## The molecular basis for distinction between GA-101 and Rituximab

Objective: Rituximab, a monoclonal antibody targeting CD20, has improved the treatment of malignant lymphomas. Therapeutic CD20 antibodies are classified as either type I or II based on different mechanisms of killing malignant B cells. To reveal the molecular basis of this distinction, we fine-mapped the epitopes recognized by Rituximab (Type I) and novel GA101 (Type II).

Results: 4300 overlapping CLIPS peptides derived from CD20 were synthesized on solid support and screened with Rituximab or GA101 with single-residue precision. This revealed that Asn-171, crucial for Rituximab, was not critical for GA101, pointing to a shift in binding orientation between the two antibodies. This shift was later confirmed by X-ray crystallography.

Conclusion: CLIPS epitope mapping identified a novel epitope on CD20. 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.
CLIPS epitope mapping has proven to be a powerful tool to study complex epitopes of the membrane embedded CD20 tetramer. CLIPS Epitope Mapping of epitopes and ligand binding sites on other membrane proteins such as ion-channels and GPCR's also shows great promise.

Epitope mapping with single-residue resolution

Rituximab   **GA101**
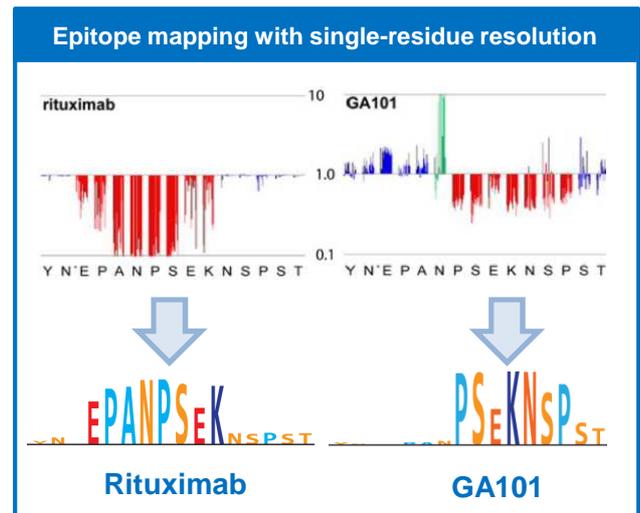
Comparison between CLIPS epitope mapping and X-ray. N_{171} indicates the critical difference between the epitopes

CLIPS epitope mapping   **X-ray**

N_{171} N_{171}

Rituximab   **GA101**   **Rituximab**   **GA101**
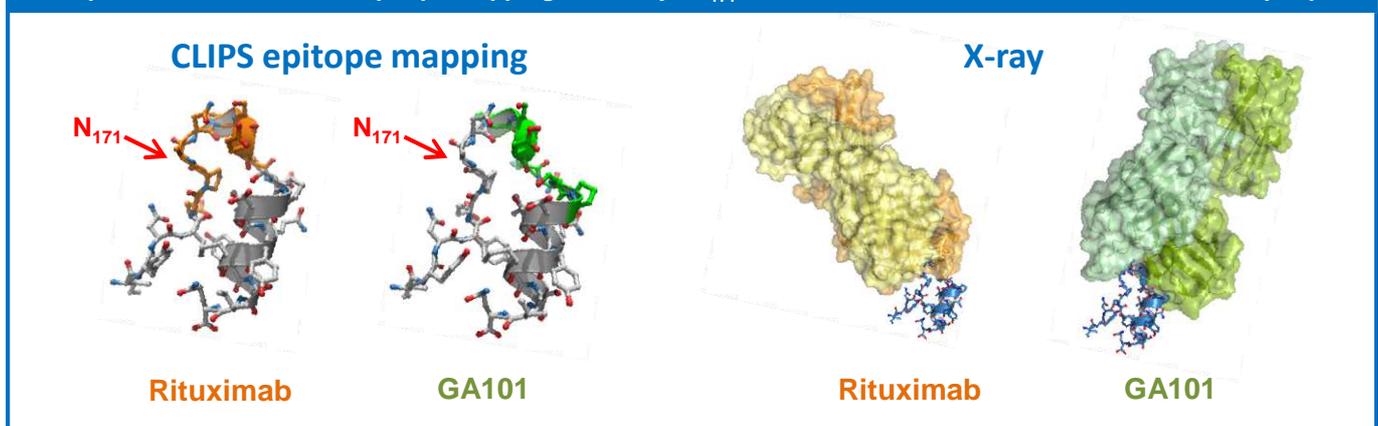
The benefits of CLIPS Precison Epitope Mapping

- Works for all epitopes
- Discontinuous, conformational, and linear
- Applicable to all kinds of target proteins
- Soluble as well as membrane integrated proteins

- Re-usable arrays fof multiple screenings
- Comparative mapping of sets of samples
- Unrivalled single-residue resolution
- Solid support for patent claims and regulatory filings

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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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