LOX-1 Antibody Recruiting Molecules: Directing the Immune System Towards Immunosuppressive Cells

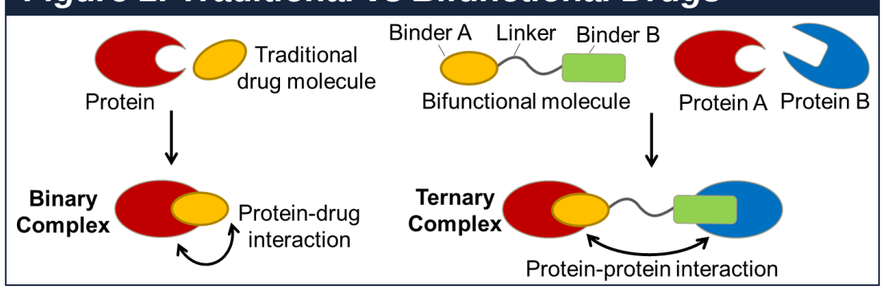
Katherine Macfarlane[‡], Dr. Svetlana Belyanskaya[¥], Dr. Carol Mulrooney[¥], Dr. Eric Shi[¥], Sopheary Op[¥], Jeff Messer[¥], Dr. Thomas Clohessy[†], Dr. Matthew Sender[Ж], Dr. Craig Jamieson[‡]

‡ Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow, G1 1XL, UK. ¥ Encoded Library Technology Department, GlaxoSmithKline, 200 Cambridge Park Drive, 6th Floor, Cambridge, MA 02140, USA.
† Medicinal Chemistry Department GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY, UK. Ж Chemical Biology, GlaxoSmithKline, 1250 S Collegeville Rd, Collegeville, PA 19426, USA.

Project Overview

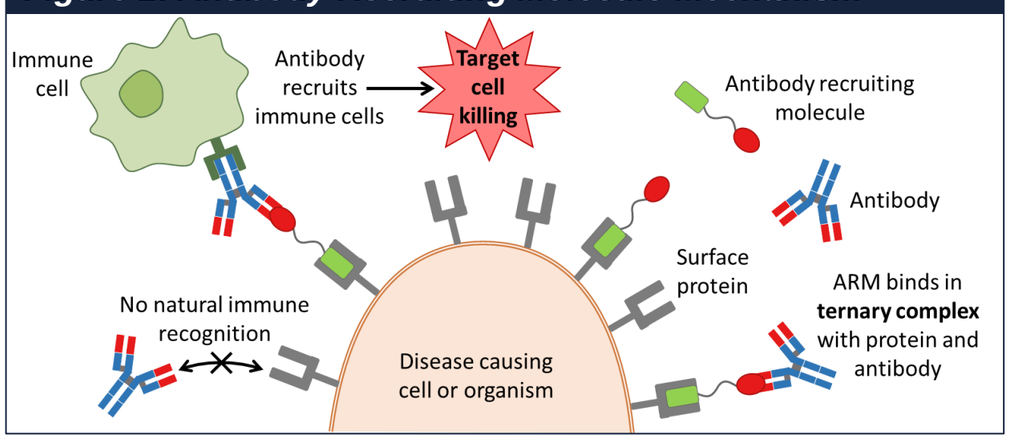
Bifunctional small molecules are molecules which can bind two different proteins simultaneously, inducing protein-protein interactions by proximity (Fig. 1). Compared to traditional small molecule drugs, which bind to a single protein target, this creates many new opportunities to influence biology Bifunctional small molecules are therefore very interesting to medicinal chemists as potential drugs.[1] In this work **DNA-encoded library technology** was used to rapidly identify and access novel bifunctional **Antibody Recruiting Molecules (ARMs)** against **Lectin-type Oxidized LDL Receptor 1 (LOX-1)**. These ARMs are designed to direct the immune system to kill immune suppressive cells which express LOX-1 on their surface, which could be beneficial to cancer and COVID-19 patients.

