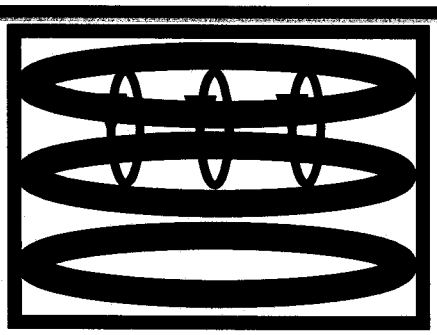


Project/Contract #:

503231

Project Acronym:

Genomes_To_Vaccines

Project Title:

Translating genome and proteome information into immune recognition

Instrument:

Specific Targeted Research Project (STREP)

6th Framework Programme, Thematic Priority 1:

"Life Sciences, Genomics and Biotechnology for Health"

Final Activity Report

Period covered: From 01 JAN 04 to 31 DEC 07

Date of preparation: 15 MAR 08

Start date of project: 01 JAN 04

Duration: 48 months

Project coordinator & organisation:

Revision 2

Søren BUUS, professor, MD, PhD
Division of Experimental Immunology
Institute of Medical Microbiology and Immunology (IMMI), University of Copenhagen
Panum 18.3.12, Blegdamsvej 3, DK-2200 N Copenhagen, DENMARK
Phone: (45) 3532 7853, Fax: (45) 3532 7696 or 7853, Email: S.Buus@immi.ku.dk

TABLE OF CONTENTS

Translating Genome and proteome information into immune recognition

A project supported by the 6th framework programme of the European Union

Final Publishable Executive Summary

The continued spread of "older" pathogen (such as TB, HIV, malaria and most recently bird flu) and the emergence of "new" pathogens (such as the recent SARS epidemic) have vividly demonstrated how vulnerable modern societies are to infectious diseases. Vaccines, which basically alert the immune system to one or more physical characteristics of a given pathogen, remain the most efficient measure to control infectious diseases.

Unfortunately, vaccine development is slow and cumbersome, and for several serious pathogens, no vaccines are available. This project emanates from the recent discovery that the immune system considers peptides (fragments of proteins) as key targets. In fact, an entire arm of the immune system – that of T cells, which essentially controls specific immune responsiveness - has been devoted to peptide recognition. It is our premises that if one could predict how the immune system handles proteins, and how it generates, selects and recognizes peptides, then one should also be able to translate genome information to immunogens, and thereby forecast immune recognition.

Although many of the mechanisms involved in antigen presentation have been described in general terms, only few have been described in sufficient details to allow for accurate prediction of their outcome. In this project, we have generated a European resource for large-scale prediction of immune epitopes, which will allow scientists and clinicians to screen whole genomes and proteomes for the presence of potential immunogenic epitopes. The ability to rapidly identify immune targets will become a platform technology enabling future rational vaccine and immunotherapy development.

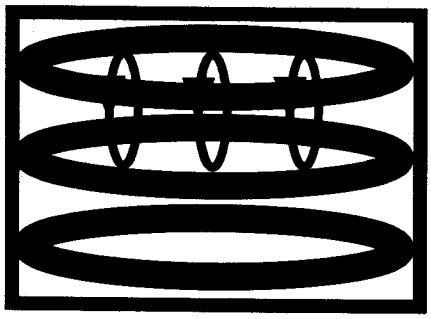
Web site: www.cbs.dtu.dk/Services/

A recent survey finds that our NetMHC predictor is the best of 147 tested world-wide (BMC Immunol 2008, 9:8)

This project has been conducted by a European consortium with participants from France, Germany and Denmark, each of them being experts of international standing within their area of responsibility. The consortium included a small privately owned biotechnology company. The co-ordinator, professor Søren Buus from the University of Copenhagen, have had extensive experience in co-ordinating international collaborations at this scale.

The project and its results are presented at a public website, which can be found at http://www.cbs.dtu.dk/researchgroups/immunology/Genomes2vaccines/

The **project logo** describes the iterative and integrative nature of the project. The three small vertical ellipses with arrows symbolises the feedback loops between actual data (upper horizontal ellipse) and predictive models (middle horizontal ellipse), which are analysed for each of three important steps involved in immune recognition. These predictive models are integrated and compared to the outcome (bottom horizontal ellipse) of the entire process as it is found in nature.



Coordinator contact details

Søren BUUS, professor, MD, PhD
Division of Experimental Immunology
Institute of Medical Microbiology and Immunology (IMMI), University of Copenhagen
Panum 18.3.12, Blegdamsvej 3, DK-2200 N Copenhagen, DENMARK
Phone: (45) 3532 7853, Fax: (45) 3532 7696 or 7853, Email: S.Buus@immi.ku.dk, Skype: S_Buus

Introduction

The basic premise of this project remains intact and as strong as when it was originally written during the application – and reprinted below. A recent review by one of the partners (van Enders, Eur J Immunol 2008, 38:609) confirms the continued importance of the major mechanisms described in this project.

Complete sequence information is accumulating for all known human pathogens, and – as so convincingly demonstrated for SARS - can rapidly be acquired for any new emerging pathogen. As this information is being expanded to multiple isolates it begins to represent pathogen diversity, and as it is being expanded to transcriptomes and proteomes it addresses pathogen expression during different disease stages. Primary sequences allows unequivocal identification of the pathogen and it is not surprising that the immune system has evolved mechanisms to access and target primary sequence information. It is an excellent target choice; the diversity of peptides is enormous and sufficient discriminatory power can be obtained already at the level of oligo-peptides. It is now well established that the immune system processes all protein available to the body and selects some of the resulting peptides for inspection. However, identifying the particular peptide target of interest in a given disease setting is a awesome task hindered by the fact that only few processes have been described in sufficient details to allow for accurate predictions of their outcome, and even fewer predictions are capable of handling entire organisms. To meet this challenge and solve how to design antigens for effective, protective immunity, our objective is to generate a suite of tools that will enable a pathogen-, genome- and host-wide search for T cell epitopes. If the extremely diverse interactions between pathogen and human host are to be handled rationally it is essential to integrate bioinformatics and high-throughput immunology. The proposed tools will use fast, high capacity, and low cost genomics and bioinformatics to identify potential antigens and epitopes. These can then be validated by slower, intermediate capacity, and more costly in vitro validation steps, before they are passed to the extremely slow, limited, and costly clinical trials preceding clinical applications (the latter is not part of this project). Rational identification of immune targets will be of considerable theoretical and practical utility. The amount of time and resources spent in antigen and epitope discovery will be significantly reduced, yet be global in an unprecedented way. Genomes of all available pathogen isolates can be searched for conserved vaccine candidates. It will also be possible to take the entire host population into account (i.e. to span all HLA specificities (see below)). All of these tools will be publicly available; scientifically by publication and web-accessible services, clinically by making tools and peptides available, and commercially through licensing.

The major activity has focused on the three most important and well-established events in antigen processing and presentation: WP1, proteasome digestion of protein antigens; WP2, TAP mediated translocation of the resulting peptides to the ER; and WP3, peptide selection

by HLA class I molecules. Initially, we developed the necessary biochemical tools to generate large-scale quantitative data representing proteasomes peptide generation, TAP mediated translocation and HLA selection. In an iterative procedure, the resulting data was used to generate and perfect bioinformatics resources to quantitatively predict these events (WP4). This unique procedure involved integration between biochemistry and bioinformatics; biochemical data directs bioinformatics, which in turn directed new and particularly valuable biochemical experiments etc.

During the project period, the number of registered HLA molecules continued its accelerating growth and the central problem of addressing and solving all HLA-I specificities deepened. For the entire 4-year project period (primo 2004 to ultimo 2007) the number of registered HLA class I molecules have increased from 874 to 1592 (corresponding to a steady annual growth rate of 16-18%). (source: www.anthonynolan.org.uk/HIG/).

Total #	Mar-04	Mar-05	Mar-06	Mar-07	Mar-08
HLA-A	255	283	341	405	495
HLA-B	497	555	648	729	833
HLA-C	122	146	173	219	264
Total	874	984	1162	1353	1592

Our aim was to describe and predict peptide-binding to HLA class I molecules with a particular emphasis on HLA-A and HLA-B molecules. Knowing that it would be impossible to directly address every single HLA-I molecules, our primary strategy was to select a subset of HLA-I, which gives maximum population coverage and represent the diversity of HLA-I molecules, and, for this subset, generate the corresponding high-quality data and prediction tools. It would seem that the current HLA-I discovery rate is threatening to outpace our discovery rate. However, major developments in our labs published during the last reporting period (Nielsen et al, PLoS One 2007, 2:786) suggested a general strategy to solve and predict all HLA-I specificities.

Even a high-risk sub-project aimed at generation high-quality HLA class II molecules succeeded. These molecules sample peptides derived from the metabolism of extracellular proteins and present them to CD4 positive helper T cells. This allows HLA class II molecules to control helper T cell responses maintaining immune memory. Some 743 different HLA-II chains have been registered (March 2008). Assuming free pairing of the relevant α and β chains, this could lead to as much as 3700 different HLA-II molecules. A recombinant approach is needed to handle this diversity, however, current rHLA-II production is primarily based upon cumbersome eukaryotic expression systems not suited for the problem at hand. We have solved thus problem during the last reporting period and generated highly active HLA class II molecules as well as a high-throughput peptide binding assay.

We have integrate the different predictive tools to mimic natural antigen processing and presentation, and made this publicly available. One of us has pioneered a technology to biochemically identify peptides resulting from natural processing and presentation. A large

number of new natural ligands has been identified during this reporting period. These natural ligands represent important data to develop and validate the integrated predictors.

All the biochemical tools have been (or will be) published. The data and the prediction tools have been made web-accessible. To exemplify the utility, we have scanned the genomes of known human pathogens (such as influenza and HIV) and collaborated with groups (not funded trough this project) testing for recognition of the predicted epitopes. In this project, we have identified a panel of highly conserved flu epitopes of relevance for the current bird flu epidemic.

Finally, through the development of a peptide array approach capable of large-scale scanning (WP6), we have generated tools that might enhance our future biochemical capacity and thereby further improve our predictive tools.

1. Project execution

Scientific and technological objectives of the project, and state-of-the-art.

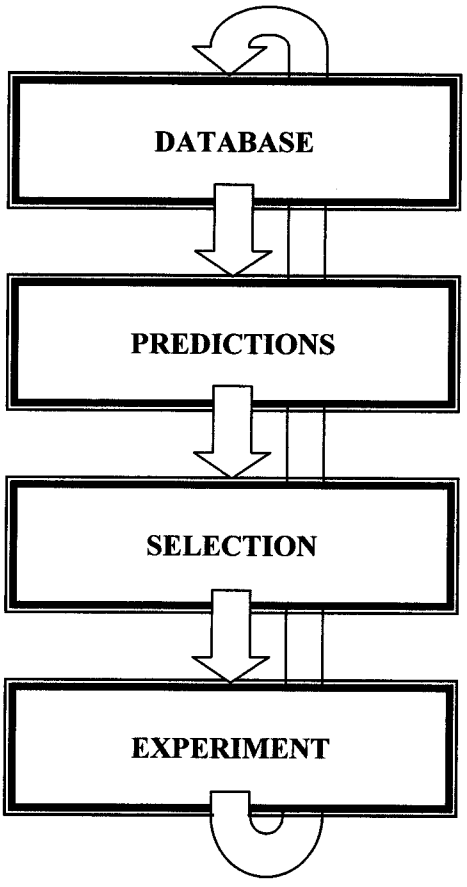
Relationship to state-of-the-art. In the original application the project was focused on the most important steps involved in the creation of T cell targets: the generation and selection of immune peptide epitopes. The primary emphasis was on HLA class I restricted presentation to cytotoxic T cells. The major mechanisms involved - and those addressed in this project – were proteasome, TAP, MHC binding. Two additional mechanisms of importance for antigen processing and presentation – those of ER aminopeptidase mediated epitope trimming and of tapasin mediated peptide-HLA quality control – were also known at the start of the project. Nonetheless, they were not included in the project. No additional mechanism of major prominence and acceptance has been identified since (van Endert, Eur J Immunol 2008, 38:609).

The past years have seen an increase in the available data on epitopes and in predictive tools. However, these predictions tend to score the outcome of the function in question (i.e they are non-quantitative) and they do not have the capability to handle entire genomes. Recently, the immune epitope database (IEDB, http://immuneepitope.org) has gone online including a comprehensive listing of data and tools. In a side-by-side comparison our artificial neural network based prediction tools prevailed.

Scientific rationale. CD8+ cytotoxic T cells (CTLs) recognizes HLA class I molecules, which have bound and presented peptides that are 8-11 amino acids long. These peptides have been generated through limited proteolytic fragmentation of all the proteins within the body. This is known as "antigen processing"; it ensures that all potential targets, even those hidden within microbial organisms or within the tertiary structure of a protein, are exposed and subject to immune surveillance. The actual epitopes are determined by the specificities of the processing events involved in fragmenting the original protein(s) generating the pool of peptides, which will eventually be offered to the HLA. These specificities include proteasomal activity leading to a cytosolic protein fragmentation (of size 4-20 amino acids) and TAP mediated translocation of the resulting peptide across the endoplasmic reticulum membrane where HLA-I peptide loading occurs. It is estimated that only 20% of the peptides, which could be generated from protein by systematic degradation, is generated successfully by the proteasome. The specificities of the proteasome and of TAP play important roles for the generation of HLA-I restricted T cell epitopes. The proteasome is believed to be directly responsible for the generation of the C-terminals of HLA-I restricted T cell epitopes. The generation of the N-terminals of the epitopes is somewhat more complicated. Compared to the peptide epitopes, the corresponding N-terminals of the proteasomal fragments are frequently extended i.e. the same epitope is included in several different N-terminally extended fragments. Since TAP tends to recognize N-terminally located motifs, this effectively means that many epitopes will have multiple different opportunities to be translocated into the ER. Once it is in the ER, N-terminal extensions of the T cell epitope can be removed by aminopeptidases located in the ER. Only then is the final epitope liberated,

and can be specifically sampled by the HLA-I for subsequent presentation. Importantly, the MHC is extremely polymorphic and the peptide binding specificity varies for the different polymorphic MHC molecules. The net effect of this complicated system is that each individual presents a unique and highly diverse peptide imprint of the ongoing protein metabolism to his or her T cells. To recognise such a diverse array of target structures, the T cells employ a receptor repertoire, which constitutes a more or less complete library of reactivities. This omnipotent T cell repertoire is educated - in the context of self-MHC molecules - to ignore peptides derived from self-proteins, and to recognise peptide derived from non-self proteins. This suggests an efficient search strategy for immunogenic epitopes; one should - like T cells do - focus on protein primary structure information as it is being interpreted by the antigen processing and presentation machinery of the immune system. Thus, the scientific rationale of this project is one of understanding and predicting how the immune system handles proteins; or more specifically, of how it generates, selects and recognises peptides. Generating these predictive tools amount to translating genomes/proteomes into immunogens and it will enable a new and rational approach to immunotherapy.

