Case report

Precision Epitope Mapping of daratumumab

Precision epitope mapping of the discontinuous epitope of daratumumab

Objective:

CD38 is a 46kDa trans-membrane glycoprotein highly expressed in hematological malignancies including multiple myeloma. Therefore, it represents a promising target for mAb-based immunotherapy. CD38 antibodies were isolated by Genmab from immunized HuMAB mice, from which daratumumab was selected. Pepscan determined the exact epitope for daratumumab using a two-stage CLIPS mapping strategy. Daratumumab is currently in Phase I/IIa clinical testing and recently received FDA break-through therapy designation.

Results:

A set of overlapping 15-mer CLIPS peptides was used to identify the core epitope (Fig 1.) Visualisation of the identified (green) region suggested that neighboring areas may be involved in binding.

To investigate this further, a focused library of discontinuous CLIPS peptides was synthesized (Fig. 2). This identified a secondary binding region (red). Visualisation confirmed that both regions formed a single discontinuous epitope.

A site-directed mutagenesis approach was used later to confirm the predicted epitope.

Conclusion:

A two-stage CLIPS mapping strategy precisely determined the discontinuous epitope on the membrane-bound CD38 for daratumumab. These results are important for regulatory as well as patent considerations.

In April 2013 Johnson & Johnson licensed daratumumab from Genmab in a deal of in total $ 1.1 billion.

Core epitope: mapping with CLIPS conformational peptides

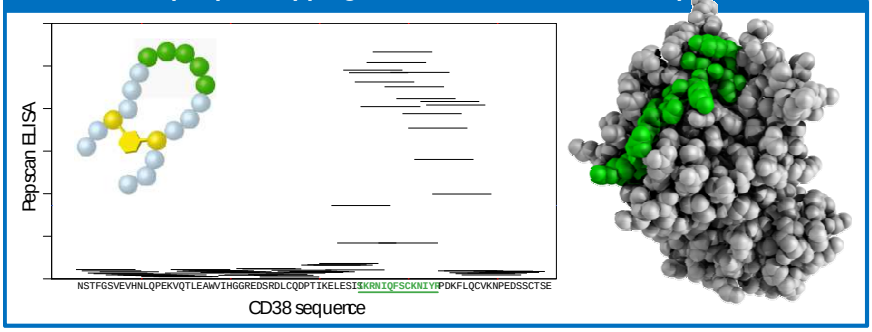

Figure 1: *Identification of the core epitope for daratumumab using single-looped CLIPS peptides. Each peptide is shown as a horizontal line that covers the amino acid residues shown below that line. The height of the line indicates the observed binding. The identified binding region is shown in green on the structure visualisation.*

Complete epitope: mapping with CLIPS discontinuous peptides

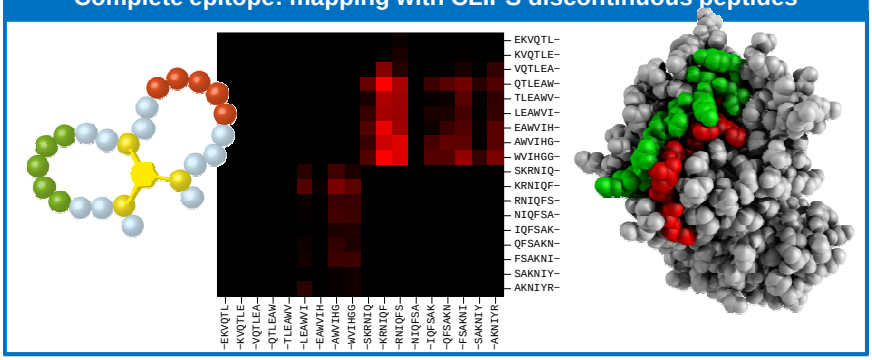

Figure 2: *Identification of a secondary binding region of the discontinuous epitope of daratumumab using double-looped combinatorial CLIPS peptides. The binding results are shown as a heat map. Each coordinate on the map combines two protein fragments (shown at the axes). For each combined peptide, the observed binding is plotted on a color scale between back and red. The newly identified region is shown in red 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

This work was performed in a collaboration with Genmab and published by de Weers *et al.*, Journal of immunology 186:1840-1848; 2011.

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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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