

Target re-utilisation at the C-terminus

A novel approach to maximise probability-of-success in known targets.

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Summary

Many biopharmaceuticals are currently re-targeting existing mAB targets in the extracellular space (ligands and receptors) with orally available molecules - a medically viable option that unfortunately has recently become crowded thus reducing commercial opportunities. How can we overcome this? Here we show two examples of extracellular receptor targeting and then discuss a third approach, the targeting of intracellular (C-terminal) receptor interaction domains to block downstream signalling, which offers new possibilities in a less crowded space.

Currently, many biopharmaceutical companies are pursuing orally available alternatives to monoclonal antibodies (mABs). This is done because companies aim to retain the clinical validity of established targets while improving pharmacology, accessibility and scalability through orally available modalities. Two categories of limitations motivate this strategy. First, mABs require injection and – as well as their smaller derivatives – hardly reach the CNS, making truly relevant therapeutic gains unrealistic in this area as the Alzheimer mABs demonstrate.

The challenge with mAB replacement

Most of the mAB replacement programs target the **extracellular space** (receptor N-terminus) to prevent ligands from binding to their receptors. This is challenging, since small molecules which prevent receptor oligomerisation are hard to screen for, and it is not easy either to identify peptides that block receptor binding sites and can be made orally bioavailable. At the same time, the extracellular target landscape for many clinically validated pathways has become increasingly saturated. Numerous research programmes now pursue similar ligand- or receptor-blocking strategies, creating intense competition while offering limited conceptual differentiation. This raises the question: are there less explored ways to exploit these targets?

One such alternative emerges from focusing on intracellular (C-terminal) signalling interfaces of

established transmembrane receptor targets. By disrupting the interaction of the receptors with their intracellular partners, one can abrogate downstream signalling. Systematic analysis of the intracellular interaction partners of established mAB targets therefore represents a promising and comparatively underexplored opportunity for target re-utilisation.

The concept — target re-utilisation via interfaces to intracellular interaction partners — is discussed in this article. In what follows we contextualize it by looking at the **two extracellular approaches** using recent examples and then discuss the **novel intracellular approach**.

Approach 1: Oral TNF-antagonist: Balinatunfib

Balinatunfib is an example for ligand targeting. TNF α is secreted by cells of the acquired and innate immune system. It forms a trimer that binds to TNF-receptor 1 and triggers inflammatory signalling inside the target cells.

The invention of mABs (first in class: etanercept, 1998) blocking this pathway by binding to the trimer has revolutionised the treatment of rheumatoid arthritis. Balinatunfib is an orally bioavailable 500 Da small molecule which interacts with the TNF α trimer in a way that keeps it in a conformation which cannot bind to its receptor in the required manner [1] (see Fig. 1): With this ligand confirmation, the receptors cannot bind the TNF α -trimer in a manner that normally would

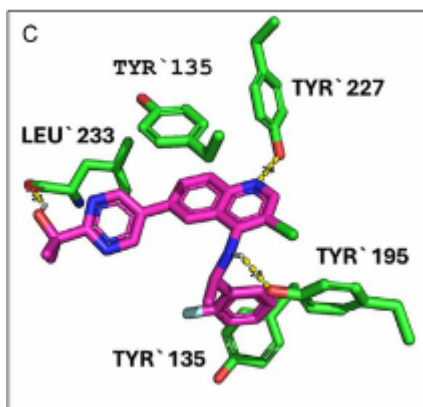


Figure 1 - Balinatunfib (pink sticks) interacting with tyrosine and leucine residues of TNF α stabilising it in a conformation unable to activate its receptor (from [1, Fig. 2c])

lead to their physiological oligomerisation that is required for down-stream signalling. Balinatunfib is currently in early stage clinical trials.

This example represents the first of the three strategies discussed in this paper: reproducing the extracellular neutralisation mechanism of antibodies by targeting the ligand itself. But it is also possible to interact with receptors using small molecules to prevent their oligomerisation.

Approach 2: IL-23 antagonist: Icotrokinra

Icotrokinra is an example for the targeting of an extracellular receptor ligand-binding domain to block ligand binding and receptor signalling.

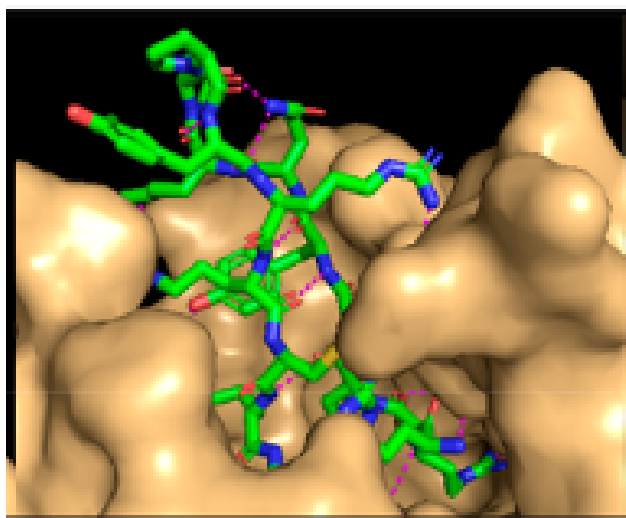


Figure 2 - CXCR4 chemokine receptor in complex with the cyclic peptide antagonist CVX15 (PDB 3OE0), reproduced from [3, Fig. 72].

Figure 2 illustrates how a cyclic peptide can bind to the extracellular domain of the chemokine receptor CXCR4.

IL-23 is a cytokine secreted by macrophages and NK-cells. Via its receptor complex, it stimulates multiple cells of the innate and acquired immune system and triggers the production of downstream cytokines. This pathway plays an important role in immune-mediated inflammatory diseases. Its blockage by mABs targeting IL-23 is a well established therapeutic principle for the diseases of this group such as psoriasis, psoriatic arthritis, or inflammatory bowel diseases.

Icotrokinra is a novel cyclic peptide with a molecular weight of 1.9 kDa with good oral bioavailability which blocks the IL-23 receptor. Its invention was very elegant, the approach is situated at the forefront of today's applied biomedical research involving the computational biophysical screening of virtual peptide libraries and sophisticated subsequent physical screening. The results of the clinical trials to show its efficacy and safety could lead to an FDA approval soon [2]. This new class of IL-23 antagonists would increase patient compliance and reduce treatment costs, thereby enabling treatment access for more patients in the mid-term.

Approach 3: Target re-utilisation via intracellular signalling interfaces

In contrast to the two previous strategies, this approach starts by looking at the intracellular C-terminal domain of the transmembrane-protein target. Instead of preventing ligand binding at the cell surface, the aim is to interrupt the intracellular protein-protein interactions required for signal propagation.

An example for the blocking of a protein-protein interface is shown in Fig. 3.

To apply this strategy, the following key questions need to be addressed:

- Does the target oligomerize?
- Are there known specific interaction partners?

- How does it interact with its associated intracellular proteins?
- Which domains mediate this interaction?
- What evidence for the interaction exists?

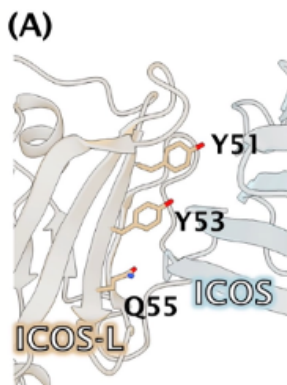


Figure 3 - Blocking the interface of the inducible T- cell costimulatory molecule ICOS and its ligand ICOS-L (PDB: 6X4G) with a circular peptide, reproduced from [4, Fig. 1].

If there is clear evidence that you have such a constellation, the next step is to design a system for target validation and also interaction inhibition screening. The goal is to demonstrate that disrupting the interaction indeed suppresses the disease-relevant signalling pathway and therefore represents a viable pharmacological intervention point.

Target validation can be achieved using a knock-down strategy in a 2D or 3D in vitro disease model using human (ideally patient cells) that allows an adequate read-out. Such models make it possible to test whether removal or disruption of the interaction partner attenuates the signalling cascade and produces the expected phenotypic effect in a disease-relevant cellular system.

Interaction inhibition screening can be achieved by the expression and in vitro constitution of the relevant domain complex for biophysical screening purposes.

Using recombinant proteins to reconstitute the interacting domains in vitro allows the development of screening assays that directly measure disruption of the protein-protein interaction. This creates a tractable experimental system for identifying molecules capable of interfering with the sig-

nalling complex. Because the goal is to prevent the interacting protein from binding to the receptor (complex), ideal libraries for screening contain molecules which are bigger than typical small molecules, such as peptide libraries, LMW libraries, or cyclic peptide libraries.

If structural information is published or can be obtained (Cryo-EM or co-crystallisation), virtual screening is possible to reduce the physical screening effort.

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Jobst Landgrebe is a physician, biochemist and biomathematician working at the interface of mathematics, cell biology and biopharma R&D strategy since 2006. His work addresses a central problem of the industry: the gap between theoretical laboratory models and the biological reality of human disease (“Epistemic Gap”)

In this role he supports biopharma R&D leaders when discovery programmes become technically or conceptually blocked—for example when targets are considered “undruggable”, when programmes converge on crowded extracellular mechanisms, or when the biological validity of a target remains uncertain.

His approach combines **biomathematical analysis**, **advanced biophysical screening strategies** and **human-centric validation systems** such as organoid models. Together these methods help test biological hypotheses earlier, design more predictive screening systems and focus discovery resources on targets with a higher probability of clinical success.



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