General approach. The project focused on the specificities of three important events involved in antigen processing and presentation to cytolytic T cells: proteasome, TAP and HLA-I. Accurate and quantitative predictions were generated by the following data-driven approach: a) generate databases containing information on natural ligands and immune epitopes, b) generate preliminary predictions), c) select data points that most efficiently complements existing data, d) generate new quantitative data, and e) update the databases. The latter marks the first step in the next iteration. The bioinformatics resources can now be updated and refined, and another new set of data points can be selected, tested, and entered into the database - and another iteration can begin. Through this process the predictions were successively improved. Thus, we established an iterative and interactive loop between bioinformatics and wet biochemistry. Basically, we integrated the two fields such that the data guide the development of the predictions, and the predictions guide the selection of new data points. This allowed us to search the entire sequence space (more than 10^{12} members) by fast and cheap computation. It is our hypothesis, that this integrated and iterative approach generated accurate prediction algorithms with a minimum of effort (i.e. in a most cost-efficient manner).



The iterative approach has been used to generate predictions for each of the important events involved in antigen processing and presentation (as symbolized in the project logo). In

an attempt to reproduce biology in silico and generate an overall prediction of immunogenicity, the individual predictions was integrated. Since natural ligands represent examples of peptides that have made it successively through all relevant in vivo processes, these ligands served as important controls of this integrated prediction approach.

Achievements by Workpackage

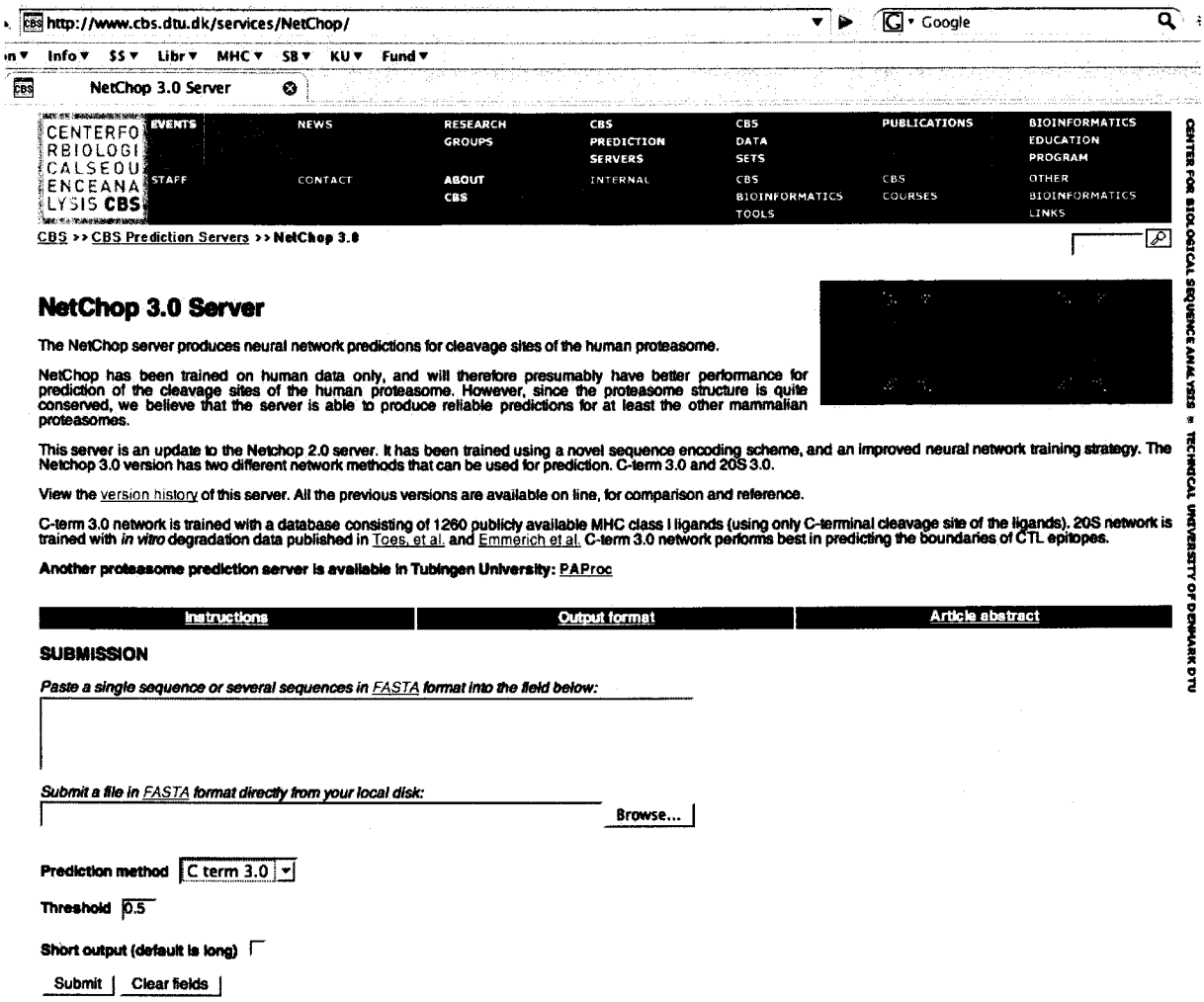
WP1 (Proteasome)

Lead participant: Project participant # 2, Johannes Gutenberg-University Mainz (JGUM.ITI) under the direction of professor Hansjörg Schild, Institute for Immunology, Obere Zahlbacher Strasse 67, D-55131 Mainz, Germany, Tel. + 49 6131 39 32451, Fax + 49 6131 39 35688, email: schild@uni-mainz.de

Objectices: The main objectives of the project were to develop the tools to measure and predict the efficiency of generation of individual peptide epitopes by the constitutive and immuno 20S and 26S proteasome. This entailed cleavage maps generated by c20S proteasomes, cleavage maps generated by i20S proteasomes, cleavage maps generated by c26S proteasomes, cleavage maps generated by i26S proteasomes, selection and verification of digestion of synthetic peptides and verification of CTL activation by cellular expression of minigenes.

Overview: In eucaryotic cells, the degradation of proteins that are misfolded, no longer needed or aged depends largely on the ubiquitin-proteasome system. Polyubiquitinylation targets proteins for degradation by the 26S proteasome. The 26S proteasome is composed of two protein complexes, called the 19S cap and the 20S core particle respectively. While the 19S cap is responsible for the recognition of polyubiquitinylated substrates, for deubiquitinylation and unfolding, the proteolytically active sites of the proteasome are found in the 20S core particle. Upon stimulation with interferon-γ, these proteolytically active subunits can be exchanged by the so-called immuno-subunits LMP2, LMP7 and MECL1. This exchange results in a change of proteasomal specificity that favors the generation of peptides that are likely to be transported by TAP [Tenzer S, at al. Cell Mol Life Sci. 2005; 62(9):1025-37].

Results: As illustrated in the interim progress reports, we have generated the various proteasome cleavage maps and made the data available to our bioinformatics partner (P4) for identification of additional peptides of interest. Partner 4 has generated web-based predictors of proteasome activity, NetChop, as illustrated below.



WP2 (TAP)

Lead participant: Project participant # 3 (INSERM), Institut National de la Santé et de la Recherche Médicale Unité 580 under the direction of Peter van Endert, professor of Immunology at the University Paris Descartes, Director of INSERM U580. Address: Inserm U580, Hôpital Necker, 161 rue de Sèvres, F-75015 Paris, France, Tel. + 33 1 44 49 25 63, Fax + 33 1 44 49 53 82, e-mail: vanendert@necker.fr

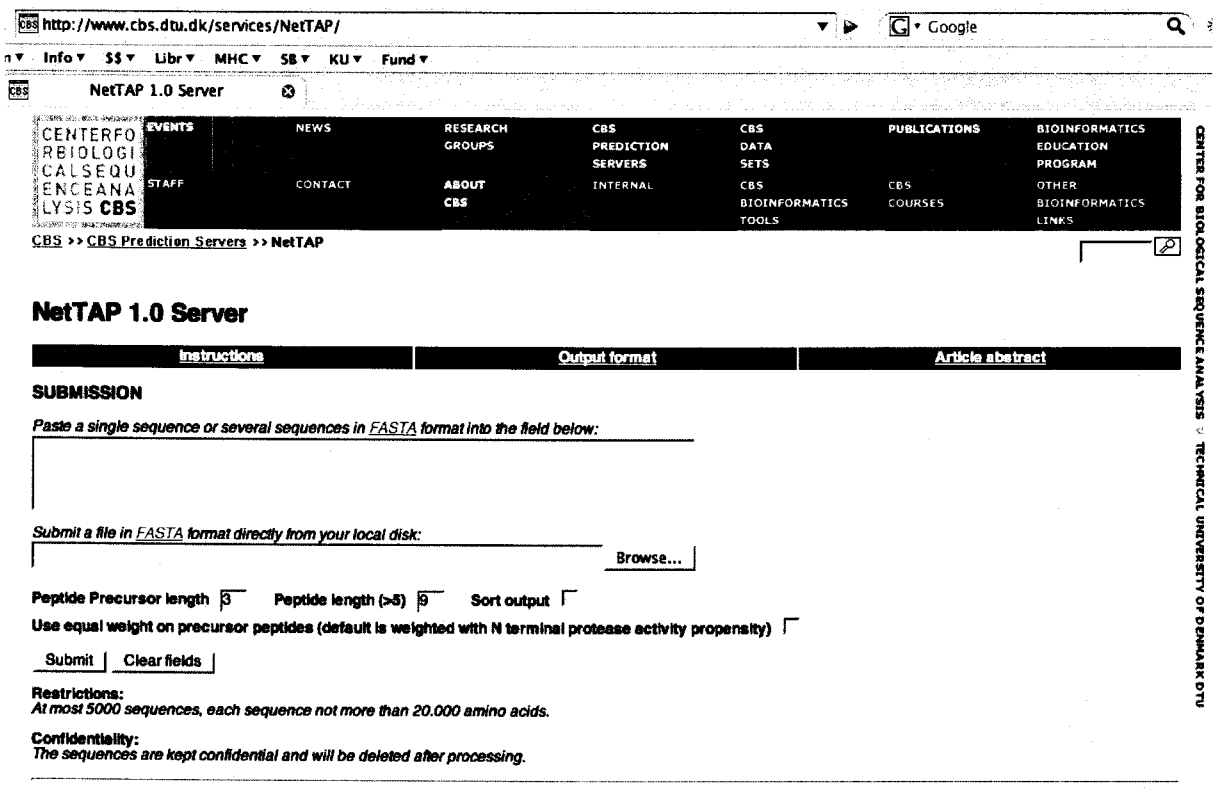
Objectives: To produce data suitable for development of high performance algorithms for prediction of peptide affinity for the TAP peptide transporters. This entailed the generation of a 96 high throughput TAP binding well format assay, development of high performance algorithm for predicting TAP affinities of 9-mer peptides, algorithms for prediction of TAP transport of epitope precursors and HLA class I allomorph-specific algorithms describing weighting of TAP affinities of 9-mer and longer peptides for epitope prediction.

Overview: Before this project, the TAP transport step was rarely been taken into account in strategies for epitope identification and selection. Nonetheless, it was clear that

human TAP is selective, and that this selectivity has an impact on the efficiency of presentation of an individual epitope. Thus, partner 3 had just shown that presentation efficiency (measured as number of epitope molecules on the cell surface/molecules in the cytosol) ws directly proportional to TAP affinity.

Human TAP selects peptides based both on sequence and on length. The nature of the amino acids in the three aminoterminal (Nt) positions and at the carboxyterminal (Ct) has a strong influence on TAP affinity. TAP transports peptides with a length between 8 or 9 and about 15 residues with equal efficiency. This length preference provides an opportunity for antigen processing to increase the array of potential epitopes by transporting extended precursors into the ER that can then be trimmed to optimally class I-adapted peptides by luminal peptidases.

Results: As illustrated in the interim progress reports we have generated a novel high-throughput assay for TAP activity and several generations of data for our bioinformatics partner to generate predictors. Eventually, we worked out both human an mouse TAP specificity. Partner 4 has generated a web-based predictors of TAP activity, NetTAP, as illustrated below.



