PROIMMUNE
www.proimmune.com

ProImmune Pro5® MHC Pentamers in Cancer Research

Increasing numbers of researchers requiring consistent tools for clinical immune monitoring for cancer are using ProImmune's Pro5® MHC Pentamer technology.

Our MHC Class I Pentamers have a high avidity for T cell receptors and so provide a vital tool in the accurate detection and separation of antigen-specific T cells for events as rare as 0.01% of total $CD8^+$ T cells. The determination of antigen-specific T cell frequencies by Pentamer staining allows in-depth evaluation of the T cell immune response.

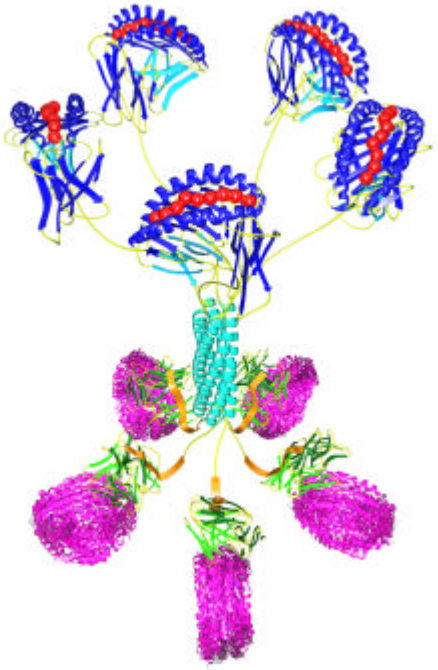
Pentamers can also be used in combination with existing technology, such as intracellular cytokine staining, to establish an accurate profile of the functionality of antigen-specific $CD8^+$ T cells, and with magnetic bead-based systems for enrichment of antigen-specific cells.

ProImmune supplies Pro5 MHC Pentamers as unlabeled or fluorescently labeled (APC or PE). All Pentamers listed are available in 50, 150 or 500 test quantities.

Researchers involved in long-running experiments benefit from the fact that our unlabeled Pentamers can be stored for up to a year and conjugated freshly for use – allowing the same batch to be used throughout a study.

ProImmune is proud to stock the widest range of cancer epitope-specific multimers of any commercial provider, in addition to our custom synthesis service (see below). This wide range is reflected in the extensive publication record of our customers.

All of our reagents are rigorously quality-checked before we send them out to you to ensure consistent and reproducible results, and as our customer you will benefit the support and guidance of our team of Ph.D – qualified Immunologists.



Catalog Pro5® MHC Pentamers for Cancer Epitopes

ALLELE	PEPTIDE SEQUENCE	EPITOPE ORIGIN
A*01:01	KSDICTDEY	Tyrosinase 243-251 (244S)
A*02:01	AILALLPAL	Prostate Stem Cell Antigen (PSCA) 105-133
A*02:01	ALLTSRLRFI	Telomerase Reverse Transcriptase (hTRT) 615-624
A*02:01	FLAEDALNTV	Epithelial Discoidin Domain Receptor 1 (EDDR1) 867-876
A*02:01	FLYDDNQRV	Topoisomerase II-alpha-b 828-836
A*02:01	GLAPPQHLIRV	p53 187-197
A*02:01	ILSLELMKL	Receptor for hyaluronic acid–mediated motility (RHAMM) 165-173
A*02:01	LTLGEFLKL	Survivin-3A 96-104
A*02:01	SLPPPGTRV	p53 149-157
A*02:01	VIMPCSWWV	Chondromodulin-I 319-327
A*02:01	YMCSFLFNL	Ewing Tumor EZH2 666-674
A*02:01	YMDGTMSQV	Tyrosinase 369-377 (371D)
A*02:01	YLQVNSLQTV	Telomerase Reverse Transcriptase (hTRT) 988-997
A*02:01	KLQDASAEV	HM1.24-aa 126-134
A*02:01	ILLWQPIPV	Prostatic Acid Phosphatase-3 (PAP-3) 135-143
A*02:01	GLMEEMSAL	Human Mena protein (overexpressed in breast cancer)
A*02:01	FVGEFFTDV	GPC3 144-152 (overexpressed in hepatocellular carcinoma)
A*02:01	YLSGANLNL	Carcinogenic Embryonic Antigen (CEA) 571-579
A*02:01	VLYRYGSFSV	gp100 (pmel17) 476-485
A*02:01	VLQELNVTV	Leukocyte Proteinase-3 (Wegener's autoantigen) 169-177
A*02:01	VISNDVCAQV	Prostate Specific Antigen-1 (PSA-1) 154-163
A*02:01	SLLMWITQV	NY-ESO-1 157-165
A*02:01	RLLQETELV	HER-2/neu 689-697
A*02:01	LIAHNQVRQV	HER-2/neu 85–94
A*02:01	LLHETDSAV	PSMA/PSM-P1 4-12
A*02:01	ALDVYNGLL	Prostatic acid phosphatase precursor (PAP) 299-307
A*02:01	YLNTVQPTCV	EGF-R 1138-1147
A*02:01	VLDGLDVLL	PRAME 100-108
A*02:01	VLAGGFFLL	PSMA 27-38
A*02:01	YLFFYRKSV	mTERT 572-580
A*02:01	FLWGPRALV	MAGEA3 271-279
A*02:01	KLFGTSGQKT	EGF-R-479 350-359
A*02:01	TLPGYPPHV	PAX-5 311-319
A*02:01	SLFLGILSV	CD20 188-196 (B cell malignancies)
A*02:01	RLVDDFLLV	Telomerase Reverse Transcriptase 865-873
A*02:01	LLLLTVLTV	MUC-1 12-20
A*02:01	LLGRNSFEV	p53 264-272
A*02:01	ALFDIESKV	PSM P2 (prostate)
A*02:01	FLGYLILGV	Prostatic Acid Phosphatase-3 (PAP-3)

