Chapter

Monospecific and Polyreactive Monoclonal Antibodies against Human Leukocyte Antigen-E: Diagnostic and Therapeutic Relevance

Mepur H. Ravindranath and Fatiha E.L. Hilali

Abstract

A monoclonal antibody (mAb) binds to an antigen recognizing an epitope (a sequence of amino acids). A protein antigen may carry amino acid sequence unique to that antigen as well as sequences found in other proteins. Human leukocyte antigens (HLA), a family of proteins expressed by the Major Histocompatibility Complex gene family represent a special case, in that it displays a high degree of polymorphism. Every HLA molecule possesses both specific (private) epitopes and epitopes shared (public) with other HLA class Ia and class Ib molecules. HLA-E is overexpressed in cancer cells more than any other HLA Class I molecules. Therefore specific localization of HLA-E with mAbs is pivotal for developing targeted therapy against cancer. However, the commercially available mAbs for immunodiagnosis are polyreactive. We have developed anti-HLA-E mAbs and distinguished monospecific from polyreactive mAbs using Luminex multiplex single antigen bead (SAB) assay. HLA-E-binding of monospecific-mAbs was also inhibited by E-restricted epitopes. The amino acid sequences in the region of the epitopes bind to CD94/NKG2A receptors on CD8+ T cells and NK cells and block their antitumor functions. Monospecific-HLA-E mAbs recognizing the epitopes sequences can interfere with the binding to restore the anti-tumor efficacy of NK cells. Also, monospecific-mAbs augment the proliferation of CD4-/CD+ cytotoxic T-lymphocytes. Therefore, anti-HLA-E monospecific-mAb can serve as a double-edged sword for eliminating tumor cells.

Keywords: human leukocyte antigen (HLA), epitope, monospecific, polyreactive, cytotoxic T-lymphocytes, inhibitory receptors, NK cells

1. Introduction

An in-depth understanding of amino acid sequences and conformations of primary antigens recognized by any monoclonal antibody (mAb) is a necessary prerequisite for clarifying the specificity and functional limitations of a mAb. A protein antigen may be glycosylated or can occur as a monomer or a dimer or a trimer.

In this regard, human leukocyte antigen (HLA) classes are a structurally identical complex family of glycosylated homo- or hetero-dimeric proteins. They are expressed on cell surface complexed with an exogenous or endogenous peptide, as trimers. Defining the monospecificity of mAb raised against one family member of HLA is challenging. Often anti-HLA mAbs are polyreactive in that they bind to sequences common to all family member antigens, which are also known as "public epitopes". It is difficult to identify mAbs binding to unique sequences or private epitopes. Identifying such monospecific mAbs are critical for defining specific functions of antigens. Although sensitive and specific assay protocols are available to define the monospecificity of mAbs, many commercial mAbs, apparently specific for a unique HLA antigen, remain without defining their monospecificity. This review aims to distinguish monospecific mAbs that recognize private epitopes from polyreactive mAbs that bind to public epitopes of one of the HLA class Ib molecules, namely HLA-E, commonly overexpressed on human cancers. A pool of mouse mAbs was developed at Terasaki Foundation Laboratory (TFL) after immunizing with HLA-E. After validating the monospecificity of anti-HLA-E mAbs, their diagnostic and therapeutic potentials have been evaluated. These include (i) immunolocalization of cell surface expression HLA-E on human cancers, (ii) upregulation of CD8+ cytotoxic T lymphocytes, and (iii) restoration of antitumor activity of CD8+ T cells, NKT cells, and NK cells by preventing binding of HLA-E expressed on cancer cells to the inhibitory receptors (CD94/NKG2A) on the immune cells.

2. Nature and characteristics of human leukocyte antigens

Human Leukocyte antigens (HLA) are a subgroup of the Major Histocompatibility Complex (MHC) gene family. The genes that encode the HLA class-I and class-II antigens are located on the short arm of human chromosome 6 [1]. Three constituent regions of the HLA gene complex are illustrated in **Figure 1**. Class, I genes are those encoding the heavy chains (HC) or α chains, of the six class I isoforms HLA-A, -B, -C, -E, -F, and -G. Extensive polymorphism of the glycosylated heavy chains of these HLA molecules are presented in **Table 1**. We carry a pair of alleles that represent each isoform derived from their mother and father (**Table 2**). Understanding HLA profiles of a patient is necessary when administering mAbs targeting a particular HLA molecule, for amino acid sequences of target HLA may cross-react with other HLA alleles of the patient. Native HLA-I

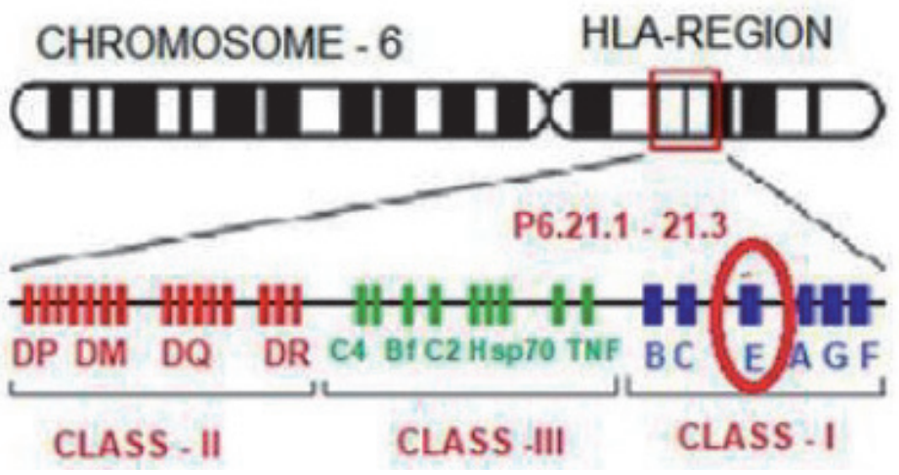

Figure 1.
Profile of the HLA gene complex on chromosome 6. All regions contain additional genes.

HLA Class I						
Gene	***A***	***B***	***C***	***E***	***F***	***G***
Alleles	**6,291**	**7,582**	**6,223**	**256**	**45**	**82**
Proteins	**3,896**	**4,803**	**3,681**	**110**	**6**	**22**

Table 1.
Numbers of HLA alleles (as of September 2020) and their proteins. See updated information at https://www.ebi.ac.uk/ipd/imgt/hla/stats.html.

PROFILES OF HLA TYPING: HLA ISOFORMS AND THEIR ALLELES					
HLA CLASS	**ISOFORMS**	**BROTHER***		**SISTER**	
I	A*	**[11:02]**	[33:01]	[01:01]	**[11:02]**
I	B*	[15:01]	[58:01]	[40:01]	[57:01]
I	C*	[15:02]	[15:02]	[03:04]	[06:02]
II	DRB1	[04:03]	[13:02]	[07:01]	[11:01]
II	DRB3,4,5	[3*03:01]	**[4*01:01]**	[3*02:02]	**[4*01:01]**
II	DQA	**[01:02]**	**[03:01]**	**[01:02]**	**[03:01]**
II	DQB	[03:01]	[06:09]	[02:02]	**[03:01]**
II	DPA	**[01:03]**	**[01:03]**	**[01:03]**	**[02:01]**
II	DPB	[02:01]	[03:01]	[01:07]	[01:11]

**Mepur H. Ravindranath (brother) and his first sister.*
The alleles in bold letters refer to alleles shared by the brother and the sister.

Table 2.
Pair of HLA alleles representing each of the commonly typed HLA isoforms.

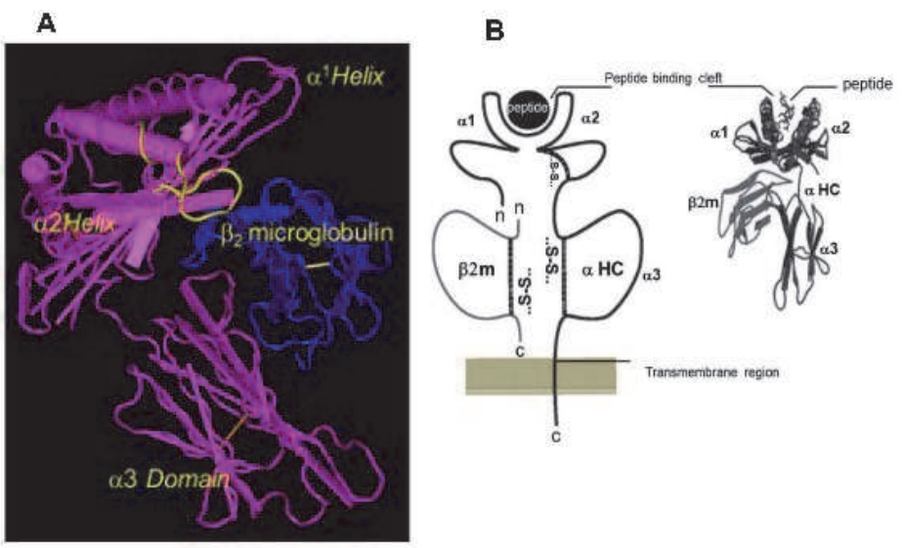

Figure 2.
(A) Conformational structure of HLA class I. the native HLA-I proteins are expressed on the cell surface as hetero-dimers, the heavy chain in combination with β2-microglobulin (β2-m). (B) the hetero-dimer on the cell surface may carry a short peptide to generate trimeric structure, designated as "closed conformer"(CC).

proteins are expressed on the cell surface as hetero-dimers, in combination with β2-microglobulin (β2-m) (**Figure 2A**). The gene encoding β2-m is situated on human chromosome 15. The hetero-dimers may also carry a peptide to form a trimer (**Figure 2B**), which is designated as "Closed Conformers (CCs)" [2]. Under the influence of cytokines (e.g. IFN- ɣ) and other activating factors (e.g. T-cell

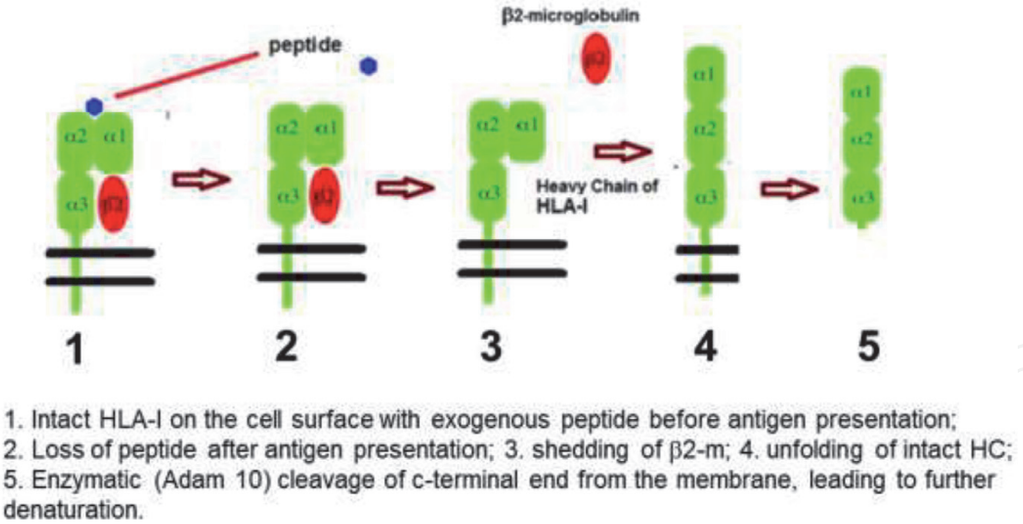

Figure 3.
The fate of HLA-I molecule after antigen presentation.

antibodies) or during inflammation, infection and tumorigenesis, the surface of metabolically active cells express only monomeric HLA heavy chains, called "Open Conformers (OCs) [3]. The examples include human T-lymphocytes activated *in vitro* and *in vivo,* as well as by EBV-transformed B-cells, CD19+ B-cells, CD8+ T cells, CD56+ NK-cells, CD14+ monocytes, extravillous trophoblasts and monocytes, dendritic cells (DCs), B-cell lines (RAJI, NALM6), and the myeloid cell line (KG-1A) [4–12]. The kinetics of conformational alterations in the naturally-occurring HLA-I OCs after activation has been investigated in healthy human T-cells [11]. The cytoplasmic c-terminal tail of naturally-occurring HLA-I OCs is tyrosine phosphorylated and plays a role in signal transduction [11].