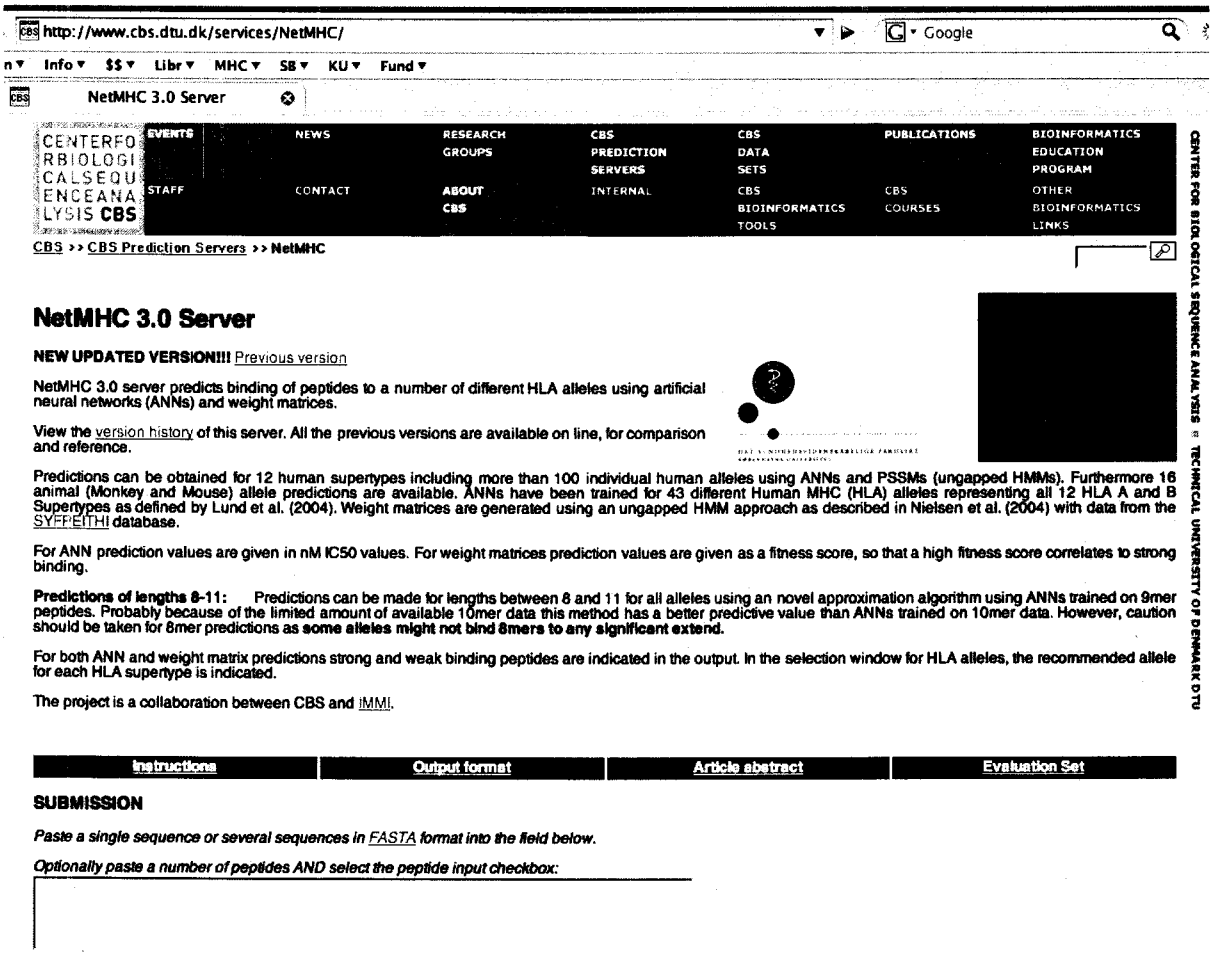
WP3 (HLA)

Lead participant: Project coordinator & participant # 1, University of Copenhagen (UKBH) under the direction of professor Soren Buus, Institute of Medical Microbiology and Immunology, Panum 18.3.12, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark, Tel. + 45 3532 7885, Fax + 45 3532 7696, e-mail: S.Buus@immi.ku.dk

Objectives: The main project objective was to develop the tools to measure and predict the efficiency of binding of individual peptide epitopes to representative HLA class I molecules. As an additional, ambitious and high-risk objective, we also aimed at developing the tools to measure and predict peptide binding to HLA class II molecules.

Overview: Peptide binding to MHC is the most selective event in antigen processing and presentation. It is also the most polymorphic specificity involved. It is therefore a particularly appropriate target for the iterative strategy proposed here.

Results: As illustrated in the interim progress reports we have generated nine class I molecules and three class II molecules covering parts of the HLA class I-space, which until now have been less well populated. We have generated the corresponding high-throughput assays. The data has been communicated this data to partner 4 for the purpose of generating and improving bioinformatics predictors of peptide-HLA-I and II binding, NetMHC[1], as illustrated below (for brevity, we do not show the corresponding class II predictor at http://www.cbs.dtu.dk/services/NetMHCII/).



[1] An independent survey of 147 different HLA class I predictors (Lin et al, BMC Immunol. 2008 Mar 16; 9(1):8 PMID: 18366636) finds that NetMHC is the"best prediction server across all HLA molecules".

In the last period we even managed to develop and publish a pan-specific predictor taking all available data into account allowing new and never previously described HLA molecules to be described by interpolating existing data. This predictor is also available at the internet as NetMHCpan – in both a class I version (http://www.cbs.dtu.dk/services/NetMHCpan/) and in a class II version (not yet released). For the sake of brevity none of these are shown here.

In addition, we have in this reporting period demonstrated and published that our HLA class I molecules are of quality that allows very simple and rapid HLA tetramer formation for the purpose of monitoring specific T cells. This is a major achievement, as it will allow basically any end-user – even those without any special biochemical expertise – to generate HLA tetramers. Figure 4 from this publication - comparing a commercial pentamer (left panel) with our new "one-pot, mix-and-read" tetramers (right panel) - is shown here:

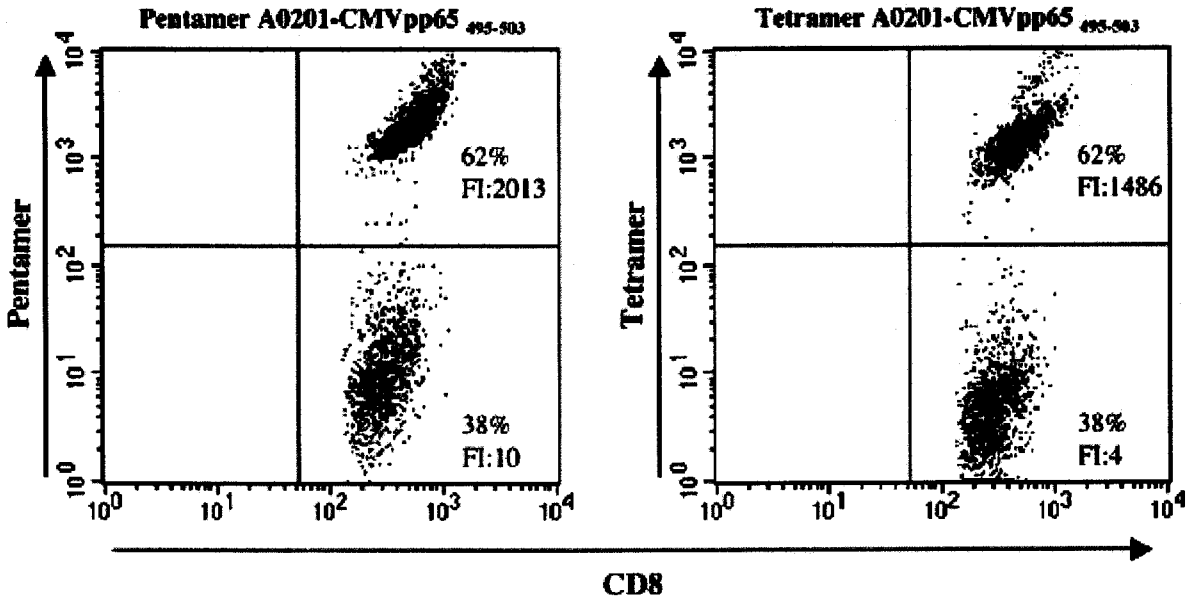


Figure 4. Tetramer staining comparison to pentamer. PBMC from a healthy donor were stimulated for 8 day with the pp65$_{495-503}$ peptide and stained with APC labeled anti-CD8 and PE-labeled HLA-A*0201-pp65$_{495-503}$ tetramer (right panel) or with PE-labeled HLA-A*0201-pp65$_{495-503}$ pentamer (left panel). The dot plot shows the gated CD8 T cells. Numbers in the upper right quadrant of each plot are the percentage of epitope specific CD8 T cells. The fluorescence intensity (FI) of the staining of the positive and negative population is given in the upper and lower right quadrant, respectively.
doi:10.1371/journal.pone.0001678.g004

WP4 (Bioinformatics, database, web)

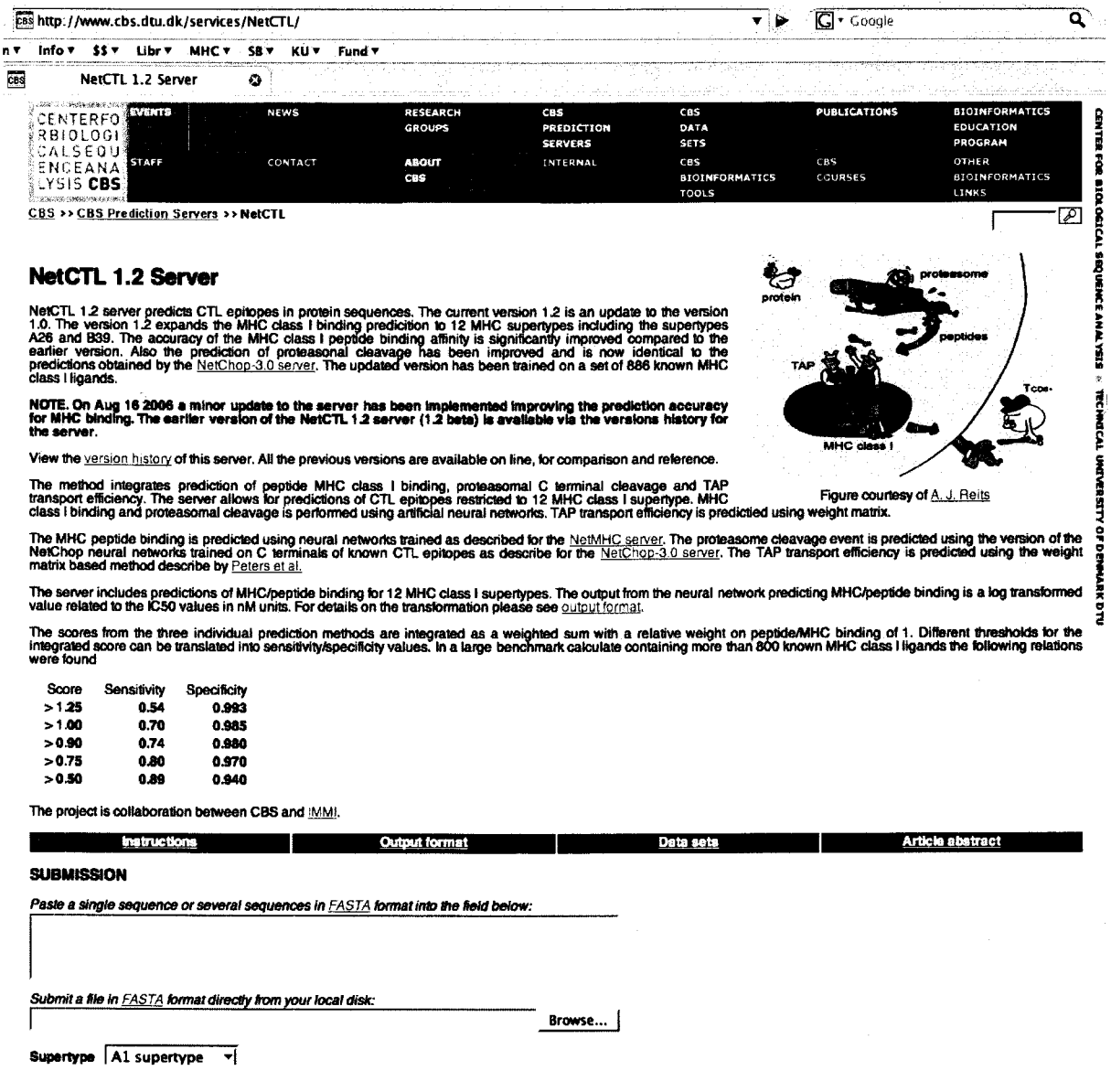
Lead participant: Project participant # 4, Technical University of Denmark (DTU) under the direction of professor Soren Brunak, Center for Biological Sequence Analysis, Kemitorvet, Building 208, DK-2800 Lyngby, Denmark, Tel. + 45 4524 2477, Fax + 45 4593 1585, e-mail: brunak@cbs.dtu.dk

Objectives: To develop a database containing the experimental results generated through this project and make it website available; to select the most information-rich data for experimentation, to generate predictions of the affinity of binding of peptides to different MHC alelles, TAP binding peptides, the specificity of different species of the proteasome,

and make these web available, and finally to integrate these prediction into a single CTL predictor.

Overview: A bioinformatics driven prediction strategy is an ideal way to represent and predict the pattern-recognition events of antigen processing and presentation. It is also an ideal way to handle the enormous amount of input data made possible by genome and proteome projects, and suggest new epitopes in computational time.

Results: Data have been generated and communicated to us by our partners. We have selected new and particularly information-rich peptides for efficient next iteration data generation. This strategy has worked (not shown here). The individual servers have been exemplified under the three previous workpackages. Shown here is the integrated CTL predictor, NetCTL, which aims at incorporation all known antigen processing and presentation events in order to predict those peptides that most likely represent CTL epitopes.