ALLELE	PEPTIDE SEQUENCE	EPITOPE ORIGIN
A*02:01	TMNGSKSPV	hMena 502-510
A*02:01	LMLGEFLKL	Survivin 96-104
A*02:01	QLCPICRAPV	Livin/ML-IAP280 175-184
A*02:01	SLSEKTVLL	CD59 glycoprotein precursor 106-114
A*02:01	SLVDVMPWL	Cytochrome p450 1B1 239-248
A*02:01	KIFGSLAFL	HER-2/neu 369-377
A*02:01	IMDQVPFSV	gp100 (pmel17) 209-217
A*02:01	ILHNGAYSL	HER-2/neu 435-443
A*02:01	ILAKFLHWL	Telomerase 540-548
A*02:01	HLSTAFARV	G250 (renal cell carcinoma) 217-225
A*02:01	GVLVGVALI	Carcinogenic Embryonic Antigen (CEA) 694-702
A*02:01	ALYVDSLFFL	PRAME PRA 300–309
A*02:01	KLQCVDLHV	Prostate Specific Antigen 146-154
A*02:01	ALQPGTALL	Prostate Stem Cell Antigen (PSCA) 14-22
A*02:01	KVAELVHFL	MAGEA3 112-120
A*02:01	KTWGQYWQV	gp100 (pmel17) 154-162
A*02:01	GLYDGMEHL	MAGEA-10 254-262
A*02:01	FLTPKKLQCV	Prostate Specific Antigen-1 (PSA-1) 141-150
A*02:01	ELAGIGILTV	MelanA / MART 26-35
A*02:01	KVLEYVIKV	MAGEA1 278-286
A*03:01	ALLAVGATK	gp100 (pmel17) 17-25
A*03:01	ATGFKQSSK	bcr-abl 210 kD fusion protein 259-269
A*03:01	KQSSKALQR	bcr-abl 210 kD fusion protein 21-29
A*24:02	EYLQLVFGI	MAGEA2 156-164
A*24:02	TFPDLESEF	MAGEA3 97-105
A*24:02	TYACFVSNL	Carcinogenic Embryonic Antigen (CEA) 652-660
A*24:02	TYLPTNASL	HER-2/neu 63-71
A*24:02	VYGFVRACL	Telomerase reverse transcriptase (hTRT) 461-469
A*24:02	AFLPWHRLF	Tyrosinase 188-196
A*24:02	CYASGWGSI	Prostate Specific Antigen-1 153-161
A*24:02	VYFFLPDHL	gp100-intron 4 170-178
B*08:01	GFKQSSKAL	bcr-abl 210 kD fusion protein 19-27
H-2 Db	ATFKNWPFL	Murine Survivin 20-28
H-2Db	Abu-Abu-L-Abu-LTVFL	Moloney murine sarcoma virus (MoMSV) GagL 85–93
H-2Db	FSNSTNDILI	VEGFR2/KDR fragment 1 614-624
H-2Db	KVPRNQDWL	gp100 (pmel17) 25-33
A*02:01	TMNGSKSPV	hMena 502-511
A*02:01	LMLGEFLKL	Survivin 96-105
A*02:01	QLCPICRAPV	Livin/ML-IAP280 175-185

PROIMMUNE
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Custom Pro5® MHC Class I Pentamers

ProImmune has over 10 years experience making custom MHC multimers specific to customers' requirements. Our technical expertise and experience in making thousands of MHC-peptide complexes means that we are able to provide custom reagents efficiently and with a high rate of success.

Our customers can expect the same consistency and reliability from our custom reagents as our regularly ordered catalog Pentamers. In addition, all labeled custom Pro5 Pentamers come with a six month performance guarantee, and unlabeled custom Pentamers have a twelve month guarantee.

MHC Alleles Available for Custom Synthesis			
Human HLA		Mouse H-2	Simian
A*01:01	B*07:02	Db	Mamu-A*01
A*02:01	B*08:01	Dd	Mamu-A*02
A*03:01	B*14:02	Kb	
A*11:01	B*15:01	Kd	
A*11:03	B*27:05	Ld	
A*24:02	B*35:01		
A*29:02	B*35:08		
A*68:01	B*40:01		
	B*54:01		
Other: Chimeric A*02:01/Kb			

ProImmune will only proceed with the synthesis of a custom Pro5 Pentamer if we are confident that the peptide will bind sufficiently to the relevant MHC allele. Usually synthesis will proceed if the affinity score is 21 or higher using the SYFPEITHI prediction algorithm, www.syfpeithi.com.

Lower scoring peptides will be considered if sufficient supporting evidence is available for binding.

Custom Pro5 Pentamers are dispatched 4-6 weeks from receipt of order.

Testimonials and case studies from Pro5® Pentamer users working in cancer research

Dr.Cath Bollard
Baylor college of Medicine, Houston, USA

"Pro5 MHC Pentamers have played an important part in our study on adoptive immunotherapy for cancer and viral infections, post-transplant. We use Pentamers to detect and quantify the populations of antigen-specific T cells in CTL lines made for clinical use. We also use them to detect antigen-specific CTL in the peripheral blood of transplant patients, pre and post-CTL-infusion. The Pentamers were chosen for their stability and for the increased intensity and specificity of the positive population compared to MHC tetramers. We have found Pentamers to be consistent and reliable, and ProImmune's customer service is excellent".

Dr. John Webb
British Columbia Cancer Agency, Canada

“Over the past six years I have been using ProImmune’s Pro5 Pentamers for the identification and characterization of tumour epitope-specific T cells. Pentamers play an important role in my work to develop new vaccine strategies to induce T cell-mediated immune responses directed against human malignancies. I am always impressed by the knowledge and friendliness of the customer service team, and find the website very user-friendly and well-organized."