HLA-I on antigen-presenting cells presents endogenous (intracellular) peptides. Importantly, viral peptides that have been broken by the proteasome are transferred to the endoplasmic reticulum (ER) via transporters (TAP). In ER, peptides are processed with OCs of HLA-I and exported to the cell surface as a trimer for presentation to T-cell receptors of CD8+ T-cells. This strategy kills the cell, thus preventing viral replication. After antigen presentation, the HLA-I is degraded (**Figure 3**). Ultimately, such degradation results in exposing the cryptic epitopes on the OCs to an individual's own immune system. Antibodies formed against the cryptic epitopes eliminate the degraded HLA from the circulation. The antibody-producing cells may remain hidden and silent for long periods. They are referred to as "long-lived B cells" [13]. Evidently, anti-HLA antibodies occur in normal and healthy individuals [14–16], as well as in the pooled and purified plasma also known as intravenous immunoglobulin (IVIg) [16, 17].

3. Diagnostic and clinical relevance of non-classical HLA class Ib antigens

Unlike classical HLA-Ia (HLA-A, HLA-B & HLA-C), non-classical HLA-Ib (HLA-E, HLA-F & HLA-G) genes and molecules are oligotrophic, with restricted and selective tissue distribution [18–20]. HLA-Ib molecules are expressed in a diverse array of cells including T and B lymphocytes, Natural Killer Cells, monocytes, macrophages, megakaryocytes, and organs i.e., lymph nodes, spleen, skin, salivary glands, thyroid, stomach, liver, kidney, urinary bladder, endometrial, and

trophoblasts. Their overexpression is reported on activated T cells bone marrow cells inflamed cells and tissues (e.g. synovial fibroblasts), tumor cells [21–24].

The HLA-Ib molecules are capable of interacting with cell-surface receptors present on specific immune-cell subsets, inducing activation or inhibition of signaling cascades within such specific immune cells as NK cells, macrophages, and dendritic cells [25–27]. Their interaction with different immunomodulatory (activating and/or inhibiting) cell-surface receptors on NK cells and macrophages signify their role in innate immunity; these receptors include CD94/NKG2, Ig-like transcript 2 (ILT2), Ig-like transcript 4 (ILT4), KIR2DL4, and CD160. These interactions are a component of innate immunity [27]; e.g., HLA-Ib is expressed during pregnancy, playing a major role in tolerance shown towards the fetus and placenta [28–34]. HLA-Ib molecules also generate a pool of antibodies *in vivo*, which may include monospecific or polyreactive (cross-reactive with other HLA-I molecule [16, 35–39]. Soluble HLA-Ib is also found in the synovial fluid and the circulation of healthy and in cancer patients [40–42].

4. Human leukocyte antigen-E (HLA-E)

4.1 Unique characteristics of HLA-E

Although several alleles of HLA-E (**Table 1**) exist, only two are extensively distributed among different ethnic groups [43]. The alleles differ by a single amino acid at position 107 [44–46]; Arginine in HLA-E^{R107} (HLAE*01:01) is replaced by glycine in HLA-E^{G107} (HLA-E*03:01) [45]. Such amino acid substitution influence thermal stability, which results in a more stable expression of cell surface HLA-E*01:03 compared to HLA-E*01:01 [44], including half-life of the molecule. HLA-E*01:01 and HLA-E*03:01 bind to different restricted sets of peptides.

HLA-E present peptides derived from HLA-Ia signal sequences (leader peptides), heat-shock protein (Hsp-60), human cytomegalovirus, Hepatitis C virus, Human Immunodeficiency Virus, Epstein Barr virus, Influenza virus, *Salmonella enteric* and *Mycobacterium* glycoproteins to T-lymphocytes [46–49]. The binding of HLA-E to the leader peptides of HLA-Ia stabilizes the HLA-E and enables migration to the cell surface [49]. When HLA-E does not reach the cell surface of a tumor cell, the cell is susceptible to lysis by NK cells. The crystallographic analyses of HLA-E structure reveals the molecular mechanisms underlying this function of HLA-E [24]. Importantly, tumor-associated HLA-E can be shed into the tumor microenvironment and circulation as soluble HLA-E (sHLA-E) [23, 50–56].

4.2 HLA-E expression on cancer cells using mAb-based diagnostic assays: Limitations and reliability

The literature (**Table 3**) on HLA-E expression on human cancers based on the commercially available diagnostic anti-HLA-E mAbs tests, reveals that none of the diagnostic mAbs were tested for their unique or monospecificity for HLA-E. If the mAb is not specific for the unique epitopes of antigen and if it binds to public epitopes or epitopes shared by a family of antigens, then data is unjustified to conclude the expression HLA-E. Principally this criterion is valid for any diagnostic or therapeutic antibody. We have undertaken efforts to examine, using Luminex multiplex SAB assay, the specificity of commercial anti-HLA-E mAbs employed in the 47 clinical studies (**Table 3**). Summary of the results [16, 21, 35–39, 96–98] is

NATURE OF HUMAN CANCER	COMMERCIAL mAbs	REFERENCES
Melanoma Cervical Cancer	3D12	Marín R et al. Immunogenetics. 54(11):767–75.2003 [57]
Melanoma	MEM-E/02	Derré L et al. J Immunol. 177:3100–7. 2006. [22]
Melanoma and other cancers	MEM-E/07	Allard M et al. PLoS One 6(6):e21118, 2011 [55]
	MEM-E/08	
Lip squamousal cell carcinoma	MEM-E/02	Goncalves et al. Human Immunol. 77(9): 785–790, 2016 [58]
Laryngeal carcinoma	MEM-E/02	Silva TG et al. Histol Histopathol. 26:1487–97. 2011 [59]
Vulvar intraepithelial carcinoma	MEM-E/02	van Esch EM et al. Int J Cancer. 135(4): 830–42, 2014 [60]
Penile Cancer	MEM-E/02	Djajadiningrat et al. J Urol. 193(4):1245–51. 2015. [61]
Glioblastomas	MEM-E/02	Mittelbronn, M. et al., J. Neuroimmunol. 189: 50–58. 2007 [62]
Glioblastomas	MEM-E/02	Kren L et al. J Neuroimmunol. 220:131–5. 2010 [63]
Glioblastomas	MEM-E/02	Kren L et al. Neuropathology. 31: 129–34. 2011 [64]
Glioblastomas stem cells	3D12	Wolpert et al. J Neuroimmunol. 250(1–2):27–34 2012 [65]
Glioblastomas	3D12	Wischhusen J et al. J Neuropathol Exp Neurol. 64:523–8. 2005 [66]
Neuroblastoma	3H2679	Zhen et al. Oncotarget. 7(28): 44340–44349, 2016. [67]
Neuroblastoma	3D12	Morandi et al. J Immunol Res. 2016:7465741, 2016. [53]
Oral Osteosarcoma	MEM-E/02	Costa Arantes et al. Oral Surg Oral Med Oral Pathol Oral Radiol. 123(6):e188-e196. 2017. [68]
Intraoral mucoepidermoid carcinoma	MEM-E/02	Mosconi C Arch Oral Biol. 83:55–62, 2017. [69]
Rectal Cancer	MEM-E/02	Reimers et al. BMC Cancer BMC Cancer. 14:486.1–12, 2014. [70]
Colorectal carcinoma	MEM-E/08	Levy et al. Int J Oncol. 32(3): 633–41. 2008 [71]
Colorectal carcinoma	MEM-E/08	Levy et al. Innate Immun. 15(2):91–100. 2009. [72]
Colorectal carcinoma	MEM-E/02	Benevolo M, et al. J Transl Med. 9:184. 2011. [73]
Colorectal carcinoma	MEM-E/02?	Bossard C et al. Int J Cancer. 131 (4): 855–863. 2012. [67]
Colorectal carcinoma	MEM-E/02?	Zhen et al., Med Oncol. 30(1):482. 2013. [74]
Colorectal carcinoma	MEM-E/02	Zeestraten et al. Br J Cancer. 110(2):459–68. 2014. [75]
Colorectal carcinoma	MEM-E/02	Guo et al. Cell Immunol. 293(1):10–6, 2015. [76]
Colorectal carcinoma	3H2679	Ozgul Ozdemir et al. Ann Diagn Pathol. 25:60–63, 2016 [77]
Colorectal carcinoma	MEM-E/02	Huang et al. Oncol Lett. 13(5):3379–3386, 2017. [78]
Colon carcinoma and leukemia (K562)	MEM-E/06	Stangl S et al. Cell Stress Chaperones. 13(2):221–30. 2008. [79]
Colon carcinoma	MEM-E/02	Zeestraten EC et al. Br J Cancer. 110(2): 459–68.2014. [75]
Hepatocellular carcinoma	MEM-E/02	Chen et al. Neoplasma. 58(5):371–376, 2011. [80]
Non-small cell Lung Carcinoma	MEM-E/02	Talebian-Yazdi et al. Oncotarget. 7(3):3477–3488, 2016. [81]

NATURE OF HUMAN CANCER	COMMERCIAL mAbs	REFERENCES
Breast cancer	MEM-E/02	de Kruijf EM et al. J Immunol. 185:7452, 2010 [82]
Breast cancer	MEM-E/02	da Silva et al. Int J Breast Cancer. 2013:250435. 2013. [83]
Ovarian cancer/ Cervical cancer	MEM-E/02	Gooden M et al.PNAS USA 108:10656, 2011. [84]
Cervical cancer	MEM-E/02	Gonçalves MA et al. Eur J Obstet Gynecol Reprod Biol. 141:70–4. 2008. [85]
Cervical cancer	MEM-E/02	Spaans VM et al., J Transl Med. 10:184. 2012. [86]
Cervical squamous and adenocarcinoma	MEM-E/02	Ferns et al. J Immunother Cancer. 4:78, 2016. [87]
Serous Ovarian Adenocarcinoma	MEM-E/02	Andersson et al. Oncoimmunology, 25;5(1):e1052213, 2015. [88]
Serous Ovarian Adenocarcinoma	MEM-E/02	Zheng et al. Cancer Sci. 106(5): 522–528, 2015. [89]
Renal Cell Carcinoma	MEM-E/02	Hanak L et al. Med Sci Monit. 15(12):CR638–43.2009. [90]
Renal Cell Carcinoma	MEM-E/02	Kren L et al., Diagnostic Pathology, 7:58, 2012 [91]
Thyroid cancer	MEM-E/02	Zanetti et al. Int J Immunopathol Pharmacol. 26(4):889–96, 2013. [92]
Hodgkin Lymphoma	MEM-E/02	Kren L, et al., Pathology, Research and Practice 208: 45–49, 2012. [93]
Chronic Lymphocytic Leukemia	3D12	McWilliams et al., Oncoimmunology. 5(10):e1226720, 2016. [94]
Chronic Lymphocytic Leukemia	3D12	Wagner et al. Cancer, 23(5):814–823, 2017. [52]
Many Cancers	3D12	Sensi M, et al. Int Immunol. 21(3):257–268. 2009. [95]

Table 3.
Expression of HLA-E on human cancer cells (biopsies or cell lines) monitored with commercial mouse anti-HLA-E mAbs (MEM-E/02, MEM-E/06, MEME/07. MEM-E/08, 3D12, 3H2679).

presented in **Figure 4** show that the commercial anti-HLA-E mAbs react with HLA-A, HLA-B and HLA-C in the following order: MEM-06 > MEM-02 > MEM-07 > MEM >08 >> > 3D12. That the mAbs are recognizing the epitopes shared with several HLA-Ia (HLA-A, HLA-B, HLA-C) antigens confirms that none of the above mAbs are specific for HLA-E. Therefore conclusions concerning the expression of HLA-E in human cancers require further validation with monospecific anti-HLA-E mAbs.

