Case report

FDA breakthrough therapy designation for two Pepscan-mapped antibodies

Precision Epitope Mapping of conformational and discontinuous epitopes

Introduction

Two Pepscan-mapped antibodies, obinutuzumab (Roche/Genentech) and daratumumab (Genmab/Johnson & Johnson), recently received breakthrough therapy designation by the FDA. Exact epitope definition is of key importance for patentability or freedom to operate assessments. Moreover, FDA and EMA guidelines require specific binding site information to be included in regulatory filings for novel antibodies. CLIPS Precision Epitope Mapping is specifically developed for the mapping of conformational and discontinuous epitopes, which occur with the majority of neutralizing antibodies. It provides unrivalled single residue resolution of the epitope and determines the contribution of each of the individual amino-acids to the binding of the antibody.

Obinutuzumab (Genentech/Roche)

CLIPS epitope mapping identified a novel conformational epitope on CD20[1]. Comparative, single-residue precision mapping of rituximab and obinutuzumab revealed that Asn-171, crucial for rituximab, was not critical for obinutuzumab, pointing to a shift in binding orientation between the two antibodies. This altered functionality can be developed into a novel cancer therapy for patients resistant to Rituxan. The absence of requirement for Asn-171 resulted in full freedom to operate for obinutuzumab.

N_{171} indicates the critical difference between the epitopes

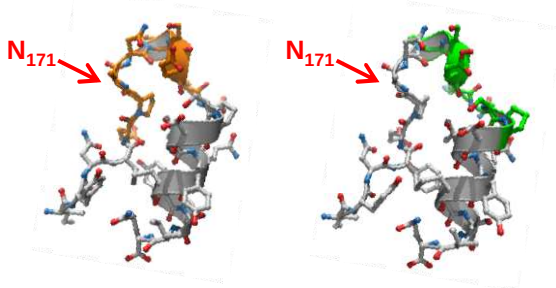

Figure 1: *A full positional-scan, in which each amino acid was replaced by all other amino acids, allowed to pin-point residue N-171 as most different in recognition of rituximab and obinutuzumab.*

Daratumumab (Genmab/J&J)

A two-stage CLIPS mapping strategy precisely determined the conformational and discontinuous epitope on the membrane-bound CD38 receptor for daratumumab [2].

First a set of overlapping 15-mer CLIPS peptides was used to identify the core epitope. Subsequently a focused library of discontinuous CLIPS peptides was synthesized. This identified a secondary binding region. Visualisation confirmed that both regions formed a single discontinuous epitope. A site-directed mutagenesis approach was used later to confirm the predicted epitope.

Epitope mapping with CLIPS discontinuous peptides

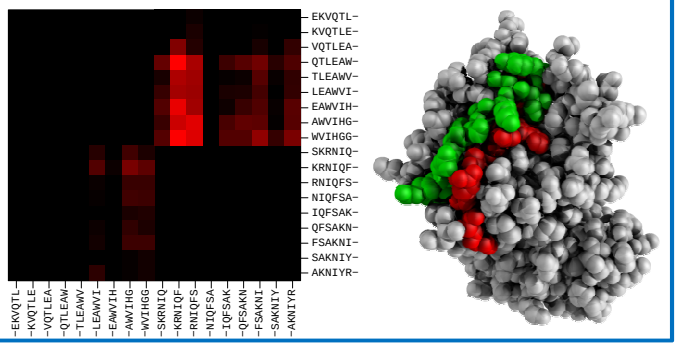

Figure 2: *Identification of the discontinuous epitope of daratumumab using double-looped combinatorial CLIPS peptides. The binding results are shown as a heat map. The identified discontinuous epitope is shown on the structure visualisation.*

The benefits of CLIPS Precision Epitope Mapping

- Works for all types of epitopes
- Discontinuous, conformational, and linear
- Applicable to all kinds of target proteins
- Soluble as well as membrane integrated proteins
- Unrivalled single residue resolution
- Solid support for patent claims and regulatory filings
- Re-usable arrays for multiple screenings
- Comparative mapping of sets of samples

1) *Study performed in a collaboration with Roche and published by Niederfellner et al., Blood 2011: 118:358-367.*

2) *Study performed in a collaboration with Genmab and published by de Weers et al., Journal of immunology 2011: 186:1840-1848.*

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Technical information CLIPS Precision Epitope Mapping

Peptide synthesis	FMOC chemistry. Maximum peptide length over 40 residues. All amino acids, including D-amino acids and non-natural amino acids.
Capacity	Ten custom high-throughput parallel synthesis robots, each 10.000 peptides per run.
Peptide library format	Proprietary 'Minicard' format with solid phase-bound peptide constructs in 455 microwells. Surface chemistry: proprietary polymeric graft optimized for low non-specific binding and high peptide construct loading.
Combinatorial library complexity	Matrix analysis, e.g. 50 x 50 = 2.500 double loop T3 CLIPS™. All matrix combinations within 40-mers possible. All overlapping single loops, usually 15 - 20-mers. All overlapping peptides of a protein, usually 15 - 20-mers. Full positional scan libraries of all epitopes.
Spatial construct complexity	Single loops on T2 CLIPS. Double loop combinations on T3 or 2 x T2 CLIPS. Sheet-like T2 CLIPS, helix-like T2 CLIPS. All loop structures with 2-6 cysteines and 1 or 2 CLIPS.
Peptide library reusability	At least 20 times, but up to 100 depending on the samples. Library storage and re-use up to years.
Binding detection	Binding of the antibodies to the CLIPS peptides is determined in an ELISA. The resulting color in each well is quantified with a CDD camera.
Binding detection sensitivity	Optimized for epitope mapping, down to $K_d=10^{-3}$
Required material and information	100 µl polyclonal serum or 100 µg antibody Linear sequence of target protein.
Project run-through time	Priority 1.5 months, Standard 3 months.
Reporting	Binding values of all peptides are quantified and stored in the PepLab™ database. A full report is provided including details on binding and specificity for each residue, optimized for registration, regulatory,and/or IP purposes. Full support is offered for IP generation and publishing.

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