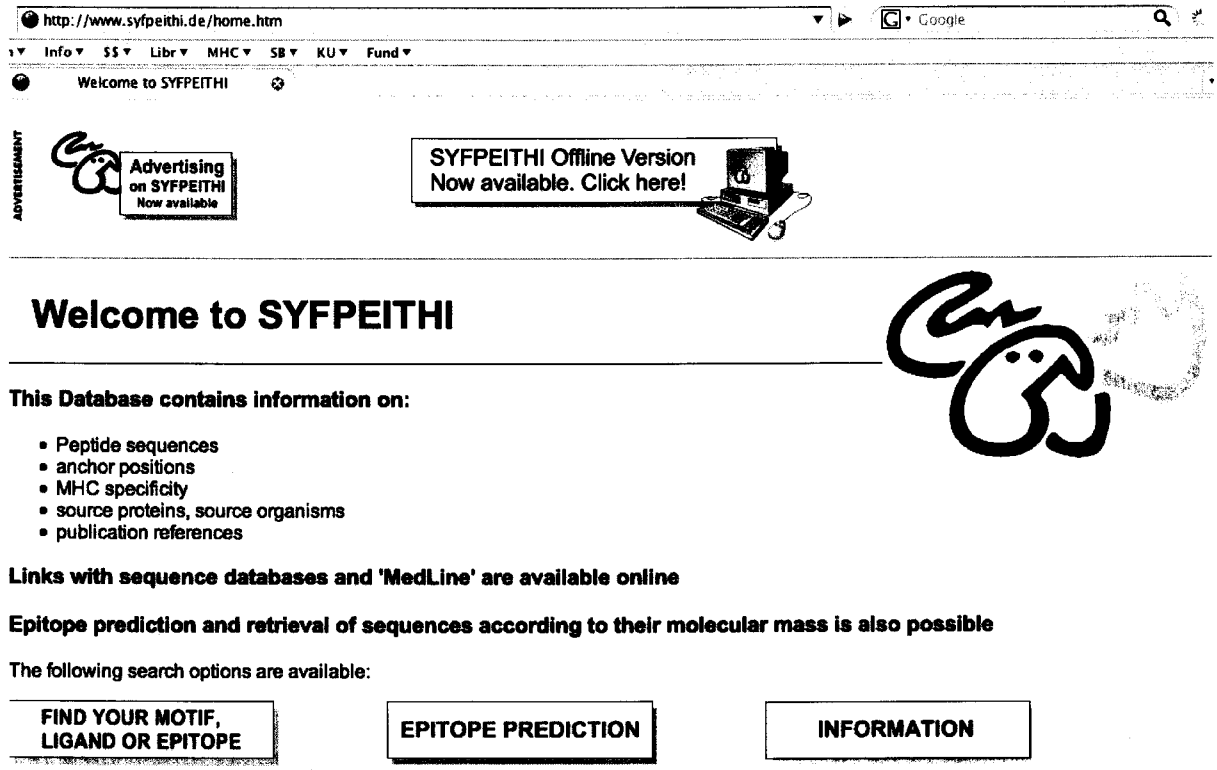
WP5 (Natural ligands)

Lead participant: Project participant # 5, University of Tübingen (UTUB) under the direction of professor Stefan Stevanovic, Department of Immunology, Institute for Cell Biology, Auf der Morgenstelle 15, D-72076 Tübingen, Germany, Tel. + 49 7071 298 7645, Fax + 49 7071 295653, e-mail: stefan.stevanovic@uni-tuebingen.de

Objectives: The main project objective is to characterise HLA-presented naturally processed peptides from different sources (HLA-defined cell lines, from HLA molecules with known and not yet known peptide motifs. For every relevant HLA molecule, the number of known ligands will be expanded to more than 50 naturally processed peptides.

Overview: "Natural ligands" – those peptides, which can be eluted off HLA molecules purified from cells – represent very valuable information. Every natural ligand identified is a example of a peptide that have made it successfully through all the events of antigen processing and presentation. Information on natural ligands are therefore particularly important when trying to integrate predictions of the individual events.

Results: Partner 5 has coordinated his effort with that of Partner 1 so that the same HLA class I specificities have been addressed in both workpackages. All the natural ligands identified have been disseminated via the database SYFPEITHI (www.syfpeithi.de) shown below:



The table below gives an overview on the "HLA ligandome" as of the end of the GenomesToVaccines project.

>100 ligands		50-99 ligands		1-49 ligands		no ligands	
A*02	B*15	A*01	B*07	A*11	B*08	A*23	B*42
A*03	B*18	A*25	B*14	A*29	B*13	A*34	B*46
A*24	B*39	A*26	B*27	A*30	B*41	A*36	B*54
	B*44	A*66	B*35	A*31	B*45	A*43	B*55
	B*49	A*68	B*37	A*32	B*47	A*74	B*56
	B*51		B*38	A*33	B*50	A*80	B*59
			B*40	A*69	B*52		B*67
					B*53		B*73
					B*57		B*81
					B*58		B*82
							B*83

Table 1. Analysis of the natural ligand repertoire of HLA antigens. Of all HLA allotypes occurring frequently in the Caucasian population, sufficient naturally processed ligands have been characzerised for accurate motif description and epitope prediction.

WP6 (Peptide arrays)

Lead participant: SME and project participant # 6, Schafer-N (SN) under the direction of the owner Mr. Claus Nielsen, Fruebjergvej 3, DK-2100 Copenhagen Ø, Denmark, Tel. + 45 3927 3800, Fax + 45 3927 3801, e-mail: peptides@schafer-n.com
This SME has made a business of synthesizing custom defined peptides on the milligram- to gram scale. The peptides are shipped freeze-dried in airtight vials with accompanying spec sheets including HPLC- and mass spec analysis. This has only been made possible through extensive automation using robots for synthesis and purification.

Objectives: To develop a cheaper technology to synthesise, purify and validate peptides in large numbers.

Overview: The T cell immune systems targets peptides, and a comprehensive analysis of T cell specificity may involve many different peptides. By way of example, a recent analysis of cytomegalovirus specific T cells used more than 13,000 peptides (J Exp Med 2008, 202:673). Such numbers of peptides would be prohibitive for most laboratories if they were made using conventional synthesis strategies. To solve this problem we aimed at developing robotized equipment that could produce large numbers of peptides in micro-reactors. We further aimed at developing techniques for synthesis of very large arrays of peptides on activated surfaces in a form that is suitable for analytical screening of peptide motifs of MHC class I molecules.

Results: Initially, we had decided to attempt to construct equipment based on commercially available dot-on-demand spraying nozzles that can deliver activated amino acids with high speed to arrays of mini reactors or directly onto activated surfaces. However, a much more promising photolithographic technique emerged during the project. Whereas the

originally planned "spray" robot could synthesize up to a few thousand peptides at a time, the photolithographic technique promised to make up to 20,000 peptides per 2 x 3 cm chip. As previously reported, we have adopted the photolithographic approach. At the conclusion of the project, we have established a much improved version of this strategy, demonstrated feasibility and started the first functional screenings using antibodies as target specificities.

Model experiments in which peptide chips were tested using commercially available antibodies to the peptides revealed a clear reactivity of the antibody against the corresponding peptide antigens synthesized on the chips. An example where a fluorochrome-labeled antibody against the well-known FLAG-peptide (H-DYKDDDDK-) was incubated with native and scrambled variants of this peptide on the chip is shown in the figure below. Enabling epitope analysis using this kind of peptide technology would be a quantum leap in our capabilities.

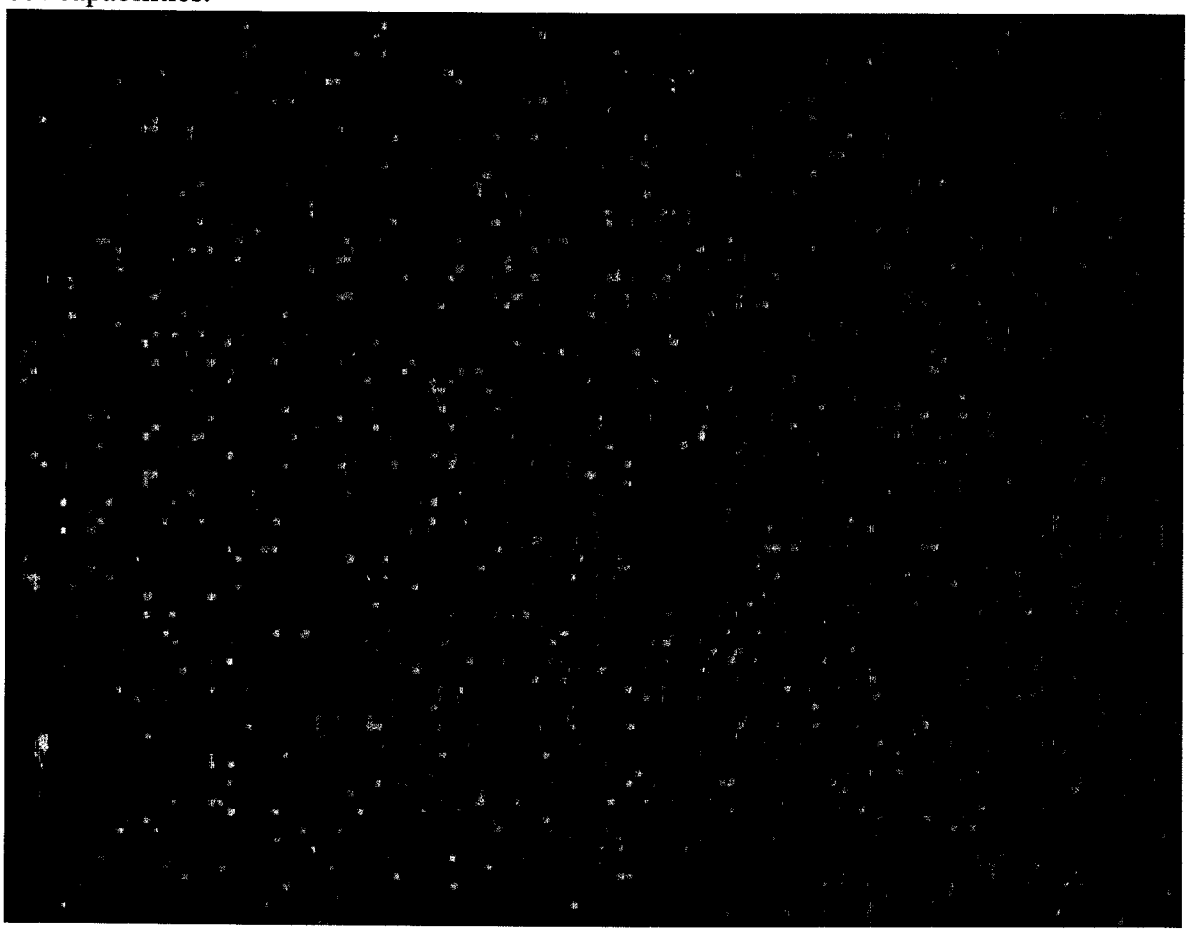


Fig. 12: Peptide array with ca. 12.000 fields containing variants of the FLAG-peptide (H-DYKDDDDK-[slide surface]). After workup in TFA, the chip was incubated with Cy3-labelled antibody against the FLAG-peptide followed by scanning of the chip in a laser-scanner at appropriate excitation wavelength. Bright fields contain a peptide that binds to the antibody.

2. Dissemination and use

General: The immune system has devoted an entire arm, T cells, to the recognition of protein fragments, or peptide epitopes. With appropriate peptides one can monitor specific immune responses, and manipulate specific immune responses (as in vaccination and immunotherapy). However, the universe of peptides is enormous, and it is by no means an easy task to identify those that are targeted by the T cell immune system. This project aimed at providing a solution to this problem.

We have successfully generated the bioinformatics tools that will allow any clinicians or scientists to perform global screenings for T cell epitopes in computational time. This entails that the immune system can be analysed at a scale that allows

- genome-wide analysis (we can encompass any size genome, transcriptome, proteome etc)
- pathogen-wide analysis (we can encompass any number of pathogen isolates and search for conserved, or non-conserved, epitopes depending on choice)
- host-wide analysis (more specifically, we can encompass the HLA diversity of the human population, which determines the specificity of the immune response of the individual)

The accompanying computational tools are freely available at our web-site.

Exploitable knowledge: Our tech transfer offices (TTO) and we are aware of the value intellectual property right, but also the difficulties of protecting methods. Patent applications were filed, but eventually discontinued.

Dissemination of knowledge: We have made all of our prediction servers freely available at the web. This will allow any interested party to scan their chosen genome/proteome – say SARS or bird flue – for the presence of T cell epitopes. In addition, our methods and results have been published (or are being prepared for publication)

Appendix 1 – Final plan for Using and Disseminating the Knowledge

Section 1 - Exploitable Knowledge and its use

Overview table Exploitable Knowledge

Partners 1 and 4 generated an integrated predictor aimed at identifying CTL epitopes and thus vaccine candidates. The tech transfer offices (TTO) at the two universities applied for patent protection and actively sought interested investors. However, renewed internal evaluation of the application questioned the degree of non-obviousness. This, together with a lack of commercial interest eventually led the TTO's to abandon the application.

Section 2 - Dissemination of knowledge

Overview table - Dissemination of knowledge

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Jan 04	Presentation, (Keystone)	Scientist and industry	International	ca 300	P1 Søren Buus
Apr 04	Presentation	Research	Norway	ca 50	P1 Søren Buus
May 04	Presentation	Research	Denmark	ca. 200	P1 Søren Buus
Juni 04	Presentation	Industry and scientist	international (ScanLab)	ca 100	P1 Søren Buus
Oct 04	Presentation	NIH and Scientists	US and International	ca 100	P1 Søren Buus
Oct 04	Presentation	Vaccine company	(Bavarian)	3	P1 and P4
Apr 05	Presentation	Politicians	Industry and scientist, Scandinavia,	ca. 200	P1 Søren Buus
May 05	Presentation	Genome Canada	Canadians	ca.12	P1 Søren Buus
Jun 05	Presentation	UK TTO's	UK,	ca. 50	P1 Søren Buus
2005	Publication: Tenzer, S., Peters, B., Bulik, S., Schoor, O., Lemmel, C., Schatz, M.M., Kloetzel, P.-M., Rammensee, H.-G., Schild, H.* and H.-G. Holzhütter. 2005. Modeling the MHC class-I pathway by combining predictions of proteasomal cleavage, TAP	Research	International	unlimited	P2 Hansjörg Schild / Stefan Tenzer

	transport and MHC class-I binding. Cell Mol Life Sci. 2005 May;62(9):1025-37.				
2005	Publication: Tenzer, S. and H. Schild. Assays of proteasome-dependent cleavage products. 2005. Methods Mol Biol. 2005;301:97-115	Research	International	unlimited	P2 Hansjörg Schild / Stefan Tenzer
20-22 Sept 2004	Presentation: Schatz,M., Peters, B., Tenzer, S., Rammensee,H.-G., Holzhütter, H.-G., and H. Schild. 2004. Broad Spectrum Analysis of N-terminal Epitope Trimming in the Cell. 35th Annual Meeting of the German Society of Immunology, Maastricht	Research	Maastricht	?	P2 Hansjörg Schild / Stefan Tenzer
20-22 Sept 2004	Poster: Tenzer, S., Peters, B., Bulik, S., Schoor, O., Lemmel, C., Schatz, M.M., Dengjel, J., Reichle, C., Krämer, B., Kloetzel, P.-M., Rammensee, H.-G., H.-G. Holzhütter and H. Schild. 2004. Modeling the MHC class-I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class-I binding. 35th Annual Meeting of the German Society of Immunology	Research	Maastricht	?	P2 Hansjörg Schild / Stefan Tenzer
8-9 Mar 2005	Poster: Tenzer, S., Peters, B., Bulik, S., Schoor,	Research	Boston University	?	P2 Hansjörg Schild / Stefan Tenzer

	O., Lemmel, C., Schatz, M.M., Kloetzel, P.-M., Rammensee, H.-G., H.-G. Holzhütter and H. Schild. 2005. Modeling the MHC class-I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class-I binding. 2nd International Immunoinformatics Symposium				
April 2-7, 2005	Poster: Genome- and HLA- wide scanning and validation of cytotoxic CD8+ T cell responses against Mycobacterium tuberculosis. Keystone Symposia Whistler, Canada Tuberculosis: Integrating Host and Pathogen Biology (D1)	Research	International	250	P4, Søren Brunak
April 9-15, 2005	Poster: An Integrative Approach to CTL Epitope Prediction. A Combined Algorithm Integrating MHC-I Binding, TAP Transport Efficiency, and Proteasomal Cleavage Predictions, Keystone Symposia Banff, Canada HIV Pathogenesis/HIV Vaccines (X7/X8)	Research	International	350	P4, Søren Brunak
June 2005 revision	Projects Web Site: http://www.cbs.dtu.dk/researchgroups/immunology/	General Public /Research	International	?	P4, Søren Brunak

June 28, 2005	Software Demonstration Immunological Web Tools ISMB 2005, Detroit, USA	Research	international	30	P4, Søren Brunak
July 2005	Publication: Larsen et al., An integrative approach to CTL epitope prediction: A combined algorithm integrating MHC class I binding, TAP transport efficiency, and proteasomal cleavage predictions, Eur J Immunol. 2005 Jul 5;35(8):2295-2303	Research	International	Impact factor: 5.0	P4, Søren Brunak
Apr 2005	Exhibition: European Day of Immunology (April 29th, 2005) Exhibition with poster walls, flyers, videos, discussions and a quizz on immunology. Flyer (ppt file) and report in our local newspaper (pdf file) are attached	*General public*	Germany	500	P5 S.Stevanovic
May 2005	Poster: OVERLAP OF HLA LIGAND REPERTOIRE FROM DIFFERENT SOURCES: IMPLICATIONS FOR IMMUNOTHERAPY, N. Hillen, T. Krüger, M. Müller, C. Lemmel, T. Weinschenk, H.-G. Rammensee, S. Stevanović ENII Meeting, Les Embiez, May 2005	*Research*	Europe	100	P5 S.Stevanovic
Apr. 2005	Flyer: European Day of Immunology (April 29th, 2005)	*General public*	Germany	500	P5 S.Stevanovic

	Exhibition with poster walls, flyers, videos, discussions and a quizz on immunology. Flyer (ppt file) and report in our local newspaper (pdf file) are attached				
2005	Publication: Krüger T, Schoor O, Lemmel C, Krämer BF, Reichle C, Eberle U, Häntschel M, Obermayr F, Dengjel J, Weinschenk T, Müller M, Hennenlotter J, Stenzl A, Rammensee HG, Stevanovic S (2005) Lessons to be learned from primary renal cell carcinomas: Novel tumor antigens and HLA ligands for immunotherapy. Cancer Immunol Immunother 54:826-836	*Research*	Worldwide	unlimited	P5 S.Stevanovic
Jun 05	Presentation, Medicon Valley visit to UK	*Scientist and industry*	UK	ca 100	P1 Søren Buus
Aug 05	Presentation, Biotech Conference, Dk	*Research*	International	ca 150	P1 Søren Buus
Sep 05	Presentation, Indian delegation, DK	*Research and politicians*	Indians	Ca. 10	P1 Søren Buus
Oct 05	Presentations, Biotech companies (Danisco NsGene, Neurosearch, Bavarian Nordic)	*Industry and scientist*	International	Ca. 10	P1 Søren Buus
Nov 05	Presentation,	*NIH and scientist*	International	Ca 100	P1 Søren Buus
Mar 06	Presentation	*Scientist*	Norway	Ca. 50	P1 and P4
Mar 06	Presentation	*Scientists*	International, Scandinavia,	Ca. 10	P1 Søren Buus
Mar 06	Presentation	*Clinicians*	Danish	Ca. 50	P1 Søren Buus
Apr 06	Presentation (Day of Immunology)	*Public*	Danish	Ca. 150	P1 Søren Buus
Aug 06	Presentation	*EU accountants*	EU	2	P1, Søren Buus

Sep 06	Presentation (EU grants)	*Administrators*	Danish	Ca. 25	P1, Søren Buus
Oct 06	Presentation (Graduate School of Immunology	*PhD Students*	Danish	Ca. 75	P1, Søren Buus
Nov 06	Presentation,	*NIH and scientist*	International	Ca 100	P1 Søren Buus
Nov 96	Presentation	*Scientist and industry*	International	Ca 150	P1, Søren Buus
Aug 05	Larsen MV, Lundegaard C, Lamberth K, Buus S, Brunak S, Lund O, Nielsen M. An integrative approach to CTL epitope prediction: a combined algorithm integrating MHC class I binding, TAP transport efficiency, and proteasomal cleavage predictions. Eur J Immunol. 2005 Aug;35(8):2295-303. PMID: 1599746	*Research*	International	unlimited	P1, Søren Buus P4, Søren Brunak / Ole Lund
Jul 05	Blicher T, Kastrup JS, Buus S, Gajhede M. High-resolution structure of HLA-A*1101 in complex with SARS nucleocapsid peptide. Acta Crystallogr D Biol Crystallogr. 2005 Aug;61(Pt 8):1031-40. Epub 2005 Jul 20. PMID: 16041067	*Research*	International	unlimited	P1, Søren Buus
Sep 05	Snabe T, Roder GA, Neves-Petersen MT, Buus S, Petersen SB. Oriented coupling of major histocompatibility complex (MHC) to sensor surfaces using light assisted immobilisation technology. Biosens Bioelectron. 2006	*Research*	International	unlimited	P1, Søren Buus

	Feb 15;21(8):1553-9. Epub 2005 Sep 1. PMID: 16139496				
Jun 06	Peters B, Bui HH, Frankild S, Nielson M, Lundegaard C, Kostem E, Basch D, Lamberth K, Harndahl M, Fleri W, Wilson SS, Sidney J, Lund O, Buus S, Sette A. A community resource benchmarking predictions of peptide binding to MHC-I molecules. PLoS Comput Biol. 2006 Jun 9;2(6):e65. Epub 2006 Jun 9. PMID: 16789818	*Research*	International	unlimited	P1, Søren Buus P4, Søren Brunak / Ole Lund
Sep 06	Wang M, Bai F, Pries M, Buus S, Prause JU, Nissen MH. Identification of MHC class I H-2 Kb/Db-restricted immunogenic peptides derived from retinal proteins. Invest Ophthalmol Vis Sci. 2006 Sep;47(9):3939-45. PMID: 16936108	*Research*	International	unlimited	P1, Søren Buus
Nov 06	Roder G, Blicher T, Justesen S, Johannesen B, Kristensen O, Kastrup J, Buus S, Gajhede M. Crystal structures of two peptide-HLA-B*1501 complexes; structural characterization of the HLA-B62 supertype. Acta Crystallogr D Biol Crystallogr. 2006 Nov;62(Pt 11):1300-10. Epub 2006 Oct 18. PMID: 17057332	*Research*	International	unlimited	P1, Søren Buus

Nov 06	Blicher T, Kastrup JS, Pedersen LO, Buus S, Gajhede M. Structure of HLA-A*1101 in complex with a hepatitis B peptide homologue. Acta Crystallograph Sect F Struct Biol Cryst Commun. 2006 Dec 1;62(Pt 12):1179-84. Epub 2006 Nov 4. PMID: 17142892	*Research*	International	unlimited	P1, Søren Buus
Dec 06	Wang M, Johansen B, Nissen MH, Thorn M, Kloverpris H, Fomsgaard A, Buus S, Claesson MH. Identification of an HLA-A*0201 restricted Bcl2-derived epitope expressed on tumors. Cancer Lett. 2006 Dec 18; [Epub ahead of print] PMID: 17182178	*Research*	International	unlimited	P1, Søren Buus
Feb 06	Iversen AK, Stewart-Jones G, Learn GH, Christie N, Sylvester-Hviid C, Armitage AE, Kaul R, Beattie T, Lee JK, Li Y, Chotiyarnwong P, Dong T, Xu X, Luscher MA, MacDonald K, Ullum H, Klarlund-Pedersen B, Skinhoj P, Fugger L, Buus S, Mullins JI, Jones EY, van der Merwe PA, McMichael AJ. Conflicting selective forces affect T cell receptor contacts in an immunodominant	*Research*	International	unlimited	P1, Søren Buus

	human immunodeficiency virus epitope. Nat Immunol. 2006 Feb;7(2):179-89. Epub 2006 Jan 1. PMID: 16388312				
12 Mar 2007	Presentation / Business proposal	*Bavarian Nordic (Vaccine Company)*	Denmark/Germany	10	P1 (Buus) and P4 (Brunak / Lund)
12 Apr 2007	Publication: Wang M, Lamberth K, Harndahl M, Røder G, Stryhn A, Larsen MV, Nielsen M, Lundegaard C, Tang ST, Dziegiel MH, Rosenkvist J, Pedersen AE, Buus S, Claesson MH, Lund O. CTL epitopes for influenza A including the H5N1 bird flu; genome-, pathogen-, and HLA-wide screening. Vaccine. 2007, 25(15):2823-31.	*Research*	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)
29 Aug 2007	Publication: Nielsen M, Lundegaard C, Blicher T, Lamberth K, Harndahl M, Justesen S, Røder G, Peters B, Sette A, Lund O, Buus S. NetMHCpan, a method for quantitative predictions of peptide binding to any HLA-A and -B locus protein of known sequence. PLoS ONE. 2007 Aug 29;2(8):e796.	*Research*	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)
29 Oct 2007	Presentation / Adminnistrators	*EU Contact Liasons*	Denmark	75	P1 (Buus)
31 Oct 2007	Publication: Larsen MV, Lundegaard C, Lamberth K, Buus S, Lund O, Nielsen M.	*Research*	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)

	Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. BMC Bioinformatics. 2007 Oct 31;8:424.				
13 Nov 2007	Presentation	*Clinicians / Scientists*	US (FHCRC, Seattle)	30	P1 (Buus)
14 Nov 2007	Presentation IEDB	*Scientists*	International	100	P1 (Buus)
28 Nov 2007	Presentation / Business proposal	*Seed Fund Investors*	Denmark	2	P1 (Buus)
27 feb 2008	Publication: Leisner C, Loeth N, Lamberth K, Justesen S, Sylvester-Hvid C, Schmidt EG, Claesson M, Buus S, Stryhn A. One-Pot, Mix-and-Read Peptide-MHC Tetramers. PLoS ONE. 2008 Feb 27;3(2):e1678.	*Research*	International	Unlimited	P1 (Buus / Stryhn)
22 Apr 2007	Presentation: Schild HJ „Non-proteasomal cleavage activities in the generation of MHC class I ligands" 3rd Charité Workshop Zeutherner See	*Research*	Germany	50	P2 (Schild)
01 Mar 2008	Publication: Schatz MM, Peters B, Akkad N, Ullrich N, Martinez AN, Carroll O, Bulik S, Rammensee HG, van Endert P, Holzhütter HG, Tenzer S, Schild H.. "Characterizing the N-terminal processing motif of MHC class I ligands." J Immunol. 180(5):3210-7	*Research*	International	Unlimited	P2 (Schild/ Tenzer)
2008	Publication: Tenzer S, Wee E, Burgevin A,	*Research*	International	Unlimited	P2, H. Schild/ S. Tenzer P1, S. Buus,