5. Anti-HLA-E mAbs: Characteristics, diagnostic and therapeutic potentials

5.1 The technology that clarifies monospecificity or polyreactivity of a mAb of MHC

Luminex multiplex assays are based on xMAP (Multi-Analyte Profiling) technology that enables simultaneous detection and quantitation of antibodies reacting to multiple proteins simultaneously, using detection mAbs [16, 17, 21, 35–39, 96–98]. The results are comparable to assays such as ELISA but with greater specificity, sensitivity and resolution. The technology employs superparamagnetic 6.5-micron microspheres with a magnetic core and polystyrene surface. The beads are

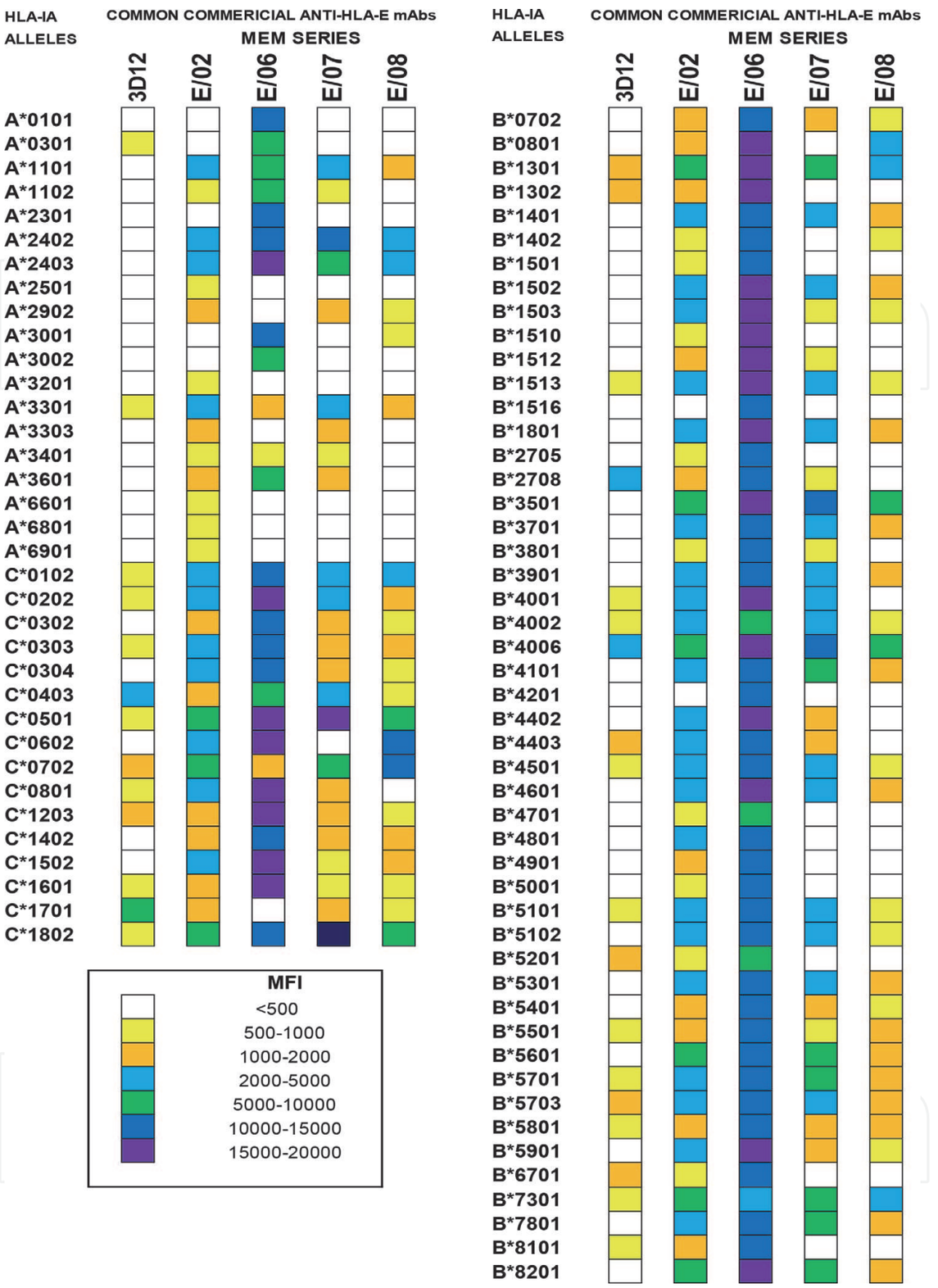

Figure 4.
HLA-IA-polyreactivity of the commercial anti-HLA-E mAbs indicates that these mAbs cannot be considered monospecific or specific for HLA-E. The mAbs were tested at a dilution of 1/300. These mAbs were used to conclude on the expression of HLA-E on human cancers.

internally dyed with precise proportions of red and infrared fluorophores. The Luminex xMAP detection systems identifies differing proportions of the red and infrared fluorophores that result in 100 unique spectral signature microspheres. The antigens are individually attached to polystyrene microspheres by a process of simple chemical coupling. The conjugation of a mAb to one or more of the antigen-coated beads allows it to be evaluated for the mono- or polyreactivity of mAb

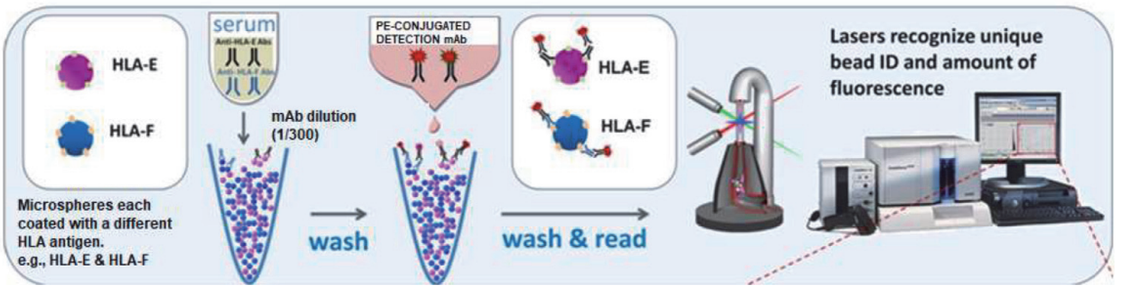

Figure 5.
Luminex single antigen bead assay is used to determine the monospecificity or polyreactivity of the mAbs as well as to determine the strength of the antibodies measured as mean fluorescent intensity (MFI) at specified dilution. The assay is also used to measure the antibody strength titrimetrically. Using peptide inhibition assay epitope affinity or specificity of a mAb can be studied to determine monospecificity or polyreactivity of the mAb. Using a mAb (e.g., HLA-I mAb, TFL-006) recognizing the most commonly shared epitope of an HLA-I (or HLA-II) in an open conformer, the commercial beads can be distinguished as those containing open conformers or closed conformers.

[96–98]. **Figure 5** illustrates the SAB Assay used for determining the monospecificity or polyreactivity of mAbs as well as evaluating the strength of the antibodies measured as mean fluorescent intensity (MFI) at specified dilution. The assay is also used to measure antibody specificity by peptide inhibition assays, to define the epitope-specificity of a mAb. Commercial HLA class I or II beadsets are commercially available as LABScreen (One Lambda Inc., now merged with Thermofisher Inc) and LIFECODES (Immucore Inc)]. The both beadsets together is useful to distinguish CCs from OCs of HLA-I molecules, using a mAb (HLA-I mAb, TFL-006) (See **Table 7** in [99]).

5.2 Development of mAbs against HLA-E

Following guidelines of the National Research Council's Committee on Methods of Producing Monoclonal Antibodies [35, 98, 100], 235 anti-HLA-E mAbs were generated immunizing mice with recombinant HCs of HLA-E^{R107} (Immune Monitoring Lab, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA) (10 mg/ml in MES buffer). In a separate mouse model, HLA-E^{G107} (heavy-chain only) was used as an immunogen. The β2m-free HC of HLA-E (50 μM in 100 mL of PBS (pH 7.4) mixed with 100 mL of TiterMaxVR Gold adjuvant (CytRx, San Diego, CA) were injected into the mouse footpad and intraperitoneum. Three immunizations were given at 12-day intervals. The B cell clones were cultured in RPMI 1640 medium w/L-glutamine and sodium bicarbonate (Sigma-Aldrich, St. Louis, MO, cat. no. R8758), 15% fetal calf serum, 0.29 mg/ml L-glutamine, Pen-Strep (Gemini-Bio, MEd Supply Partners, Atlanta, GA, cat. no. 400–110) and 1 mM sodium pyruvate (Sigma, cat. no. S8636). Several clones were grown using Hybridoma Fusion and Cloning Supplement (HFCS) (Roche Applied Science, Indianapolis, IN, cat. no. 11363735001). The purified-mAbs from HLA-E hybridoma culture supernatants and ascites of hybridoma immunized in BALB/c mice were examined for HLA-I reactivity using Luminex SAB Assay.

5.3 Characterizing the diversity of anti-HLA-E mAbs using single antigen bead (SAB) assay

The HLA-I reactivity of the mAbs was examined by their dose-dependent binding to microbeads coated with 31 HLA-A, 50 HLA-B, and 16 HLA-C antigens and with recombinant single alleles of HLA-E, -F, and -G [35, 98, 100]. The HLA-Ia microbeads have built-in control beads: positive beads coated with human IgG and negative beads coated with serum albumin (human or bovine). For HLA-Ib, the control beads

(both positive and negative) were added separately. PE-conjugated anti-human IgG-detection mAbs were used for immunolocalization of mAb bound to HLA antigens coated on beads [35–37, 96–100]. **Table 4** summarizes the diverse types of mAbs observed after immunizing with heavy chains of HLA-E. Group 1 consists of mAbs that are only bound to HLA-E. Anti-HLA-E mAbs were also characterized for their IgG subclasses, using monoclonal IgG specific for the Fc portion of the subclasses

Fluorophore intensity was measured in a specialized flow cytometer (Luminex) together with microbead identifiers, and the fluorescence measurement classified by the bead identifier. Fluorescent intensity generated by Luminex Multiplex Flow Cytometry (LABScan 100) was analyzed using the same computer software and protocols. For each analysis, at least 100 beads were counted. The "trimmed mean" is obtained by trimming a percentage of the high and low ends of distribution and finding the mean of the remaining distribution. Trimmed mean fluorescence intensity (MFI) for the SAB reactions are obtained from output (CSV) file generated by flow analyzer, and it was adjusted for background signal using the formula (sample #N bead – sample negative control bead) [35–37, 96–100]. The MFI was compared with the negative control mean and the standard deviation of MFI recorded. The purpose of MFI is to define the affinity of mAbs to HLAs and the intensity or strength of the mAbs.

5.4 The diversity anti-HLA-E mAbs

Of the 235 hybidomas generated, mAbs secreted by 214 hybridomas were reactive to HLA-E. These mAbs included both monospecific [35, 98] and polyreactive (with other HLA-Ia and HLA-Ib molecules) [98, 101]. **Table 5**, **A** presents category 1 correspond to monospecific mAbs reacting restrictively to mAbs with HLA-E and failing to recognize HLA-F, HLA-G, HLA-A, HLA-B, and HLA-C. Category 2 refers to HLA-Ib specific anti-HLA-E mAbs (**Table 5**, **B**). Category 3 presents anti-HLA-E mAbs reactive with several HLA-Ia molecules (HLA-A, HLA-B, and HLA-C) but not reactive to HLA-F and HLA-G (**Table 5**, **C**). Category 4 presents mAbs recognizing both HLA-Ib and HLA-Ia molecules (**Table 5**, **D**).

mAbs formed after immunizing HLA-E							
	HLA Class Ia			**HLA Class Ib**			
	HLA-A	**HLA-B**	**HLA-C**	**HLA-E**	**HLA-F**	**HLA-G**	
Group 1	(−)	(−)	(−)	(+)	(−)	(−)	24 TFL-monospecific anti-HLA-E mAbs
Group 2	(−)	(−)	(−)	(+)	(+)	(−)	TFL-anti-HLA-E/F mAbs
Group 3	(−)	(−)	(−)	(+)	(−)	(+)	TFL-anti-HLA-E/G mAbs
Group 4	(−)	(−)	(−)	(+)	(+)	(+)	TFL-anti-HLA-Ib sepecific mAbs
Group 5	(+)	(+)	(+)	(+)	(−)	(−)	Reactivity of the mAbs 3D12, MEM-E/02 & MEM-E/07 & TFL series
Group 6	(+)	(+)	(+)	(+)	(+)	(−)	Reactivity of the mAb MEM-E/06 & TFL-series
Group 7	(+)	(+)	(+)	(+)	(−)	(+)	Reactivity of the mAb MEM-E/08 & TFL series
Group 8	(+)	(+)	(+)	(+)	(+)	(+)	Reactivity of the mAb TFL-006, TFL-007 & other TFL mAbs

Table 4.
The diverse HLA-E monospecific and polyreactive mAbs generated after immunizing mice with a recombinant heavy chain of HLA-E^{R107} & HLA-E^{G107}.

Nature of mAbs	mAb specificity	number of mAbs	Examples of TFL mAbs	Antigen (heavy chain only) tested on beads	Subclass	HLA-E	HLA-F	HLA-G	HLA-A	HLA-B	HLA-C
						Reactivity in MFI					
A		**Antigen immunized: β2-microglobulin-free heavy chain of HLA-E^{R107}**									
HLA-E Monospecific mAbs (Category 1)	**HLA-E**	16	TFL-145, TFL-33, TFL, 34, TFL-73, TFL-74	HLA-E^{R}	IgG1	4 K–22 K	0	0	0	0	0
		3	TFL-001	HLA-E^{R}	IgG2a	0.9 K - 4 K	0	0	0	0	0
			TFL-016								
			TFL-013								
		Antigen immunized: β2-microglobulin-free heavy chain of HLA-E^{G107}									
		5	TFL-185	HLA-E^{G}	IgG1	19 K	0	0	0	0	0
			TFL-184								
			TFL-186								
			TFL-226								
			TFL-254								
B		**Antigen immunized: β2-microglobulin-free heavy chain of HLA-E^{R}107**									
HLA-IB polyreactive and HLA-IA and non-reactive HLA-E mAbs (Category 2)	**HLA-Ib specific mAbs**	1	TFL-050	HLA-E^{R}	IgG2b	4 K	3 K	2 K	0	0	0
		Antigen immunized: β2-microglobulin-free heavy chain of HLA-E^{G}107									
		3	TFL-208, TFL-209, TFL-223,	HLA-E^{G} HLA-E^{R}	IgG1	21 K	8 K	20 K	0	0	0
		4	TFL-164	HLA-E^{G}	IgG2b	14 K–15 K	8 K–9 K	24 K–25 K	0	0	0
			TFL-165								
			TFL-162								
			TFL-161								
	E + G+	1	TFL-191	HLA-E^{G}	NK	1 K	0	1 K	0	0	0
	E + F+	1	TFL-228	HLA-E^{G}	IgG1	19 K	1 K	0	0	0	0

Nature of mAbs	mAb specificity	number of mAbs	Examples of TFL mAbs	Antigen (heavy chain only) tested on beads	Subclass	HLA-E	HLA-F	HLA-G	HLA-A	HLA-B	HLA-C
						Reactivity in MFI					
C	**Antigen immunized: β2-microglobulin-free heavy chain of HLA-E^{R}107**										
HLA-IA Polyreactive HLA-E mAbs (Categroy 3)	**E + B + C+**	31	TFL-059	HLA-E^{G} HLA-E^{R}	IgG1 (n = 12) IgG2A (n = 9) IgG2b (n = 9) IgG3 (n = 1)	8 K–20 K	0	0	0	1 K–17 K	1 K–7 K
			TFL-143								
			TFL-158								
			TFL-076								
			TFL-159								
	E + A + B + C+	68	TFL-119	HLA-E^{G} HLA-E^{R}	IgG1 (n = 27) IgG2A (n = 23) IgG2b (n = 17) IgG3 (n = 1)	11 k–22 k	0	0	1 K–4 K	1 K–24 K	1 K–13 K
			TFL-142								
			TFL-153								
			TFL-118								
			TFL-133								
			TFL-141								
			TFL-095								
	E^{G} + B+	3	TFL-173	HLA-E^{G}	IgG1	12 K	0	0	0	1 K	0
			TFL-174								
			TFL-175								
	E + B+	1	TFL-219	HLA-E$^{G/R}$	IgG1	21	0	0	0	2 K	0
	E^{G} + A + B + C+	6	TFL-167	HLA-E^{G}	IgG1	15 K-25	0	0	1 K–9 K	1 K 20 K	1 K–20 K
			TFL-170								
			TFL-169								
			TFL-166								

Nature of mAbs	mAb specificity	number of mAbs	Examples of TFL mAbs	Antigen (heavy chain only) tested on beads	Subclass	HLA-E		HLA-F	HLA-G	HLA-A	HLA-B	HLA-C
						Reactivity in MFI						
			TFL-168									
			TFL-205									
	E + A + B + C+	35	TFL-243	HLA-EG/R	IgG1 (n = 22) IgG2A (n = 6) IgG2b (n = 6) IgG3 (n = 1?)	13 K–26 K		0	0	1 K–9 K	1 K–24 K	1 K–20 K
			TFL-246									
			TFL-244									
			TFL-245									
			TFL-172									
			TFL-171									

Nature of mAbs	Immunogen used	mAb specificity	number of mAbs	Examples of TFL mAbs	Subclass	HLA-E		HLA-F	HLA-G	HLA-A	HLA-B	HLA-C
						Reactivity in MFI						
						E^{R107}	E^{G107}					
D. Category 4. HLA- IA and IB polyreactive anti-HLA-E mAbs. (n = 36)												
HLA = IA Polyreactive HLA-IB mAbs (Category 4)	**$HLA\text{-}E^{G}$**	**E+/F+/G+**	4	TFL-232	IgG3	13–22	21	2 to 10	11 to 21	1 to 13	1 to 20	1 to 20
				TFL-177	IgG1 (n = 3)		0					
				TFL-176								
				TFL-198					0			
		E+/G+	16	TFL-236	IgG1 (n = 14)	18–22 (n = 13)	0	0	18–22 (n = 11)	1 to 9	1 to 25	1 to 24
				TFL-238								
				TFL-256	IgG3	22	27		1			
				TFL-229	IgG2b	30	22		18			
		E+/F+	10	TFL-210	IgG1 (n = 10)	18–21	17–19	5 to 11	0	1 to 15	1 to 20	1 to 22

Nature of mAbs	mAb specificity	number of mAbs	Examples of TFL mAbs	Antigen (heavy chain only) tested on beads	Subclass	HLA-E	HLA-F	HLA-G	HLA-A	HLA-B	HLA-C
						Reactivity in MFI					
				TFL-211							
				TFL-212							
				TFL-235							
	HLA-E^{R}	**E+/F+/G+**	3	TFL-049	IgG2b	15–22	8 To 12	2 to 7	1 to 10	1 to 10	1 to 17
				TFL-006	IgG2a (n = 2)						
				TFL-007							
		E+/G+	2	TFL-103	IgG1 (n = 2)	17,18	0	4	1 to 6	1 to 11	1 to 11
				TFL-104							
		E+/F+	1	TFL-063	IgG2b	22	3	0	2-Jan	1 tp 7	3 to 8

mAbs in Bold are highly polyreactive,

Table 5.
Different categories of mAbs (n = 212) formed after immunizing mice with HLA-E open conformer (β2-microglobulin-free heavy chain) of HLA-E^{R107} or HLA-E^{G107}.

5.5 Unique (private) and common (public) epitopes of HLA-E

The international immunogenetics project (http://www.ebi.ac.uk; or http://www.ebi.ac.uk/ipd/imgt/hla/intro.html) updates HLA genes and sequence alleles yearly. We have compared the entire amino acid sequences of HLA-E (**Figure 6**) with 511 alleles of HLA-A, 846 alleles of HLA-B, 275 alleles of HLA-C, 2 alleles of HLA-F, and 2 alleles of HLA-G sequences(see **Table 1**). Amino acid sequences unique to HLA-E (private epitopes) and common amino acid sequences (public epitopes) can be identified by comparing the amino acid sequences of HLA-E with thousands of HLA-Ia and Ib antigens (**Table 6**). Anti-HLA-E mAbs could bind to HLA-E restricted (monospecific) or HLA-I amino acid sequences. Several HLA-E sequences are shared with HLA-A loci or HLA-C loci or specific alleles such as A*3306 or B*8201. **Table 7** shows HLA-E restricted amino acid sequences found in α1 and α2 helices, which were used for peptide inhibition assays. **Figure 7A** illustrates locations of private and public epitopes. **Figure 7B** shows allele-specific amino acid sequences in α1 & α2 helical groove and **Figure 7C** shows shared peptide amino acid sequences.

Peptide inhibition analyses were performed to confirm the monospecificity of HLA-E mAbs. Various concentrations of HLA-E-restricted peptides (serially diluted from the initial concentration of 100 μL to 100 μL) were added to the mAbs (7 μL). The mAbs were further diluted with 14 μL PBS-BSA (pH 7.0; final dilution 1/1200),

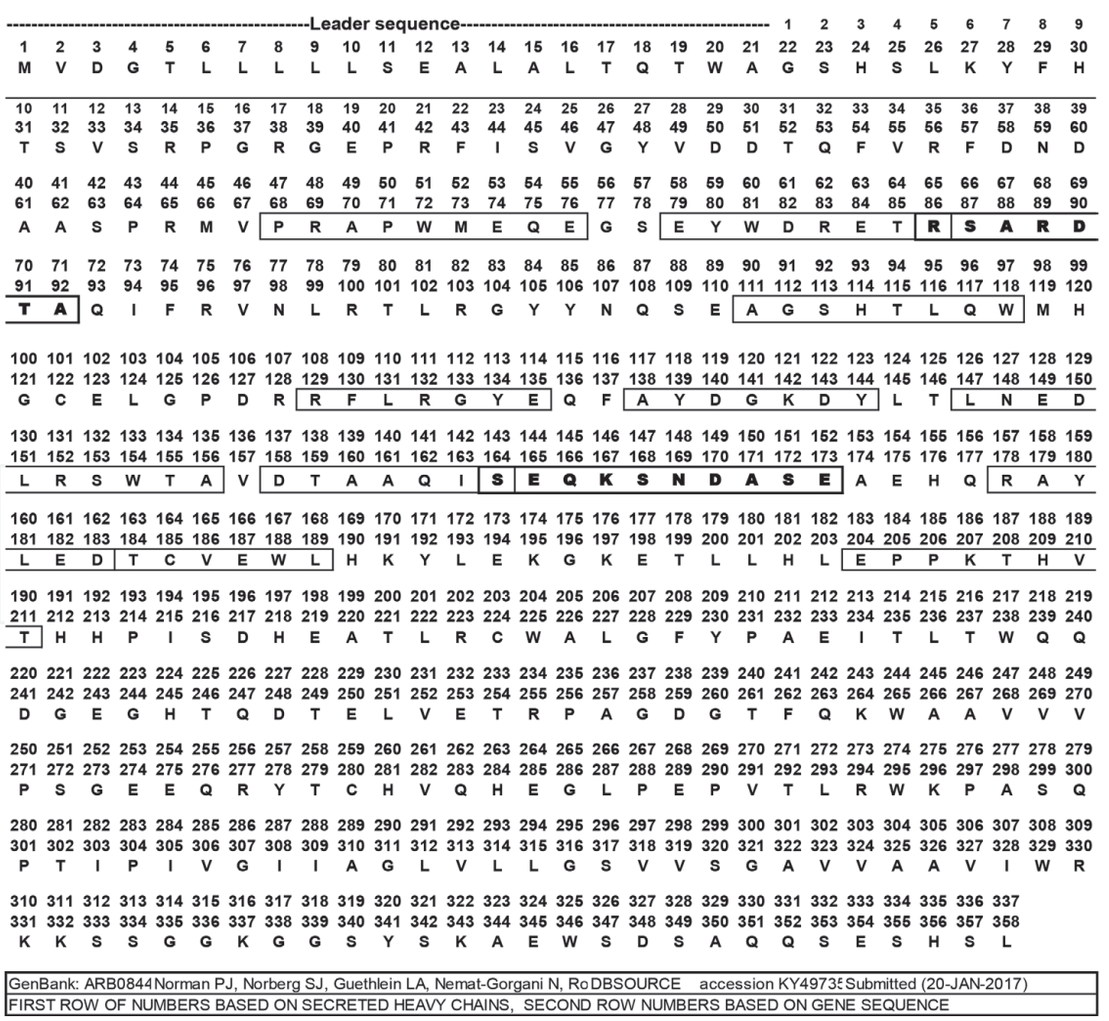

Figure 6.
Amino acid sequence of HLA-E^{R107}. Two sets of serial numbers provide one to include leader sequence and another after deleting leader sequence. Sequences in the boxes refer to either specific (private) or shared (public) epitopes. The box with bold letters was used to test for peptide inhibition in our experiments using TFL-monospecific mAbs.

Comparison of the amino acid sequences of HLA-E with other HLA-I antigens							
		HLA alleles					
HLA-E peptide sequences	Number of amino acids	Classical HLA-Ia			Non-classical HLA-Ib		Specificity
		A	B	Cw	F	G	
^{47}PRAPWMEQE^{55}	9	1	0	0	0	0	A*3306 restricted
^{59}EYWDRETR^{65}	8	5	0	0	0	0	A-restricted
^{65}RSARDTA^{71}	6	**0**	**0**	**0**	**0**	**0**	**E-monospecific**
^{90}AGSHTLQW^{97}	8	1	10	48	0	0	Multispecific
^{108}RFLRGYE^{123}	7	24	0	0	0	0	A-restricted
^{115}QFAYDGKDY^{123}	9	1	104	75	0	0	Multispecific
^{117}AYDGKDY^{123}	7	491	831	271	21	30	Highly Multispecific
^{126}LNEDLRSWTA^{135}	10	239	219	261	21	30	Multispecific
^{137}DTAAQI^{142}	6	0	824	248	0	30	Multispecific
^{137}DTAAQIS^{143}	7	0	52	4	0	30	Multispecific
^{143}SEQKSNDASE^{152}	10	**0**	**0**	**0**	**0**	**0**	**E-monospecific**
^{157}RAYLED^{162}	6	0	1	0	0	0	B*8201-restricted
^{163}TCVEWL^{168}	6	282	206	200	0	30	Multispecific
^{182}EPPKTHVT^{190}	8	0	0	19	0	0	C-restricted

Table 6.
Identifying HLA-E specific epitope or amino acid sequences: Peptide sequences specific and shared between HLA-E and HLA class Ia alleles: Monospecific (HLA-E restricted) versus polyreactive epitopes.

and then exposed to 2 mL of beads. The two different HLA-E-restricted peptides, RSARDTA and SEQKSNDASE were synthesized and purified by GenScript Corporation (Piscataway, NJ). The assay was performed in triplicate. Dosimetric peptide inhibition analysis was performed for mAb TFL-033. Before dosimetric peptide inhibition, the mAb TFL-033 was dosimetrically titrated to assess their strength (MFI), and protein-G purified culture supernatants and ascites compared. Then, concentrated Protein-G purified from ascites is titrated and the protein content is measured. Titrimetric inhibition was done with ascites protein-G concentrate. A summary of the peptide inhibition experiments is presented in **Figure 8**. Results confirm that TFL-003 binding to HLA-E can be inhibited dosimetrically using two HLA-E-restricted epitopes. The level of inhibition differed between the two epitopes.

5.6 Diagnostic potential of HLA-E monospecific mAbs

Immunolocalization of HLA-E on human melanoma cancer tissues was performed using culture supernatants (s) or ascites (a) of TFL monospecific mAbs (TFL-033, TFL-034, TFL-074, and TFL-216), and staining is compared with commercial anti-HLA-E mAb (MEM-E/02) [35, 98]. Titration of Protein-G purified culture supernatants and ascites concentrates of different anti-HLA-E monospecific mAbs are shown in **Table 8**. As revealed in **Figure 4**, the MEM-02 cross-reacts with several HLA class Ia alleles. Although it stains melanoma tissues, due to the paucity of HLA-E specificity, specific localization of HLA-E was confirmed with monospecific anti-HLA-E mAbs (**Figure 9A**). Similarly, immune-localization of HLA-E on human

Peptide [# 1] specific for HLA-E							Peptide [# 2] specific for HLA-E									
HLA Class Ib	**α1**	**α1**	**α1**	**α1**	**α1**	**α1**	**HLA Class Ib**	**α2**	**α2**	**α2**	**α2**	**α2**	**α2**	**α2**	**α2**	**α2**
	65	**66**	**67**	**68**	**69**	**70**		**143**	**144**	**145**	**146**	**147**	**148**	**149**	**150**	**151**
E*01010101	***R***	***S***	***A***	***R***	***D***	***T***	**E*01010101**	***S***	***E***	***Q***	***K***	***S***	***N***	***D***	***A***	***S***
G*01010101	***R***	N	T	K	A	H	**G*01010101**	**S**	K	R	**K**	C	E	A	**A**	N
F*01010101	G	Y	A	K	A	N	**F*01010101**	T	Q	R	F	Y	E	A	E	E
A*110101	**R**	N	V	K	**A**	Q	**A*110101**	T	K	R	**K**	W	E	A	**A**	H
B *1401	Q	I	C	K	T	N	**B *1401**	T	Q	R	**K**	W	E	A	**A**	R
B *350101	Q	I	F	K	T	N	**B *350101**	T	Q	R	**K**	W	E	A	**A**	R
B *40060101	Q	I	S	K	T	N	**B *40060101**	T	Q	R	**K**	W	E	A	**A**	R
B *530101	Q	I	F	K	T	N	**B *530101**	T	Q	R	**K**	W	E	A	**A**	R
B *5801	R	N	M	K	**A**	S	**B *5801**	T	Q	R	**K**	W	E	A	**A**	R
CW*050101	Q	K	Y	K	R	Q	**CW*050101**	T	Q	R	**K**	W	E	A	**A**	R
CW*080101	Q	K	Y	K	R	Q	**CW*080101**	T	Q	R	**K**	W	E	A	**A**	R
CW*1802	Q	K	Y	K	R	Q	**CW*1802**	T	Q	R	**K**	W	E	A	**A**	R
Qa-1(murine eq:HLA-E)	W	K	**A**	**R**	D	M	**Qa-1(murine eq:HLA-E)**	**S**	K	H	**K**	**S**	E	A	V	D

Table 7.
Identifying HLA-E specific epitope or amino acid sequences: Comparing the two HLA-E restricted sequences with other HLA-I amino acid sequences at the same position.

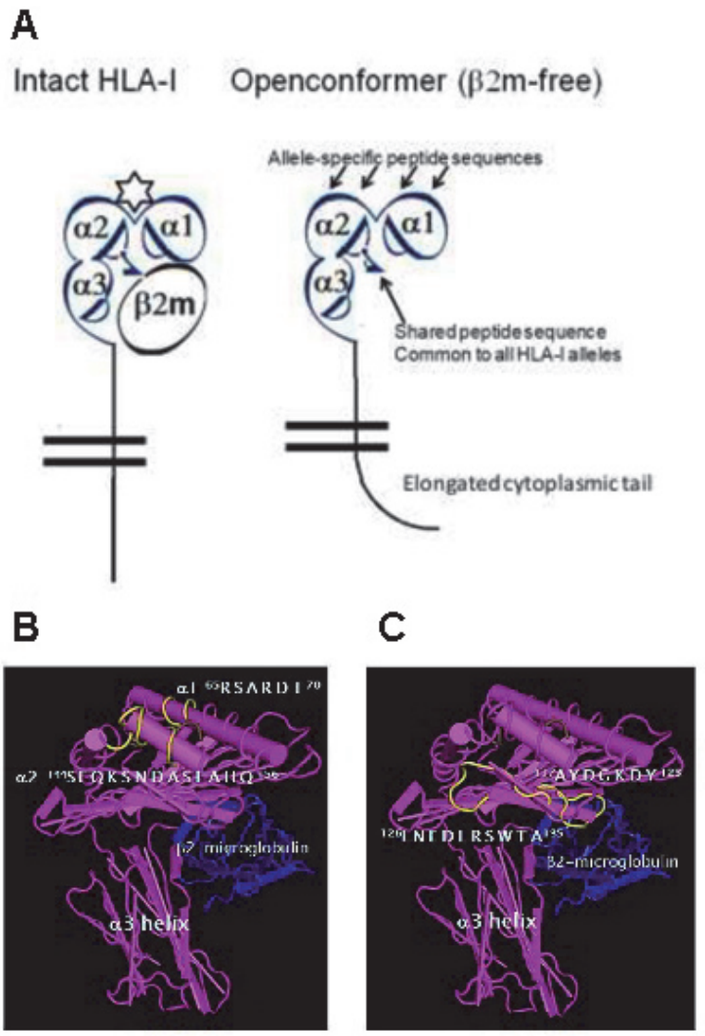

Figure 7.
Diagrammatic illustration of the structure of HLA-E, closed (intact trimer) and open conformers and specific (private) and shared (public) epitopes. (A) Illustrates the locations of allele-specific sequence (private epitope) and shared peptide (public epitopes) sequence. HLA-E with β2-microglobulin (in blue) showing (B) the allele-specific amino acid sequences (private epitopes) in α1 & α2 helical groove and (C) shared peptide amino acid sequences (public epitopes).

gastric diffused carcinoma paraffin tissue sections was observed after staining with the diluted ascites of monospecific mAb TFL-033a and MEM-E/02. The reliability of HLA-E tissue localization with monospecific immunostaining of human gastric adenocarcinoma (A, B) with TFL-033 and MEM-E/02 with that obtained for gastric diffuse carcinoma (C, D) control, stained without primary mAbs. MEM-E/02 failed to stain any cells while TFL-033a showed intense and widely distributed staining indicating the overexpression of intact HLA-E (**Figure 9C**). Immunostaining was performed on human breast ductal adenocarcinoma with TFL monospecific-mAbs and results obtained using monospecific anti-HLA-E mAb TFL-216, generated by immunizing HLA-E^G, is presented in **Figure 9D**.

Detailed immunodiagnostic analyses were performed using a tissue microarray (TMA) of normal gastric mucosal and primary gastric cancer tissues [98]. Three tissue microarrays (TMAs; US Biomax, Rockville, MD) were carefully selected. The tissue sections of all TMA were 1.5 mm in diameter and 5 μm thick. In TMA of normal gastric mucosa and of primary gastric cancer, which contained 30 adenocarcinomas, 40 diffuse carcinomas and ten normal gastric mucosae were immunostained. TMA array included: well-differentiated, moderately differentiated, poorly differentiated, and undifferentiated cancer. In addition, TMA also

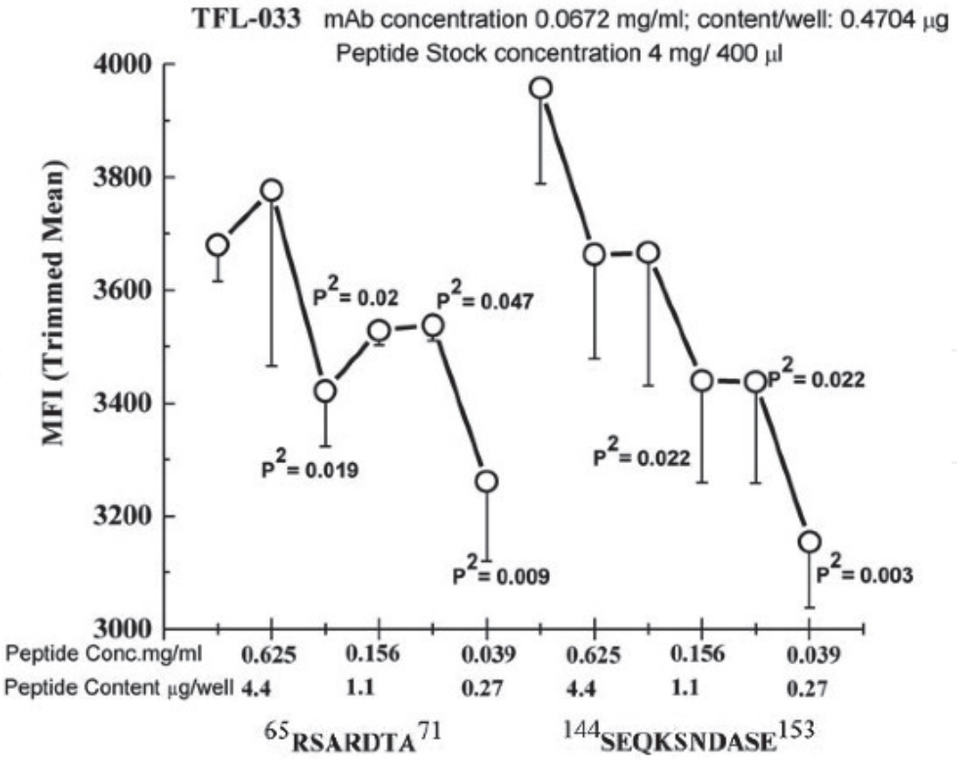

Figure 8.
Dosimetric inhibition of purified culture supernatants of TFL-033 with two HLA-E-restricted peptides, ^{65}RSARDTA^{71} and ^{143}SEQKSNDASE^{152}, at concentrations ranging from 4.4 to 0.27 mg/well. Although both peptides showed inhibition, the α2 helical peptide SEQKSNDASE showed better dosimetric inhibition than the other peptide. Peptide concentration and peptide content (µG/well) in parenthesis are shown. Pair-sample or equal-variant t-tests were carried out in this investigation using a graphic website (www.originlab.com). (Source: U. S. Patent No 10,656,158 B2 (*U.S. patent application No. 13/507,537)* issued on May 19, 2020, to Dr. Mepur H. Ravindranath*) see also Int J cancer. 2014;134(7):1558–70. DOI: 10.1002/ijc.28484.*

included Stages I to IV of metastatic gastric cancer with 5 peritoneal, 3 liver, 27 lymph node metastases. TMA was immunostained with TFL-033 mAbs (culture supernatants and ascites), controls were stained without primary mAbs [98]. The diagnostic potential of HLA-E-monospecific mAb TFL-033 for different kinds and stages of gastric cancer is illustrated in **Figure 4a** in International Journal of Cancer [98]. The observations confirm that specific identification and localization of MHC antigens, stringently require monospecific mAbs. The conclusion is highly reliable compared to the use of polyreactive commercial mAbs (MEM-E/02) [36, 98], presented in **Figure 4**. Importantly, characterizations of monospecificity should include (1) multiantigen coated solid matrix assays, e.g., Luminex multiplex SAB assay; (2) titrimetric inhibition with the private epitope of the antigen. Only such monospecific mAbs are reliable for diagnosis and therapeutic purposes.

5.7 Differences in the immunoregulatory potentials of HLA-E monospecific versus polyreactive mAbs

5.7.1 Potential of polyclonal anti-HLA-E mAbs in immune regulation

Immunoregulatory properties of both monospecific (TFL-033) and polyreactive (TFL-006 & TFL-007) anti-HLA-E mAbs were examined for their ability to suppress or activate CD3/CD4+, CD3/CD8+ T cells, T-regs, and CD3+/CD19/20+ B cells. The results show that the polyreactive anti-HLA-E mAbs (TFL-006/TFL-007) are immunosuppressive comparable to IVIg, used in immunotherapy of several diseases [16, 17]. Indeed the anti-HLA antibody profile of IVIg from different sources showed

Sample	Dilution	TFL-033	TFL-034	TFL-073	TFL-074
Culture Supernatant	*Neat*	11273	11601	7781	8493
Protein-G purified Culture supernatant	*(1:10)*	4424	2730	1974	2507
Protein-G purified Culture supernatant Concentrate	*(1:10)*	11953	10364	7708	8467
	(1:20)	9423	8146	6861	7500
	(1:40)	8167	6347	5324	5883
	(1:80)	6203	4622	3792	4176
	(1:160)	4139	1379	2683	2438
	(1:320)	2862	626	1454	943
	(1:640)	1434	198	590	474
	(1:1280)	694	98	275	220
Protein-G purified Ascites Concentrate (Eluate # 2)	*(1:50)*	17898			
	(1:100)	16246			
	(1:200)	14004			
	(1:400)	12520			

Table 8.
Titration of protein-G purified culture supernatant and ascites concentrates of different HLA-E monospecific mAbs. These concentrates were used for immunolocalization, peptide inhibition studies as well as for their effects on T-lymphoblasts.

both HLA-Ia and HLA-Ib reactivities [16, 17]. IVIg preparations were reported to suppress CD4+ T cells [102–113], CD20+ B cells [108–113] and expand CD4 + CD25+ T-regs [114, 115]. The polyreactive anti-HLA-E mAbs performed the major immunoregulatory functions better than IVIg [101, 116–118]. These functions are (1) suppression of CD19+ B lymphocyte blastogenesis, proliferation, and suppression of production of anti-HLA-I and anti-HLA-II IgG Abs, (2) suppression of blastogenesis and proliferation of CD4+ as well as CD8+ T lymphocytes, and (3) expansion of CD4 +. CD25+ and FoxP3+ T-regs. The monospecific mAbs, when used as controls failed to perform these functions. Peptide inhibition analyses revealed that mAbs TFL-006 and TFL-007 bind to shared amino acid sequences of HLA-I molecules (^{117}AYDGKDYLT^{125}, ^{126}LNEDLRSWTAV^{136}, and ^{137}DTAAQI^{142}) (**Figure 7C**). Possibly such binding affinity of polyreactive but not monospecific mAbs contributes to the unique immunoregulatory functions mimicking IVIg [101, 118].

5.7.2 Therapeutic potential of anti-HLA-E monospecific mAbs

In contrast to polyreactive anti-HLA-E mAb, monospecific mAbs (TFL-033) recognized HLA-E- specific amino acid sequences (^{65}RSARDT^{70} and^{154}AESADNSKQES^{144}) on the α1 and α2 helices (**Figure 7B**).

5.7.2.1 Monospecific mAbs promote the proliferation of CD8+ T lymphocytes

To test whether monospecific anti-HLA-E mAbs suppress proliferation of the CD3+, CD4+, or CD8+ T cells, human T lymphocytes (both CD4+ and CD8+) isolated from whole blood of a normal male donor with Ficol Hypaque (31) were treated either with phytohaemagglutinin (PHA, EY Laboratories, San Mateo, CA) at a final concentration of 2.25 mL/mL or not exposed to PHA (31). The mAbs (monospecific mAbs TFL-033, TFL-034, TFL-073, TFL-074, and TFL-216, polyreactive mAb TFL007, and negative control antibodies) were separately added to cells in culture within 2 hours after adding

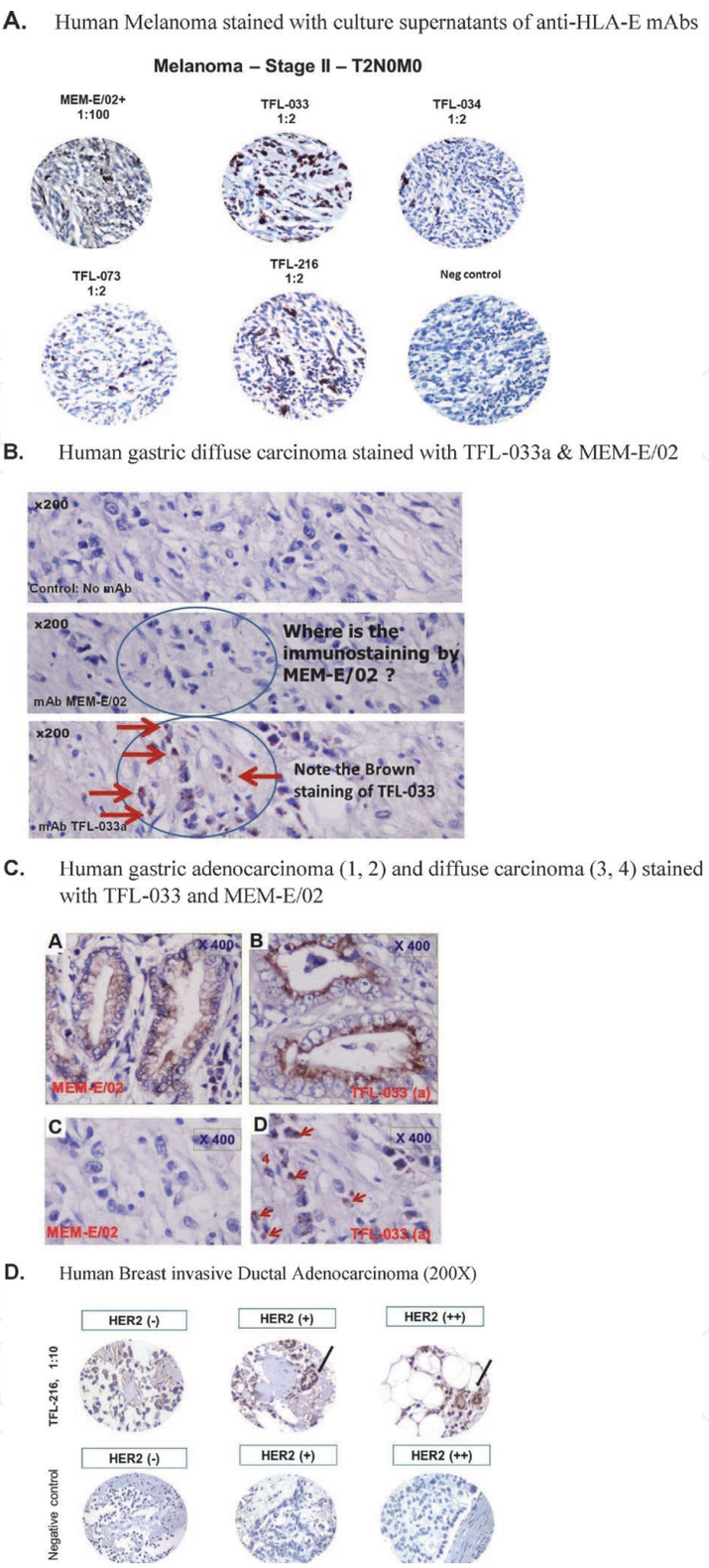

Figure 9.
*Immunolocalization of HLA-E in cancer tissues with culture supernatants (s) or ascites (A) of TFL monospecific mAbs compared with staining by MEM-E/02, an HLA-E mAb that shows cross-reactivity to HLA class Ia alleles. (**A**) Human melanoma paraffin tissue sections stained with the culture supernatants of TFL monospecific MAbs and MEM-E/02. (**B**) Human gastric cancer (diffused carcinoma) paraffin tissue sections stained with the diluted ascites of monospecific MAb TFL-033a and MEM-E/02. (**C**). Immunostaining of human gastric adenocarcinoma (A, B) and gastric diffuse carcinoma (**C, D**) control, stained without primary mAbs. Note the differences in staining between the two antibodies; MEM-E/02 failed to stain any cells while TFL-033a showed intense and widely distributed staining indicative of overexpression of intact HLA-E. (**D**) Human breast ductal adenocarcinoma stained with monospecific anti-HLA-E mAb TFL-216 generated by immunizing HLA-E^{G}. (source*: U. S. Patent No 10,656,158 B2 (*U.S. patent application No. 13/507,537)* issued on May 19, 2020, to Dr. Mepur H. Ravindranath*) see also Int J cancer. 2014;134(7):1558–70. DOI: 10.1002/ijc.28484.*

Presence or absence of CD4/CD8	**CD3+ NAÏVE T-CELLS**				**CD3+ LYMPHOBLASTS**							
	No PHA		**With PHA**		**No PHA**				**With PHA**			
	CD4+/ CD8-	**CD4-/ CD8+**	**CD4+/ CD8-**	**CD4-/ CD8+**	**CD4+/ CD8-**	**CD4-/ CD8+**	**CD4+/ CD8+**	**CD4-/ CD8-**	**CD4+/ CD8-**	***CD4-/ CD8+***	**CD4+/ CD8+**	**CD4-/ CD8-**
No mAb *[n = 5]*												
Mean	3063	547	1249	475	197	*65*	141	52	867	*325*	128	289
SD	149	86	99	37	33	*14*	35	15	115	*126*	43	84
2-tail p [<]		*<0.0001*							***0.001***	*0*	*NS*	*0*
mAb TFL-033 (IgG1) [n = 3]												
[1/30]												
Mean	3185	755	1170	536	223	***163***	153	99	1129	***505***	152	412
SD	180	146	58	12	40	*27*	80	13	86	*23*	16	20
2-tail p [<]	*NS*	*NS*	*NS*	***0.009***	*NS*	***0.015***	*NS*	*0.005*	***0.010***	***0.016***	*NS*	*0.014*
[1/150]												
Mean	3238	681	1149	508	252	***120***	205	68	1266	***572***	157	412
SD	14	64	21	22	30	*17*	13	9	80	*31*	14	16
2-tail p [<]	*NS*	*NS*	*NS*	***NS***	*0.047*	***0.001***	*0.020*	*NS*	***0.001***	***0.003***	*NS*	*0.001*
mAb TFL-007 (Polyreactivec anti-HLA-E, IgG2a) [n = 3]												
[1/10]												
Mean	2876	451	1183	444	164	*63*	145	52	676	*317*	100	222
SD	136	72	19	26	33	*2*	3	17	79	*25*	4	29
2-tail p [<]	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*0.027*	*NS*	*NS*	*NS*
[1/50]												

Presence or absence of CD4/CD8	**CD3+ NAÏVE T-CELLS**				**CD3+ LYMPHOBLASTS**							
	No PHA		**With PHA**		**No PHA**				**With PHA**			
	CD4+/ CD8-	**CD4-/ CD8+**	**CD4+/ CD8-**	**CD4-/ CD8+**	**CD4+/ CD8-**	**CD4-/ CD8+**	**CD4+/ CD8+**	**CD4-/ CD8-**	**CD4+/ CD8-**	***CD4-/ CD8+***	**CD4+/ CD8+**	**CD4-/ CD8-**
Mean	3,088	667	1,075	491	230	*107*	193	80	892	*443*	122	339
SD	65	16	55	48	23	*7*	17	4	26	*18*	8	21
2-tail p [<]	*NS*	*0.018*	*0.013*	*NS*	*NS*	*NS*	*0.019*	*0.006*	*NS*	*NS*	*NS*	*NS*

Table 9.
TFL-033 promotes T-lymphoblast proliferation of CD8+ naïve T cells and T-Lymphoblasts in the absence or the presence of PHA. The proliferation of CD4+ T lymphoblasts occurs only after PHA activation.

PHA (final 200 mL) (31). Detailed experimental protocol is described elsewhere (31). The effects of mAbs (monospecific mAb TFL-033 and polyreactive mAb TFL-007) on untreated (no PHA) and PHA-treated T lymphocytes in these categories of T cells: CD4+/CD8-, CD4-/CD8 +, CD4 + /CD8 +, and CD4-/CD8- are presented in **Table 9**. There was a significant increase in numbers of CD4-/CD8+ T lymphoblasts among the PHA-treated T lymphoblasts under the influence of TFL-033 s at 1:30 and 1:150). Numbers of PHA-untreated T lymphoblasts increased for almost all mAbs, TFL-033 s at 1/30 and 1/150, TFL-034 s at 1/10 and 1/50, TFL-073 s at 1/50, TFL-074 s at 1/10 [35]. An increase in PHA-untreated T lymphoblasts clarifies the functional potential of HLA-E monospecific mAbs in augmenting CD4−/ CD8+ T lymphoblasts. A significant increase in numbers of PHA-treated CD3+/CD4-/CD8+ lymphoblasts suggests that monospecific monoclonal mAbs, particularly TFL-003 confers the potential to augment cytotoxic T cells. Results prompt investigating humanized version TFL-003 on proliferation cytotoxic T-cells.

5.7.2.2 HLA-E expressed on cancer cells can directly bind to CD8+ T cells and NK cells and suppress their tumor-killing activity

Cancer cells lose their cell surface HLA-Ia alleles (HLA-A, HLA-B, and HLA-C) and upregulate the surface expression of HLA-Ib molecules (HLAE, HLA-F, and HLA-G) [57, 82, 119–128]. The upregulation of HLA-E gene expression is correlated with immunolocalization and overexpression of cell surface HLA-E [71, 91, 128–132]. HLA-E gene expression in some cancers [e.g., melanoma] is ranked 19th among overexpressed genes [133]. HLA-E overexpression and loss of HLA-Ia in

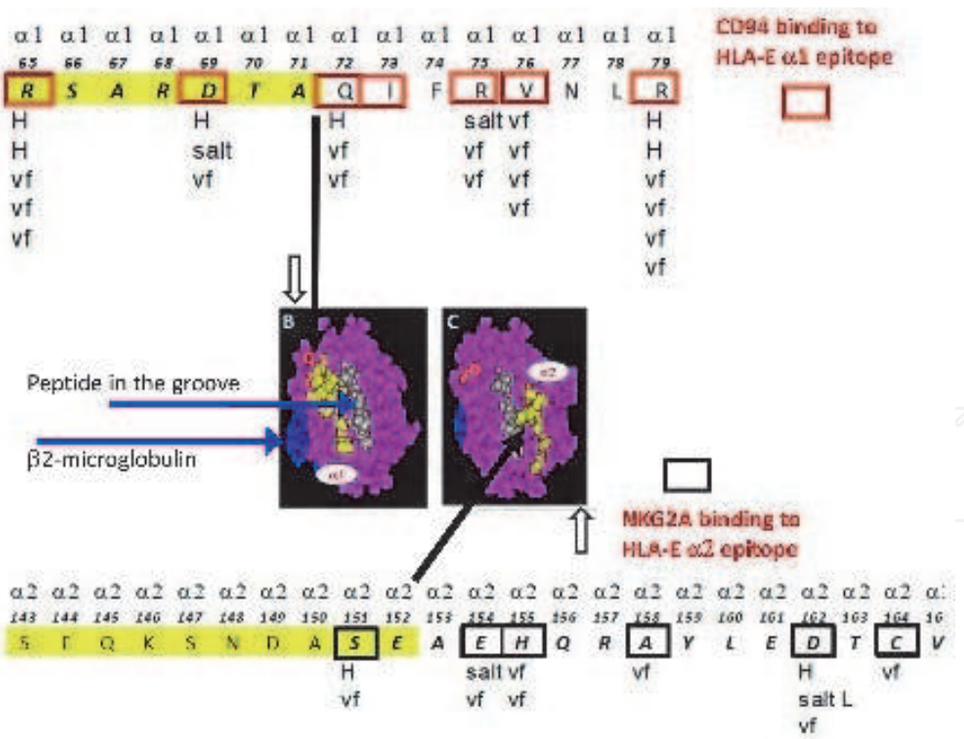

Figure 10.
*Binding of HLA-E to the inhibitory receptors CD94 and NKG2A on both CD8+ CTLs and NKT cells. The structural configuration of the binding of HLA-E and the inhibitory receptors, leading to the arrest of the anti-tumor activity function of CD8+ and NKT cells. The interaction between HLA-E and the inhibitory receptors involves the binding of amino acids located on the α1 and α2 helices of HLA-E to specific amino acids on CD94 and NKG2A. The amino acid sequences on HLA-E recognized by the inhibitory receptors are unique and specific for HLA-E and they are also recognized by HLA-E monospecific mAbs. The binding involves H-bonding (H), van der Waal forces (vf), and salt linkages (salt) of the amino acids of HLA-E a1 and a2 helices and CD94 and NKG2A inhibitory receptors. (*Modified from Ravindranath et al. Monoclon Antib Immunodiagn Immunother. 2015,34(3):135–53*).*

cancer cells are correlated with disease progression and poor prognosis [60, 82, 130, 134]. Disease progression is attributed to the suppression of the tumor-killing activity of CD8+ cytotoxic T lymphocytes (CTLs) and NKT cells.

Cell surface and soluble HLA-E are capable of binding to the inhibitory receptors CD94 and NKG2A on both CTLs (CD3+/CD8+), NK cells (CD2+, CD7+, CD11b+, CD11c+, CD90+, perforin+, & granzyme A+) and NKT cells (plus CD8+) [25, 27, 135, 136]. These cells are capable of destroying tumor cells. These cells interact with MHC-I ligands (HLA-E) on tumor cells through inhibitory receptors. The binding of above mentioned immune cells to HLA-E overexpressed on tumor cells cell surface may explain why the cancer patients failed to respond to NK cell therapies.

Interaction between HLA-E and inhibitory receptors involves the binding of HLA-E specific amino acids located on α1 and α2 helices (**Table 7**) to specific amino acids on CD94 and NKG2A (**Figure 10**) [22, 27, 135, 136]. This specific interaction is attributed to the loss of anti-tumor activity of CD8+ CTLs as well as that of NK or NKT cells [22, 27, 135, 136]. We have used the synthetic peptides of these sequences to ascertain the specific binding affinity of anti-HLA-E mAbs (**Figure 8**). The ability of monospecific anti-HLA-E mAbs to bind at the site of epitopes of CD94 and NKG2A on HLA-E favor the use of the monospecific anti-HLA-E mAbs to mask binding sites of inhibitory receptors on HLA-E. Such blocking of HLA-E may help restore the antitumor efficacy of NK cells and CD8+ T cells that were lost due to the interaction of inhibitory receptors and HLA-E. Possibly humanized monospecific anti-HLA-E may be potentially considered for anti-cancer NK therapy.

6. Conclusion

The anti-HLA-E mAbs TFL- 033, TFL-034, TFL-073, and TFL-074 due to their monospecificity are advantageous than the commercial anti-HLA-E mAbs for specific identification and localization of HLA-E on the surface of human cells, particularly in different cancer types. Our observations stress the need for characterization of monospecificity and epitope specificity of any mAb, after analyzing binding affinity on a multiplex solid matrix assays coated with the desired antigen (in question) and the closely related antigens and inhibition of the binding affinity using peptides sequences specific for the antigen in question. This is an important criterion to be followed for all clinical diagnostic and therapeutic antibodies. If specific epitopes are exposed to antigen located on the cell surface, it would be a more valuable diagnostic tool, than those binding to specific but cryptic epitopes.

The HLA-E monospecific antibodies (e.g., TFL-033) are capable of augmenting proliferation of non-activated CD8+ T cells and activated CD8+ T-lymphoblasts. TFL-033 binds to a unique epitope of HLA-E, a region that is involved in binding to inhibitory receptors (CD94 and NKG2A) present on CD3+/CD8+ T cells (Cytotoxic T cells) and CD3-/CD8+ NKT cells and NK cells. The binding of HLA-E to inhibitory receptors results in the suppression of anti-tumor cytotoxic functions of these immune cells. *Since TFL-033 can also upregulate anti-tumor cytotoxic T cell lymphoblasts and also capable of blocking the interaction between cancer-associated HLA-E and inhibitory receptors CD94/NKG2A, the mAb can be considered as a double-edged sword to eliminate cancer cells.* Therefore, TFL-033 could be a valuable therapeutic agent for passive immunotherapy of human cancer, provided the mAb is humanized.

In contrast to monospecific mAbs, HLA-I polyreactive anti-HLA-E monoclonal Abs (TFL-006 and TFL-007) mimic not only HLA-I reactivity of IVIg but also performs several critical immunoregulatory functions of IVIg, better than IVIg *per se*. These functions include suppression of blastogenesis and proliferation of CD4+ T cells and CD8+ T cells, effective inhibition of production of anti-HLA-I and

HLA-II Abs. HLA-I polyreactive anti-HLA-E monoclonal Abs (TFL-006 and TFL-007) are capable of upregulating T-regs. T-regs acting alone is capable of suppressing CD4+ T cells, CD8+ T cells, and antibody.

Acknowledgements

We wish to express sincere thanks to Professor Dr. Edwin L. Cooper, *Distinguished Professor, at* Collaborative Centers for Integrative Medicine, University of California, Los Angeles, for critically editing this manuscript. We wish to dedicate this chapter to Professor Edwin L Cooper. We also wish to thank Professor Mark Terasaki and Terasaki Family Foundation for providing financial support to carry out the research reported in this chapter at Terasaki Foundation Laboratory (TFL), Santa Monica, CA. We wish to thank the following research scientists at TFL, Drs. Dong Zhu, Toshiyuki Sasaki, Vadim Jucaud, Mr. Tho Pham, Mr. Satoru Kawakita, Mr. Curtis Y. Maehara, and Mrs. Judy Hopfield were directly involved in the experiments reported in this chapter.

A patent was filed based on our research on monospecific anti-HLA-E mAb TFL-033 and a U. S. Patent No 10,656,158 B2 was issued on May 19, 2020, to Dr. Mepur H. Ravindranath & Late Professor Paul Ichiro Terasaki. Hybridoma of TFL-033 is deposited with ATCC Patent Depository (ID: PTA-125908) at Manassas, Virginia 20110, USA.

Conflict of interest

The authors declare no conflict of interest.

Author details

Mepur H. Ravindranath[1*†] and Fatiha E.L. Hilali[2]

1 Children's Hospital, Formerly, Terasaki Foundation Laboratory, Los Angeles, CA

2 28 DB Dighoussi Zitoune Meknes, Morocco

*Address all correspondence to: thiruranganath04@gmail.com

†Formerly, Recipient of Fulbright Award at the University of Madras India and came to the University of California, Los Angeles, and appointed as Adjunct Associate Professor in the Department of Anatomy. Senior Research Scientist (2008-2018), Terasaki Foundation Laboratory, Santa Monica, CA, Director, Department of Glycoimmunomics (1990-2008), John Wayne Cancer Institute, Santa Monica CA. Adjunct Faculty, University of California, Los Angeles (1983-1990), Faculty and Fulbright Recipient (1983-1984), University of Madras, Chennai, Tamilnadu, India 60005 (1972-1983).

References

[1] Marsh SGE, Parham P, Barber LD. The HLA. Facts Book. Academic Press. San Diego, 2000, 400p.

[2] Arosa FA, Esgalhado AJ, Padrão CA, Cardoso EM. Divide, Conquer, and Sense: CD8+CD28- T Cells in Perspective. Front Immunol 2017;7:665, DOI:10.3389/fimmu. 2016.00665.

[3] Arosa FA, Santos SG, Powis SJ. Open conformers: the hidden face of MHC-I molecules. Trends Immunol 2007;28(3): 115–123, DOI:10.1016/j.it. 2007 01.002.

[4] Schnabl E, Stockinger H, Majdic O, Gaugitsch H, Lindley IJ, Maurer D, et al., (1990) Activated human T lymphocytes express MHC class I heavy chains not associated with beta 2microglobulin. J Exp Med, 1990;171(5): 1431–1442. DOI: 10.1084/ jem.171.5.1431.

[5] Schumacher TN, Heemels MT, Neefjes JJ, Kast WM, Melief CJ, Ploegh HL. (1990) Direct binding of peptide to empty MHC class I molecules on intact cells and in vitro. Cell 1990;62(3);563–567. DOI:10.1016/0092-8674(90) 90020-F

[6] Benjamin RJ, Madrigal JA, Parham P. Peptide binding to empty HLA-B27 molecules of viable human cells. Nature, 1991;351(6321);74–77. DOI: 10.1038/ 351074a0.

[7] Majdic O, Schnabl E, Stockinger H, Gadd S, Maurer D, Radaszkiewics T, et al. LA45, an activation-induced human lymphocyte antigen with strong homology to MHC class I molecules. In Leukocyte Typing IV. Oxford University Press, 1989, 511p.

[8] Madrigal JA, Belich MP, Benjamin RJ, Little AM, Hildebrand WH, Mann DL, et al., Molecular definition of a polymorphic antigen (LA45) of free HLA-A and -B heavy chains found on the surfaces of activated B and T cells. J Exp Med 1991;174(5);1085–1095, DOI: 10.1084/jem.174.5.1085.

[9] Raine T, Brown D, Bowness P, Hill Gaston JS, Moffett A, Trowsdale J, et al. Consistent patterns of expression of HLA class I free heavy chains in healthy individuals and raised expression in spondyloarthropathy patients point to physiological and pathological roles. Rheumatology (Oxford) 2006;45(11); 1338–1344, DOI: 10.1093/ rheumatology/kel305

[10] Cardoso EM, Esgalhado AJ, Patrao L, Santos M, Neves VP, Martinez J, et al. Distinctive CD8+ T cell and MHC class I signatures in polycythemia vera patients. Ann. Hematol. 2018;97(9): 1563–1575, DOI: 10.1007/s00277-018-3332-7

[11] Santos SG, Powis SJ, Arosa FA. Misfolding of major histocompatibility complex class I molecules in activated T cells allows cis-interactions with receptors and signaling molecules and is associated with tyrosine phosphorylation. J Biol Chem. 2004; 279 (51):53062–53070. DOI: 10.1074/jbc. M408794200.

[12] Goodridge JP, Lee N, Burian A, Pyo CW, Tykodi SS, Warren EH, Yee C, Riddell SC, Geraghty DE. HLA-F and MHC-I open conformers cooperate in an MHC-I antigen cross-presentation pathway. J. Immunol. 2013;191:1567–1577, DOI:10.4049/jimmunol.1300080.

[13] Brynjolfsson SF, Persson Berg L, Olsen Ekerhult T, Rimkute I, Wick MJ, Mårtensson IL, Grimsholm O. Long-Lived Plasma Cells in Mice and Men. Front Immunol. 2018;9:2673. DOI: 10.3389/fimmu.2018.02673.

[14] Morales-Buenrostro LE, Terasaki PI, Marino-Vázquez LA, Lee JH, El-Awar N, Alberú J. "Natural" human leukocyte

antigen antibodies found in nonalloimmunized healthy males. Transplantation. 2008;86(8):1111–1115. doi: 10.1097/TP.0b013e318186d87b.

[15] Ravindranath MH, Kaneku H, El-Awar N, Morales-Buenrostro LE, Terasaki PI. Antibodies to HLA-E in nonalloimmunized males: pattern of HLA-Ia reactivity of anti-HLA-E-positive sera. J Immunol. 2010;85(3): 1935–1948. DOI: 10.4049/jimmunol.1000424.

[16] Ravindranath MH, Terasaki PI, Pham T, Jucaud V, Kawakita S. Therapeutic preparations of IVIg contain naturally occurring anti-HLA-E antibodies that react with HLA-Ia (HLA-A/-B/-C) alleles. Blood. 2013;121 (11);2013-28. DOI: 10.1182/blood-2012. 08-447771.

[17] EL Hilali F, Jucaud V, EL Hilali H, Bhuiyan MH, Mancuso A, LiuSullivan N, Elidrissi A, Mazouz H. Characterization of the Anti-HLA Class I and II IgG Antibodies in Moroccan IVIg Using Regular Beads and Ibeads in Luminex Multiplex Single Antigen Immunoassay Int. J. Immunol. 2017; 5(4): 53–65. DOI: 10.11648/j. iji.20170504.11

[18] Geraghty DE, Koller BH. Orr HT. A human major histocompatibility complex class I gene that encodes a protein with a shortened cytoplasmic segment. Proc Natl Acad Sci USA. 1987; 84(24): 9145–9149. DOI: 10.1073/ pnas.84.24.9145

[19] Koller BH, Geraghty DE, Shimizu Y, DeMars R, Orr HT. HLA-E. A novel HLA class I gene expressed in resting T lymphocytes. J Immunol. 1988; 141(3): 897–904.

[20] Rodgers JR, Cook RG. MHC class Ib molecules bridge innate and acquired immunity. Nat Rev Immunol. 2005; 5 (6): 459–471. DOI: 10.1038/nri1635.

[21] Lee N, Ishitani A, Geraghty DE. HLA-F is a surface marker on activated lymphocytes. Eur J Immunol. 2010; 40 (8): 2308–2318. DOI:10.1002/ eji.201040348.

[22] Lee N, Llano M, Carretero M, Ishitani A, Navarro F, López-Botet M, Geraghty DE. HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. Proc Natl Acad Sci U S A. 1998;95(9):5199–5204. DOI: 10.1073/ pnas.95.9.5199.

[23] Derré L, Corvaisier M, Charreau B, Moreau A, Godefroy E, Moreau-Aubry A, Jotereau F, Gervois N. Expression and release of HLA-E by melanoma cells and melanocytes: potential impact on the response of cytotoxic effector cells. J Immunol. 2006;177(5):3100–7. DOIi: 10.4049/jimmunol.177.5.3100.

[24] O'Callaghan CA. Molecular basis of human natural killer cell recognition of HLA-E (human leucocyte antigen-E) and its relevance to clearance of pathogen-infected and tumour cells. Clin Sci (Lond). 2000;99(1):9–17. DOI: 10.1042/cs0990009.

[25] Kochan G, Escors D, Breckpot K, Guerrero-Setas D. Role of non-classical MHC class I molecules in cancer immunosuppression. Oncoimmunology. 2013;2(11):e26491. DOI: 10.4161/ onci.26491.

[26] Sullivan LC, Hoare HL, McCluskey J, Rossjohn J, Brooks AG. A structural perspective on MHC class Ib molecules in adaptive immunity. Trends Immunol. 2006;27(9): 413–420. DOI: 10.1016/j. it.2006.07.006.

[27] Llano M, Lee N, Navarro F, et al. HLA-E-bound peptides influence recognition by inhibitory and triggering CD94/NKG2 receptors: preferential response to an HLA-G-derived nonamer. Eur J Immunol. 1998; 28(9): 2854–2863. DOI: 10.1002/(SICI)1521-4141(199809)

[28] Foroni I, Couto AR, Bettencourt BF, Santos M, Lima M, Bruges-Armas J. HLA-E, HLA-F, and HLA-G — The Non-Classical Side of the MHC Cluster. Chapter 3, pages 61-109, HLA, and associated important diseases. Intech, Edited by Yongzhi Xi, https://www.intechopen.com/books/hlaand-associated-important-diseases.

[29] Carosella ED, Moreau P, Le Maoult J, Le Discorde M, Dausset J, Rouas-Freiss N. HLA-G molecules: from maternal-fetal tolerance to tissue acceptance. Adv Immunol. 2003;81:199–252. DOI: 10.1016/s0065-2776(03)81006-4.

[30] Le Rond S, Le Maoult J, Créput C, Menier C, Deschamps M, Le Friec G, Amiot L, