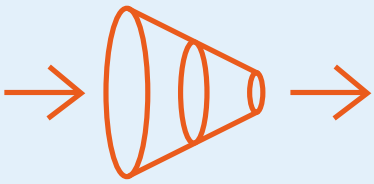
IMMUNITRACK

Deimmunisation using NeoScreen®

Project Overview for Immunogenicity Screening



Reducing the immunogenicity of biotherapeutics without compromising their function will improve the safety and efficacy profiles of drugs, thereby increasing the likelihood of a succesfull clinical outcome.

This can be accomplished by performing amino acid substitutions that reduce binding of drug-derived epitopes to MHC II, thereby lowering the risk of triggering a CD4 T cell-mediated anti-drug antibody response.

Immunitrack has a unique *in vitro* platform, coined NeoScreen®, that can screen for immunogenic epitopes and subsequently propose and confirm amino acid substitutions that will reduce epitope binding to MHC molecules.

INTRODUCTION

Predicting the Immune Response

How do you know whether your biologic will provoke an unwanted immune response in patients? Part of the answer lies in how the immune system recognises epitopes. An ideal biologic should combine maximum efficacy with low (or zero) propensity for recognition by the immune system.

Reducing the immunogenicity of biologics without compromising their function will improve the safety and efficacy profiles of such drugs, thereby increasing the likelihood of a successful clinical outcome. The immune processes leading to formation of anti-drug antibodies (ADAs) are complex, but decisively linked to the binding of drug-derived peptides (epitopes) to MHC II molecules. Assessing and reducing the binding of potential epitopes to MHC II minimises the likelihood of ADA formation.

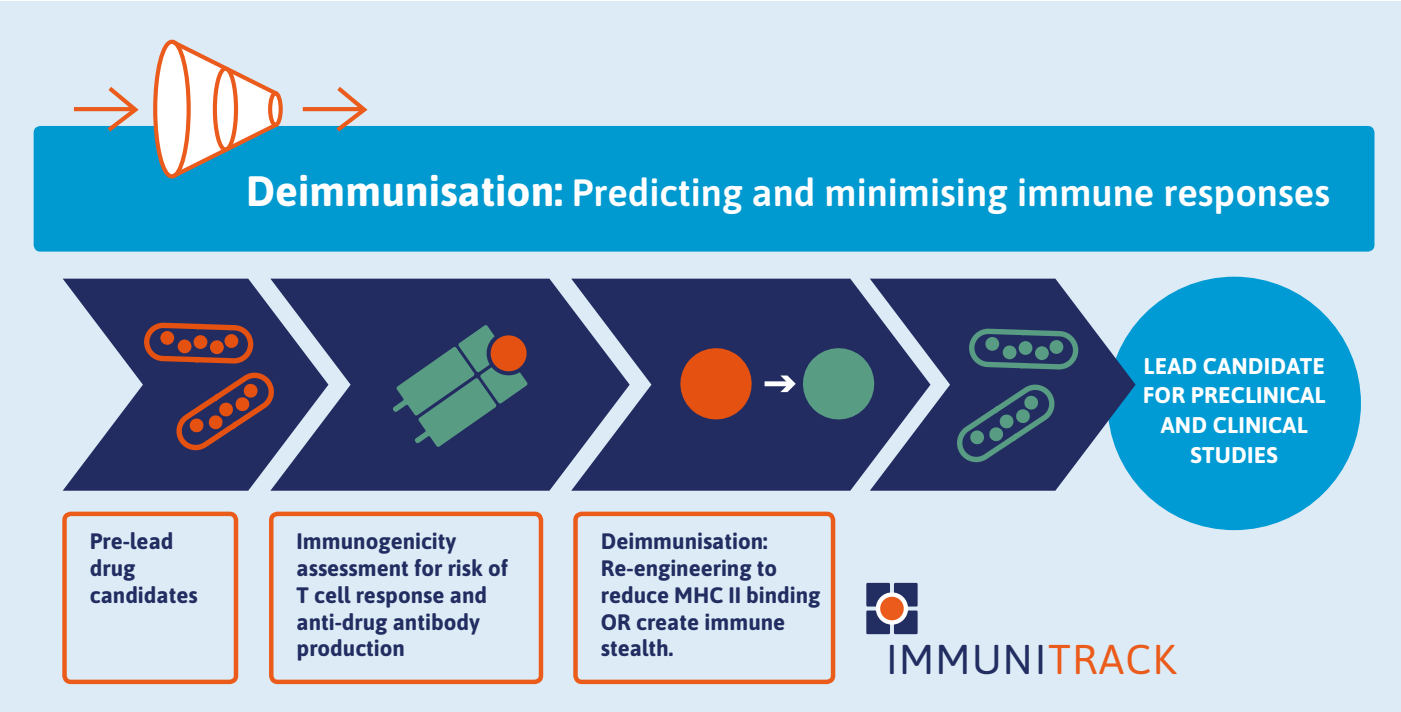
Immunitrack has a unique *in vitro* platform that can screen for potential immunogenic epitopes in biologics and subsequently identify amino acid substitutions that reduce epitope binding to MHC molecules.