	Stewart-Jones G, Friis L, Lamberth K, Chang CH, Harndahl M, Gerstoft J, Fugger L, Jones EY, McMichael AJ, Buus S, Schild H, van Endert P, Iversen AKN. "Antigen dose shape HIV - specific CTL-response hierarchies." submitted				P3, P. van Endert
2008	Publication: Nacarino Martinez A, Tenzer S, Schild H. "T-Cell Epitope Processing (The epitope flanking regions matter)" Methods in Molecular Biology, in press	*Research*	International	Unlimited	P2, H. Schild/ S. Tenzer
2008	Publication: Cardinaud S, Rohrlich PS, Tourdot S, Bouziat R, Mattei S, Février M, Weiss L, Langlade-Demoyen P, Burgevin A, Fiornetino S, van Endert P, Lemonnier FA. Design of a HIV-1 derived, HLA-B07.02-restricted polyepitope construct (submitted)	*Research*	International	Unlimited	P3, Van Endert
2008	Publication: Burgevin A, Saveanu L, Kim Y, Barilleau E, Sette A, van Endert P, Peters B. A detailed analysis of the murine TAP transporter substrate specificity (submitted)	*Research*	International	Unlimited	P3, Van Endert
2008	Publication: Tenzer S, Wee E, Burgevin A,	*Research*	International	Unlimited	P3, Van Endert

	Stewart-Jones G, Lamberth K, Chang C, Harndahl M, Gerstoff J, Fugger L, Yvonne Jones E, McMichael A, Buus S, Schild H, van Endert P, Iversen AKN. Antigen processing determines HIV-specific CTL immunodominanc e. (submitted)				
Nov 30 2007	Newspaper Article: Personlig Vaccine. Weekend Avisen (in Danish)	*General*	Denmark	5 Million	P4. Brunak
Dec 15 2007	Publication: Lundegaard C, Lund O, Kesmir C, Brunak S, Nielsen M. Modeling the adaptive immune system: predictions and simulations. Bioinformatics. 2007 Dec 15;23(24):3265-75	*Scientific*	International	Unlimite d	P4. Brunak
2008	Publication: Claus Lundegaard, Ole Lund, and Morten Nielsen. Accurate approximation method for prediction of class I MHC affinities for peptides of length 8, 10 and 11 using prediction tools trained on 9mers. Bioinformatics. Accepted	*Scientific*	International	Unlimite d	P4. Brunak
June 2008	Claus Lundegaard, Kasper Lamberth, Mikkel Harndahl, Søren Buus, Ole Lund and Morten Nielsen. NetMHC-3.0: Accurate web accessible predictions of Human, Mouse,	*Scientific*	International	Unlimite d	P4. Brunak and P1 Buus

	and Monkey MHC class I affinities for peptides of length 8-11. Nucl. Acid Res. Accepted				
22 Mar 2007	Presentation S. Stevanovic: "Peptides of tumor antigens presented by MHC molecules" Lecture Workshop: From innate to specific immunity. Voss (Norway)	*Scientific*	Europe	200	P5, S. Stevanovic
04 Oct 2007	Presentation S. Stevanovic: „Protein antigens in tumor diagnosis and therapy" Sofia school of protein science 2007 Sofia (Bulgaria)	*Scientific*	Europe	100	P5, S. Stevanovic
26 Nov 2007	Presentation S. Stevanovic: "HLA-presented peptides in cancer immunotherapy" Seminar at IRIC Montreal (Canada)	*Scientific*	International	100	P5, S. Stevanovic
2008	Publication: Weinzierl AO, Rudolf D, Hillen N, Tenzer S, van Endert P, Schild H, Rammensee HG, Stevanovic S (2008) Features of TAP-independent MHC class I ligands revealed by quantitative mass spectrometry. Eur J Immunol (in press)	*Scientific*	International	unlimited	P5, S. Stevanovic

Appendix 2 – Deliverables and milestones list

Table 1: Deliverables List

Del. no.	Deliverable name	Work package no.	Date due	Actual/Forecast delivery date	Lead contractor
WP1.D1	Proteasome assay	1	18 months	18 months	2
WP1.D2	Proteasome predictor	1	48 months	48 months	4
WP2.D3	TAP assay	2	18 months	18 months	3
WP2.D4	TAP predictor	2	48 months	48 months	4
WP3.D5	MHC class I assays	3	18 months	18 months	1
WP3.D6	MHC class I predictors	3	48 months	48 months	4
WP4.D7	Database	4	18 months	18 months	4
WP4.D8	Integrated predictors	4	48 months	48 months	4
WP4.D9	Web interface	4	18 months	18 months	4
WP5.D10	Natural ligands	5	48 months	48 months	5
WP6.D11	Peptides	6	48 months	48 months	6
WP7.D12	Management	7	48 months	48 months	1

Table 2: Milestones List

Mile stone no.	Mile stone name	Work package no.	Date due	Actual Forecast delivery date	Lead contractor
WP1.M1	Cleavage maps generated by c20S proteasomes	1	0-18 months	18 months	2
WP1.M2	Cleavage maps generated by i20S proteasomes	1	18-36 months	18 monhs	2
WP1.M3	Verification of pro. Digestion of synthetic peptides	1	18-48 months	18-48 months	2
WP1.M4	Verification of pro. CTL activation by cell express.	1	36-48 months	36-48 months	2
WP2.M1	96 high throughput TAP binding well format assay	2	18 months	18 months	3
WP2.M2	High performance algorithm for predicting TAP affinities of 9-mer peptides	2	36 month	36 month	3
WP2.M3	Algorithms for prediction of TAP transport of epitope precursors	2	36 month	36 month	3
WP2.M4	HLA class I allomorph-specific algorithms describing weighting of TAP affinities of 9-mer and longer peptides for epitope prediction	2	48 month	48 month	3
WP3.M1	Generation of HLA-I molecules	3	continuous	continuous	1
WP3.M2	Generation of HLA-I data	3	continuous	continuous	1
WP3.M3	Generation of HLA-I predictors	3	continuous	continuous	1
WP4.M1	First version of database become available via the web	4	18 months	18 months	4
WP4.M2	First generation of MHC, TAP, Proteasome prediction tools release on the website	4	36 months	36 months	4

WP4.M3	Second generation of MHC, TAP, Proteasome prediction tools release on the website	4	36 months	36 months	4
WP4.M4	Third generation of MHC, TAP, Proteasome prediction tools release on the website	4	48 months	48 months	4
WP4.M5	Integrated (CTL) prediction tools developed	4	48 months	48 months	4
WP4.M6	Final version of database/prediction tool released at the website	4	48 months	48 months	4
WP5.M1	T cell epitope prediction procedures developed and available on www	4	continuous	continuous	5
WP5.M2	Such predictions with natural ligand data will validate methods	5	continuous	continuous	5
WP6.M1	Construction and testing of prototype robots for preparative synthesis	6	18 months	30 months	6
WP6.M2	Construction and testing of prototype robots for analytical synthesis	6	36 months	36 months	6
WP6.M3	Develop a high speed parallel preparative purification system	6	18 months	Superseded	6
WP6.M4	Design and testing of analytical peptide arrays for MCH class1 motif-screening	6	36 months	36	6
WP6.M5	Generating peptide arrays for consortium	6	36-48 months	40	6

Appendix 1 - Plan for Using and Disseminating the Knowledge

Section 1 - Exploitable Knowledge and its use

Overview table Exploitable Knowledge

As described in the last report, partners 1 and 4 generated an integrated predictor aimed at identifying CTL epitopes and thus vaccine candidates. The tech transfer offices (TTO) at the two universities applied for patent protection and actively sought interested investors. However, renewed internal evaluation of the application questioned the degree of non-obviousness. This, together with a lack of commercial interest eventually led the TTO's to abandon the application.

Section 2 - Dissemination of knowledge

Overview table - Dissemination of knowledge

Date	Type	Type of audience	Countries addressed	Size of audience	Partner responsible
12 Mar 2007	Presentation / Business proposal	Bavarian Nordic (Vaccine Company)	Denmark/Germany	10	P1 (Buus) and P4 (Brunak / Lund)
12 Apr 2007	Publication: Wang M, Lamberth K, Harndahl M, Røder G, Stryhn A, Larsen MV, Nielsen M, Lundegaard C, Tang ST, Dziegiel MH, Rosenkvist J, Pedersen AE, Buus S, Claesson MH, Lund O. CTL epitopes for influenza A including the H5N1 bird flu; genome-, pathogen-, and HLA-wide screening. Vaccine. 2007, 25(15):2823-31.	Research	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)
29 Aug 2007	Publication: Nielsen M, Lundegaard C, Blicher T, Lamberth K, Harndahl M, Justesen S, Røder G, Peters B, Sette A, Lund O, Buus S. NetMHCpan, a method for quantitative predictions of peptide binding to any HLA-A and -B locus protein of known sequence. PLoS ONE. 2007 Aug 29;2(8):e796.	Research	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)

29 Oct 2007	Presentation / Adminnistrators	EU Contact Liasons	Denmark	75	P1 (Buus)
31 Oct 2007	Publication: Larsen MV, Lundegaard C, Lamberth K, Buus S, Lund O, Nielsen M. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. BMC Bioinformatics. 2007 Oct 31;8:424.	Research	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)
13 Nov 2007	Presentation	Clinicians / Scientists	US (FHCRC, Seattle)	30	P1 (Buus)
14 Nov 2007	Presentation IEDB	Scientists	International	100	P1 (Buus)
28 Nov 2007	Presentation / Business proposal	Seed Fund Investors	Denmark	2	P1 (Buus)
27 feb 2008	Publication: Leisner C, Loeth N, Lamberth K, Justesen S, Sylvester-Hvid C, Schmidt EG, Claesson M, Buus S, Stryhn A. One-Pot, Mix-and-Read Peptide-MHC Tetramers. PLoS ONE. 2008 Feb 27;3(2):e1678.	Research	International	Unlimited	P1 (Buus / Stryhn)
22 Apr 2007	Presentation: Schild HJ „Non-proteasomal cleavage activities in the generation of MHC class I ligands" 3rd Charité Workshop Zeutherner See	Research	Germany	50	P2 (Schild)
01 Mar 2008	Publication: Schatz MM, Peters B, Akkad N, Ullrich N, Martinez AN, Carroll O, Bulik S, Rammensee HG, van Endert P, Holzhütter HG, Tenzer S, Schild H.. "Characterizing the N-terminal processing motif of MHC class I ligands." J Immunol. 180(5):3210-7	Research	International	Unlimited	P2 (Schild/ Tenzer)
2008	Publication: Tenzer S, Wee E, Burgevin A, Stewart-Jones G, Friis L, Lamberth K, Chang CH, Harndahl M, Gerstoft J, Fugger L, Jones EY, McMichael AJ, Buus S,	Research	International	Unlimited	P2, H. Schild/ S. Tenzer P1, S. Buus, P3, P. van Endert

	Schild H, van Endert P, Iversen AKN. "Antigen processing determines HIV-specific CTL immunodominance". (submitted)				
2008	Publication: Nacarino Martinez A, Tenzer S, Schild H. "T-Cell Epitope Processing (The epitope flanking regions matter)" Methods in Molecular Biology, in press	Research	International	Unlimited	P2, H. Schild/ S. Tenzer
2008	Publication: Cardinaud S, Rohrlich PS, Tourdot S, Bouziat R, Mattei S, Février M, Weiss L, Langlade-Demoyen P, Burgevin A, Fiornetino S, van Endert P, Lemonnier FA. Design of a HIV-1 derived, HLA-B07.02-restricted polyepitope construct (submitted)	Research	International	Unlimited	P3, Van Endert
2008	Publication: Burgevin A, Saveanu L, Kim Y, Barilleau E, Sette A, van Endert P, Peters B. A detailed analysis of the murine TAP transporter substrate specificity (submitted)	Research	International	Unlimited	P3, Van Endert
Nov 30 2007	Newspaper Article: Personlig Vaccine. Weekend Avisen (in Danish)	General	Denmark	5 Million	P4. Brunak
Dec 15 2007	Publication: Lundegaard C, Lund O, Kesmir C, Brunak S, Nielsen M. Modeling the adaptive immune system: predictions and simulations. Bioinformatics. 2007 Dec 15;23(24):3265-75	Scientific	International	Unlimited	P4. Brunak
2008	Publication: Claus Lundegaard, Ole Lund, and Morten Nielsen. Accurate approximation method for prediction of class I MHC affinities for	Scientific	International	Unlimited	P4. Brunak

	peptides of length 8, 10 and 11 using prediction tools trained on 9mers. Bioinformatics. *Accepted*				
June 2008	Claus Lundegaard, Kasper Lamberth, Mikkel Harndahl, Søren Buus, Ole Lund and Morten Nielsen. NetMHC-3.0: Accurate web accessible predictions of Human, Mouse, and Monkey MHC class I affinities for peptides of length 8-11. Nucl. Acid Res. *Accepted*	Scientific	International	Unlimited	P4. Brunak and P1 Buus
22 Mar 2007	Presentation S. Stevanovic: "Peptides of tumor antigens presented by MHC molecules" Lecture Workshop: From innate to specific immunity. Voss (Norway)	Scientific	Europe	200	P5, S. Stevanovic
04 Oct 2007	Presentation S. Stevanovic: „Protein antigens in tumor diagnosis and therapy" Sofia school of protein science 2007 Sofia (Bulgaria)	Scientific	Europe	100	P5, S. Stevanovic
26 Nov 2007	Presentation S. Stevanovic: "HLA-presented peptides in cancer immunotherapy" Seminar at IRIC Montreal (Canada)	Scientific	International	100	P5, S. Stevanovic
2008	Publication: Weinzierl AO, Rudolf D, Hillen N, Tenzer S, van Endert P, Schild H, Rammensee HG, Stevanovic S (2008) Features of TAP-independent MHC class I ligands revealed by quantitative mass spectrometry. Eur J Immunol (in press)	Scientific	International	unlimited	P5, S. Stevanovic

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Jan 04	Presentation, (Keystone)	Scientist and industry	International	ca 300	P1 Søren Buus
Apr 04	Presentation	Research	Norway	ca 50	P1 Søren Buus
May 04	Presentation	Research	Denmark	ca. 200	P1 Søren Buus
Juni 04	Presentation	Industry and scientist	international (ScanLab)	ca 100	P1 Søren Buus
Oct 04	Presentation	NIH and Scientists	US and International	ca 100	P1 Søren Buus
Oct 04	Presentation	Vaccine company	(Bavarian)	3	P1 and P4
Apr 05	Presentation	Politicians	Industry and scientist, Scandinavia,	ca. 200	P1 Søren Buus
May 05	Presentation	Genome Canada	Canadians	ca.12	P1 Søren Buus
Jun 05	Presentation	UK TTO's	UK,	ca. 50	P1 Søren Buus
2005	Publication: Tenzer, S., Peters, B., Bulik, S., Schoor, O., Lemmel, C., Schatz, M.M., Kloetzel, P.-M., Rammensee, H.-G., Schild, H.* and H.-G. Holzhütter. 2005. Modeling the MHC class-I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class-I binding. Cell Mol Life Sci. 2005 May;62(9):1025-37.	Research	International	unlimited	P2 Hansjörg Schild / Stefan Tenzer
2005	Publication: Tenzer, S. and H. Schild. Assays of proteasome-dependent cleavage products. 2005. Methods Mol Biol. 2005;301:97-115	Research	International	unlimited	P2 Hansjörg Schild / Stefan Tenzer
20-22 Sept 2004	Presentation: Schatz,M., Peters, B., Tenzer, S., Rammensee,H.-G., Holzhütter,	Research	Maastricht	?	P2 Hansjörg Schild / Stefan Tenzer

	H.-G., and H. Schild. 2004. Broad Spectrum Analysis of N-terminal Epitope Trimming in the Cell. 35th Annual Meeting of the German Society of Immunology, Maastricht				
20-22 Sept 2004	Poster: Tenzer, S., Peters, B., Bulik, S., Schoor, O., Lemmel, C., Schatz, M.M., Dengjel, J., Reichle, C., Krämer, B., Kloetzel, P.-M., Rammensee, H.-G., H.-G. Holzhütter and H. Schild. 2004. Modeling the MHC class-I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class-I binding. 35th Annual Meeting of the German Society of Immunology	Research	Maastricht	?	P2 Hansjörg Schild / Stefan Tenzer
8-9 Mar 2005	Poster: Tenzer, S., Peters, B., Bulik, S., Schoor, O., Lemmel, C., Schatz, M.M., Kloetzel, P.-M., Rammensee, H.-G., H.-G. Holzhütter and H. Schild. 2005. Modeling the MHC class-I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class-I binding. 2nd International	Research	Boston University	?	P2 Hansjörg Schild / Stefan Tenzer

	Immunoinformatics Symposium				
April 2-7, 2005	Poster: Genome- and HLA- wide scanning and validation of cytotoxic CD8+ T cell responses against Mycobacterium tuberculosis. Keystone Symposia Whistler, Canada Tuberculosis: Integrating Host and Pathogen Biology (D1)	Research	International	250	P4, Søren Brunak
April 9-15, 2005	Poster: An Integrative Approach to CTL Epitope Prediction. A Combined Algorithm Integrating MHC-I Binding, TAP Transport Efficiency, and Proteasomal Cleavage Predictions, Keystone Symposia Banff, Canada HIV Pathogenesis/HIV Vaccines (X7/X8)	Research	International	350	P4, Søren Brunak
June 2005 revision	Projects Web Site: http://www.cbs.dtu.dk/researchgroups/immunology/	General Public /Research	International	?	P4, Søren Brunak
June 28, 2005	Software Demonstration Immunological Web Tools ISMB 2005, Detroit, USA	Research	international	30	P4, Søren Brunak
July 2005	Publication: Larsen et al., An integrative approach to CTL epitope prediction: A combined algorithm integrating MHC	Research	International	Impact factor: 5.0	P4, Søren Brunak

	class I binding, TAP transport efficiency, and proteasomal cleavage predictions, Eur J Immunol. 2005 Jul 5;35(8):2295-2303				
Apr 2005	Exhibition: European Day of Immunology (April 29th, 2005) Exhibition with poster walls, flyers, videos, discussions and a quizz on immunology. Flyer (ppt file) and report in our local newspaper (pdf file) are attached	*General public*	Germany	500	P5 S.Stevanovic
May 2005	Poster: OVERLAP OF HLA LIGAND REPERTOIRE FROM DIFFERENT SOURCES: IMPLICATIONS FOR IMMUNOTHERAPY, N. Hillen, T. Krüger, M. Müller, C. Lemmel, T. Weinschenk, H.-G. Rammensee, S. Stevanović ENII Meeting, Les Embiez, May 2005	*Research*	Europe	100	P5 S.Stevanovic
Apr. 2005	Flyer: European Day of Immunology (April 29th, 2005) Exhibition with poster walls, flyers, videos, discussions and a quizz on immunology. Flyer (ppt file) and report in our local newspaper (pdf file) are attached	*General public*	Germany	500	P5 S.Stevanovic
2005	Publication:	*Research*	Worldwide	unlimited	P5 S.Stevanovic

	Krüger T, Schoor O, Lemmel C, Krämer BF, Reichle C, Eberle U, Häntschel M, Obermayr F, Dengjel J, Weinschenk T, Müller M, Hennenlotter J, Stenzl A, Rammensee HG, Stevanovic S (2005) Lessons to be learned from primary renal cell carcinomas: Novel tumor antigens and HLA ligands for immunotherapy. Cancer Immunol Immunother 54:826-836				
Jun 05	Presentation, Medicon Valley visit to UK	*Scientist and industry*	UK	ca 100	P1 Søren Buus
Aug 05	Presentation, Biotech Conference, Dk	*Research*	International	ca 150	P1 Søren Buus
Sep 05	Presentation, Indian delegation, DK	*Research and politicians*	Indians	Ca. 10	P1 Søren Buus
Oct 05	Presentations, Biotech companies (Danisco NsGene, Neurosearch, Bavarian Nordic)	*Industry and scientist*	International	Ca. 10	P1 Søren Buus
Nov 05	Presentation,	*NIH and scientist*	International	Ca 100	P1 Søren Buus
Mar 06	Presentation	*Scientist*	Norway	Ca. 50	P1 and P4
Mar 06	Presentation	*Scientists*	International, Scandinavia,	Ca. 10	P1 Søren Buus
Mar 06	Presentation	*Clinicians*	Danish	Ca. 50	P1 Søren Buus
Apr 06	Presentation (Day of Immunology)	*Public*	Danish	Ca. 150	P1 Søren Buus
Aug 06	Presentation	*EU accountants*	EU	2	P1, Søren Buus
Sep 06	Presentation (EU grants)	*Administrators*	Danish	Ca. 25	P1, Søren Buus
Oct 06	Presentation (Graduate School of Immunology	*PhD Students*	Danish	Ca. 75	P1, Søren Buus
Nov 06	Presentation,	*NIH and scientist*	International	Ca 100	P1 Søren Buus
Nov 96	Presentation	*Scientist and industry*	International	Ca 150	P1, Søren Buus
Aug 05	Larsen MV,	*Research*	International	unlimited	P1, Søren Buus

	Lundegaard C, Lamberth K, Buus S, Brunak S, Lund O, Nielsen M. An integrative approach to CTL epitope prediction: a combined algorithm integrating MHC class I binding, TAP transport efficiency, and proteasomal cleavage predictions. Eur J Immunol. 2005 Aug;35(8):2295-303. PMID: 1599746				P4, Søren Brunak / Ole Lund
Jul 05	Blicher T, Kastrup JS, Buus S, Gajhede M. High-resolution structure of HLA-A*1101 in complex with SARS nucleocapsid peptide. Acta Crystallogr D Biol Crystallogr. 2005 Aug;61(Pt 8):1031-40. Epub 2005 Jul 20. PMID: 16041067	*Research*	International	unlimited	P1, Søren Buus
Sep 05	Snabe T, Roder GA, Neves-Petersen MT, Buus S, Petersen SB. Oriented coupling of major histocompatibility complex (MHC) to sensor surfaces using light assisted immobilisation technology. Biosens Bioelectron. 2006 Feb 15;21(8):1553-9. Epub 2005 Sep 1. PMID: 16139496	*Research*	International	unlimited	P1, Søren Buus
Jun 06	Peters B, Bui HH, Frankild S, Nielson M,	*Research*	International	unlimited	P1, Søren Buus P4, Søren Brunak / Ole

	Lundegaard C, Kostem E, Basch D, Lamberth K, Harndahl M, Fleri W, Wilson SS, Sidney J, Lund O, Buus S, Sette A. A community resource benchmarking predictions of peptide binding to MHC-I molecules. PLoS Comput Biol. 2006 Jun 9;2(6):e65. Epub 2006 Jun 9. PMID: 16789818				Lund
Sep 06	Wang M, Bai F, Pries M, Buus S, Prause JU, Nissen MH. Identification of MHC class I H-2 Kb/Db-restricted immunogenic peptides derived from retinal proteins. Invest Ophthalmol Vis Sci. 2006 Sep;47(9):3939-45. PMID: 16936108	*Research*	International	unlimited	P1, Søren Buus
Nov 06	Roder G, Blicher T, Justesen S, Johannesen B, Kristensen O, Kastrup J, Buus S, Gajhede M. Crystal structures of two peptide-HLA-B*1501 complexes; structural characterization of the HLA-B62 supertype. Acta Crystallogr D Biol Crystallogr. 2006 Nov;62(Pt 11):1300-10. Epub 2006 Oct 18. PMID: 17057332	*Research*	International	unlimited	P1, Søren Buus
Nov 06	Blicher T, Kastrup JS, Pedersen LO, Buus S, Gajhede	*Research*	International	unlimited	P1, Søren Buus

	M. Structure of HLA-A*1101 in complex with a hepatitis B peptide homologue. Acta Crystallograph Sect F Struct Biol Cryst Commun. 2006 Dec 1;62(Pt 12):1179-84. Epub 2006 Nov 4. PMID: 17142892				
Dec 06	Wang M, Johansen B, Nissen MH, Thorn M, Kloverpris H, Fomsgaard A, Buus S, Claesson MH. Identification of an HLA-A*0201 restricted Bcl2-derived epitope expressed on tumors. Cancer Lett. 2006 Dec 18; [Epub ahead of print] PMID: 17182178	*Research*	International	unlimited	P1, Søren Buus
Feb 06	Iversen AK, Stewart-Jones G, Learn GH, Christie N, Sylvester-Hviid C, Armitage AE, Kaul R, Beattie T, Lee JK, Li Y, Chotiyarnwong P, Dong T, Xu X, Luscher MA, MacDonald K, Ullum H, Klarlund-Pedersen B, Skinhoj P, Fugger L, Buus S, Mullins JI, Jones EY, van der Merwe PA, McMichael AJ. Conflicting selective forces affect T cell receptor contacts in an immunodominant human	*Research*	International	unlimited	P1, Søren Buus

	immunodeficiency virus epitope. Nat Immunol. 2006 Feb;7(2):179-89. Epub 2006 Jan 1. PMID: 16388312				
12 Mar 2007	Presentation / Business proposal	*Bavarian Nordic (Vaccine Company)*	Denmark/Germany	10	P1 (Buus) and P4 (Brunak / Lund)
12 Apr 2007	Publication: Wang M, Lamberth K, Harndahl M, Røder G, Stryhn A, Larsen MV, Nielsen M, Lundegaard C, Tang ST, Dziegiel MH, Rosenkvist J, Pedersen AE, Buus S, Claesson MH, Lund O. CTL epitopes for influenza A including the H5N1 bird flu; genome-, pathogen-, and HLA-wide screening. Vaccine. 2007, 25(15):2823-31.	*Research*	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)
29 Aug 2007	Publication: Nielsen M, Lundegaard C, Blicher T, Lamberth K, Harndahl M, Justesen S, Røder G, Peters B, Sette A, Lund O, Buus S. NetMHCpan, a method for quantitative predictions of peptide binding to any HLA-A and -B locus protein of known sequence. PLoS ONE. 2007 Aug 29;2(8):e796.	*Research*	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)
29 Oct 2007	Presentation / Adminnistrators	*EU Contact Liasons*	Denmark	75	P1 (Buus)
31 Oct 2007	Publication: Larsen MV, Lundegaard C, Lamberth K,	*Research*	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)

	Buus S, Lund O, Nielsen M. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. BMC Bioinformatics. 2007 Oct 31;8:424.				
13 Nov 2007	Presentation	*Clinicians / Scientists*	US (FHCRC, Seattle)	30	P1 (Buus)
14 Nov 2007	Presentation IEDB	*Scientists*	International	100	P1 (Buus)
28 Nov 2007	Presentation / Business proposal	*Seed Fund Investors*	Denmark	2	P1 (Buus)
27 feb 2008	Publication: Leisner C, Loeth N, Lamberth K, Justesen S, Sylvester-Hvid C, Schmidt EG, Claesson M, Buus S, Stryhn A. One-Pot, Mix-and-Read Peptide-MHC Tetramers. PLoS ONE. 2008 Feb 27;3(2):e1678.	*Research*	International	Unlimited	P1 (Buus / Stryhn)
22 Apr 2007	Presentation: Schild HJ „Non-proteasomal cleavage activities in the generation of MHC class I ligands" 3rd Charité Workshop Zeutherner See	*Research*	Germany	50	P2 (Schild)
01 Mar 2008	Publication: Schatz MM, Peters B, Akkad N, Ullrich N, Martinez AN, Carroll O, Bulik S, Rammensee HG, van Endert P, Holzhütter HG, Tenzer S, Schild H.. "Characterizing the N-terminal processing motif of MHC class I ligands." J Immunol.	*Research*	International	Unlimited	P2 (Schild/ Tenzer)