Figure 1: Traditional vs Bifunctional Drugs



Step 1

Figure 2: Antibody Recruiting Molecule Mechanism



1. Target Selection

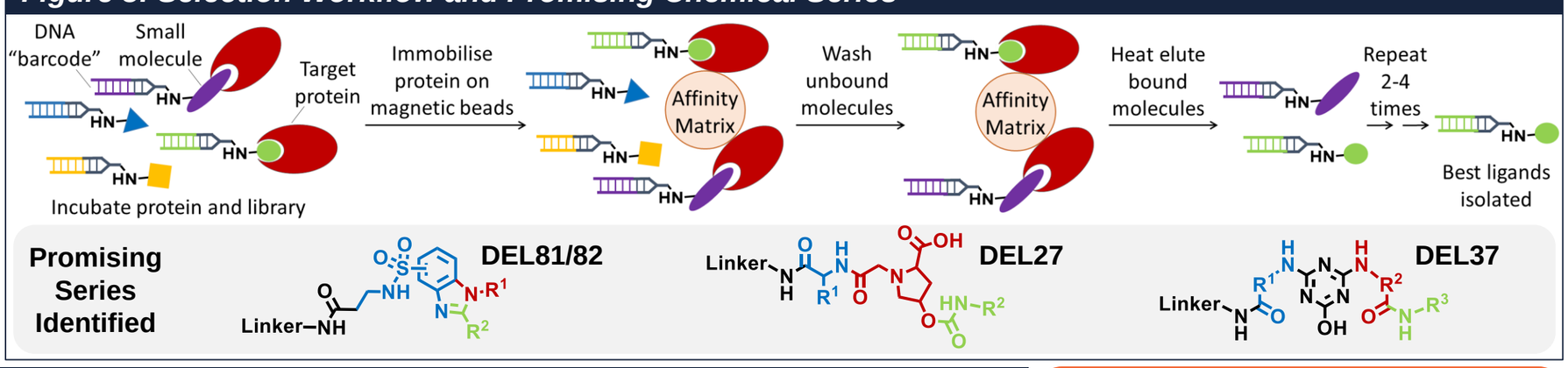
- **LOX-1** is highly overexpressed on immunosuppressive **Polymorphonuclear Myeloid-Derived Suppressor Cells (PMN-MDSCs)**[2]
- Selective killing of these immune suppressive cells could be beneficial in oncology[3]
  - May improve checkpoint inhibitor response[4,5]
  - Are also associated with acute respiratory distress in COVID-19 patients[6]
- **Antibody Recruiting Molecules (ARMs)** are one approach to cell killing (Fig. 2)[7]
  - ARM binds a surface receptor, and simultaneously to an antibody in a ternary complex
  - Antibody then recruits immune cells and complement, resulting in cell killing
- Current LOX-1 binders are unsuitable for ARM development,[8,9] novel binders are required

Step 2

2. DNA-Encoded Library Selection

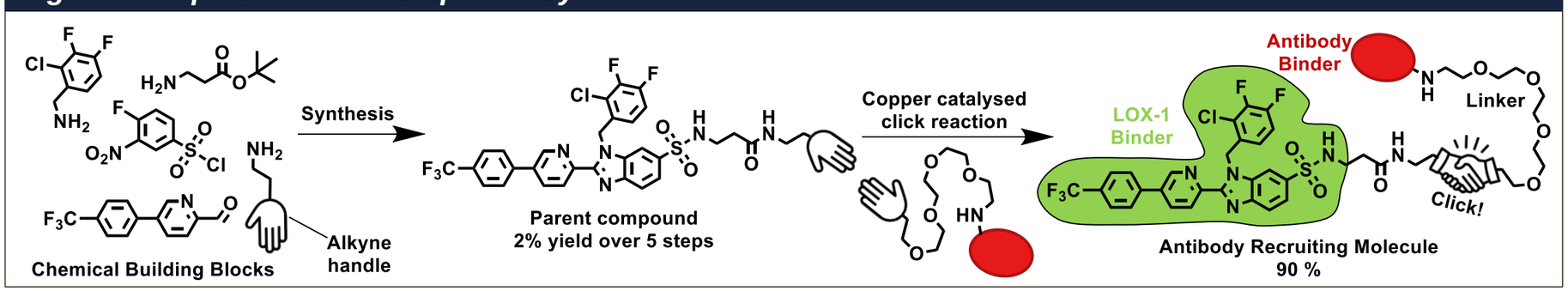
- DNA-encoded libraries: small molecules attached to a unique DNA "barcode"[10]
- **Billions** of compounds tested simultaneously for LOX-1 binding in one experiment (Fig. 3)
- DNA tags sequenced to identify binders
- Three chemical series were selected from 13,000 LOX-1 specific hits
- **17 compounds** chosen for synthesis

Figure 3: Selection Workflow and Promising Chemical Series



Step 3

Figure 4: Representative Compound Synthesis



3. ARM Synthesis

- Off-DNA synthesis was required for further biological testing (Fig. 4)
- Parent compounds were synthesised with an alkyne handle, which facilitates a copper catalysed "click" reaction
- Produces ARMs from parent compounds in one very reliable step

Step 4

4. Testing ARMs for Biological Activity

- Binding of parent compounds to LOX-1 first tested by Affinity Selection Mass Spectrometry (ASMS), and thermal shift (Fig. 6)
- **Small molecule ELISA** developed to test ARMs for ternary complex formation between LOX-1, ARM, and antibody[11] (below, Fig. 5)
- Closely simulates antibody recruitment at cell surface (Fig. 3)
- **Competition assays** were carried out with the parent LOX-1 binding compounds to assess specificity (Fig. 5)

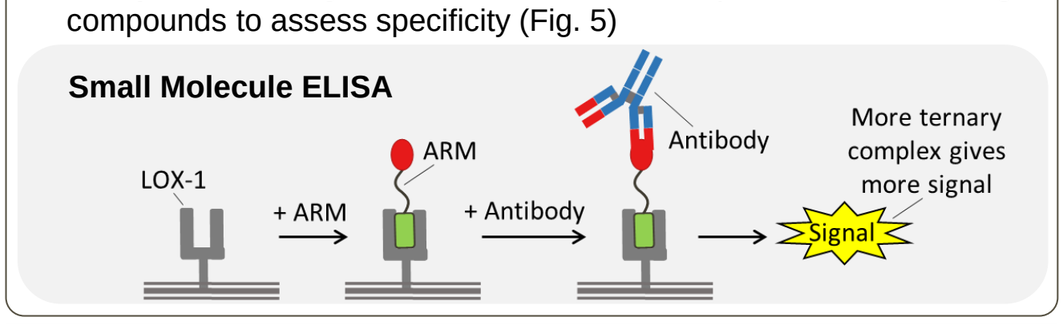
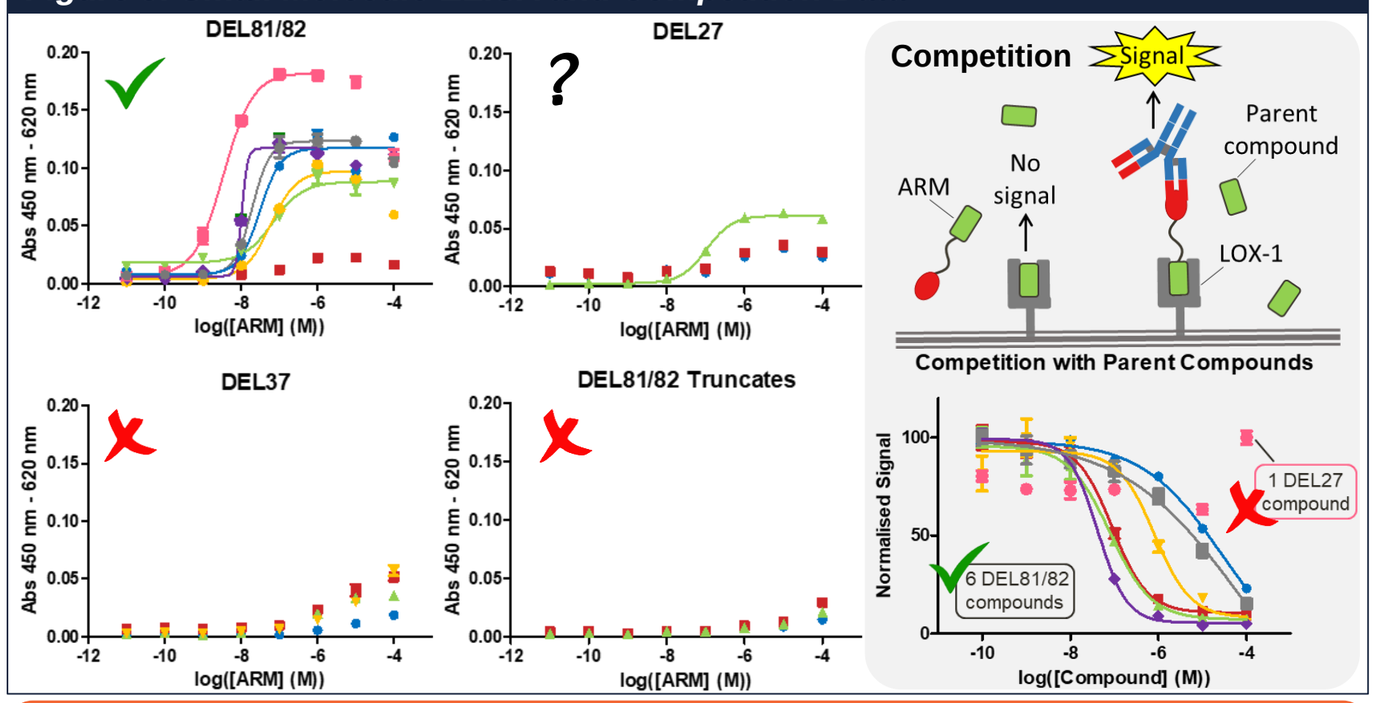
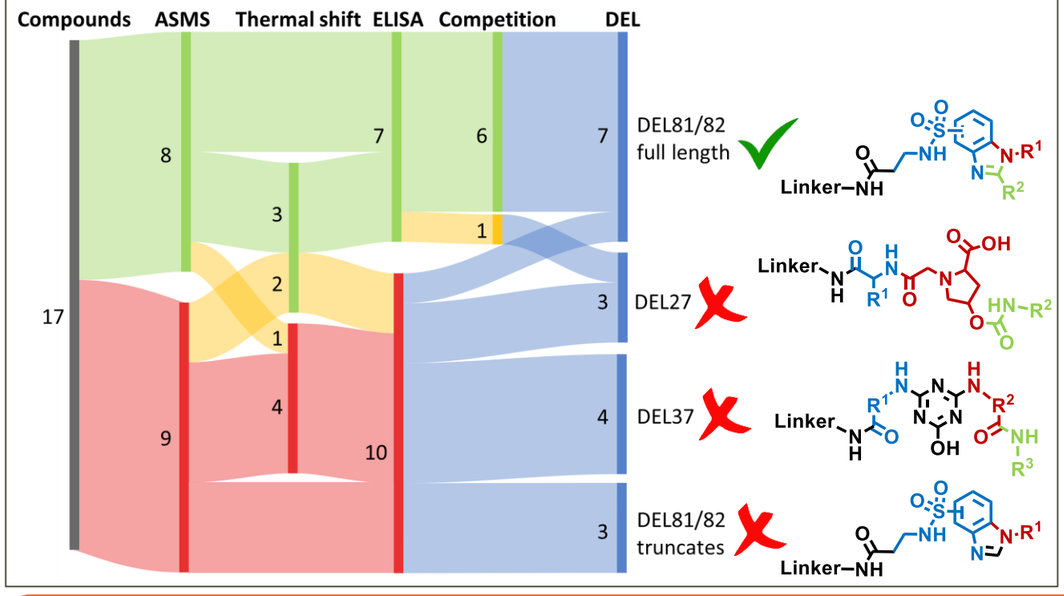


