targeting TNFRs (e.g. CD40 and 4-1BB)
  - Driven by the conformation of disulfide bonds in the hinge region.
  - Specific to the human IgG2 isotype.

- Prior "Antibody Hinge" technology:
  - Relies on the sequence of the hIgG2 isotype (extra-disulfides present).
  - Not applicable for other IgG subclasses.
    - Different subclasses have different properties and potential MoAs

- Key publication:
  - Orr, C.M., Fisher, H., Yu, X. *et al. Hinge disulfides in human IgG2 CD40 antibodies modulate receptor signaling by regulation of conformation and flexibility*. Sci Immun **7**, 73, (2022).

# Antibody Clamp

## We are seeking a licensing or collaboration partner

CANCER RESEARCH HORIZONS

**Problem:** Cancer immunotherapy needs new approaches, as progress has stalled and efficacy is limited

- 70-80% of cancer patients are ICI unresponsive

**Solution:** Immunostimulatory antibodies (ISAs) target & bind co-stimulatory receptors expressed on immune cells to elicit agonistic effects, including (i) boosted anti-tumour immune responses & (ii) synergy with ICIs

- BUT many ISAs are stalling in clinical trials, highlighting an unmet need for more agonistic & less toxic ISAs

**Technology:** **Antibody Clamp is positioned to unlock powerful & safe agonists for broad clinical impact**

- Simple mutations restrict Fab arm mobility, resulting in compact Abs that increase receptor clustering/agonism
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**Intellectual Property status:** Protected by Dec 2024 PCT filing

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

# Antibody Clamp for enhanced immunotherapy agonism

**The Ab Clamp is a novel, simple & flexible plug-&-play technology with potential to increase Ab agonism for cancer immunotherapy**

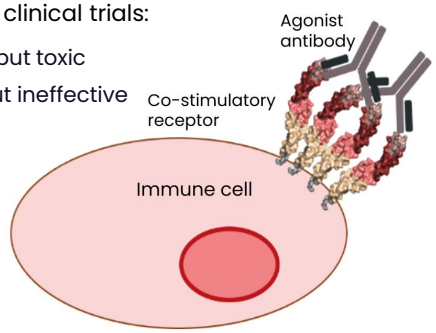
- Restricts Fab arm mobility via simple mutations
- More compact Abs increase receptor clustering and agonism
- Can be deployed in any isotype and any antibody

## Problem: Cancer immunotherapy needs new approaches

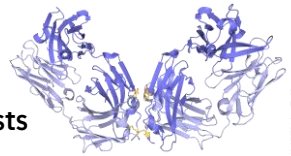
- Immunotherapies have revolutionised cancer treatment, through targeting inhibitory immune receptors (e.g. PD-1)
- But progress has stalled & their efficacy is limited
  - 70-80% of cancer patients are ICI unresponsive

## Approach: Immunostimulatory antibodies (ISAs)

- Target and bind co-stimulatory receptors expressed on immune cells to elicit agonistic effects, resulting in:
  - Boosted anti-tumour immune responses
  - Synergy with ICIs
- BUT, many ISAs are stalling in clinical trials:
  - Urelumab = effective but toxic
  - Utomilumab = safe but ineffective
- Therefore, there is an unmet need for more agonistic & less toxic ISAs



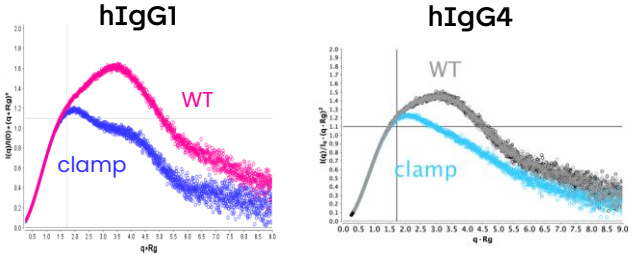

## Solution: Ab Clamp unlocks powerful & safe agonists



- Ab Clamp involves simple Ab modifications
- Clamped ISAs are potentially more effective and less toxic
- Ab Clamp can be applied to evoke agonism across isotypes in a wide range of targets
- Simpler to implement than other Ab engineering methods
  - Avoids increased instability, immunogenicity and clearance observed with Fc region Ab modifications
- Potential to synergise with other Ab engineering methods
  - e.g. bispecificity/multi-valency

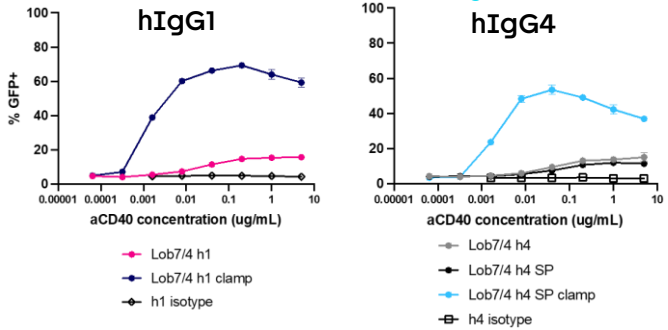
## Technology Stage of Development

- Ab Clamp has been successfully applied to reduce flexibility of multiple ISAs to receptors in the TNFR and IgSF families:
  - CD40, 4-1BB, OX40, CD27 & CD28
- POC data validates that Clamped ISAs elicit increased agonism
- Compelling *in vivo* data demonstrating improved agonism of Clamped v WT Chi Lob 7/4 (CD40)
- Awarded a CRH translational grant to perform head-to-head comparison experiments:
  - PK and efficacy for Clamped v WT Urelumab (4-1BB) and Chi Lob 7/4 (CD40) in CRC tumour model
  - Toxicity of Clamped vs WT 4-1BB ISAs in a PBMC humanized mouse model (versus Urelumab)
  - Benchmarking increased Ab agonism against other Ab engineering approaches




**SAXS shows Clamp restricts Ab flexibility**

**Which evokes increased Ab agonism**




## Opportunity Summary

**Seeking**: partners for co-development/licensing, including those with stalled or in-development ISAs

**IP**: protected by Dec 2024 PCT filing - covers all isotypes & a broad range of receptors (e.g. CD40, 4-1BB, CD28, CD27, DR4, DR5)

- Background IP in Ab engineering (*PCT/IB2015/052166*) & 4-1BB/OX40 Abs (*PCT/EP2016/076747*)

**Team**: research group are world specialists in antibody engineering


Lead contact
**Gregor Lawrence, MSc**
Cancer Research Horizons
Business Development Associate
gregor.lawrence@cancer.org.uk

Academic contact
**Prof. Mark Cragg**
University of Southampton
Professor in Experimental Cancer Biology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