	180(5):3210-7				
2008	Publication: Tenzer S, Wee E, Burgevin A, Stewart-Jones G, Friis L, Lamberth K, Chang CH, Harndahl M, Gerstoft J, Fugger L, Jones EY, McMichael AJ, Buus S, Schild H, van Endert P, Iversen AKN. "Antigen dose shape HIV - specific CTL-response hierarchies." submitted	*Research*	International	Unlimited	P2, H. Schild/ S. Tenzer P1, S. Buus, P3, P. van Endert
2008	Publication: Nacarino Martinez A, Tenzer S, Schild H. "T-Cell Epitope Processing (The epitope flanking regions matter)" Methods in Molecular Biology, in press	*Research*	International	Unlimited	P2, H. Schild/ S. Tenzer
2008	Publication: Cardinaud S, Rohrlich PS, Tourdot S, Bouziat R, Mattei S, Février M, Weiss L, Langlade-Demoyen P, Burgevin A, Fiornetino S, van Endert P, Lemonnier FA. Design of a HIV-1 derived, HLA-B07.02-restricted polyepitope construct (submitted)	*Research*	International	Unlimited	P3, Van Endert
2008	Publication: Burgevin A, Saveanu L, Kim Y, Barilleau E, Sette A, van Endert P, Peters B. A detailed analysis of the murine TAP	*Research*	International	Unlimited	P3, Van Endert

	transporter substrate specificity (submitted)				
2008	Publication: Tenzer S, Wee E, Burgevin A, Stewart-Jones G, Lamberth K, Chang C, Harndahl M, Gerstoff J, Fugger L, Yvonne Jones E, McMichael A, Buus S, Schild H, van Endert P, Iversen AKN. Antigen processing determines HIV-specific CTL immunodominanc e. (submitted)	*Research*	International	Unlimite d	P3, Van Endert
Nov 30 2007	Newspaper Article: Personlig Vaccine. Weekend Avisen (in Danish)	*General*	Denmark	5 Million	P4. Brunak
Dec 15 2007	Publication: Lundegaard C, Lund O, Kesmir C, Brunak S, Nielsen M. Modeling the adaptive immune system: predictions and simulations. Bioinformatics. 2007 Dec 15;23(24):3265-75	*Scientific*	International	Unlimite d	P4. Brunak
2008	Publication: Claus Lundegaard, Ole Lund, and Morten Nielsen. Accurate approximation method for prediction of class I MHC affinities for peptides of length 8, 10 and 11 using prediction tools trained on 9mers. Bioinformatics. Accepted	*Scientific*	International	Unlimite d	P4. Brunak
June 2008	Claus Lundegaard,	*Scientific*	International	Unlimite d	P4. Brunak and P1 Buus

	Kasper Lamberth, Mikkel Harndahl, Søren Buus, Ole Lund and Morten Nielsen. NetMHC-3.0: Accurate web accessible predictions of Human, Mouse, and Monkey MHC class I affinities for peptides of length 8-11. Nucl. Acid Res. Accepted				
22 Mar 2007	Presentation S. Stevanovic: "Peptides of tumor antigens presented by MHC molecules" Lecture Workshop: From innate to specific immunity. Voss (Norway)	*Scientific*	Europe	200	P5, S. Stevanovic
04 Oct 2007	Presentation S. Stevanovic: „Protein antigens in tumor diagnosis and therapy" Sofia school of protein science 2007 Sofia (Bulgaria)	*Scientific*	Europe	100	P5, S. Stevanovic
26 Nov 2007	Presentation S. Stevanovic: "HLA-presented peptides in cancer immunotherapy" Seminar at IRIC Montreal (Canada)	*Scientific*	International	100	P5, S. Stevanovic
2008	Publication: Weinzierl AO, Rudolf D, Hillen N, Tenzer S, van Endert P, Schild H, Rammensee HG, Stevanovic S (2008) Features of TAP-independent MHC class I ligands revealed by quantitative mass spectrometry. Eur J Immunol (in	*Scientific*	International	unlimited	P5, S. Stevanovic

	press)				

Appendix 1 - Plan for Using and Disseminating the Knowledge

Section 1 - Exploitable Knowledge and its use

Overview table Exploitable Knowledge

As described in the last report, partners 1 and 4 generated an integrated predictor aimed at identifying CTL epitopes and thus vaccine candidates. The tech transfer offices (TTO) at the two universities applied for patent protection and actively sought interested investors. However, renewed internal evaluation of the application questioned the degree of non-obviousness. This, together with a lack of commercial interest eventually led the TTO's to abandon the application.

Section 2 - Dissemination of knowledge

Overview table - Dissemination of knowledge

Date	Type	Type of audience	Countries addressed	Size of audience	Partner responsible
12 Mar 2007	Presentation / Business proposal	Bavarian Nordic (Vaccine Company)	Denmark/Germany	10	P1 (Buus) and P4 (Brunak / Lund)
12 Apr 2007	Publication: Wang M, Lamberth K, Harndahl M, Røder G, Stryhn A, Larsen MV, Nielsen M, Lundegaard C, Tang ST, Dziegiel MH, Rosenkvist J, Pedersen AE, Buus S, Claesson MH, Lund O. CTL epitopes for influenza A including the H5N1 bird flu; genome-, pathogen-, and HLA-wide screening. Vaccine. 2007, 25(15):2823-31.	Research	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)
29 Aug 2007	Publication: Nielsen M, Lundegaard C, Blicher T, Lamberth K, Harndahl M, Justesen S, Røder G, Peters B, Sette A, Lund O, Buus S. NetMHCpan, a method for quantitative predictions of peptide binding to any HLA-A and -B locus protein of known sequence. PLoS ONE. 2007 Aug 29;2(8):e796.	Research	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)

29 Oct 2007	Presentation / Adminnistrators	EU Contact Liasons	Denmark	75	P1 (Buus)
31 Oct 2007	Publication: Larsen MV, Lundegaard C, Lamberth K, Buus S, Lund O, Nielsen M. Large-scale validation of methods for cytotoxic T-lymphocyte epitope prediction. BMC Bioinformatics. 2007 Oct 31;8:424.	Research	International	Unlimited	P1 (Buus) and P4 (Brunak/Lund)
13 Nov 2007	Presentation	Clinicians / Scientists	US (FHCRC, Seattle)	30	P1 (Buus)
14 Nov 2007	Presentation IEDB	Scientists	International	100	P1 (Buus)
28 Nov 2007	Presentation / Business proposal	Seed Fund Investors	Denmark	2	P1 (Buus)
27 feb 2008	Publication: Leisner C, Loeth N, Lamberth K, Justesen S, Sylvester-Hvid C, Schmidt EG, Claesson M, Buus S, Stryhn A. One-Pot, Mix-and-Read Peptide-MHC Tetramers. PLoS ONE. 2008 Feb 27;3(2):e1678.	Research	International	Unlimited	P1 (Buus / Stryhn)
22 Apr 2007	Presentation: Schild HJ „Non-proteasomal cleavage activities in the generation of MHC class I ligands" 3rd Charité Workshop Zeutherner See	Research	Germany	50	P2 (Schild)
01 Mar 2008	Publication: Schatz MM, Peters B, Akkad N, Ullrich N, Martinez AN, Carroll O, Bulik S, Rammensee HG, van Endert P, Holzhütter HG, Tenzer S, Schild H.. "Characterizing the N-terminal processing motif of MHC class I ligands." J Immunol. 180(5):3210-7	Research	International	Unlimited	P2 (Schild/ Tenzer)
2008	Publication: Tenzer S, Wee E, Burgevin A, Stewart-Jones G, Friis L, Lamberth K, Chang CH, Harndahl M, Gerstoft J, Fugger L, Jones EY, McMichael AJ, Buus S,	Research	International	Unlimited	P2, H. Schild/ S. Tenzer P1, S. Buus, P3, P. van Endert

	Schild H, van Endert P, Iversen AKN. "Antigen processing determines HIV-specific CTL immunodominance". (submitted)				
2008	Publication: Nacarino Martinez A, Tenzer S, Schild H. "T-Cell Epitope Processing (The epitope flanking regions matter)" Methods in Molecular Biology, in press	Research	International	Unlimited	P2, H. Schild/ S. Tenzer
2008	Publication: Cardinaud S, Rohrlich PS, Tourdot S, Bouziat R, Mattei S, Février M, Weiss L, Langlade-Demoyen P, Burgevin A, Fiornetino S, van Endert P, Lemonnier FA. Design of a HIV-1 derived, HLA-B07.02-restricted polyepitope construct (submitted)	Research	International	Unlimited	P3, Van Endert
2008	Publication: Burgevin A, Saveanu L, Kim Y, Barilleau E, Sette A, van Endert P, Peters B. A detailed analysis of the murine TAP transporter substrate specificity (submitted)	Research	International	Unlimited	P3, Van Endert
Nov 30 2007	Newspaper Article: Personlig Vaccine. Weekend Avisen (in Danish)	General	Denmark	5 Million	P4. Brunak
Dec 15 2007	Publication: Lundegaard C, Lund O, Kesmir C, Brunak S, Nielsen M. Modeling the adaptive immune system: predictions and simulations. Bioinformatics. 2007 Dec 15;23(24):3265-75	Scientific	International	Unlimited	P4. Brunak
2008	Publication: Claus Lundegaard, Ole Lund, and Morten Nielsen. Accurate approximation method for prediction of class I MHC affinities for	Scientific	International	Unlimited	P4. Brunak

	peptides of length 8, 10 and 11 using prediction tools trained on 9mers. Bioinformatics. *Accepted*				
June 2008	Claus Lundegaard, Kasper Lamberth, Mikkel Harndahl, Søren Buus, Ole Lund and Morten Nielsen. NetMHC-3.0: Accurate web accessible predictions of Human, Mouse, and Monkey MHC class I affinities for peptides of length 8-11. Nucl. Acid Res. *Accepted*	Scientific	International	Unlimited	P4. Brunak and P1 Buus
22 Mar 2007	Presentation S. Stevanovic: "Peptides of tumor antigens presented by MHC molecules" Lecture Workshop: From innate to specific immunity. Voss (Norway)	Scientific	Europe	200	P5, S. Stevanovic
04 Oct 2007	Presentation S. Stevanovic: „Protein antigens in tumor diagnosis and therapy" Sofia school of protein science 2007 Sofia (Bulgaria)	Scientific	Europe	100	P5, S. Stevanovic
26 Nov 2007	Presentation S. Stevanovic: "HLA-presented peptides in cancer immunotherapy" Seminar at IRIC Montreal (Canada)	Scientific	International	100	P5, S. Stevanovic
2008	Publication: Weinzierl AO, Rudolf D, Hillen N, Tenzer S, van Endert P, Schild H, Rammensee HG, Stevanovic S (2008) Features of TAP-independent MHC class I ligands revealed by quantitative mass spectrometry. Eur J Immunol (in press)	Scientific	International	unlimited	P5, S. Stevanovic

Final Publishable Executive Summary

The continued spread of "older" pathogen (such as TB, HIV, malaria and most recently bird flu) and the emergence of "new" pathogens (such as the recent SARS epidemic) have vividly demonstrated how vulnerable modern societies are to infectious diseases. Vaccines, which basically alert the immune system to one or more physical characteristics of a given pathogen, remain the most efficient measure to control infectious diseases.

Unfortunately, vaccine development is slow and cumbersome, and for several serious pathogens, no vaccines are available. This project emanates from the recent discovery that the immune system considers peptides (fragments of proteins) as key targets. In fact, an entire arm of the immune system – that of T cells, which essentially controls specific immune responsiveness - has been devoted to peptide recognition. It is our premises that if one could predict how the immune system handles proteins, and how it generates, selects and recognizes peptides, then one should also be able to translate genome information to immunogens, and thereby forecast immune recognition.

Although many of the mechanisms involved in antigen presentation have been described in general terms, only few have been described in sufficient details to allow for accurate prediction of their outcome. In this project, we have generated a European resource for large-scale prediction of immune epitopes, which will allow scientists and clinicians to screen whole genomes and proteomes for the presence of potential immunogenic epitopes. The ability to rapidly identify immune targets will become a platform technology enabling future rational vaccine and immunotherapy development.

Web site: www.cbs.dtu.dk/Services/

A recent survey finds that our NetMHC predictor is the best of 147 tested world-wide (BMC Immunol 2008, 9:8)

3rd executive summary

Publishable executive summary

The continued spread of "older" pathogen (such as TB, HIV, malaria and most recently bird flu) and the emergence of "new" pathogens (most prominently illustrated by the recent SARS epidemic) have vividly demonstrated how vulnerable modern societies are to infectious diseases. Vaccines, which basically alert the immune system to one or more physical characteristics of a given pathogen, remain the most efficient measure to control infectious diseases. Unfortunately, vaccine development is slow and cumbersome, and for several serious pathogens, no vaccines are available. This project emanates from the recent discovery that the immune system considers peptides (fragments of proteins) as key targets. In fact, an entire arm of the immune system – that of T cells, which essentially controls specific immune responsiveness - has been devoted to peptide recognition. It follows that if one could predict how the immune system handles proteins, and how it generates, selects and recognizes peptides, then one should also be able to translate genomes/proteomes to immunogens, and thereby forecast immune recognition. Although many of the mechanisms involved in antigen presentation have been described in general terms, only few have been described in sufficient details to allow for accurate prediction of their outcome. In this project, we have integrated cell biology with bioinformatics to create a European resource, which should allow scientists and clinicians to screen whole genomes and proteomes for the presence of potential immunogenic epitopes. The ability to rapidly identify immune targets should emerge as a platform technology enabling rational developments of vaccines and immunotherapy in the future.

This 3rd activity report describes the development of improved biochemical and bioinformatics resources enabling high-throughput epitope identification. To exemplify this, these tools have been applied to the identification of vaccine candidates relevant to the current bird flu scare as well as to HIV. In additional, the consortium have made major advances towards a peptide-based microarray system aimed at identifying peptides of interest such as immune peptide epitopes.