Case Study:
Pro5® Pentamers identify allo-restricted T cells in study to advance research into cancer immunotherapy

Stronen *et al*. (2009). Dendritic cells engineered to express defined allo-HLA peptide complexes induce antigen-specific cytotoxic T cells efficiently killing tumor cells. Scand J Immunol. 69: 319-328

Many tumor-associated antigens (TAA) are derived from self proteins that are expressed at low levels in normal tissues. Cancer patients therefore do not produce a cytotoxic T lymphocyte (CTL) response to tumors over-expressing these TAA as they are recognized as self proteins. If CTL can be generated for TAA that are presented by foreign MHC molecules and transferred to the cancer patient, self-tolerance could be avoided. These allo-restricted $CD8^+$ T cells could be used as an immunotherapy to kill the patient cancer.

Stronen *et al.* investigated the use of dendritic cells (DC) as antigen-presenting cells to generate highly specific, functional allo-restricted T cells. Monocyte derived dendritic cells from HLA-A*02:01 negative individuals were transfected with A*02:01 and loaded with MART-1 (ELAGIGILTV) peptide. The transfected DC were co-cultured with monocyte-depleted PBMCs from the A*0201 negative donor and reactive T cells identified using a Pro5 A*02:01/MART-1 Pentamer. Pentamer-positive cells were sorted by FACS or magnetic bead isolation and then expanded *in vitro* (figure 1). These MART-1 Pentamer positive T cells effectively killed A*02:01 melanoma tumor cell lines indicating that the sorted and expanded cells remain peptide specific.

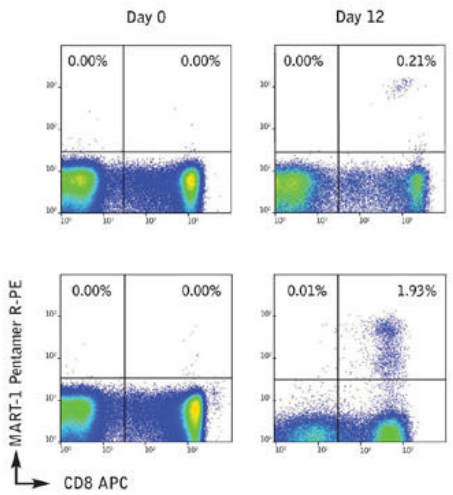


Figure 1:

Induction of MART-1-positive cytotoxic T lymphocytes (CTL) from human leucocyte antigen (HLA)-A2-negative donors. Data plots are representative of PBMC from HLA-A*02:01 donors showing anti-CD8 and A2/MART-1 Pentamer immediately after isolation (left) or after 12 days of co-culture with MART-1 peptide-pulsed A2-monocyte-derived dendritic cells (right).

The validity of this novel methodology was confirmed by generating cell lines for peptides from 2 other leukemia associated self-antigens for CD33 and CD19. Peptides were selected using binding algorithms to predict potential epitopes. Custom Pro5 Pentamers were synthesized for both complexes (A*02:01/CD33 (9-17) LLWAGALAM and A*02:01/CD19 (279-287) VLWHWLLRT). Pentamer-positive cells could be detected *ex vivo* after 19 days in culture and these cells were successfully expanded whilst retaining peptide specificity against peptide pulsed A2 transfected EBV-LCLs.

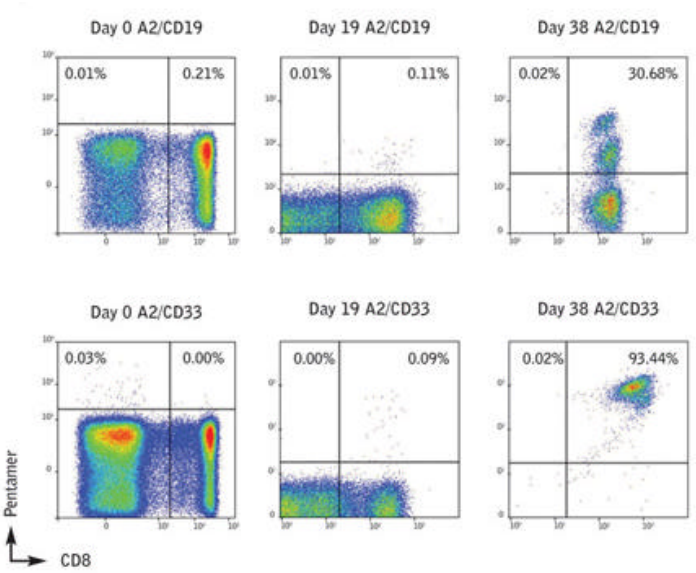


Figure 2:

Allo-restricted T cells specific for CD19 (279-287) and CD33 (9-17). Data plots are representative of PBMC from HLA-A*02:01 donors showing anti-CD8 and A2/CD19 or A2/CD33 Pentamer immediately after isolation (left), after 19 days (centre) or 38 days (right) of co-culture with specific peptide-pulsed A2-monocyte-derived dendritic cells.

These data show that it is possible to isolate allo-restricted, antigen-specific T cells and expand these cells to high numbers whilst still retaining functional specificity. The use of both catalogue and custom Pro5 Pentamers to identify and confirm specificity of the allo-restricted T cells was essential in this study.

Case Study:
ProVE® MHC Pentamers used for validation of novel T cell epitopes in colorectal cancer patients vaccinated with TroVax®, a novel cancer vaccine

Harrop *et al*. (2008). Vaccination of colorectal cancer patients with TroVax given alongside chemotherapy (5-fluorouracil, leukovorin and irinotecan) is safe and induces potent immune responses.
Cancer Immunology and Immunotherapy.(2008) 57: 977-86.

Scientists at Oxford BioMedica have used ProImmune's PEPscreen® peptides and ProVE MHC Pentamers to detect novel T cell epitopes in patient samples following TroVax administration. Using ProVE Pentamers they confirmed the MHC restriction of the T cell epitopes identified and demonstrated that the T cells were truly antigen-specific.

TroVax consists of a recombinant vaccinia virus (MVA) encoding the tumour-associated antigen 5T4, which is rarely detected on normal tissues but is expressed at high levels on a broad range of solid tumours. The presence of the 5T4 antigen correlates with poor prognosis.

In Oxford BioMedica's Phase I/II trial for colorectal cancer, 94% of patients responded to the TroVax antigen. A positive correlation was found between the magnitude of the immune response and time to disease progression. Based on this information, it was of great importance for Oxford BioMedica to analyze the 5T4-specific T cell response induced by TroVax in further detail. In particular, researchers wanted to understand the magnitude, specificity and phenotype of these immune responses.

Two subsequent Phase II studies in metastatic colorectal cancer reiterated the high frequency of responses observed in the earlier Phase I/II. In the immunological analysis of these studies, Oxford BioMedica used a ProImmune PEPscreen 9mer peptide library in IFN-gamma ELISPOT assays to begin the validation process for 5T4 epitopes implicated in earlier MHC-peptide binding studies. This identified patients who showed no detectable 5T4-specific cellular responses prior to treatment, but who mounted very strong responses following TroVax vaccination (Figure 1). However, the IFN-gamma ELISPOT assay indicates the frequency of cells that secrete IFN-gamma following peptide stimulation, but does not reveal their phenotype.

In order to confirm the nature of the immune responses in patients that had received TroVax, Oxford BioMedica wished to identify the presence of increased antigen-specific T cell populations in these patients. They turned again to ProImmune for the rapid synthesis of a ProVE MHC Pentamer Library. ProVE Pentamers can be generated quickly and affordably for the detection of single antigen-specific T cells in flow cytometry and enable the conclusive validation of new T cell epitopes.

ProImmune supplied ten 5T4-specific ProVE MHC Pentamers that were used to validate the responses detected by IFN-gamma ELISPOT and to confirm the MHC restriction of the 5T4 T cell epitopes (Figure 2). The precursor frequencies detected using the ProVE MHC Pentamers were approximately 2-fold greater than those detected using the IFN-gamma ELISPOT assay against the same peptide antigen, demonstrating a good correlation between these two assays.

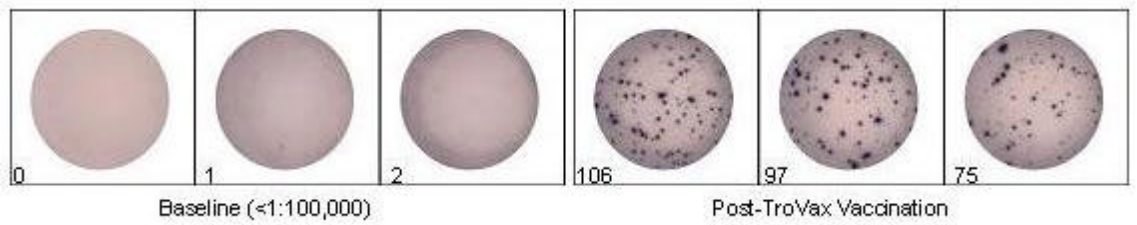


Figure 3: Patients received a total of 6 intramuscular TroVax vaccinations: 2 before, 2 during and 2 after chemotherapy. Blood samples were taken prior to the initial vaccination and 2 weeks after completion of chemotherapy. A 96-well culture plate was coated with an anti-IFN-gamma antibody (1-D1K). 200,000 PBMCs were plated per well and incubated overnight at 37°C with 5µg/ml peptide. Cells were removed and the plate washed prior to addition of a biotinylated anti-IFN-gamma detection antibody (7-B6-1). Upon addition of streptavidin-ALP, followed by a precipitating substrate for ALP, spots developed and were counted. The figure shows results for a single patient. Prior to treatment, an average of one spot could be detected per 200,000 cells. However, following a combination of chemotherapy and TroVax treatment, an average of 93 spots per 200,000 could be detected, indicating that a strong response to 5T4-specific peptide had been mounted.

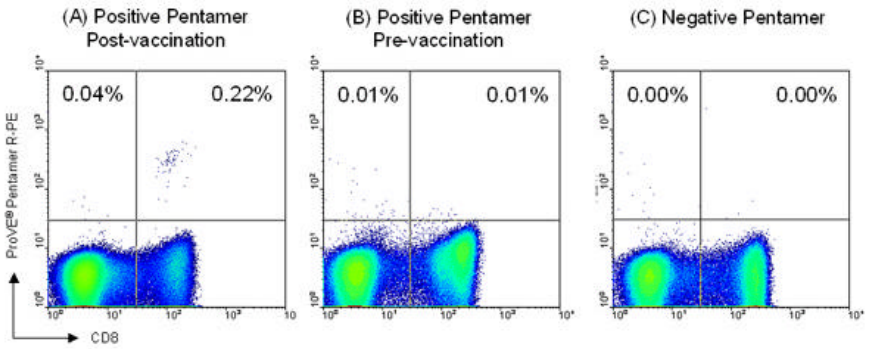


Figure 4: Patient blood samples were taken prior to the initial vaccination and 2 weeks after completion of chemotherapy. For flow cytometry staining, 2 x 10^6 PBMC were incubated with 1 test (0.5µg) ProVE Pentamer for 10 minutes at room temperature, followed by 1 test R-PE-labeled Pro5® Fluorotag and 1 test FITC-labeled anti-CD8 antibody (clone RPA-T8) for 20 minutes at 4ºC. Samples were analyzed by flow cytometry and 500,000 live events collected. Results are shown for the same patient as in figure 3. A clear population of 5T4-specific $CD8^+$ T cells was detected in the sample taken after completion of chemotherapy using an A*02:01-restricted ProVE Pentamer (C: 0.22% of live gate). Such antigen-specific cells were not present prior to vaccination with TroVax (B). No antigen-specific cells were detected at either time-point when an A*01:01-restricted ProVE Pentamer (negative Pentamer) was used for staining (A).

