Dissertation

Profiling of Neuroblastoma cell epitopes as target presentation on corresponding dendritic cells

eingereicht von

Mag. rer. nat. Christoph Ludwig Jandl

Mat.Nr.: 9510677

zur Erlangung des akademischen Grades

Doctor scientiae medicae

(Dr. scient. med.)

an der

Medizinischen Universität Graz

ausgeführt an der

Klinischen Abteilung für Pädiatrische Hämatologie Onkologie

unter der Leitung von

Prof. Dr. Wolfgang Schwinger

Graz, Mai 2010

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwende habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 31.05.2010

In der Wissenschaft gleichen wir alle nur den Kindern, die am Rande des Wissens hie und da einen Kiesel aufheben, während sich der weite Ozean des Unbekannten vor unseren Augen erstreckt.
(Sir Isaac Newton)

Danksagung

Zuallererst möchte ich mich recht herzlich bei **Univ.-Prof. Dr. Wolfgang Schwinger** und bei **PD. DI. Dr. Ruth Birner – Grünberger** bedanken. Ihrer intensiven Betreuung verdanke ich viele neue Erkenntnisse im wissenschaftlichen Bereich.

Bei der Steirischen Kinderkrebshilfe möchte ich mich herzlichst für die finanzielle Unterstützung bedanken, ohne die diese Arbeit nicht möglich gewesen wäre.

Für seine wertvolle Unterstützung und die Erlaubnis zur Benutzung der Räumlichkeiten und Techniken möchte ich mich bei Univ.-Prof. Dr. Christian Urban bedanken.

Ein ganz besonders lieber Dank gilt meiner Ehefrau **Kerstin**, die mich bei dieser Arbeit tatkräftig unterstützte und mit viel Geduld mir immer liebevoll zur Seite stand. Auch haben meine **Kinder Laurin, Niels und Olivia** auf ihre Art zum Gelingen dieser Arbeit beigetragen.

Meinen Eltern und Großeltern möchte ich dafür danken, dass sie mich bei der Verwirklichung meines wissenschaftlichen Werdeganges mit allen Kräften unterstützt haben.

An alle MitarbeiterInnen im Labor für die hilfreiche Zusammenarbeit, die wertvollen Tipps und Ratschläge und die angenehme Atmosphäre.

Zahlreiche weitere Personen haben zum Gelingen dieser Arbeit beigetragen. Nicht nur bei der Durchsicht und Korrektur der Arbeit, sondern auch durch ihre Unterstützung und Freundschaft. Ihnen allen gilt mein herzlichster Dank.

Zusammenfassung

Das Neuroblastom ist der häufigste extrakranielle Tumor im Kindesalter welcher seinen Ursprung in den sympathischen Neuralleistenzellen hat. Die Amplifikation des N – myc Onkogens ist ein wichtiger prognostischer Indikator für einen schweren Krankheitsverlauf. Auf Grund signifikanter Toxizität erlauben derzeitige Chemotherapien keine Intensivierung der Behandlung mehr. Eine gangbare Lösung wäre die Immuntherapie, welche spezifischer und weniger toxisch zu sein verspricht. Da bisherige Versuche eine immungerichtete Antwort gegen Neuroblastomzellen zu etablieren wenig Erfolg zeigten, sollte in der vorliegenden Arbeit untersucht werden, ob die Verarbeitung von Neuroblastomgewebe zu einer tumorspezifischen Epitop-Präsentation durch die MHC Klasse I Moleküle dendritischer Zellen führt.

Zu diesem Zweck, wurden unreife dendritische Zellen mit Neuroblastom Zell - Lysaten, aus der N-myc positiven Neuroblastom-Zelllinie Kelly und der N-myc negativen SK-N-AS, beladen. Weiteres sollte geklärt werden, ob ein spezifisches Muster der Antigenpräsentation zwischen N-myc positiven und N-myc negativen Neuroblastom – Zelllinien existiert. Verschiedene Peptide wurden von der Oberfläche der behandelten dendritischen Zellen eluiert und durch Massenspektroskopie nachgewiesen. Mit der Unterstützung von Computer-Programmen (SYFPEITHI , SVMHC und NetMHCIIpan) die es erlauben die Bindungswahrscheinlichkeit zwischen MHC Molekülen und den identifizierten Peptiden vorhersagen zu können, wurden die relevanten Peptide ermittelt. Voraussetzung dafür waren eine Länge von 8-11 Aminosäuren bei einer Bindung mit dem MHC-I Komplex und 14-18 Aminosäuren bei MHC-II Komplex bei gleichzeitig hoher Bindungswahrscheinlichkeit zwischen dem Peptid und dem MHC Komplex. Von jenen Peptiden welche ausschließlich an der Oberfläche von Tumor-Lysat behandelten Zellen gefunden wurden waren 7 MHC Klasse I und 23 MHC-Klasse II Peptide identifizierbar. Nur eines der 30 Peptide wurde in 2 biologischen Replikaten gefunden. Die fehlende Reproduzierbarkeit der Ergebnisse weist auf eine mögliche Heterogenität des Präsentationssystems hin. Zur Kontrolle wurden unreife dendritische Zellen mit Lysat aus weißen Blutzellen oder Phosphat gepufferter Kochsalzlösung (PBS) behandelt.

Um einen Bezug zu neuronalem Gewebe, und damit eine mögliche Zugehörigkeit der Peptide zum verwendeten Tumormaterial herstellen zu können, wurden die dazugehörigen Proteine untersucht. Die „Human NCBI nonredundant public database" lieferte die hierfür notwendigen Daten.

Gefunden wurden aus den MHC – Klasse I Peptiden 5 und aus den MHC – Klasse II Peptiden 6 verschiedene tumorspezifische Proteine. Bei 3 Proteinen (GABBR2-, TPI1- und DNAH14 - Protein) der MHC – II Peptide ließ sich eine Beteiligung neuronalen Gewebes nachweisen.

Die höhere Gesamtzahl der analysierten MHC-Peptide des Lysates aus weißen Blutzellen (n = 131) im Vergleich zu jenen mit PBS (n = 11) deutet auf einen Aufnahme - Mechanismus und / oder eine verstärkte Stimulation der MHC-Peptid-Präsentation hin. Im Gegensatz zu den MHC Klasse II-Liganden scheinen die identifizierten MHC Klasse I-Peptide offensichtlich nicht neuronalen Ursprungs zu sein. Weder die mit Kelly noch die mit SK-N-AS behandelten reifen dendritischen Zellen zeigten ein nachweisbares Muster tumorspezifischer MHC-Epitope. Aus diesem Grund, war kein signifikanter Unterschied zwischen N-myc positiver oder negativer Zelllinie nachweisbar. Zusammenfassend belegen diese Daten, dass ein reproduzierbares Muster der Neuroblastom - vermittelten MHC Klasse I - Peptid-Präsentation nicht unterstützt werden kann. Das deutet darauf hin, dass die adaptive T-Zell Immunantwort eine untergeordnete Rolle in der Abwehr des Neuroblastoms spielen könnte.

Abstract

Neuroblastoma is the most common extracranial tumor of childhood originating from sympathetic neural crest cells. Amplification of the N-myc oncogene is an important predictor of poor prognosis. Existing therapies do not allow any treatment intensification because of the significant toxicity. In contrast, immune therapy seems to be more specific and potentially less toxic.

To explore whether processing of neuroblastoma leads to tumor-specific epitope presentation by MHC class I molecules, immature dendritic cells were loaded with neuroblastoma cell lysates originating from the N-myc positive neuroblastoma cell line Kelly and the N-myc negative SK-N-AS. A further question was whether there was a difference in the pattern of antigen presentation between N-myc positive and N-myc negative neuroblastoma cell cultures.

Distinct peptides were eluted from the surface of lysate pulsed dendritic cells and detected by mass spectroscopy. For MHC prediction studies, programs with different analyzation methods (SYFPEITHI , SVMHC and NetMHCIIpan) were used to examine the identified peptides.

Among them, seven predicted MHC class I and 23 MHC class II ligands were exclusively but not reproducibly found on tumor-lysate treated cells as compared to white blood cell-lysate or phosphate buffered saline treated cells. The higher total number of analyzed MHC peptides derived from white blood cell-lysate (n=131) as compared to phosphate buffered saline (n=11) treated controls suggests an uptake mechanism and/or a stimulation of enhanced MHC peptide presentation. In contrast to the MHC class II ligands, the identified MHC class I peptides appeared not to be of neuronal origin.

Neither Kelly nor SK-N-AS treated maturated dendritic cells showed a detectable tumor specific pattern of MHC epitopes. Therefore no significant difference between N-myc positive or negative cell lines was detectable.

In conclusion our data do not support a reproducible pattern of neuroblastoma mediated MHC class I peptide presentation, suggesting that the cytotoxic T-cell system may play a minor role in the immunological defense against neuroblastoma.

Publications

This thesis is based on the following submitted publication:

Title: Neuroblastoma associated MHC - peptide pattern presented by tumour-pulsed matured dendritic cells

Christoph L. Jandl[1], Ruth Birner-Gruenberger[2], Christian Urban[1], Petra Sovinz[1], Konrad Rosskopf [3] and Wolfgang Schwinger[1] (2010)

List of tables

Introduction

Neuroblastoma is an embryonal malignancy of early childhood and the most common extra-cranial solid tumor originating from sympathetic neural crest cells. Neuroblastoma accounts for 6-8% of childhood cancers and over 15% of pediatric cancer deaths *(1)*. More than 50% of children present with metastatic disease at diagnosis *(2)*.

A strong predictor of poor prognosis is the amplification of the N-myc oncogene, which is used internationally as a parameter for treatment stratification in clinical neuroblastoma trials *(3-4)*. Current treatment options include chemotherapy, high-dose chemotherapy with autologous stem cell rescue, radiotherapy, surgery and immune modulation. Although about 80% of high-risk patients achieve remission *(5)*, the majority of the patients relapses and succumbs to therapy-resistant tumors resulting in long-term survival rates of less than 50% *(6)*.