How Can Immunitrack Help?

The Major Histocompatibility Complexes (MHC) are critical to the adaptive immune response.

Peptide binding to MHC II and subsequent presentation to CD4 T cells are decisive events that occur upstream of any immune response. MHC II/peptide binding assays are recommended by FDA and EMEA guidelines as a way to assess the immunogenicity risk of a biologic. Due to the low precision of current MHC II predictive algorithms we do not recommend relying on *in silico* immunogenicity assessments.

Immunitrack has world-leading expertise in assessing epitope binding to MHC I and MHC II molecules. Our NeoScreen® technology supports clients and partners all over the world in vaccine development or deimmunisation efforts.



Project overview

In a deimmunisation project, highly sensitive affinity and stability assays will be applied to assess the binding of peptides to MHC II molecules. Peptide MHC interactions have traditionally been assessed by affinity assays but we, and others, have found that stability of the peptide/MHC complex is a better indicator of potential immunogenicity. The difference between these two assay types is shown in Figure 1. We recommend carrying out both assay types.

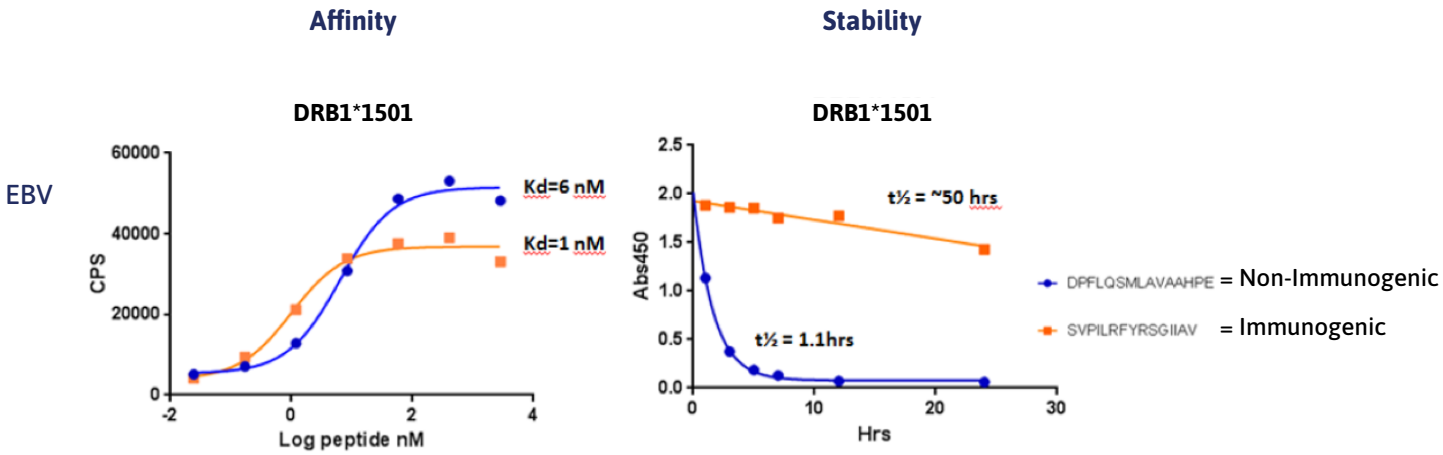


Figure 1: Affinity measurement of two Epstein-Barr virus (EBV)-derived peptides on DRB1*1501 by alpha screen assay reveals nearly identical Kd values (1 and 6 nM respectively). The same two peptides analysed in a temperature driven stability assay reveals dramatic differences in stability. The orange peptide is a known CD4 T cell epitope from EBV.

Overview – a deimmunisation project and a follow-up project

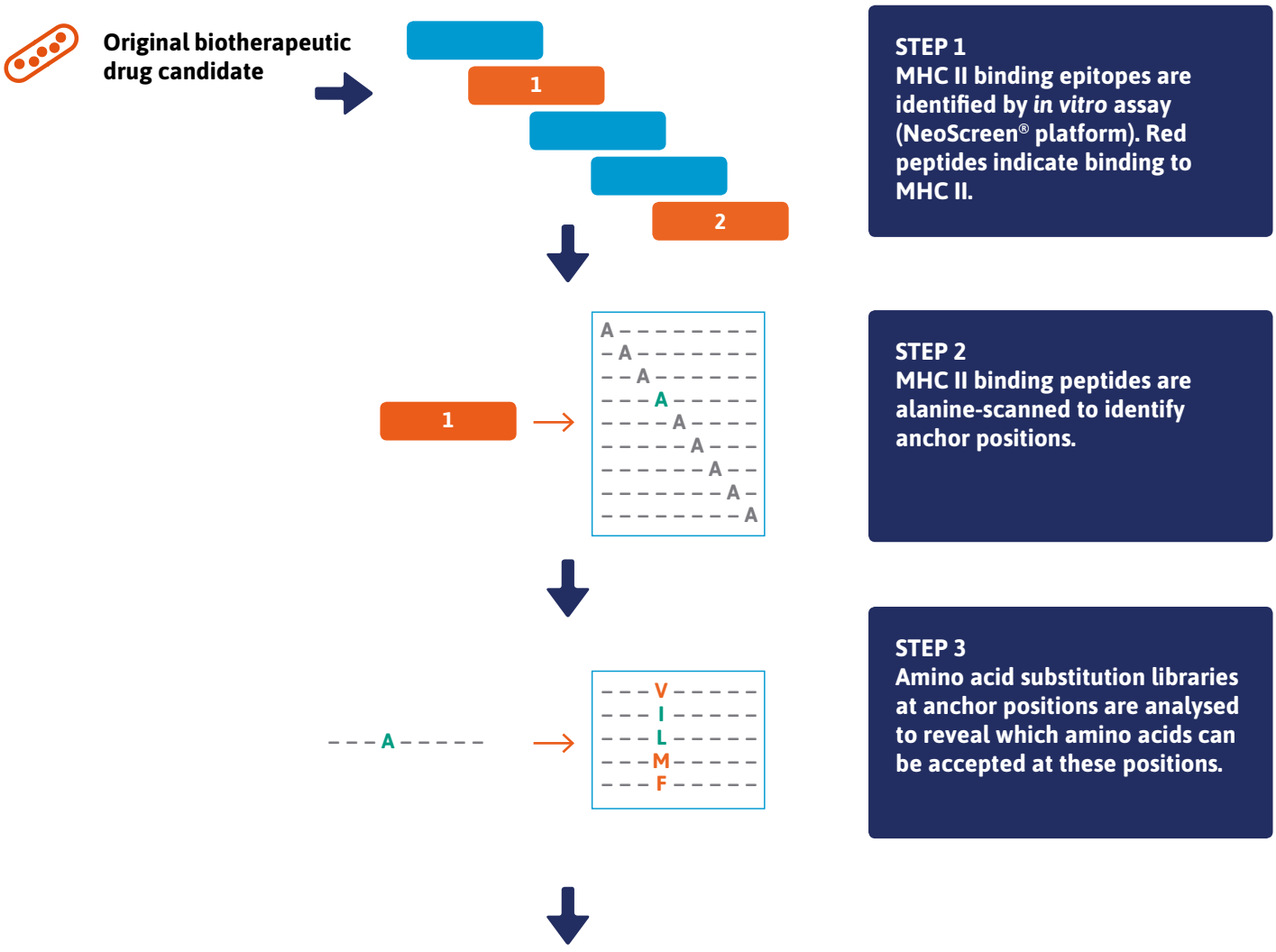


Figure 2: Step 1. Workflow for screening for immunogenic epitope mapping. Step 2. Subsequent mapping of anchoring positions. Step 3. Determination of amino acids that can be inserted at the anchoring positions without generating new immunogenic epitopes.

Step 1

In a deimmunisation project, the biologic will be divided into overlapping peptides that will then be analysed for stability and/or affinity on a representative panel of MHC II molecules, see Table 1. For preclinical assessment we can also run assays on MHC alleles from both BL6 and BALBc mice. Stability (and affinity) assays will determine which of the overlapping peptides constitute a potential CD4 T cell epitope (Figure 2 step 1).

Allele	Frequency %	Ethnic group
DRB1*0101	15	Caucasian
DRB1*0301	23	Caucasian
DRB1*0401	17	Caucasian
DRB1*0701	20	Caucasian
DRB1*0801	5	Caucasian
DRB1*1101	11	Caucasian
DRB1*1301	10	Caucasian
DRB1*1501	25	Caucasian
DRB3*0101	18	Caucasian
DRB3*0202	16	Caucasian
DRB4*0101	28	Caucasian
DRB5*0101	16	Caucasian
DPA1*0103/DPB1*0401	20-60	Caucasian*
DPA1*0103/DPB1*0402	20-60	Caucasian*

Table 1: Proposed MHC II alleles and their approximate frequency in a Caucasian population. Data from www.allelefrequencies.net/ or * J Immunol. 2002 Dec 15;169(12):6928-34. DOI: 10.4049/jimmunol.169.12.6928

Step 2

In a follow-up project (Figure 2; step 2), potential CD4 T cell epitopes are alanine-scanned to reveal anchor positions. Figure 3 shows the alanine scan results from a peptide (N3) that had previously been identified by cellular assays to be a potential CD4 epitope. Initial analysis revealed that it bound with reasonable stability to DRB1*0101 and with high stability to DRB3*0101. When Immunitrack carries out an MHC II stability assay, a known reference peptide is included and used to calculate a stability in percent relative to the reference peptide. A value of 100 indicates that a given peptide has the same stability as the reference peptide.

Figure 3 shows the stability of the wild type peptide. Arrows 2, 3 and 4 indicate anchor positions where an alanine substitution decreases binding.

In a follow-up experiment positions 4, 5 and 12 (arrow 2, 3 and 4) could be substituted with any of the remaining 19 amino acids to create a "library" of accepted amino acids at these positions. The final product should be a molecule with retained biological activity but with low or no MHC II binding.

	N3	N3_1	N3_2	N3_3	N3_4	N3_5	N3_6	N3_7	N3_8	N3_9	N3_10	N3_11	N3_12	N3_13	N3_14	N3_15
DRB1*0101	58	58	68	50	7	44	43	51	55	62	63	53	58	72	54	49
DRB1*0301	17	7	7	8	5	9	8	8	8	7	7	6	7	7	8	7
DRB1*0401	6	5	8	4	9	2	6	13	6	7	19	9	33	11	4	2
DRB1*0701	45	40	59	26	5	11	33	122	41	63	42	53	11	43	42	28
DRB1*1101	16	14	12	13	13	18	13	9	13	13	11	14	12	11	15	14
DRB1*1301	10	11	10	9	8	10	10	10	10	10	10	11	15	11	10	10
DRB1*1501	33	32	33	33	33	32	33	33	32	33	47	33	34	31	35	35
DRB3*0101	247	232	311	148	24	53	196	150	284	299	236	249	31	229	232	179
DRB3*0202	4	4	5	5	4	6	6	14	6	9	14	6	6	8	4	3
DRB4*0101	21	21	21	21	19	21	21	24	21	28	22	21	17	23	26	19
DRB5*0101	35	36	41	30	8	17	23	61	35	44	31	40	55	47	33	21

Figure 3: Alanine scanning of one 15-mer epitope presented by a subset of MHC II molecules. Alanine replacements at position 3 or position 8 results in loss of binding.
Arrow 1 = Wild type peptide Arrow 2, 3 and 4 = Anchor positions

Step 3

In a last step (Figure 2; step 3), identified anchor positions can be substituted with 20 amino acids to reveal substitutions that can be used without causing binding to relevant MHC II molecules (Figure 2; step 3).

The three-step process can be combined with MHC-associated peptide proteomics (MAPPS).

Peptide synthesis and processing

Peptides can be supplied in microscale in microplates by JPT Peptide Technologies, Germany. Microscale peptides are crude with a typical purity of around 50-80% for a 9-mer. The peptides are economically attractive and perform well in our assays. In each 96 well plate, 2x3 co-synthesised reference peptides serve as quality control for peptide synthesis. LC-MS purity analysis can be performed for all peptides.

Peptides will be dissolved in a minimal amount of DMSO heated to 50° C for 5 minutes followed by further dilution into assay compatible buffer. If peptides contain cysteines, a low concentration of reducing agent will be included to minimise the formation of disulphide bonds. Immunitrack will do its best to monitor and aid solubility of peptides but is not obliged to carry out any corrective actions if peptides are not soluble.

Mapping anchoring positions: Vatreptacog case

- Many novel protein-based therapeutics (biologics) are not naturally occurring proteins. They may be enhanced by mutation or fused to other functional entities.
- In either case they may be recognised as “non-self” by the immune system.
- Our technology can be used to determine whether the biologic will be perceived as non-self by the immune system.

Case story

- In 2012 Novo Nordisk discontinued a phase 3 clinical trial on Vatreptacog alfa, an optimised variant of their blockbuster Novoseven containing 3 mutations. A few patients developed antibodies against the biologic and the program was cancelled, resulting in the loss of 3 billion Danish Krone.
- Retrospective analysis using our assays revealed that the M to Q mutation gave rise to a “non-self”peptide that bound to at least one particular MHC molecule. This knowledge could have allowed Novo to do a This knowledge could have allowed Novo Nordisk to conduct a more thorough risk assessment at an earlier point in the project.

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NovoSeven:   GQLLDRGATALELMVLNVPRLMTQD
                          ↓ ↓
Vatreptacog:GQLLDRGATALVLQVLNVPRLMTQD
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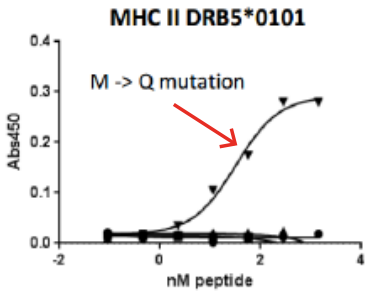

Reporting

The obtained stabilities to disclose anchor positions will be reported in a table format with comments. An excel file containing the raw data will also be supplied.

Warranties and Liabilities

Not for clinical use. Reagents and data are for research purposes only, and not for use in diagnostic procedures or human testing. Not for resale. All analyses performed by Immunitrack are experimental in nature, and Immunitrack can therefore not provide any warranty in relation to the results obtained and provided.

Are you developing new biologics such as antibodies, nanobodies or therapeutic enzymes?

Contact us and learn how we can help you in selecting drug candidates that are least immunogenic, deimmunise your drugs and provide safety data that can be used in your IND filling.

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