Administration of TroVax vaccine clearly elicits potent cellular immune responses in the patient studied, demonstrated by the expansion of antigen-specific T lymphocytes that recognize specific epitopes of 5T4. ProVE MHC Pentamers provide a powerful means to elucidate a detailed profile of cellular immune responses in patients undergoing immunotherapy. As this study shows, CTL responses to single epitopes can be determined clearly, confirming the applicability of ProVE MHC Pentamers in the clinical development of new immunother

Case Study:
Epitope Discovery in Prostate Cancer: The Use of H-2Db/HCIRNKSVI (PSA 65-73) Custom Pro5® MHC Class I Pentamer in the Characterization of an Immunodominant CTL Epitope of PSA in Mice

Pavlenko, M. *et al.* (2005) Identification of an immunodominant H-2Db-restricted CTL epitope of human PSA. Prostate 64: 50-59

Prostate cancer is a serious condition affecting 1 in 6 men. Prostate specific antigen (PSA) expression is increased in prostate cancer and so is exploited not only for diagnosing and monitoring prostate cancer, but also as a potential target for immunotherapy. By working with Custom Pro5 Pentamers from ProImmune, Pavlenko *et al.* have identified and validated an immunodominant cytotoxic T lymphocyte (CTL) epitope of PSA in C57BL/6 mice. A combined bioinformatics approach using the SYFPEITHI website (www.syfpeithi.com), and biochemical MHC-peptide stabilization assays was used to define the candidate epitope (H-2Db/HCIRNKSVI) and a custom Pro5 Pentamer was synthesized.

PSA-specific CTLs were induced by immunizing mice with a plasmid expressing PSA (pVax-PSA). By using both functional assays, intracellular cytokine s taining and detection of PSA-specific $CD8^+$ T cells with Pro5 Pentamer staining, the authors were able to demonstrate correlating frequencies of both IFN-? positive and Pentamer positive T cells following *in vitro* stimulation with the specific peptide.

The authors conclude that “Pentamer technology enables detection of T cell receptor specific T-cell populations and allows, in combination with functional assays, the discrimination between anergy and tolerance induction during effector responses. H-2Db Pentamers assembled with this peptide are an efficient tool for the monitoring of PSA-specific CTL responses after DNA vaccination”.

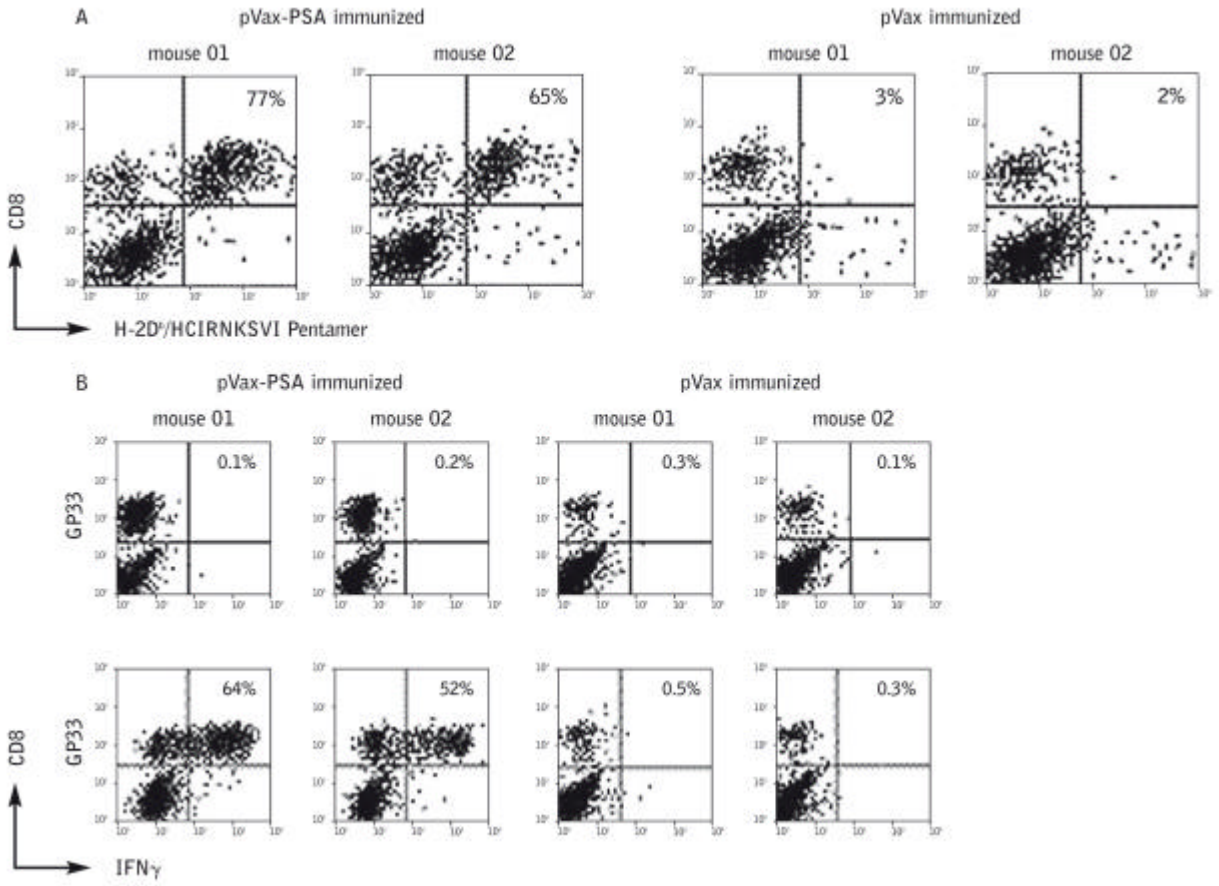


Figure 5: 12 days after immunization as described, the splenocytes were restimulated for 5 days *in vitro* with 1nM of psa65-73 (HCIRNKSVI) peptide (A). The PSA-specific T cells were detected by staining with the H-2Db/HCIRNKSVI Pentamer (B). The splenocytes were stimulated for 5hr with 10nM of the psa65-73 or GP33 (negative control) peptides and stained with the antibodies against CD8 and IFN-gamma.

