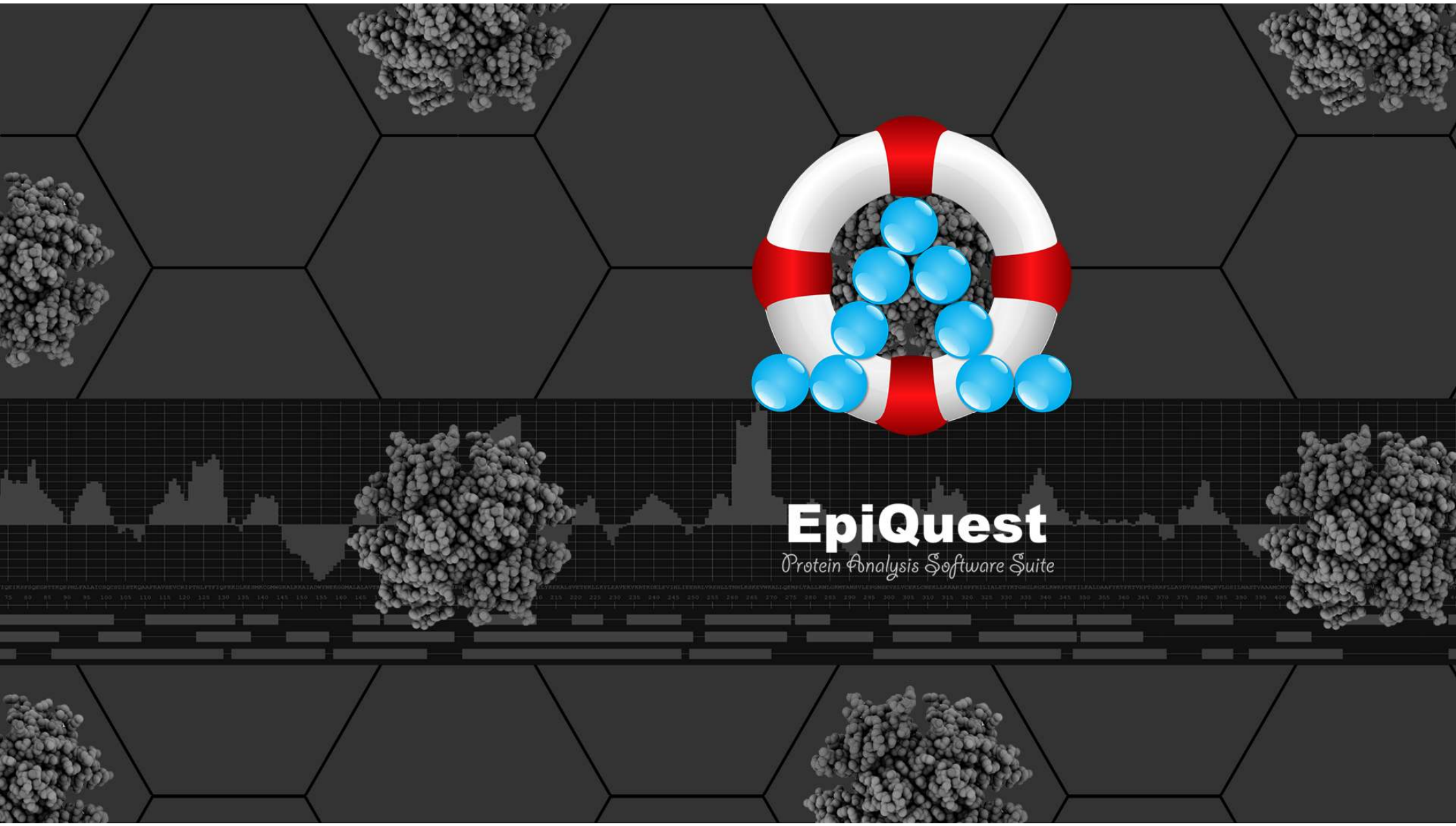


EpiQuest™ How To

How to compare epitopes, and select an immunogenic one, by using EpiQuest-B

Often we would like to know upfront whether:

- the given peptide epitopes are strongly immunogenic;
- to achieve good response in at least one animal many have to be immunized, or
- all animal respond strongly and just a couple will be sufficient to prepare a hyperimmune serum;
- the epitope (region) is worse to be included into recombinant vaccine, as it will be highly immunogenic for many immunized individuals;
- The epitope is likely to be responded to by most animals/patients and thus it is suitable as antigen in an assay for immune response.