Figure 5: Small Molecule ELISA and Competition Data



Step 5

Figure 6: Summary of Assay Data



5. Biological Data Analysis

- DEL37 and DEL81/82 truncates gave negative results in all biological tests
- Binding of eight parent compounds from DEL81/82 and DEL27 series confirmed by **ASMS**
- Ten parent compounds also gave usable **thermal shift** data
  - Of these, five confirmed, with moderate correlation to ASMS
- Six DEL81/82 ARMs and one DEL27 ARM gave signal in the **small molecule ELISA** assay
  - Excellent correlation to ASMS and thermal shift (Fig. 6)
- 6/7 DEL81/82 ARMs signals were **competable** by the parent compounds, suggesting LOX-1 binding occurs at a specific binding site
- The DEL27 signal was **not competable**, likely does not have a specific binding site
- DEL81/82 was therefore the most promising series to progress

Conclusions and Outlook

Six promising ARMs have been identified, which demonstrate the required ternary complex formation with their two protein targets. They are all from the same chemical series, DEL81/82. Ongoing work focuses on further improving these compounds, and running biological assays to demonstrate killing of LOX-1 expressing cells.

(1) Gerry, C. J.; Schreiber, S. L. *Nature Chem. Bio*. **2020**, *16*, 369 (2) Chai, E.; Zhang, L.; Li, C. *Cancer Manag. Res.* **2019**, *11*, 7307 (3) Yang, Z. *et al. J. Hematol. Oncol.* **2020**, *13*, 10 (4) Youn, J. I. *et al. Sci. Rep.* **2020**, *10*, 9050 (5) Kim, H. R. *et al. Am. J. Respir. Crit. Care. Med.* **2019**, *199*, 243 (6) Rowlands, M.; Segal, F.; Hartl, D. *Front. Immunol.* **2021**, *12*, 697405 (7) Achilli, S.; Berthet, N.; Renaudet, O. *RSC Chem. Biol*., **2021**, *2*, 713–724 (8) Schnapp, G. *et al. Commun. Chem.* **2020**, *3*, 1 (9) Thakkar, S. *et al. Sci. Rep.* **2015**, *5*, 16740 (10) Madsen, D. *et al. Progress in Medicinal Chemistry*, Vol. 59, p. 181, Elsevier **2020** (11) Catalano, M. *et al. Anal. Chem.* **2020**, *92*, 10822–10829.

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Contact email: katherine.macfarlane@strath.ac.uk