Case Study:
ProImmune's Pro5® MHC Pentamers are used to demonstrate that allogeneic T cells may be targeted to B cell leukemia

Abrahamsen, *et al.*, (2010) Targeting B cell leukemia with highly specific allogenieic T cells with a public recognition motif. Leukemia 2010 Nov;24(11):1901-9.

The intelligent design of immunotherapy for B cell leukemia represents a significant challenge. Any potentially cancer–reactive T cells will have been deleted from the repertoire of the patient during maturation of their immune system, as the B cell antigens borne by the leukemia cells are by their nature self antigens.

However, T cell receptors are inherently reactive towards MHC. Building on this, Abrahamsen *et al.* investigated whether foreign HLA molecules bearing self B cell peptide antigens could provoke a response from patient-derived T cells against their own B cell antigens, and so potentially eliminate cancerous B cells. It has been suggested that such alloreactive T cells will respond to a wide repertoire of peptides, so the team first sought to address the important question of peptide specificity: would these T cells respond to any peptide in the context of foreign MHC, or be sufficiently restricted to a single B cell antigen to be of potential therapeutic value?

T cells reactive with a peptide derived from the B cell antigen CD20 (SLFLGILSV) in the context of HLA-A*02:01 could be readily obtained from HLA-A*02:01-negative donors. Abrahamsen *et al.* used their established method (Stronen *et al.*, Scand J Immunol. 2009, 69:319-28) of co-culturing T cells from A*02:01-negative donors with A*02:01-positive CD20 peptide –pulsed antigen presenting cells to successfully generate A*02:01/CD20-specific T cell lines. A custom ProImmune Pro5 A*02:01/ SLFLGILSV MHC class I Pentamer was used to identify the antigen-specific T cell population.

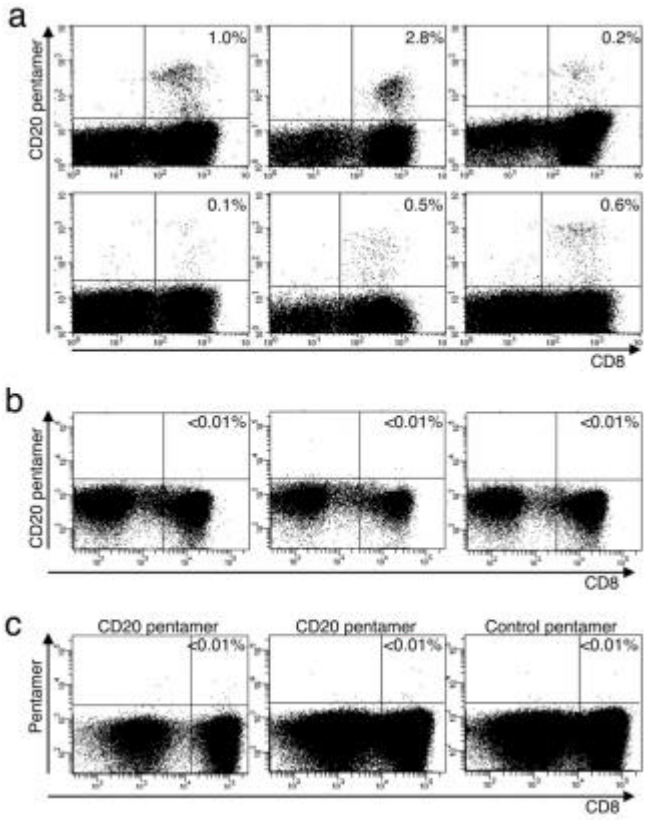


Figure 5: Representative flow cytometry analysis plots to show (a) A*02:01/SLFLGILSV Pro5 Pentamer-reactive cells from A*02:01-negative donors on day19 after the start of co-culture with A*02:01-transfected, SLFLGILSV peptide-pulsed dendritic cells. (b) A*02:01/SLFLGILSV Pentamer staining of freshly-isolated PBMC from the same donor and (c) A*02:01-positive donor PBMC subject to the same treatment as those in (a).

The same method used with A*02:01-positive donors failed to expand a similar population of antigen-specific T cells (Figure 5c), confirming that A*02:01-positive individuals are tolerant of this self antigen.

To demonstrate the specificity of their antigen-reactive T cells, Abrahamsen *et al.* used a library of 42 A*02:01 Pentamers, the majority of which were ProImmune ProVE Pentamers, each displaying an irrelevant peptide with homology to SLFLGILSV. The two cell lines tested stained positively with the A*02:01/SLFLGILSV Pentamer, but negatively for the irrelevant peptides, confirming their antigen specificity. In order to assess the fine specificity of their T cell lines, the team took a scanning alanine substitution approach, using a panel of A*02:01 multimers. The motif of four central amino acids from the SLFLGILSV epitope were found to be most critical for binding, since binding of the cells to Pentamer was most reduced when these residues were substituted for alanine. Different isolated T cell line clones showed variable dependency on other residues, but all relied strongly on the core four amino acids for multimer binding.

As the most stringent test of specificity, Abrahamsen *et al.* next measured the reactivity of their T cell lines towards cells co-expressing A*02:01 and CD20. The team used various experimental scenarios to show that their cells were indeed selectively antigen reactive. As an example, the T cell lines were reactive to HEK 293 cells only when the HEK 293 cells were cotransfected with A*02:01 and CD20, and crucially not with A*02:01 alone or with A*02:01 and irrelevant control antigen. This evidence indicated that the CTL lines were recognizing naturally processed and presented CD20 antigen.

In a final proof of principle, the CD20-reactive T cells were tested for their ability to kill cells from the peripheral blood of chronic lymphoid leukemia (CLL) patients. Encouragingly, robust levels of specific lysis were observed with A*02:01-positive target cells, but A*02:01-negative CLL cells were spared.

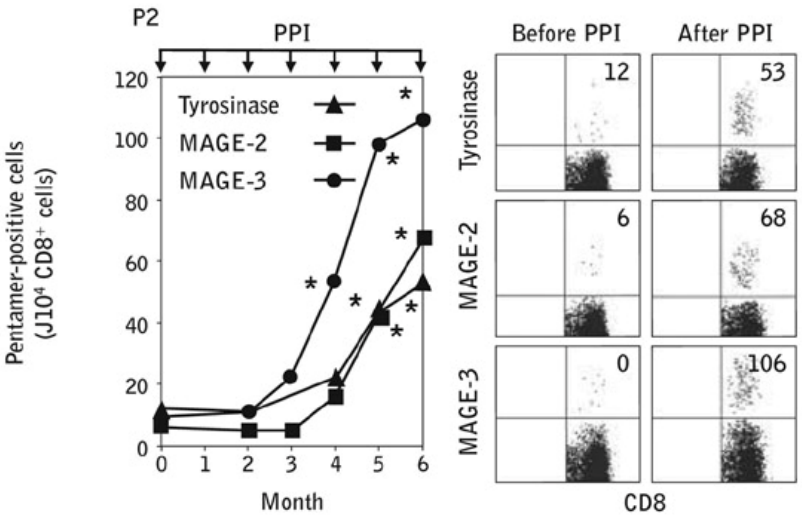
The high functional avidity of the CTLs isolated and characterized by Abrahamsen *et al.* using ProImmune MHC Pentamer technology is truly encouraging. The use of alloreactive T cells to induce a target-specific graft-versus-

leukemia response without simultaneously inducing graft-versus-host disease marks a promising start for the future of cancer immunotherapy.

This work was carried out in the Olweus Lab, Oslo University Hospital www.ous-research.no/olweus

Case Study:
Pro5® Pentamers integral in successful research into novel immunization strategy

Yagi *et al.* (2006). Induction of Therapeutically Relevant Cytotoxic T Lymphocytes in Humans by Percutaneous Peptide Immunization. Cancer Research 66 p10136-10144



In this study by Yagi *et al.* a novel percutaneous immunization strategy was used to deliver a peptide vaccine to melanoma patients. This method of percutaneous peptide immunization (PPI) used epidermal Langerhans cells as antigen presenting cells to deliver the vaccine effectively to the immune system. The outer layer of the epidermis was removed in order to increase the permeability of the skin to the peptide vaccine and allowed the maturation of the Langerhans cells for enhanced antigen presentation.Pro5 MHC Pentamers for MAGE-2 (A*24:02 / EYLQLVFGI), and tyrosinase (A*24:02 / AFLPWHRLF), and a custom Pro5 MHC Pentamer for MAGE-3 (A*24:02 / IMPKAGLLI), were used to monitor frequencies of antigen-specific T cells in patients after immunization. After several rounds of immunization, staining with Pro5 MHC Pentamers indicated that antigen specific responses increased over the course of 6 months in both melanoma patients and healthy controls.

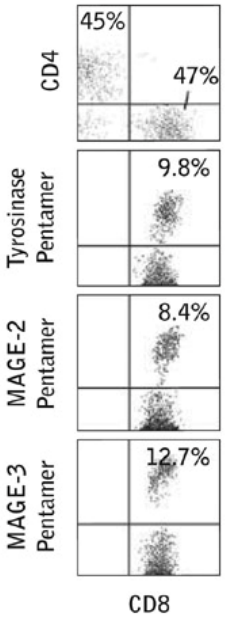


Figure 6: Induction of Pentamer-positive CD8+ T cells. PBMCs obtained 7 days after each PPI were immunophenotyped with Pentamers for tyrosinase, MAGE-2, and MAGE-3 and analyzed by flow cytometry.

Further analysis of tumor infiltrating leukocytes in one of the melanoma patients showed that the MAGE-2, MAGE-3 and tyrosinase specific $CD8^+$ cells were present in these samples.

Figure 7: *In vivo* infiltration of CTLs into melanoma lesions induced by PPI. Flow cytometric profiles of cell suspensions obtained from a regressing s.c. nodule in patient 2 during PPI, showing the percentage of CD4+ and CD8+ cells among CD45+ cell-gated leukocytes and the percentage of Pentamer+ cells among the CD8+ CD45+ cell-gated populations.

The presence of these Pentamer-positive cytotoxic T cells coincided with a decrease in tumor size and progression indicating that the percutaneous peptide vaccine delivered to barrier-disrupted skin was effective, as well as being safe and non-invasive.

Case Study:
New adjuvant drives unprecedented cytotoxic T cell response providing a potent vaccine development platform

Wells *et al*. (2008) Combined Triggering of Dendritic Cell Receptors Results in Synergistic Activation and Potent Cytotoxic Immunity J. Immunology 181: 3422-3431

Wells *et al*. investigated novel combinations of vaccine adjuvants and using Pro5 MHC Class I Pentamers, showed that optimal antigen-specific responses can be achieved using well-tolerated compounds that result in dendritic cell activation through the activation of toll-like receptors.

Initially using the ovalbumin H-2Kb/SIINFEKL epitope as a model, Pentamer staining indicated that a vaccine adjuvant referred to as a 'combined adjuvant for synergistic activation of cellular immunity' (CASAC) provided the greatest antigen-specific response as indicated by H-2Kb/SIINFEKL Pentamer+/CD8+ T cell staining. The vaccination also induced a strong memory response upon re-injection of SIINFEKL peptide as measured by Pentamer staining. The CASAC adjuvant contained several key components including two toll-like receptor agonists (e.g. CpG DNA+monophosphoryl lipid A), IFN-gamma and CD40 antibody or a class II MHC peptide to induce IL-12 production from dendritic cells. This was combined with SIINFEKL peptide in an emulsion.

The efficacy of the CASAC adjuvant was further tested on a mouse melanoma model using the TRP-2 tumour epitope (H-2Kb/SVYDFFVWL), which is known to bind with a low affinity to the H-2Kb allele. Mice were injected with B16 melanoma cells and then immunized with TRP-2 peptide suspended in either CASAC or the most potent adjuvant combination previously described (anti-CD40 and a single TLR agonist). Pentamer-binding CD8+ cells were only detected in mice that had received the CASAC adjuvant. Protection from tumours was maintained after further challenge with B16 melanoma cells.

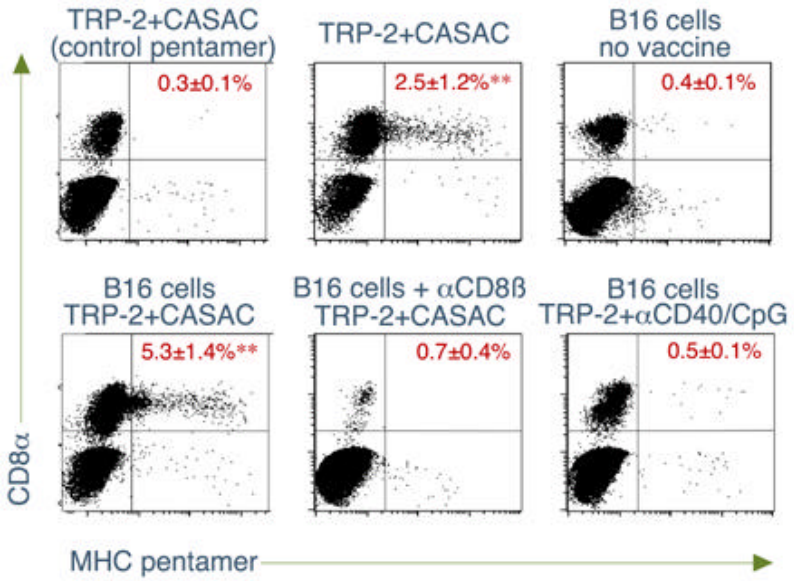


Figure 8.

CASC induces potent tumor protection in a melanoma treatment model mediated partly but not entirely by tumor-Ag specific T cells. The figure shows staining of TRP-2 specific CD8 T cells in blood of mice using TRP-2(180-188) H-2Kb Pentamer. SIINFEKL Pentamer was used for the negative control stain. Mean percentage ± SEM from 6-8 mice is shown.

**p<0.005 as determined by unpaired Student's t tests on the mean values.

The use of Pro5 MHC Pentamers to measure antigen-specific T cell responses accurately enabled the investigators to demonstrate that a combinatorial adjuvant approach may provide more effective protection than current anti-cancer vaccine strategies.

Case Study:
ProImmune's Pro5® MHC Pentamers are used to investigate immunotherapy prospects for high-grade ovarian cancer

Webb, *et al.*, (2010) Profound elevation of CD8+ T cells expressing the intraepithelial lymphocyte marker CD103 (aE/ß7 Integrin) in high-grade serous ovarian cancer Gynecological Oncology 2010 Sep;118(3):228-36.

Tumor-infiltrating CD8+ T cells are strongly associated with increased progression-free survival in high-grade serious epithelial ovarian cancer (EOC). Characterizing these T cells is an essential first step in the design of an effective immunotherapy for the disease.

With this in mind, Dr. John Webb and his colleagues at the British Columbia Cancer Agency in Vancouver, Canada, analyzed the infiltrating T cells in tumour ascites from 13 high-grade EOC patients. They were particularly interested in monitoring the cell-surface expression of the integrin CD103, as its expression has previously been found on T cells infiltrating various epithelial tumors. They used a tumor epitope-specific Pro5 MHC Class I Pentamer as part of their work, which provides the first description of CD103+ T cells in any ovarian cancer.

The team found a range of CD103 expression on CD8+ lymphocytes (CTL) from tumor ascites: some patient samples had CD103 expression on fewer than 10% of their CTL, while others had over 70% CD103+ CTL.

In the samples tested, CD103 levels did not correlate with expression of its ligand E cadherin in the tumor microenvironment, but a significant correlation between levels of the cytokine TGFß and CD103 expression on CTL was apparent. This confirmed previous work demonstrating that CTL will up-regulate CD103 in response to the presence of TGFß.

Next, the phenotype of the tumor-infiltrating CD103-expressing CTL was analyzed, as other studies have shown that CD103 expression is required for their anti-tumor activity. The cells carry a surface marker expression pattern consistent with being antigen-experienced, conforming to an effector-memory phenotype. Notably, CD103+ CTL constitutively produced IL-10, suggesting they may act in an immuno-regulatory capacity.

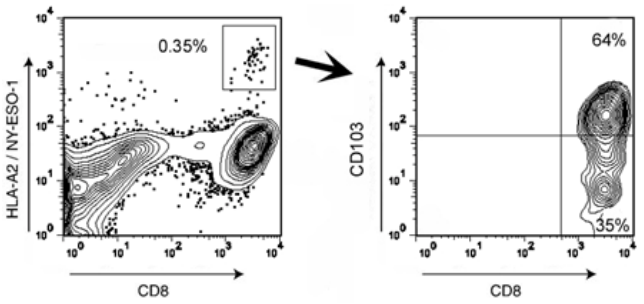


Figure 9: CD8+ T cells specific for the tumor antigen NY-ESO-1 are predominantly CD103+. Flow cytometry analysis of T cells derived from the malignant ascites of an HLA-A2+ EOC patient (IROC013) who demonstrated reactivity to an HLA-A2-restricted epitope of NY-ESO-1. The analysis shows the frequency of CD8+ lymphocytes staining positive with an A*02:01/SLLMWITQV(NY-ESO-1157–165) Pro5 Pentamer. Pentamer-positive CD8+ T cells were further characterized for CD103 expression (right panel). All events were first gated on total lymphocyte populations by forward and side scatter.

The CD103+ CTL that Webb *et al* describe in this study appear to be naturally arising, tumor-specific lymphocytes. Further work on these cells in ovarian cancer, and on characterization of similar infiltrating lymphocytes in other tumour types, will be invaluable for development of adoptive immunotherapy. ProImmune Pro5 Pentamers were crucial for this work as they made the highly refined analysis of epitope-specific T cells possible.

This work was carried out in the Deeley Research Centre at the British Columbia Cancer Research Centre www.bccrc.ca/dept/drc

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