In this release of “how to” we will explain how the EpiQuest - B can help you to compare epitopes. EpiQuest-B was created to determine the Antigenicity Index (and profile) of proteins, as well as stand alone peptide epitopes, and thus allow to get answers to many of these questions. High AGI usually means that the epitope will perform as immunodominant, and will elicit a strong response in most of recipients

Let’s say, someone determined several epitopes and you need to know whether they will be suitable for your purposes without performing actual immunizations.

ERBB2_HUMAN , Precursor
UniProtKB/Swiss-Prot: P04626.1

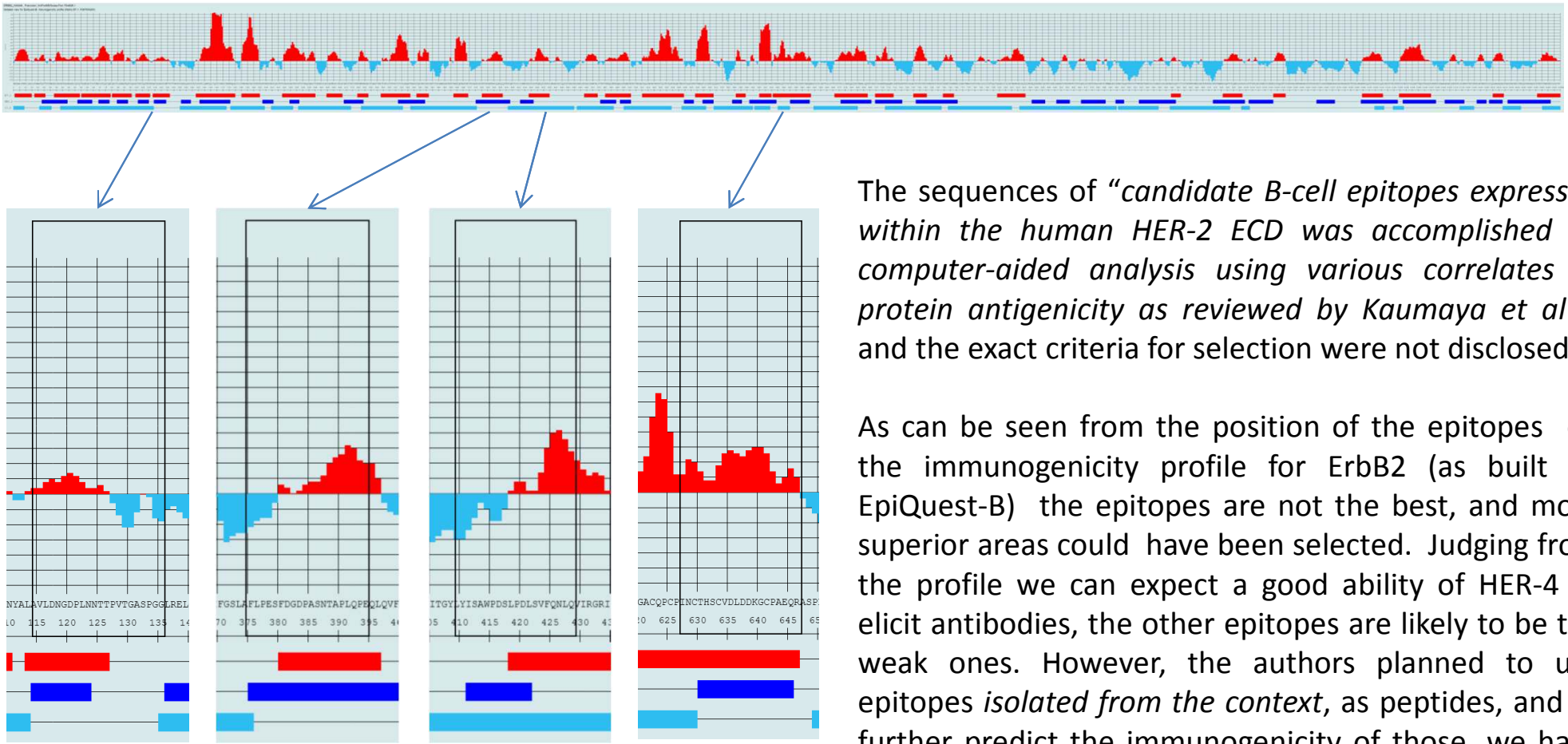
Epitope	Start	End	Length	Sequence
HER1	115	136	22	AVLDNGDPLNNTTPVTGASPGG
HER2	376	395	20	FLPESFDGDPASNTAPLQPE
HER3	410	429	20	LYISAWPDSLPDLSVFQNLQ
HER4	628	647	20	INCTHSCVDLDDKGCPAEQR

MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNAS
LSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYAL**AVLDNGDPLNNTTPVTGASPGG**LREL
QLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGESSE
DCQSLTRTVCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFE
SMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHL
REVRAVTSANIQEFAGCKKIFGSLA**FLPESFDGDPASNTAPLQPE**QLQVFETLEEITGY**LYISAWPDSLP**
DLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLALIHHNTHLCFVHTVPWDQLFRNPH
QALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHC
LPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPCP**INC**
THSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRRLLQETELVEPL
TPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENTSPKANKEILDE
AYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHVRENRGRLGSQDLLNWCMQIAKGMSYLEDVR
LVHRDLAARNVLVKSPNHVKITDFGLARLLDIDETEYHADGGKVPIKWMALESILRRRFTHQSDVWSYGV
TVWELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRPRFRELVSEFSRMA
RDPQRFVVIQNEDLGPASPLDSTFYRSLLEDDDMGDLVDAEEYLVPQQGFFCPDPAPGAGGMVHHRHRSS
STRSGGGDLTLGLEPSEEEAPRSPLAPSEGAGSDVFDGDLGMGAAKGLQSLPTHDPSPLQRYSEDPTVPL
PSETDGYVAPLTCSPQPEYVNQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGA
VENPEYLTPQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV

So, the Task:

By some methods four epitopes were chosen from the sequence of human erbB2*. Can we predict which of these will be immunogenic, and suitable as part of the vaccine or epitope to raise an erbB2-reactive hyperimmune serum?

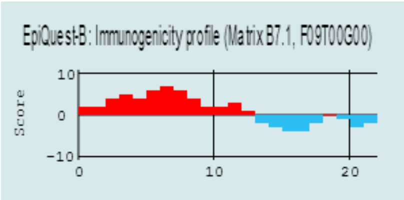
* *The epitopes were taken from a published work of Dakappagari et al, Cancer Res 2000;60:3782-3789*

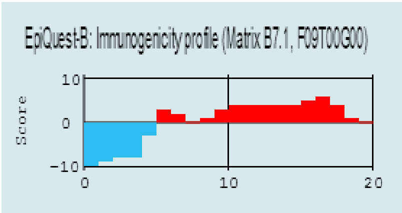


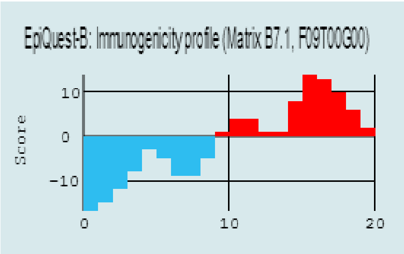
The sequences of "*candidate B-cell epitopes expressed within the human HER-2 ECD was accomplished by computer-aided analysis using various correlates of protein antigenicity as reviewed by Kaumaya et al*",* and the exact criteria for selection were not disclosed.

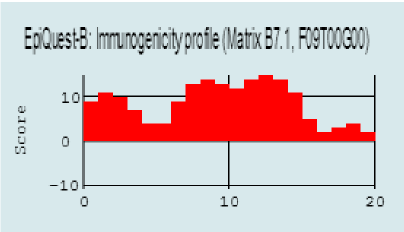
As can be seen from the position of the epitopes on the immunogenicity profile for ErbB2 (as built by EpiQuest-B) the epitopes are not the best, and more superior areas could have been selected. Judging from the profile we can expect a good ability of HER-4 to elicit antibodies, the other epitopes are likely to be the weak ones. However, the authors planned to use epitopes *isolated from the context*, as peptides, and to further predict the immunogenicity of those, we have to analyse the profile of peptides (seen further)

**Kaumaya, P. T. P., Kobs-Conrad, S., DiGeorge, A. M., and Stevens, V. De novo engineering of protein immunogenic and antigenic determinants. In: G. M. Anantharamaiah and C. Basava (Eds.), Peptides, Design, Synthesis & Biological Activity. pp. 133–164. Boston: Birkhauser, 1994*









The sequences were analysed as individual peptides using the default settings (profile on the left). The program was asked to report all positive peptides (at current cutoff level), and found one immunogenic sequence within each peptide (the positions are given for the peptide below).

#	Start	End	Length	AGI	AGR	Sequence
HER1	1	13	13	48	3	AVLDNGDPLNNTT
HER2	6	20	15	45	3	FDGDPASNTAPLQPE
HER3	10	20	11	*64*	*5*	LPDLSVFQNLQ
HER4	1	20	20	*176*	*8*	INCTHSCVDLDDKGCPAEQR

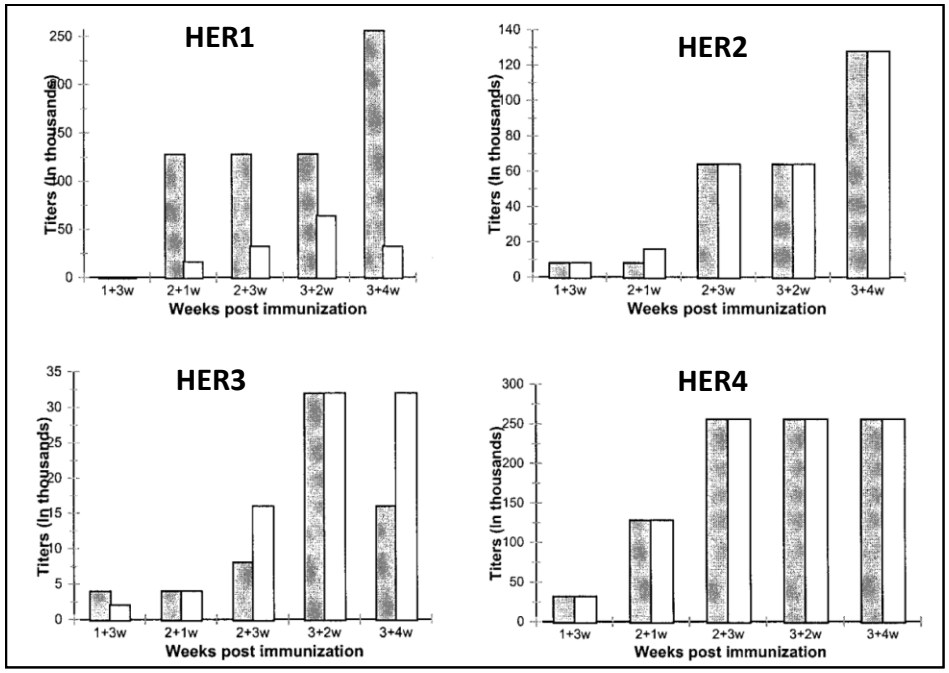
From data on immunogenicity (AGI), we can predict that the response will be a good one to HER4, and quite weak to the other peptides.

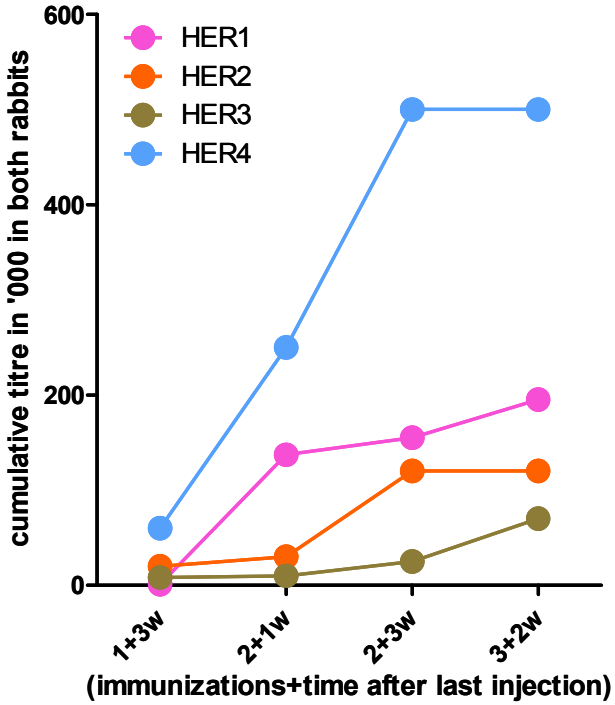
HER4>>(HER3≥HER2=HER1)

Moreover, we would expect that response to HER4 will be relatively high in all immunized animals, and to the other epitopes there will be a variation of relatively weak responses.

Let's see whether our predictions were correct, as the authors have immunized with each peptide 2 rabbits and have analyzed the dynamics of the response

Below is the titre for every peptide, used, in coupled form, to immunize 2 rabbits per antigen (data from *Dakappagari et al, Cancer Res 2000;60:3782-3789*). Please, note that the scale is different in each graph, and any significant titre after first, second and even third immunizations is observed only for epitope HER4, just as we have predicted. It should be stated that even after 3 immunizations only epitope HER4 gives high titre and in both rabbits.

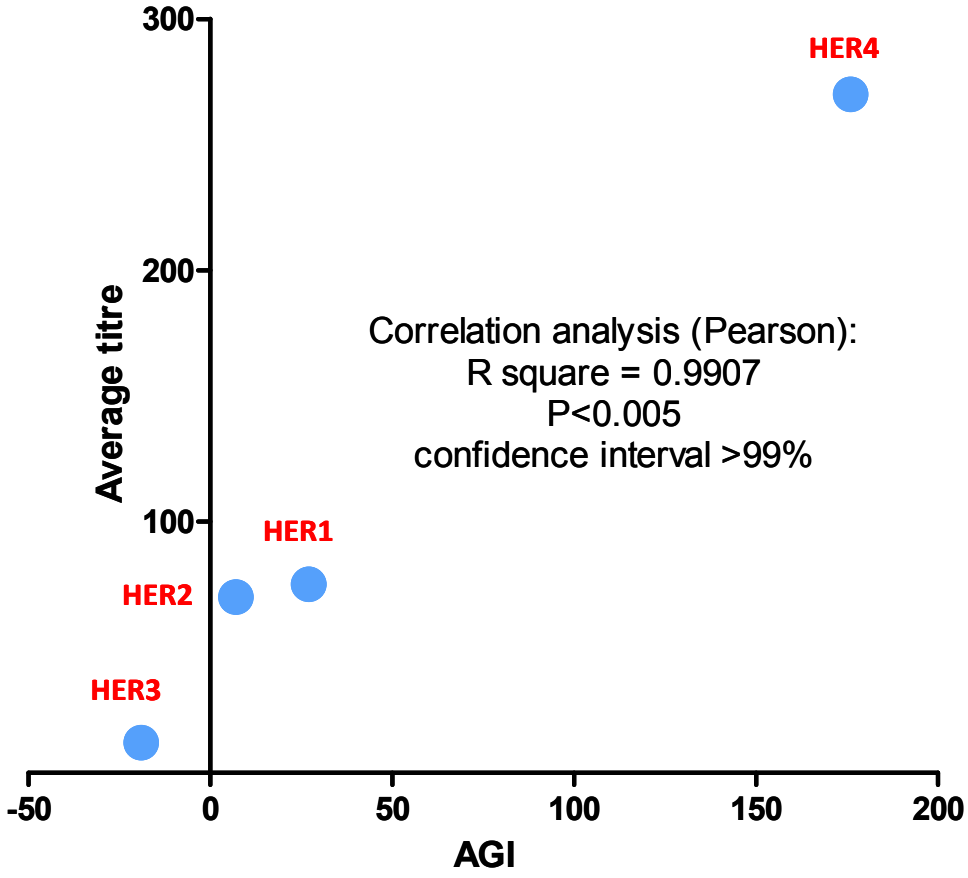




The graph on the right shows the same data translated into graphs for kinetics of immune response to every epitope. As predicted, after second immunization and 3 weeks of development, the titre to HER4 already reaches the maximum which is critically higher than for any other epitope.

In this example, we have used the analysis of given epitopes (peptide, linear, which will be used as individual peptides coupled to a carrier). The analysis was performed using the default settings of EpiQuest-B, and relative immunogenicity of the peptides was based on the antigenicity index of the best positive fragment. This is an easy approach, which allows to quickly assess the potential immunogenicity of peptides, and to select the best ones.

In actuality, every sequence of 5-6 aa may elicit immune response, so within 20aa peptides low and negative immunogenicity areas also contribute to antibody formation to epitope. If not stimulate, they may also distract immune response (by competition), or negatively affect antibody binding to the immunogenic part of epitope. Therefore, it would be correct to estimate AGI for the entire 20-22 amino acid peptide. Which we have done using own desktop version of the program (soon this feature will be available online). On the graph below you may see a cumulative AGI for each peptide plotted against average titre of serum obtained after 3 immunizations. As can be seen, the two parameters correlate with high significance.



In conclusion

We have illustrated here how to predict immunogenicity of a selected peptide epitope using EpiQuest-B.

The example is based on actual epitopes, used in immunization, and shows that the analysis allows to predict which epitope will be strongly immunogenic, and which – likely to produce poor response. It is clear that HER4, when used for the preparation of specific serum will give a good result, and only a few animals should be immunized. This epitope is the only one that will provide good protection and memory response in most recipients, when included in the vaccine.