

Rapid Mimotope Optimization for Pharmacokinetic Analysis of the Novel Therapeutic Antibody IMAB362

M. Daneschdar*, H.U. Schmoltdt*, L. M. Plum**, Y. Kühne**, M. Fiedler*, A. Masch***, K. Schnatbaum†, J. Jansong†, J. Zerweck†, H. Wenschuht, U.Reimert, Ö. Türeci**** and U. Sahin*

* BioNTech AG, Mainz, Germany

** Institute for Translational Oncology and Immunology (TRON), Mainz, Germany

*** Department of Internal Medicine III, Experimental and Translational Oncology, Johannes Gutenberg-University, Mainz, Germany

**** Ganymed Pharmaceuticals AG, Mainz, Germany

† JPT Peptide Technologies, Volmerstrasse 5, 12489 Berlin, Germany

The detection of therapeutic antibodies targeting membrane proteins in the course of (pre-)clinical development is often challenging due to the unavailability of the target molecule in its native form. As an alternative in this study, mimotopes for the antibody IMAB362 (Claudiximab) were discovered and optimized using phage display and peptide microarrays. The best mimotope was successfully used for the peptide ELISA-based quantification of IMAB362 in serum samples. The described process efficiently provides mimotopes for targets which are difficult to produce or handle.

Introduction

IMAB362 (anti-Claudin 18.2) is a highly tumor-specific monoclonal IgG1 antibody currently in clinical development for the treatment of advanced gastro-esophageal and stomach cancer [1, 2]. The antibody is directed against the cancer-specific cell surface target Claudin 18 isoform 2 (CLDN18.2), a 27.7 kDa gastric differentiation protein that spans the cell membrane with four transmembrane helices.

For the development of IMAB362 for clinical use, an assay system for the reliable detection and quantification of the antibody in sera is needed. Typically, ELISA based assays utilizing the corresponding antigen as a recombinant protein is applied for this purpose. However, transmembrane proteins are difficult to produce and handle due to their nature as membrane-embedded structures [3]. To overcome these problems, the goal of the present study was to develop peptide mimotopes as easier-to-prepare structures for IMAB362 detection by peptide ELISA.

Results

Initial IMAB362 binding peptides were identified by phage display technology. After three selection rounds several peptides were identified and confirmed in a phage ELISA to specifically bind the variable domain of IMAB362.

Table 1. Binding characteristics of parental and matured peptides.

Pep-tide	Sequence	Apparent K_D , ELISA [nM]	Apparent K_D , BLI [nM]
1a	ACHLNYPGYCG	5.80	810
1b	ACHLNYPGWCG	0.28	n.d.
1c	ACHLGYPGYCG	0.26	108
2a	ACHYSYPGVCG	4.44	100
2b	ACHYSYPGWCG	2.12	n.d.
2c	ACHYGYPGVCG	0.15	52
3a	ACHLGYPGRCG	1.61	320
3b	ACHYGYPRCG	0.57	n.d.
3c	ACHLGYPGWCG	0.13	92

Shown are the apparent binding constants for the parental and matured IMAB362 binding peptides.

The full peptide structure was Biotin-Ttds-sequence-OH. All peptides were cyclized by Cys-Cys-disulfide bond formation. Ttds is a linker with the following structure: 1,13-diamino-4,7,10-trioxatridecan-succinamic acid. Amino acids that were exchanged compared to the original sequence are underlined.

Three peptides (sequences 1a, 2a and 3a, Table 1) were selected for further analysis.

Peptide microarrays represent a highly efficient approach for peptide optimization because thousands of peptides can be screened in parallel requiring only small amounts of precious analyte. In addition, assays on peptide microarrays can be set up rapidly as the readout is usually achieved by standardized fluorescence based methods allowing low background on glass surfaces [4].

Using peptide microarrays, substitutional analyses were performed for each of the three starting peptides (by exchanging each amino acid of the respective peptide sequence by each of the other naturally occurring amino acids except Cys). The obtained data (two analyses are depicted in Fig. 1) showed that several substitutions of amino acids within the starting peptides by alternative amino acids resulted in stronger binders (indicated by darker colours).

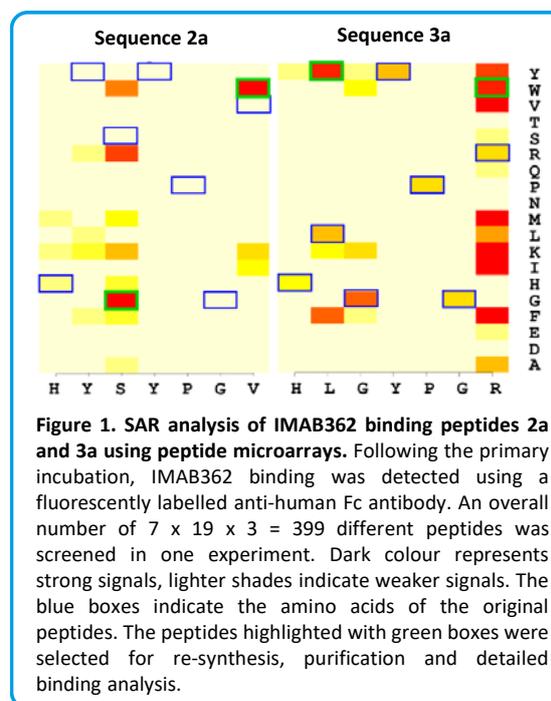


Figure 1. SAR analysis of IMAB362 binding peptides 2a and 3a using peptide microarrays. Following the primary incubation, IMAB362 binding was detected using a fluorescently labelled anti-human Fc antibody. An overall number of $7 \times 19 \times 3 = 399$ different peptides was screened in one experiment. Dark colour represents strong signals, lighter shades indicate weaker signals. The blue boxes indicate the amino acids of the original peptides. The peptides highlighted with green boxes were selected for re-synthesis, purification and detailed binding analysis.

For example, for sequence number 3a, substitutions of leucine by tyrosine and of arginine by tryptophan appeared most promising

with regard to achieving better binding. The best options for optimizing sequence number 2a were the substitution of serine by glycine and of valine by tryptophan. The peptides displaying the highest signal intensities as result of the substitutions (marked with green boxes in Fig. 1) were selected for re-synthesis, purification and detailed binding analysis.

Two complementary methods were used to characterize the interaction of IMAB362 and the mimotope peptides. An ELISA method was used to determine the thermodynamic behavior resulting in apparent affinity values. For detailed kinetic analysis BLI (biolayer interferometry) was applied to the strongest binding peptides. Table 1 shows the results of the measurements. As expected, the general SAR as observed by the microarrays was unambiguously confirmed with the purified peptides, and the affinity of each of the three starting peptides (1a, 2a and 3a) could be improved. The binding of peptide 1a was improved by a factor of more than 20 (5.80 to 0.26 nM, 1a to 1c), while peptide 3a was improved by a factor of more than 10 (1.61 to 0.13 nM, 3a to 3c). The strongest improvement of binding affinity was achieved for peptide 2a: Substitution of a single amino acid (glycine for serine) improved binding affinity by a factor of 30 (4.44 to 0.15 nM, 2a to 2c). The best peptide 2c exhibits a very strong binding to IMAB362 of 52 nM (BLI) and 0.15 nM (ELISA). With these binding characteristics, 2c completely fulfilled the criteria for the desired application. Therefore, it was refrained from additional optimization rounds to further improve binding affinity, and 2c was selected as the optimal mimotope for IMAB362 detection in biological samples by peptide ELISA.

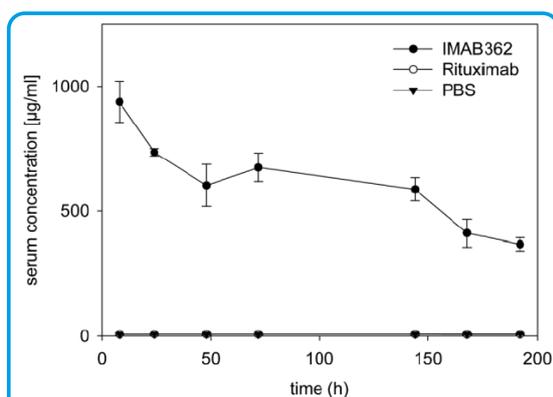


Figure 2. Pharmacokinetic analysis of IMAB362. IMAB362, Rituximab or PBS buffer were intravenously injected into BALB/cJ mice. Blood samples were taken over a period of 8 consecutive days and analyzed via peptide ELISA with the 2c mimotope peptide for antibody capturing. Median values of two mice per time point for each group are shown and SD is presented as error bars for each time point.

A number of experiments were done to confirm the applicability and robustness of the developed ELISA assay. First, it was shown that co-incubation with murine or human serum had almost no effect on IMAB362 binding to the best peptide variant 2c (data not shown). Secondly, incubation with a control antibody (an antibody with identical chimerized backbone compared to IMAB362, but directed against a different Claudin molecule) did not result in undesired assay signal (data not shown). The estimated lower limit of quantification (LLQ) value of the ELISA assay, defined as 6 times standard deviation above background, was 0.3ng/mL. The linear range of the assay goes up to approximately 300 ng/mL.

Finally, a pharmacokinetic study was done to analyze the performance of the developed IMAB362 ELISA in a pre-clinical setting. Only minimal analyte volumes were needed per time point, because the serum samples could be diluted by a factor of 1:50,000 prior to the measurement in the linear range of the assay (approximately 0.3 to 300 ng/mL). Figure 2 shows the IMAB362 concentration determined in the pharmacokinetic assay. As expected, the intramurine IMAB362 concentration decreases over time. No signal was detected in the Rituximab and buffer control mice indicating the high specificity of the assay for IMAB362.

Conclusions

In summary, mimotopes for the peptide ELISA-based detection of the antibody IMAB362 in biological samples were discovered and optimized. Initial hits were generated by phage display. Subsequently, the peptides were systematically optimized by means of peptide microarrays. The discovered peptides could be confirmed and further characterized by independent assays such as ELISA and BLI. The resulting peptides showed an improved binding by a factor of up to 30 compared to the initial hits with apparent binding constants in the low nanomolar to picomolar range. The best mimotope (2c, 0.15 nM) was successfully used for detection of IMAB362 in samples from a mouse pharmacokinetic study by peptide ELISA [5].

References

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The Author



Matin Daneschdar
 matin.daneschdar@biontech.de
 BioNTech AG
 An der Goldgrube 12
 55131 Mainz, Germany

Dr. Matin Daneschdar is Deputy Head of the 'New Scaffold' Unit at BioNTech AG in Mainz, Germany. He studied biology at the Georg-August-University in Göttingen and obtained his PhD in the division of biochemistry and organic chemistry at the Technical University of Darmstadt. At BioNTech AG, Dr. Matin Daneschdar is responsible for the lead discovery platform and the development of peptide based lead candidates.

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