Due to the significant toxicity there is not much space for treatment intensification of the existing therapies *(7-8)*. In order to improve survival, new approaches are needed. Immune therapy seems to be more specific and potentially less toxic than conventional therapies. Therefore a multimodal approach including immune therapy has been suggested as a promising treatment option for the future (9). The term "immune therapy" includes a variety of approaches such as cytokine -, vaccine -, antibody - and cellular therapies, respectively.

Therapeutic vaccination with dendritic cells presenting tumor-specific antigens (TSAs) is an important investigational study *(10)*. Dendritic cells are efficient stimulators of T- and B-lymphocytes. T-lymphocytes are stimulated by antigens processed and presented by antigen presenting cells (APCs) such as dendritic cells. T-cell-receptors (TCRs) have the ability to recognize fragments of antigens bound to molecules of the major histocompatibility complex (MHC) on the surface of dendritic cells *(11)*. The major histocompatibility complex (MHC) class I molecules are known to bind short antigenic peptides derived mostly from intracellular proteins and present them to cytotoxic CD8+ T lymphocytes (*12*).

In the present study we investigated whether processing of neuroblastoma cell detritus by dendritic cells leads to tumor-specific epitope presentation by MHC class I molecules and a reproducible pattern of tumor antigens (TAs). The second study question was whether there was a difference in the pattern of antigen presentation between N-myc positive and N-myc negative neuroblastoma cell cultures.

Material and Methods

Cell lines and culture conditions

Buffy coat preparations of whole blood units from anonymous donors were obtained from the Department of Blood Group Serology and Transfusion Medicine (Medical University of Graz, Austria). Peripheral Blood Mononuclear Cells (PBMC) were extracted from buffy coats by density gradient centrifugation over Ficoll – Paque ^{TM} Plus (GE Healthcare, Uppsala, Sweden) and were washed twice with Dulbecco's phosphate-buffered saline solution (DPBS; Gibco (Invitrogen), Paisley, UK). Monocytes were isolated by MACS-technique, using anti-CD14 conjugated magnetic microbeads following the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany), counted and cultured at $1x10^6$ cells/mL in DC-medium [RPMI 1640 (Sigma – Aldrich, St. Louis, USA) supplemented with 10% FCS and 50 U/mL Pen Strep (both Gibco (Invitrogen), Paisley, UK)] in 225 cm^2 cell culture flasks at 37°C, 5% CO_2. All DC - media were supplemented with 800 U/mL granulocyte–macrophage colony-stimulating factor (GM-CSF) (Bayer, Leverkusen, Germany) and 500 U/mL recombinant human IL-4 (Immunotools, Friesoythe, Germany).

On day 3, half of the volume of fresh DC-medium (including cytokines), was added to the cell cultures. After 5 days, immature DCs were harvested and centrifuged (Beckman 6KR; 1300 rpm; 6 min). The pellet was resuspended in fresh DC-medium (including cytokines) and cultured at $1x10^6$ cells/mL.

The N-myc positive neuroblastoma cell line Kelly [(CatNr.: 92110411) ECACC, European Collection of Cell Cultures)] was cultured in RPMI 1640 (Sigma – Aldrich, St. Louis, USA) supplemented with 2mM Glutamine, 10% Foetal Bovine Serum (FBS) and 50 IU/mL Pen Strep [both from Gibco (Invitrogen), Paisley, UK] at 37°C, 5% CO_2.

The N-myc negative neuroblastoma cell line SK-N-AS [(CatNr.: 94092302) ECACC, European Collection of Cell Cultures)] was cultured in minimum essential eagle medium [MEM Medium (Sigma – Aldrich, St. Louis, USA)] supplemented with 10% non essential amino acids [NEAA Glutamine (Sigma – Aldrich, St. Louis, USA)], 10% Foetal Bovine Serum (FBS) , 2 mM Glutamine and 50 IU/mL Pen Strep [all three from Gibco (Invitrogen), Paisley, UK)] at 37°C, 5% CO_2.

Tumor cells or white blood cells (WBC) were lysed by mechanical disruption using the MagnaLyser (Roche, Basel, Swiss) according to the manufacturer's instructions. The effect of the cell damage was proven by cytospin, smears and light microscopy.

Equal volume of tumor-lysate or as control DPBS or WBC-lysate, respectively, was added to iDCs at 1:1 cell equivalent ratio. After incubation for 18 h at 37°C and 5% CO_2, iDCs were stimulated with a combination of the following reagents for 24 h: 10 ng/mL IL-6, 10 ng/mL IL-1β (Sigma – Aldrich, St. Louis, USA), 10 ng/mL TNF-α (Immunotools, Friesoythe, Germany), and 1 µg/mL PGE2 (Cayman Chemical, Ann Arbor, USA). Subsequently pulsed DCs were harvested and counted.

FACS analyses

At day 0, 5 and 7, cells were harvested, and antibody staining of $1x10^5$ cells was measured using a FACS-Calibur, equipped with 488 nm argon laser (BD Biosciences, San Jose, California USA). Immunofluorescent staining was performed according to the manufacturer's protocols. The following commercial monoclonal antibodies (mAb) were used: FITC-conjugated mAb against CD14, CD1a, and HLA-DR, PE-conjugated mAb against CD86, CD83, CD11c and HLA – ABC and PerCP-conjugated CD45 (all BD Biosciences).
Isotype controls were FITC conjugated mouse IgG2a and IgG1, PerCP-conjugated mouse IgG1 and IgG2a and PE-conjugated mouse IgG1 (all BD Biosciences). Data of 10000 events each were acquired using CellQuestsoftware (BD Biosciences) and analyzed using Paint-a-gate ^{TM} – software (BD Biosciences).

Morphological cell analysis

Together with FACS screenings, cell cultures were analyzed by light microscopy on a Nikon Diaphot 300 microscope at 40x and 20x magnification. The used software was Nikon NIS-D.

Mild acid elution of peptides

MHC class I bound peptides were acid extracted from the surface of matured and tumor-lysate pulsed dendritic cells as described *(13).* Briefly, up to $1x10^8$ cells were washed three times with PBS and incubated with 0.1 M citrate-phosphate buffer pH 3.0 for one minute. The cells were then centrifuged for 10 min at 10000 x g and the supernatant passed through 10 kDa cutoff filters and lyophilized.

The isolation method of the peptides used in this study was described originally for the isolation of MHC class I – peptides (14). However, our study demonstrated in accordance with previous studies that the given method is not strictly selective for MHC class I – peptides (13).

MHC peptide sequencing by LC-MS/MS

Isolated MHC peptide preparations were dried by vacuum centrifugation and stored at -20°C. Prior to LC-MS/MS analysis samples were solubilised in 50 µL 0.1% formic acid and separated by nano-HPLC (Agilent 1200 system, Vienna, Austria) equipped with a Zorbax 300SB-C18 enrichment column (5 µm, 5 x 0.3 mm) and a Zorbax 300SB-C18 nanocolumn (3.5 µM, 150 x 0.075 mm). 40 µL of sample were injected and concentrated on the enrichment column for 6 min using 0.1% formic acid as isocratic solvent at a flow rate of 20 µL/min. The column was then switched in the nanoflow circuit, and the sample was loaded on the nanocolumn at a flow rate of 300 nL/min. Separation was carried out using the following gradient, where solvent A is 0.3% formic acid in water and solvent B is a mixture of acetonitrile and water (4 : 1, by vol.) containing 0.3% formic acid: 0-6min: 10% B; 6-120 min: 10-60% B; 120-122 min: 60-95% B, 122-130 min: 95% B; 130-132 min: 95-10% B; 132-140 min: re-equilibration at 10% B. The sample was ionized in the nanospray source equipped with nanospray tips (PicoTipTM Stock# FS360-75-15-D-20, Coating: 1P-4P, 15+/- 1 µm Emitter, New Objective, Woburn, MA, USA). It was analyzed in a Thermo LTQ-FT mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) operated in positive ion mode, applying alternating full scan MS (m/z 200 to 2000) in the ion cyclotron and MS/MS by collision induced dissociation of the 5 most intense peaks in the ion trap with dynamic exclusion enabled.

The LC-MS/MS data were analyzed by searching the human NCBI nonredundant public database with Spectrum mill Rev. A.03.03.084 SR4 (Agilent, Vienna, Austria) and Mascot 2.2 (MatrixScience, London, UK). Detailed search criteria were: no enzyme; max. missed cleavage sites: 2; no fixed modification; variable modification: oxidised methionine; precursor mass tolerance +/- 0.05 Da; product mass tolerance +/- 0.7 Da. Peptide hits were subjected to automatic validation by Spectrum mill: for precursor charge of 2: score threshold 11.0, percent scored peak intensity (%SPI) threshold 60.0, Fwd-Rev score threshold 2.0 and rank 1-2 score threshold 2.0; for precursor charge of 1, 3 or 4: score threshold 13.0, %SPI threshold 70.0, Fwd-Rev score threshold 2.0 and rank 1-2 score threshold 2.0; for precursor charge of 5: score

threshold 15.0, %SPI threshold 70.0, Fwd-Rev score threshold 2.0 and rank 1-2 score threshold 2.0.

Epitope prediction analysis

For MHC class I prediction studies, SBS Epitoolkit v.1 (www.epitoolkit.org.) from the Wilhelm Schickard Institute for Computer Science, Div. for Simulation of Biological Systems, was used. This program includes five different methods for the prediction of peptides binding to MHC class I and two methods for MHC class II. Two methods (SYFPEITHI and SVMHC) were used to analyze the identified peptides.

SYFPEITHI is based on position-specific scoring matrices. The manually generated matrices are based on the occurrence of amino acids in naturally processed MHC ligands from the SYFPEITHI database and expert knowledge. By contrast, SVMHC uses support vector machine classifications to predict MHC-binding peptides. SVMHC is trained on known MHC-binding peptides from the SYFPEITHI database and randomly generated nonbinders (15-17). At the time of the analysis the method included prediction for 26 MHC class I types from the MHCPEP database or alternatively 6 MHC class I types from the higher quality SYFPEITHI database.

For MHC class II prediction studies, NetMHCIIpan (www.cbs.dtu.dk/services/NetMHCIIpan) was used. At the time of the analysis the NetMHCIIpan server predicted binding of peptides to more than 500 HLA-DR alleles using artificial neural networks (ANNs). The prediction values are given in nM IC50 values.

HLA-DR locus covers more than 90% of MHC Class II molecules expressed on Antigen Presenting Cells. Out of 500, 51 HLA-DR alleles namely HLA-DRB1*(0101, 0102, 0301, 0305, 0306, 0307, 0308, 0309, 0311, 0401, 0402, 0404, 0405, 0408, 0410, 0423, 0426, 0701, 0703, 0801, 0802, 0804, 0806, 0813, 0817, 1101, 1102, 1104, 1106, 1107, 1114, 1120, 1121, 1128, 1301, 1302, 1304, 1305, 1307, 1311, 1321, 1322, 1323, 1327, 1328, 1501, 1502, 1506) and HLA-DRB5*(0101, 0105) were chosen for analysis of sequenced peptides between 15 and 18 amino acids length by NetMHCIIpan. These HLA-DR alleles were the same as used in ProPred (TEPITOPE) another top rated MHC class II prediction program.

Results

PBMC were extracted from buffy coats and CD14 $^{+}$ cells were isolated by MACS-technique. Monocytes were cultured, treated with cytokines until their differentiation into iDC. After incubation with different cell - lysates and inflammatory cocktail, cells differentiated into mDC and were harvested. The phenotypic maturation was monitored by flow cytometry for the detection of specific cell surface markers. In total 33 samples, 10 of each WBC (positive control), Kelly (N-myc positive) and SK-NAS (N-myc negative) derived matured dendritic cells and 3 DPBS (background control) treated samples were isolated by direct acid elution. After elution, isolated peptides were subsequently identified by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and database search. Validated peptides were screened for binding to MHC molecules by specific MHC prediction software.

Characteristics of mild acid-eluted peptides

A total of 583 distinct peptides were isolated and sequenced over all samples. The length distribution of the acid eluted peptides ranged from 7 to 41. 99 peptides (17%) were 8–11 amino acids (aa) long, 216 (37%) were 14-18 aa long, while 2 (0.3%) peptides were 7 aa in length and 265 (46%) were larger peptides including peptides 12-13 aa long, respectively.

Epitope prediction analysis MHC class I

Peptides within a length of 8 to 11 amino acids are able to bind MHC class I molecules. 99 identified peptides were in this length range and analyzed by SYFPEITHI and SVMHC algorithms to predict their binding affinities to MHC class I molecules. 27 of the 99 peptides were predicted MHC-I ligands or potential T-Cell epitopes (Supplementary Table 1). 7 peptides were exclusively presented by dendritic cells incubated with either Kelly or SK-N-AS tumor lysates but not by the control cells, which were incubated with WBC lysates or phosphate buffered saline (Table 1).

Table 1: Identified tumor specific MHC class I peptides

Peptide Nr.	Sequence	Name	NCBI Identifier	Score SYFPEITI* MHC I ligands	Score SVMHC* MHC I ligands	Lysate
1	VVDNGSGMCK	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	25.0 A*0301	0.886 A*03	Kelly
2	YALPHAILR	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	17.0 A*1101	0.522 A*1101	Kelly
3	ISKTNTQTY	HLA class I histocompatibility antigen, B-47 alpha chain	P30485 GI:231408	----------	-0.037 A*01	Kelly
4	VVAGTNYFIK	cystatin B	NP_000091 GI:4503117	23.0 A*0301	0.499 A*03	SK-N-AS
5	VAPEEHPVL	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	23.0 B*5101	0.161 B*5101	SK-N-AS
6	LKPSEAPEL	similar to Lymphocyte-specific protein 1	BAG52478 GI:193787272	21.0 B*3902	----------	SK-N-AS
7	DRNKDQEVNF	S100 calcium-binding protein A6	NP_055439 GI:7657532	16.0 B*3801	----------	SK-N-AS

Epitope prediction analysis MHC class II

Detected peptides which are between 14–18 amino acids long are potentially associated with MHC-II molecules *(18)*. 37 percent or 216 of the sequenced peptides were in the respective length range. Of those, the NetMHCIIpan program predicted that 127 peptides (Supplementary Table 2) were able to bind HLA class II molecules. 23 peptides were exclusively found on the tumor-debris-treated dendritic cells (Table 3).

Furthermore, peptides were divided into strong binders (SB) and weak binders (WB). The used cut-off was an affinity of 50 nM IC50. Peptides depicting an affinity of 50 nM and below were defined as strong binders. Between 51 and 440 nM affinity peptides were defined as weak binders above non binders. Six tumor specific peptides were predicted to be strong binders (Table 4, numbers 1-6).

Table 3: Identified tumor specific MHC class II peptides

Peptide Number	HLA - type	Peptide	Core	Prediction Score (1-log50K(aff))	Affinity (nm)	Binding Level	Protein Name	NCBI GI number	Lysate
1	DRB1_0101	LIKMSSPYMNNLIILGG	LIKMSSPYM	0.735	17.62	SB	gamma-aminobutyric acid B receptor, 2, isoform CRA_a	119579293	Kelly
2	DRB1_0405	IVLLPNEIDGLQKL	IVLLPNEID	0.757	13.79	SB	serine (or cys) prot. inhibitor, clade B, member 4	28076869	SK-N-AS
3	DRB1_0101	NGAFTGEISPGMIK	FTGEISPGM	0.732	18.19	SB	triosephosphate isomerase 1 isoform 2	226529917	SK-N-AS
4	DRB1_0701	IIGNSMGLVNAYSHKFI	LVNAYSHKF	0.704	24.67	SB	hCG22803, isoform CRA_e	119590144	SK-N-AS
5	DRB5_0101	FIVNTNVPRASVPDG	FIVNTNVPR	0.680	31.83	SB	macrophage migration inhibitory factor	148608029	SK-N-AS
6	DRB1_0405	SPEYVNLPINGNGKQ	YVNLPINGN	0.655	41.91	SB	glutathione S-transferase	2204207	SK-N-AS
7	DRB1_0102	YELPDGQVITIGNERFR	VITIGNERF	0.628	55.75	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS
8	DRB1_0101	KDRSSFYVNGLTLGGQK	YVNGLTLGG	0.621	60.57	WB	profilin 1	4826898	SK-N-AS
9	DRB1_0101	LPKPPKPVSKMRMATPLL	KMRMATPLL	0.600	75.96	WB	CD74/ROS fusion protein	161176974	SK-N-AS
10	DRB1_0101	VKLPDGYEFKFPNRLNL	YEFKFPNRL	0.585	89.54	WB	galectin-1	4504981	SK-N-AS
11	DRB1_0408	FVKRQFMNKSLSGPGQ	VKRQFMNKS	0.552	127.76	WB	actin related protein 2/3 complex	84626115	SK-N-AS
12	DRB1_0401	YELPDGQVITIGNER	YELPDGQVI	0.550	129.49	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS
13	DRB1_0102	DAPRAVFPSIVGRPRHQG	FPSIVGRPR	0.526	169.47	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS
14	DRB1_1501	GDDAPRAVFPSIVG	RAVFPSIVG	0.507	207.59	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS
15	DRB1_0102	VYFTNELKQMQDKYSK	LKQMQDKYS	0.491	247.60	WB	tumor necrosis factor (ligand) superfamily, member 10	4507593	SK-N-AS
16	DRB1_0101	WISKQEYDESGPSIVH	YDESGPSIV	0.486	259.19	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS
17	DRB1_0101	SSKDAIKKKLTGIKHELQ	LTGIKHELQ	0.471	304.57	WB	cofilin 1 (non-muscle)	5031635	SK-N-AS
18	DRB5_0101	TAKTEWLDGKHVVFG	TEWLDGKHV	0.469	312.79	WB	peptidylprolyl isomerase A-like, isoform CRA_c	119581485	SK-N-AS
19	DRB1_0102	AKTEWLDGKHVVFG	TEWLDGKHV	0.464	331.43	WB	peptidylprolyl isomerase A-like, isoform CRA_c	119581485	SK-N-AS
20	DRB1_0101	IHARELFDSRGNPT	LFDSRGNPT	0.461	340.03	WB	enolase 1 variant	62897945	SK-N-AS
21	DRB1_0101	RAAVPSGASTGIYE	VPSGASTGI	0.460	343.62	WB	enolase 1 variant	62897945	SK-N-AS
22	DRB1_1321	LPKPPKPVSKMRMATPL	VSKMRMATP	0.451	381.44	WB	CD74/ROS fusion protein	161176974	SK-N-AS
23	DRB1_0101	IHAREIFDSRGNPT	IFDSRGNPT	0.448	392.79	WB	enolase 1 variant	62897945	SK-N-AS

Function and source of proteins associated with tumour specific peptides which were predicted to bind to MHC class I or II molecules

Sequenced peptides were matched to the human nonredundant NCBI protein database (http://www.ncbi.nlm.nih.gov/sites/entrez). The gene ontology functions of the identified proteins containing the sequenced peptides were extracted. Five different proteins were identified from seven tumor specific predicted MHC class I peptides (Table 2) and six proteins from six tumor specific predicted MHC class II peptides (Table 4).

Table 2: Function and neuronal expression of protein sources of identified tumor specific MHC class peptides:

Human Protein Atlas data:							
Peptide Nr / Sequence		**Protein Name**	**Gene**	**Protein expression in neuronal cells (Normal Tissue)**			**Function**
				Cerebral cortex	**Lateral ventricle**	**Hippocampus**	
1 2 3	VVDNGSGMCK YALPHAILR VAPEEHPVL	mutant beta-actin (beta'- actin)	ACTB	moderate	moderate	moderate	Cellular component movement
4	ISKTNTQTY	HLA class I histocompatibility antigen, B-47 alpha chain	HLAB	no data	no data	no data	Immune system
5	VVAGTNYFIK	cystatin B	CSTB	negative	negative	negative	Cysteine-type endopeptidase inhibitor activity; Protease binding, protein binding;
6	LKPSEAPEL	similar to Lymphocyte-specific protein 1	LSP1	negative	negative	negative	Actin binding
7	DRNKDQEVNF	S100 calcium-binding protein A6	S100A6	weak	moderate	moderate	Signal transduction

The Human Protein Atlas (http://www.proteinatlas.org/index.php) based on immunohistochemistry data was searched for expression of the identified proteins in neuronal cells. The combined results for the identified tumor specific peptides and their associated proteins are summarized in Tables 2 and 4. If the protein was not found in the Human Protein Atlas, the ArrayExpress Gene Expression Atlas (http://www.ebi.ac.uk/microarray-as/atlas) was searched for mRNA expression data.

Table 4: Function and expression of protein sources of tumor specific strong binding MHC class II peptides

Human Protein Atlas data:							
Peptide Nr. / Sequence		**Protein Name**	**Gene**	**Protein expression in neuronal cells (Normal Tissue)**			**Function**
				Cerebral cortex	**Lateral ventricle**	**Hippocampus**	
1	LIKMSSPYMNNLIILGG	gamma-aminobutyric acid B receptor, 2, isoform CRA_a	GABBR2	strong	moderate	moderate	G-protein coupled receptor activity, GABA-B receptor activity, binding, receptor
2	IVLLPNEIDGLQKL	serine (or cys) prot. inhibitor, clade B, member 4	SERPINB4	none	none	none	protein binding, serine-type endopeptidase inhibitor activity
3	NGAFTGEISPGMIK	triosephosphate isomerase 1 isoform 2	TPI1	strong	strong	strong	triose-phosphate isomerase activity
5	FIVNTNVPRASVPDG	macrophage migration inhibitory factor	MIF	weak	weak	weak	isomerase activity, immune response
6	SPEYVNLPINGNGKQ	glutathione S-transferase	GSTP1	negative	moderate	weak	glutathione transferase activity, protein binding
ArrayExpress Gene Expression Atlas data:							
Peptide Nr / Sequence		**Protein Name**	**Gene**	**Protein expression in neuronal cells (Normal Tissue)**			**Function**
				Superior cervical ganglion	**Trigeminal ganglion**	**Dorsal root ganglion**	
4	IIGNSMGLVNAYSHKFI	hCG22803, isoform CRA_e	DNAH14	strong	strong	strong	ATP binding, microtubule motor activity, nucleotide binding

Discussion

Several recent studies focused on tumor antigens expressed by neuroblastoma cells like MYC-N, GD2 and NY-SO-1 *(19-21)*. Unfortunately most of them were ordinary self-peptides or not exclusively expressed on neuroblastoma cells *(22)*. Studies using antigen specific cytotoxic T-cells did not hold promise and could not transfer in vitro success into in vivo studies *(23)*. This existing but ineffective immune response proves that the immune system can recognize neuroblastoma *(22)*. Attempts to establish tumor specific immune response by infusion of tumor pulsed dendritic cells failed to induce significant tumor response (24-25). The reason may be an antigen presentation without appropriate tumor specificity by dendritic cells as demonstrated in this study.

The failure of the immune system to eradicate the tumor led to the investigation of several possible mechanisms of immune escape, such as the down regulation of MHC class I molecules or the binding to deactivating co-stimulatory molecules, which is a common mechanism of dendritic cells *(26-27)*. Furthermore regulatory T-cells (T-regs) may develop tolerance to tumor antigens. These cells play a role in protection against autoimmunity by inhibiting T-cells *(28)*. In addition, inhibitory cytokines may repress any generated immune response. In context with neuroblastoma, MHC class I chain related protein A (MICA), transforming growth factor-b (TGF-b), interleukin-10 and macrophage migration inhibitory factor (MIF) were previously described as possible inhibitory cytokines *(29-31)*.

This study was designed to explore the MHC-epitopes of matured dendritic cells pulsed with tumor - lysate in order to elucidate whether dendritic cells are able to process neuroblastoma cell detritus and present a reproducible pattern of detectable tumor associated antigens on their surface. Moreover, neuroblastomas with MYC-amplification are associated with poorer outcome compared to neuroblastomas lacking MYC-amplification. Thus we applied lysates of two different neuroblastoma cell lines, Kelly (N-myc positive) and SK-N-AS (N-myc negative) to dendritic cells.

Our data revealed 315 distinct peptides eluted from the surface of treated dendritic cells and detected by mass spectroscopy, which were possible MHC class I or II epitopes because of their respective length range between 8 and 18 aa. Among them, prediction programs confirmed 154 peptides as possible MHC ligands. Only few of them were specifically found on dendritic cells treated with tumor cell lysate. Most peptides were identified on dendritic cells incubated with detritus of either tumor cell lines or WBC, respectively, while the DPBS treated control cells displayed only few peptides. Solely 7 predicted MHC class I peptides (Table 1)

and 23 predicted MHC class II peptides (Table 3) were exclusively found on dendritic cells treated with tumor detritus. However, only one of these 30 peptides was detected in 2 out of 10 biological replicates underlying the irreproducibility and heterogeneity of the presentation system. In the context of MHC-I molecules, the epitopes presented to cytotoxic T cells are products of intracellular proteasomal cleavage. These MHC-I peptide products are not random and a function of the precise sequence of the proteins processed by the proteasome *(32)*.

Our data indicate that neuroblastoma MHC-mediated peptide presentation is not reproducible. None of the five proteins identified as sources of the seven predicted MHC class I peptides exclusively found on dendritic cells treated with tumor detritus was reported to be expressed exclusively or even strongly by neuronal cells (Table 2). In contrast, three of the protein sources of the six strong binding MHC class II peptides exclusively found on dendritic cells treated with tumor detritus were reported to be strongly expressed in neuronal cells (Table 4 peptides 1, 3 and 4). Protein 1 as part of the Gamma-aminobutyric acid B receptor 2 is a multi-pass membrane protein encoded by the GABABR2 gene. Dysfunction of these receptors underlies several of well-characterized neuropathological conditions such as epilepsy, anxiety and neurodegenerative diseases *(33)*. Protein 4 is an axonemal dynein heavy chain and a part of the microtubule-associated motor protein complex which is composed of several heavy, light, and intermediate chains *(34)*. Its mRNA was found to be highly expressed in neuronal tissue including superior cervical ganglion and trigeminal ganglion. In contrast, protein 2 encoded by the SERPINB4 gene is not expressed in normal neuronal cells and may be involved in cancer behavior such as invasion or metastasis of squamous cell carcinoma *(35)*.

Neither Kelly nor SK-N-AS treated mDCs showed a detectable tumor specific pattern of MHC epitopes. Therefore no significant difference between N-myc positive or negative cell lines was detectable.

The presented experiments demonstrate the identification of MHC class I and II peptides in tissue treated matured dendritic cells. The number of analyzed MHC peptides derived from WBC-detritus (n=131) and DPBS (n=11) controls supports an uptake mechanism and/or a stimulation of enhanced MHC peptide presentation. The DPBS control contained a low number of peptides expected to origin from dendritic cells. In contrast, the WBC detritus treated control represented a large number of distinct peptides. Thus this difference provides evidence that processed and presented proteins arise from the cell detritus.

Only few of the analyzed peptides were specific for tumor samples. Most were also found in WBC and DPBS treated controls. There was no apparent correlation between the MHC peptide class I associated proteins and neuronal tissue. Our data do not support a reproducible pattern

of neuroblastoma mediated MHC class I - peptide presentation. Thus the cytotoxic T-cell system probably plays a minor role in the immunological defense against neuroblastoma. Maybe the lack of sufficient epitope presentation is the limiting factor. This conclusion is supported by Gambini *et al.* who could not detect HLA class I peptide expression on tumor cells from Opsoclonus-myoclonus syndrome related neuroblastic tumors *(36)*. Further investigation is necessary to determine the specificity of the identified peptides.

References

1. Maris JM, Hogarty MD, Bagatell R, Cohn SL 2007 Neuroblastoma. Lancet 369:2106-2120.
2. Reynolds CP 2004 Detection and treatment of minimal residual disease in high-risk neuroblastoma. Pediatr Transplant 8 Suppl 5:56-66.
3. Brodeur GM 2003 Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer 3:203-216.
4. Schwab M, Westermann F, Hero B, Berthold F 2003 Neuroblastoma: biology and molecular and chromosomal pathology. Lancet Oncol 4:472-480.
5. Matthay KK, Reynolds CP, Seeger RC, Shimada H, Adkins ES, Haas-Kogan D, Gerbing RB, London WB, Villablanca JG 2009 Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a children's oncology group study. J Clin Oncol 27:1007-1013.
6. Van Maerken T, Ferdinande L, Taildeman J, Lambertz I, Yigit N, Vercruysse L, Rihani A, Michaelis M, Cinatl J, Jr., Cuvelier CA, Marine JC, De Paepe A, Bracke M, Speleman F, Vandesompele J 2009 Antitumor activity of the selective MDM2 antagonist nutlin-3 against chemoresistant neuroblastoma with wild-type p53. J Natl Cancer Inst 101:1562-1574.
7. Berthold F, Boos J, Burdach S, Erttmann R, Henze G, Hermann J, Klingebiel T, Kremens B, Schilling FH, Schrappe M, Simon T, Hero B 2005 Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial. Lancet Oncol 6:649-658.
8. Verdeguer A, Munoz A, Canete A, Pardo N, Martinez A, Donat J, Gomez P, Bureo E, Fernandez JM, Cubells J, Maldonado M, Sastre A 2004 Long-term results of high-dose chemotherapy and autologous stem cell rescue for high-risk neuroblastoma patients: a report of the Spanish working party for BMT in children (Getmon). Pediatr Hematol Oncol 21:495-504.
9. Navid F, Armstrong M, Barfield RC 2009 Immune therapies for neuroblastoma. Cancer Biol Ther 8:874-882.
10. Decker WK, Xing D, Li S, Robinson SN, Yang H, Yao X, Segall H, McMannis JD, Komanduri KV, Champlin RE, Shpall EJ 2006 Double loading of dendritic cell MHC

class I and MHC class II with an AML antigen repertoire enhances correlates of T-cell immunity in vitro via amplification of T-cell help. Vaccine 24:3203-3216.

11. Banchereau J, Steinman RM 1998 Dendritic cells and the control of immunity. Nature 392:245-252.
12. Germain RN 1994 MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell 76:287-299.
13. Gebreselassie D, Spiegel H, Vukmanovic S 2006 Sampling of major histocompatibility complex class I-associated peptidome suggests relatively looser global association of HLA-B*5101 with peptides. Hum Immunol 67:894-906.
14. Sugawara S, Abo T, Kumagai K 1987 A simple method to eliminate the antigenicity of surface class I MHC molecules from the membrane of viable cells by acid treatment at pH 3. J Immunol Methods 100:83-90.
15. Donnes P, Elofsson A 2002 Prediction of MHC class I binding peptides, using SVMHC. BMC Bioinformatics 3:25.
16. Feldhahn M, Thiel P, Schuler MM, Hillen N, Stevanovic S, Rammensee HG, Kohlbacher O 2008 EpiToolKit--a web server for computational immunomics. Nucleic Acids Res 36:W519-522.
17. Rammensee H, Bachmann J, Emmerich NP, Bachor OA, Stevanovic S 1999 SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 50:213-219.
18. Lippolis JD, White FM, Marto JA, Luckey CJ, Bullock TN, Shabanowitz J, Hunt DF, Engelhard VH 2002 Analysis of MHC class II antigen processing by quantitation of peptides that constitute nested sets. J Immunol 169:5089-5097.
19. Himoudi N, Yan M, Papanastasiou A, Anderson J 2008 MYCN as a target for cancer immunotherapy. Cancer Immunol Immunother 57:693-700.
20. Modak S, Cheung NK 2007 Disialoganglioside directed immunotherapy of neuroblastoma. Cancer Invest 25:67-77.
21. Rodolfo M, Luksch R, Stockert E, Chen YT, Collini P, Ranzani T, Lombardo C, Dalerba P, Rivoltini L, Arienti F, Fossati-Bellani F, Old LJ, Parmiani G, Castelli C 2003 Antigen-specific immunity in neuroblastoma patients: antibody and T-cell recognition of NY-ESO-1 tumor antigen. Cancer Res 63:6948-6955.
22. Gray JC, Kohler JA 2009 Immunotherapy for neuroblastoma: turning promise into reality. Pediatr Blood Cancer 53:931-940.

23. Coughlin CM, Fleming MD, Carroll RG, Pawel BR, Hogarty MD, Shan X, Vance BA, Cohen JN, Jairaj S, Lord EM, Wexler MH, Danet-Desnoyers GA, Pinkus JL, Pinkus GS, Maris JM, Grupp SA, Vonderheide RH 2006 Immunosurveillance and survivin-specific T-cell immunity in children with high-risk neuroblastoma. J Clin Oncol 24:5725-5734.
24. Caruso DA, Orme LM, Amor GM, Neale AM, Radcliff FJ, Downie P, Tang ML, Ashley DM 2005 Results of a Phase I study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children with Stage 4 neuroblastoma. Cancer 103:1280-1291.
25. Geiger JD, Hutchinson RJ, Hohenkirk LF, McKenna EA, Yanik GA, Levine JE, Chang AE, Braun TM, Mule JJ 2001 Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression. Cancer Res 61:8513-8519.
26. Kroczek RA, Mages HW, Hutloff A 2004 Emerging paradigms of T-cell co-stimulation. Curr Opin Immunol 16:321-327.
27. Ochsenbein AF 2005 Immunological ignorance of solid tumors. Springer Semin Immunopathol 27:19-35.
28. Johnson BD, Jing W, Orentas RJ 2007 CD25+ regulatory T cell inhibition enhances vaccine-induced immunity to neuroblastoma. J Immunother 30:203-214.
29. Bin Q, Johnson BD, Schauer DW, Casper JT, Orentas RJ 2002 Production of macrophage migration inhibitory factor by human and murine neuroblastoma. Tumour Biol 23:123-129.
30. Raffaghello L, Prigione I, Airoldi I, Camoriano M, Levreri I, Gambini C, Pende D, Steinle A, Ferrone S, Pistoia V 2004 Downregulation and/or release of NKG2D ligands as immune evasion strategy of human neuroblastoma. Neoplasia 6:558-568.
31. Rivoltini L, Arienti F, Orazi A, Cefalo G, Gasparini M, Gambacorti-Passerini C, Fossati-Bellani F, Parmiani G 1992 Phenotypic and functional analysis of lymphocytes infiltrating paediatric tumours, with a characterization of the tumour phenotype. Cancer Immunol Immunother 34:241-251.
32. Ginodi I, Vider-Shalit T, Tsaban L, Louzoun Y 2008 Precise score for the prediction of peptides cleaved by the proteasome. Bioinformatics 24:477-483.
33. Moult PR 2009 Neuronal glutamate and GABAA receptor function in health and disease. Biochem Soc Trans 37:1317-1322.

34. Vaughan KT, Mikami A, Paschal BM, Holzbaur EL, Hughes SM, Echeverri CJ, Moore KJ, Gilbert DJ, Copeland NG, Jenkins NA, Vallee RB 1996 Multiple mouse chromosomal loci for dynein-based motility. Genomics 36:29-38.
35. Murakami A, Nakagawa T, Fukushima C, Torii M, Sueoka K, Nawata S, Takeda O, Ishikawa H, Sugino N 2008 Relationship between decreased expression of squamous cell carcinoma antigen 2 and E-cadherin in primary cervical cancer lesions and lymph node metastasis. Oncol Rep 19:99-104.
36. Gambini C, Conte M, Bernini G, Angelini P, Pession A, Paolucci P, Donfrancesco A, Veneselli E, Mazzocco K, Tonini GP, Raffaghello L, Dominici C, Morando A, Negri F, Favre A, De Bernardi B, Pistoia V 2003 Neuroblastic tumors associated with opsoclonus-myoclonus syndrome: histological, immunohistochemical and molecular features of 15 Italian cases. Virchows Arch 442:555-562.

Curriculum Vitae

Personal Data

Name:	Christoph Ludwig Jandl
Date / Place of Birth:	December 22th 1975, Graz, Austria
Citizenship:	Austria
Steady Address:	Badstraße 29, 8144 Haselsdorf - Tobelbad, Austria,
Email:	christoph.jandl@medunigraz.at

Education

Since 02/2008 ***Medical University Graz, Austria***
Doctoral thesis at the Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology/ Oncology

10/1996 – 11/2003 ***Karl Franzens University Graz, Austria***
Master thesis of molecular microbiology at the Department of molecular Biosciences
Master Thesis:"Untersuchung terbinafinsensitiver *erg1*- Allele"

Work Experience

Since 07/2007 **Activities during doctoral thesis at *Medical University Graz***
Participation in a research project of Univ. Prof. Dr. Wolfgang Schwinger at the Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology/Oncology

10/2006 – 01/2007 **Activities after master thesis at *Karl Franzens University Graz***
Participation in a research project of Dr.Martin Grube at the Institute of Plant Sciences

09/2004 – 10/2006 **Activities after master thesis at *Magna Steyr automotive engineering Graz***
Participation in Quality control at the division of paint shop, surface paint inspection

03/2004 – 04/2004 **Internship after master thesis at "*Kinderwunsch Institut" Dr. Schenk Dobl***

05/1998 – 06/1998 **Internship at *Medical University Bergmannsheil, Bochum***

06/1996 – 07/1996 **Internship at *ITT Automotive Italy* (GALFA), *Torino***

Supplements

Table S1: Characteristics of identified predicted MHC I ligands

Peptide Nr.	Sequence	Protein Name	NCBI Identifier	Score SYFPEITI* MHC I ligands	Score SVMHC* MHC I ligands	MH^+ Matched (Da)	MH^+ Error (Da)	Lysate
1	VVDNGSGMK	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	25.0 A*0301	0.886 A*03	1009.4441	159.934	Kelly
2	YALPHAILR	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	17.0 A*1101	0.522 A*1101	1053.6204	-0.0003	Kelly
3	ISKTNTQTY	HLA class I histocompatibility antigen, B-47 alpha chain	P30485 GI:231408	----------	-0.037 A*01	1055.5368	-0.0004	Kelly
4	VVAGTNYFIK	cystatin B	NP_000091 GI:4503117	23.0 A*0301	0.499 A*03	1111.6146	0.0037	SK-N-AS
5	VAPEEHPVL	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	23.0 B*5101	0.161 B*5101	990.5255	0.0021	SK-N-AS
6	LKPSEAPEL	similar to Lymphocyte-specific protein 1	BAG52478 GI:193787272	21.0 B*3902	----------	983.5408	0.0011	SK-N-AS
7	DRNKDQEVNF	S100 calcium-binding protein A6	NP_055439 GI:7657532	16.0 B*3801	----------	1264.5917	-0.0001	SK-N-AS

Peptide Nr.	Sequence	Protein Name	NCBI Identifier	Score SYFPEITI* MHC I ligands	Score SVMHC* MHC I ligands	MH^+ Matched (Da)	MH^+ Error (Da)	Lysate
8	EVPESVFLHL	tumor necrosis factor, alpha-induced protein 2	AAP12649 GI:30039657	31.0 A*2402	----------	1169.6201	0.0012	WBC
9	DVHGPDWHL	AHNAK nucleoprotein isoform 1	NP_001611 GI:61743954	26.0 A*2601	----------	1075.4956	0.0010	WBC
10	APEEHPVLL	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	24.0 B*0702	0.498 B*07	1004.5411	0.0012	WBC
11	MSLKQTPLSR	clathrin, light polypeptide isoform b	NP_009028 GI:6005995	24.0 A*1101	----------	1160.6456	0.0011	WBC
12	VIDNGSGMCK	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	23.0 A*0301	0.973 A*03	1023.4598	0.0007	WBC
13	NEVFNDVRL	F-actin capping protein alpha-1 subunit	NP_006126 GI:5453597	23.0 B*4402	0.252 B*44	1105.5636	0.0015	WBC
14	LLKNSPLVSR	MDH2	CAG38785 GI:49168580	22.0 A*0301	----------	1126.6943	0.0017	WBC
15	DEPLLKHWEF	major histocompatibility complex, class II, DR alpha, isoform CRA_a	EAX03629 GI:119624034	22.0 B*4402	----------	1313.6525	0.0015	WBC

Peptide Nr.	Sequence	Protein Name	NCBI Identifier	Score SYFPEITI* MHC I ligands	Score SVMHC* MHC I ligands	MH^+ Matched (Da)	MH^+ Error (Da)	Lysate
16	KMCMKKAVI	protein arginine methyltransferase 3, isoform CRA_d	EAW68336 GI:119588742	19.0 A*0201	----------	1138.6145	160.084	WBC
17	FGANANRKF	vacuolar proton ATPase	CAA50592 GI:313014	----------	0.265 A*2402	1024.5323	0.0010	WBC
18	YLLPAIVHI	DEAD box polypeptide 17 isoform p82 variant	BAD92832 GI:62088770	30.0 A*0201	1.365 A*0201	1038.6346	0.0049	WBC Kelly
19	SLKPSEAPEL	similar to Lymphocyte-specific protein 1	BAG52478 GI:193787272	26.0 A*0201	0.242 A*0201	1070.5728	0.0009	WBC SK-N-AS
20	VISLEGKPL	cofilin 1 (non-muscle)	NP_005498 GI:5031635	18.0 A*0201	----------	955.5823	0.0015	WBC SK-N-AS
21	MAADGDFKIK	galectin-1	NP_002296 GI:4504981	16.0 A*6801	----------	1095.5503	0.0006	WBC DPBS
22	VVDNGSGMCK	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	25.0 A*0301	0.886 A*03	1009.4441	-0.0006	WBC Kelly SK-N-AS
23	LVVYPWTQRF	gamma-G globin	CAA39189 GI:31725	22.0 A*2601	0.782 B*1501	1308.7099	0.0020	WBC Kelly SK-N-AS

Peptide Nr.	Sequence	Protein Name	NCBI Identifier	Score SYFPEITI* MHC I ligands	Score SVMHC* MHC I ligands	MH^+ Matched (Da)	MH^+ Error (Da)	Lysate
24	ALIYNEALKG	S100 calcium-binding protein A6	NP_055439 GI:7657532	22.0 A*0301	----------	1091.6095	0.0014	WBC Kelly SK-N-AS
25	RVAPEEHPVL	mutant beta-actin (beta'-actin)	CAA45026 GI:28336	19.0 A*0201	1.0 A*0201	1146.6266	0.0007	WBC Kelly SK-N-AS
26	ISLKQAPLVH	clathrin, light polypeptide A isoform b	NP_009027 GI:6005993	17.0 A*1101	----------	1105.6728	0.0013	WBC Kelly SK-N-AS
27	IAVDGEPLGR	peptidylprolyl isomerase A-like, isoform CRA_c	EAW61081 GI:119581485	17.0 A*6801	----------	1026.5578	0.0001	WBC Kelly SK-N-AS DPBS

Table S2: Characteristics of identified predicted MHC II ligands

14 mer MHC class II binding peptides:

HLA - allele	Peptide - Sequence	Core	Prediction Score (1-log50K(aff))	Affinity (nm)	Binding Level	Protein Name	NCBI Accession Number	Lysate	MH^+ Matched (Da)	MH^+ Error (Da)
DRB1_0405	IVLLPNEIDGLQKL	IVLLPNEID	0.757	13.79	SB	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 4	28076869	SK-N-AS	1564.9309	0.0045
DRB1_0101	NGAFTGEISPGMIK	FTGEISPGM	0.732	18.19	SB	triosephosphate isomerase 1 isoform 2	226529917	SK-N-AS	1421.7093	0.0018
DRB1_1501	GDDAPRAVFPSIVG	RAVFPSIVG	0.507	207.59	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS	1400.7169	0.0025
DRB1_0102	AKTEWLDGKHVVFG	TEWLDGKHV	0.464	331.43	WB	peptidylprolyl isomerase A-like, isoform CRA_c	119581485	SK-N-AS	1586.8326	0.0024
DRB1_0101	IHARELFDSRGNPT	LFDSRGNPT	0.461	340.03	WB	enolase 1 variant	62897945	SK-N-AS	1612.8190	0.0022
DRB1_0101	RAAVPSGASTGIYE	VPSGASTGI	0.460	343.62	WB	enolase 1 variant	62897945	SK-N-AS	1378.6961	0.0002
DRB1_0101	IHAREIFDSRGNPT	IFDSRGNPT	0.448	392.79	WB	enolase 1 variant	62897945	SK-N-AS	1612.8190	0.0022
DRB1_0102	LGALALIYNEALKG	LALIYNEAL	0.762	13.07	SB	S100 calcium-binding protein A6	7657532	WBC	1445.8362	0.0107
DRB5_0101	FPSVSLQEASSFFR	LQEASSFFR	0.720	20.75	SB	pHL E1F1	1050983	WBC	1601.7958	0.0019
DRB1_0102	LGGSAVISLEGKPL	VISLEGKPL	0.697	26.41	SB	cofilin 1 (non-muscle)	5031635	WBC	1340.7784	0.0003
DRB1_0102	ESEIIDFFLGASLK	IDFFLGASL	0.685	30.36	SB	PREDICTED: hypothetical protein isoform 1	169204454	WBC	1568.8207	0.0061
DRB1_0101	VNGLDVTSLRPFDL	VTSLRPFDL	0.685	30.13	SB	FLJ00343 protein	21748542	WBC	1545.8271	0.0010
DRB1_0404	VTYVPVTTFKNLQT	VTTFKNLQT	0.682	31.08	SB	similar to H. sapiens ribosomal protein L31 (RPL31)	189053096	WBC	1610.8788	0.0007
DRB1_0101	SFTRGSDSLIKGKK	FTRGSDSLI	0.668	36.50	SB	prohibitin 2	6005854	WBC	1523.8540	-0.0005
DRB5_0101	NWRPAQPLKNRQIK	WRPAQPLKN	0.650	44.27	SB	carbonic anhydrase II	4557395	WBC	1749.0031	0.0398
DRB1_1501	AAIVGYKDSPSVWA	IVGYKDSPS	0.625	57.98	WB	profilin 1	4826898	WBC	1463.7529	0.0018
DRB1_0101	SKIDSEGGVSANHT	IDSEGGVSA	0.568	106.99	WB	highly similar to Peroxisomal multifunctional enzyme type	194388790	WBC	1401.6605	0.0007
DRB1_0102	NAINKCPLLKPWAL	CPLLKPWAL	0.561	116.13	WB	similar to Fructose-bisphosphate aldolase A	194378468	WBC	1580.8981	0.0025
DRB1_0101	HAAMADTFLEHMCR	HAAMADTFL	0.552	127.49	WB	similar to Pyruvate kinase isozyme M1	194377282	WBC	1632.7080	0.0057
DRB1_0101	VHVTDLSGKETICR	VTDLSGKET	0.516	188.53	WB	ribosomal protein S14	5032051	WBC	1557.8054	-0.0008
DRB1_0101	NVINGGSHAGNKLA	VINGGSHAG	0.515	189.70	WB	enolase 1 variant	62897945	WBC	1351.7077	0.0008
DRB1_0405	IYKGFESPSDNSSA	YKGFESPSD	0.506	208.65	WB	actin related protein 2/3 complex subunit 5	5031593	WBC	1501.6805	0.0010

DRB1_0101	SDRAPFAAPLPFAE	FAAPLPFAE	0.494	238.71	WB	TYMP protein	30354553	WBC	1488.7482	0.0038
DRB1_0301	MATGDLDQDGRISF	LDQDGRISF	0.488	253.34	WB	L-plastin	167614506	WBC	1525.6951	0.0024
DRB1_0102	IGGLAMACHDSFLK	IGGLAMACH	0.599	76.67	WB	S100 calcium binding protein A11	5032057	Kelly, WBC	1462.7181	0.0017
DRB1_0101	YDNEFGYSNRVVDL	YDNEFGYSN	0.517	185.07	WB	glyceraldehyde-3-phosphate dehydrogenase	31645	SK-N-AS, Kelly, WBC	1690.7707	0.0026
DRB1_0405	YNELRVAPEEHPVL	YNELRVAPE	0.499	226.11	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS, Kelly, WBC	1665.8595	0.0024
DRB1_0405	MSNLDSNRDNEVDF	MSNLDSNRD	0.493	240.34	WB	S100 calcium-binding protein A4	4506765	SK-N-AS, Kelly, WBC, DPBS	1655.6966	0.0035
DRB1_0101	ADDRVNPCIGGVIL	VNPCIGGVI	0.450	382.10	WB	similar to Fructose-bisphosphate aldolase A	194378468	SK-N-AS, Kelly, WBC, DPBS	1441.7468	0.0009
DRB1_0701	MLTELEKALNSIID	LEKALNSII	0.654	42.19	SB	S100 calcium-binding protein A8	21614544	SK-N-AS, WBC	1589.8455	0.0056
DRB1_0101	DPFNPFELTNHAVL	FELTNHAVL	0.643	47.40	SB	cathepsin C	17933077	SK-N-AS, WBC	1613.7958	0.0027
DRB1_0101	DSPSVWAAVPGKTF	WAAVPGKTF	0.624	58.45	WB	profilin 1	4826898	SK-N-AS, WBC	1461.7373	0.0031
DRB1_0101	RVFQSLPHENKPLT	FQSLPHENK	0.615	64.43	WB	cystatin B	4503117	SK-N-AS, WBC	1665.9071	0.0003
DRB1_0401	LVADENPFAQGALK	LVADENPFA	0.576	98.68	WB	gelsolin isoform a precursor	4504165	SK-N-AS, WBC	1472.7744	0.0024
DRB1_0101	IVNTNVPRASVPDG	VNTNVPRAS	0.539	147.41	WB	macrophage migration inhibitory factor	148608029	SK-N-AS, WBC	1438.7649	0.0025
DRB1_0101	SDRAPFAAPSPFAE	FAAPSPFAE	0.491	247.86	WB	Thymidine phosphorylase	17390355	SK-N-AS, WBC	1462.6961	0.0010

15 mer MHC class II binding peptides:

HLA - allele	Peptide - Sequence	Core	Prediction Score (1-log50K(aff))	Affinity (nm)	Binding Level	Protein Name	NCBI Accession Number	Lysate	MH^+ Matched (Da)	MH^+ Error (Da)
DRB5_0101	FIVNTNVPRASVPDG	FIVNTNVPR	0.680	31.83	SB	macrophage migration inhibitory factor	148608029	SK-N-AS	1585.8333	0.0002
DRB1_0405	SPEYVNLPINGNGKQ	YVNLPINGN	0.655	41.91	SB	glutathione S-transferase	2204207	SK-N-AS	1629.8231	0.0048
DRB1_0401	YELPDGQVITIGNER	YELPDGQVI	0.550	129.49	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS	1703.8599	0.0084
DRB1_0101	LNLIGGLAMACHDSF	LNLIGGLAM	0.790	9.69	SB	S100 calcium binding protein A11	5032057	WBC	1561.7501	0.0028
DRB1_0101	WVSQRTATAGTASPP	WVSQRTATA	0.686	30.04	SB	monoglyceride lipase isoform 1	6005786	WBC	1529.7707	0.0028
DRB1_0701	PELAVQKVVVHPLVL	VQKVVVHPL	0.681	31.51	SB	PSMD7 protein	33875323	WBC	1641.0098	0.0025
DRB1_0101	PMTLTTLEGGNLEAK	LTTLEGGNL	0.681	31.47	SB	lipocalin 1 precursor	4504963	WBC	1574.8094	0.0017
DRB1_0101	NGKEVAAQVKAPLVL	AAQVKAPLV	0.615	64.44	WB	Parkinson disease protein 7	31543380	WBC	1536.9108	0.0016
DRB1_0101	WNGQKLVTTVTEIAG	LVTTVTEIA	0.588	86.17	WB	CAP, adenylate cyclase-associated protein 1	55859738	WBC	1616.8642	0.0027
DRB1_0101	FSGEGQSLRKKGRKP	FSGEGQSLR	0.570	105.40	WB	ubiquitin fusion-degradation 1 like protein	1654346	WBC	1674.9398	-0.0279
DRB1_0101	LAENGRLSNTQGVVS	LSNTQGVVS	0.561	115.28	WB	mitochondrial F1 complex, O subunit	54696534	WBC	1544.8027	0.0006
DRB1_0102	LVADENPFAQGALKS	LVADENPFA	0.560	117.33	WB	gelsolin isoform a precursor	4504165	WBC	1559.8064	0.0041
DRB1_0405	RIRSKKNEIMVAPDK	IRSKKNEIM	0.532	157.48	WB	dynein, light chain, roadblock-type 1	56203991	WBC	1785.0163	-0.0006
DRB1_0405	LEGKVLPGVDALSNI	VLPGVDALS	0.531	160.48	WB	phosphoglycerate kinase 1	4505763	WBC	1524.8632	0.0027
DRB1_0421	TVYFSEQWVSSLNER	FSEQWVSSL	0.511	199.16	WB	kinesin-related protein	1155084	WBC	1844.8814	0.0040
DRB1_0701	MLTELEKALNSIIDV	LEKALNSII	0.666	37.05	SB	belonging to S100 family (unnamed protein)	29888	SK-N-AS, WBC	1688.9139	0.0056
DRB5_0101	TAKTEWLDGKHVVFG	TEWLDGKHV	0.469	312.79	WB	peptidylprolyl isomerase A-like, isoform CRA_c	119581485	SK-N-AS, WBC	1687.8802	0.0019
DRB1_0101	IAPAFSSMSNKYPQA	FSSMSNKYP	0.627	56.43	WB	thioredoxin-like 1	4759274	SK-N-AS, WBC	1611.7836	0.0038
DRB1_0101	LVSNLNPERVTPQSL	LVSNLNPER	0.627	56.34	WB	polypyrimidine tract-binding prot. 1 isoform a	4506243	SK-N-AS, WBC	1666.9123	0.0029
DRB1_0408	VKRQFMNKSLSGPGQ	VKRQFMNKS	0.528	164.86	WB	actin related protein 2/3 complex	84626115	SK-N-AS, WBC	1676.8901	0.0031

16 mer MHC class II binding peptides:

HLA - allele	Peptide - Sequence	Core	Prediction Score (1-log50K(aff)	Affinity (nm)	Binding Level	Protein Name	NCBI Accession Number	Lysate	MH^+ Matched (Da)	MH^+ Error (Da)
DRB1_0101	WISKQEYDESGPSIVH	YDESGPSIV	0.486	259.19	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS	874.8919	0.0036
DRB1_0102	VYFTNELKQMQDKYSK	LKQMQDKYS	0.491	247.60	WB	TNF(ligand) superfamily, member 10	4507593	SK-N-AS	022.0001	0.0032
DRB1_0408	FVKRQFMNKSLSGPGQ	VKRQFMNKS	0.552	127.76	WB	actin related protein 2/3 complex	84626115	SK-N-AS	823.9585	0.0015
DRB1_0305	VLSDRAPFAAPSPFAE	VLSDRAPFA	0.463	333.30	WB	Thymidine phosphorylase	17390355	WBC	674.8486	0.0039
DRB1_0405	PAYHSSLMDPDTKLIG	YHSSLMDPD	0.472	300.77	WB	actin related protein 2/3 complex	84626115	WBC	744.8574	0.0028
DRB1_0305	VLSDRAPFAAPLPFAE	VLSDRAPFA	0.488	254.50	WB	TYMP protein	30354553	WBC	700.9006	0.0045
DRB1_0101	ELRPTLNELGISTPEE	LRPTLNELG	0.537	149.22	WB	Cytochrome c oxidase subunit 5A	117098	WBC	797.9229	0.0206
DRB1_0101	LRIRSKKNEIMVAPDK	IRSKKNEIM	0.554	124.57	WB	dynein, light chain, roadblock-type 1	56203991	WBC	898.1004	0.0022
DRB1_0101	EAINYMAADGDFKIKC	INYMAADGD	0.596	78.92	WB	galectin-1	4504981	WBC	788.8295	0.0032
DRB1_0102	TYLISSIPLQGAFNYK	LISSIPLQG	0.665	37.38	SB	prolactin-induced protein	4505821	WBC	814.9687	0.0024
DRB1_0405	LTWQGLIVPDNPPYDK	WQGLIVPDN	0.683	30.70	SB	similar to Ubiquitin-conjugating enzyme E2	194389690	WBC	855.9589	0.0006
DRB1_0101	GASLKPEFVDIINAKQ	FVDIINAKQ	0.455	361.31	WB	triosephosphate isomerase 1, isoform CRA_b	119609128	SK-N-AS, WBC	729.9483	0.0025
DRB1_0102	DIEKAVQSLDKNGVDL	VQSLDKNGV	0.507	206.74	WB	actin related protein 2/3 complex subunit 5	5031593	SK-N-AS, WBC	743.9123	0.0036
DRB1_0102	RPRHQGVMVGMGQKDS	MVGMGQKDS	0.532	158.80	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS, WBC	782.8850	0.0008
DRB1_0101	ISWYDNEFGYSNRVVD	YDNEFGYSN	0.570	104.96	WB	glyceraldehyde-3-phosphate dehydrogenase	31645	SK-N-AS, WBC	963.8821	0.0164
DRB1_0101	LAENGRLSNTQGVVSA	LSNTQGVVS	0.571	103.89	WB	mitochondrial F1 complex, O subunit	54696534	SK-N-AS, WBC	615.8398	0.0013
DRB1_0405	ASPEYVNLPINGNGKQ	YVNLPINGN	0.644	47.32	SB	glutathione S-transferase	2204207	SK-N-AS, Kelly, WBC	700.8602	0.0041
DRB1_1501	GTCQDAAIVGYKDSPS	IVGYKDSPS	0.474	297.21	WB	profilin 1	4826898	SK-N-AS, Kelly, WBC, DPBS	611.7319	0.0018
DRB1_0101	FAGDDAPRAVFPSIVG	FAGDDAPRA	0.559	117.97	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS, Kelly, WBC, DPBS	618.8224	0.0019
DRB1_0408	YVNGLTLGGQKCSVIR	YVNGLTLGG	0.574	100.31	WB	profilin 1	4826898	SK-N-AS, Kelly, WBC, DPBS	707.9211	-0.0013

17 mer MHC class II binding peptides:

HLA - allele	Peptide - Sequence	Core	Prediction Score (1-log50K(aff))	Affinity (nm)	Binding Level	Protein Name	NCBI Accession Number	Lysate	MH^+ Matched (Da)	MH^+ Error (Da)
DRB1_0101	LIKMSSPYMNNLIILGG	LIKMSSPYM	0.735	17.62	SB	GABA B receptor, 2, isoform CRA_a	119579293	Kelly	1864.0071	16.0188
DRB1_0701	IIGNSMGLVNAYSHKFI	LVNAYSHKF	0.704	24.67	SB	hCG22803, isoform CRA_e	119590144	SK-N-AS	1863.9786	0.0023
DRB1_0102	YELPDGQVITIGNERFR	VITIGNERF	0.628	55.75	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS	1874.8919	0.0036
DRB1_0101	KDRSSFYVNGLTLGGQK	YVNGLTLGG	0.621	60.57	WB	profilin 1	4826898	SK-N-AS	1869.9817	0.0001
DRB1_0101	VKLPDGYEFKFPNRLNL	YEFKFPNRL	0.585	89.54	WB	galectin-1	4504981	SK-N-AS	2050.1120	0.0021
DRB1_1321	LPKPPKPVSKMRMATPL	VSKMRMATP	0.451	381.44	WB	CD74/ROS fusion protein	161176974	SK-N-AS	1891.1020	0.0032
DRB1_0701	FVDPWTVQTSSAKGIDY	VQTSSAKGI	0.749	15.15	SB	IFP53	32709	WBC	1913.9280	0.0178
DRB1_0102	AKVAVLGASGGIGQPLS	VAVLGASGG	0.718	21.10	SB	MDH2	49168580	WBC	1524.8744	0.0035
DRB1_0405	LTWQGLIVPDNPPYDKG	WQGLIVPDN	0.672	34.73	SB	Ubiquitin-conjugating enzyme E2, catalytic domain (UBCc)	194389690	WBC	1912.9803	0.0038
DRB1_0306	VFEVSLADLQNDEVAFR	LQNDEVAFR	0.641	48.50	SB	ribosomal protein S3A, isoform CRA_f	119625398	WBC	1951.9760	0.0075
DRB1_0101	VIILNHPGQISAGYAPV	ILNHPGQIS	0.640	49.32	SB	similar to H. sapiens EEF1A1	158258715	WBC	1748.9694	0.0017
DRB1_0301	LVLSDRAPFAAPLPFAE	LVLSDRAPF	0.626	56.92	WB	TYMP protein	30354553	WBC	1813.9847	0.0034
DRB1_0102	VIKMGVAAHKKSHEESH	VIKMGVAAH	0.564	111.96	WB	S100 calcium-binding protein A8	21614544	WBC	1887.9858	0.0020
DRB1_0101	TVQELLPSSFGPEDLQK	VQELLPSSF	0.554	124.69	WB	cytidine deaminase	11386157	WBC	1887.9698	0.0031
DRB1_0101	VNITPAEVGVLVGKDRS	VNITPAEVG	0.453	372.91	WB	profilin 1	4826898	WBC	1753.9807	0.0048
DRB1_0101	ARYASICQQNGIVPIVE	YASICQQNG	0.445	403.47	WB	similar to Fructose-bisphosphate aldolase A	194378468	WBC	1860.9636	0.0019
DRB1_0405	VLALSVETDYTFPLAEK	VLALSVETD	0.455	365.80	WB	ribosomal protein P0 variant	62896495	WBC	1896.0001	0.0069
DRB1_0102	ARYASICQQNGLVPIVE	YASICQQNG	0.454	367.38	WB	similar to Fructose-bisphosphate aldolase A	194378468	WBC	1860.9636	0.0019
DRB1_0102	EQTFGGVNYFFDVEVGR	VNYFFDVEV	0.551	129.01	WB	cystatin S precursor	4503109	Kelly, WBC	1963.9185	0.0037
DRB1_0101	ISWYDNEFGYSNRVVDL	YDNEFGYSN	0.546	135.91	WB	glyceraldehyde-3-phosphate dehydrogenase	31645	Kelly, WBC	2076.9661	-0.0056
DRB1_0101	LLNNDNLLREGAAHAFA	LLREGAAHA	0.708	23.68	SB	F-actin capping protein alpha-1 subunit	5453597	SK-N-AS, WBC	1838.9508	0.0036
DRB1_0101	NGKEVAAQVKAPLVLKD	AAQVKAPLV	0.635	51.72	WB	Parkinson disease protein 7	31543380	SK-N-AS, WBC	1780.0327	0.0016

DRB1_0408	YVNGLTLGGQKCSVIRD	YVNGLTLGG	0.561	115.73	WB	profilin 1	4826898	SK-N-AS, WBC	1822.9480	0.0041
DRB1_0101	ISFKANDIEKAVQSLDK	FKANDIEKA	0.536	151.34	WB	actin related protein 2/3 complex subunit 5	5031593	SK-N-AS, WBC	1906.0280	0.0045
DRB1_0301	DGPVVTDPKAPNVVVTR	VVTDPKAPN	0.506	210.28	WB	Rho GDP dissociation inhibitor (GDI) beta	56676393	SK-N-AS, WBC	1763.9650	0.0013
DRB1_0101	GGDFTRHNGTGGKSIYG	FTRHNGTGG	0.486	261.45	WB	peptidylprolyl isomerase A-like, isoform CRA_c	119581485	SK-N-AS, WBC	1723.8147	0.0009
DRB1_0701	LSEDKKNIILEEGKEIL	IILEEGKEI	0.452	377.83	WB	cofilin 1 (non-muscle)	5031635	SK-N-AS, WBC	1971.1008	-0.0019
DRB1_0405	LASPEYVNLPINGNGKQ	YVNLPINGN	0.633	53.13	WB	glutathione S-transferase	2204207	SK-N-AS, Kelly, WBC	1813.9443	0.0022
DRB1_0301	LVLSDRAPFAAPSPFAE	LVLSDRAPF	0.626	56.92	WB	Thymidine phosphorylase	17390355	SK-N-AS, Kelly, WBC	1787.9327	0.0021
DRB1_0101	TEQREAVFPFQPGSVAE	FPFQPGSVA	0.581	93.24	WB	galectin-1	4504981	SK-N-AS, Kelly, WBC	1891.9185	0.0029
DRB1_0101	IVNTNVPRASVPDGFLS	VNTNVPRAS	0.484	266.65	WB	macrophage migration inhibitory factor	148608029	SK-N-AS, WBC, DPBS	1785.9494	0.0043
DRB1_0101	ISKQEYDESGPSIVHRK	YDESGPSIV	0.485	262.61	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS, Kelly, WBC, DPBS	1973.0087	-0.0053

18 mer MHC class II binding peptides:

HLA - allele	Peptide - Sequence	Core	Prediction Score (1-log50K(aff))	Affinity (nm)	Binding Level	Protein Name	NCBI Accession Number	Lysate	MH^+ Matched (Da)	MH^+ Error (Da)
DRB1_0101	LPKPPKPVSKMRMATPLL	KMRMATPLL	0.600	75.96	WB	CD74/ROS fusion protein	161176974	SK-N-AS	2004.1861	0.0047
DRB1_0102	DAPRAVFPSIVGRPRHQG	FPSIVGRPR	0.526	169.47	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS	1960.0624	0.0002
DRB1_0101	SSKDAIKKKLTGIKHELQ	LTGIKHELQ	0.471	304.57	WB	cofilin 1 (non-muscle)	5031635	SK-N-AS	2024.1863	0.0299
DRB1_0701	ESDDSILRLAKADGIVSK	LRLAKADGI	0.708	23.57	SB	ribosomal protein S21	4506699	WBC	1917.0287	0.0039
DRB1_0102	AKVAVLGASGGIGQPLSL	VAVLGASGG	0.707	23.88	SB	MDH2	49168580	WBC	1637.9585	0.0034
DRB1_0101	VMNFANVFAVLFNGCTGI	FANVFAVLF	0.691	28.25	SB	cation-chloride cotransporter-interacting protein	9502260	WBC	1916.9397	16.0320
DRB5_0101	ETTLVANQLREAASRLKW	LREAASRLK	0.580	94.41	WB	similar to Homo sapiens mannosidase, alpha, class 2B, member 1 (MAN2B1)	158256576	WBC	2086.1404	-0.0004
DRB1_1501	GTCQDAAIVGYKDSPSVW	IVGYKDSPS	0.553	126.65	WB	profilin 1	4826898	WBC	1896.8796	0.0015
DRB1_0408	YVNGLTLGGQKCSVIRDS	YVNGLTLGG	0.550	130.70	WB	profilin 1	4826898	WBC	1909.9800	0.0008
DRB1_0102	RETNLDSLPLVDTHSKRT	LDSLPLVDT	0.506	208.90	WB	vimentin variant	62896523	WBC	2082.0938	-0.0012
DRB5_0101	IRAKRAKEAAEQDVEKKK	IRAKRAKEA	0.492	243.01	WB	BBC1	29383	WBC	2098.2091	0.0093
DRB1_0101	ELLQEFIDSDAAAEAMGK	FIDSDAAAE	0.486	259.81	WB	secretoglobin, family 2A, member 1	4505171	WBC	1937.9161	0.0038
DRB1_0101	VGGASLKPEFVDIINAKQ	GASLKPEFV	0.471	305.53	WB	triosephosphate isomerase 1, isoform CRA_b	119609128	WBC	1886.0382	0.0033
DRB1_0101	ISKQEYDESGPSIVHRKC	YDESGPSIV	0.469	312.38	WB	mutant beta-actin (beta'-actin)	28336	WBC	2076.0179	0.0451
DRB1_0408	PGHLQEGFGCVVTNRFDQ	FGCVVTNRF	0.466	323.60	WB	SERPINE1 mRNA binding protein 1, isoform CRA_e	119626898	WBC	2003.9392	0.0007
DRB1_0101	DKDGNGYISAAELRHVMT	YISAAELRH	0.555	123.42	WB	calmodulin	825635	SK-N-AS, WBC	1976.9495	0.0019
DRB1_0101	AGFAGDDAPRAVFPSIVG	FAGDDAPRA	0.587	86.86	WB	mutant beta-actin (beta'-actin)	28336	SK-N-AS, Kelly, WBC	1746.8810	0.0026
DRB1_0101	VIILNHPGQISAGYAPVL	ILNHPGQIS	0.627	56.46	WB	similar to Homo sapiens eukaryotic translation elongation factor 1 alpha 1 (EEF1A1)	158258715	SK-N-AS, Kelly, WBC, DPBS	1862.0534	0.0033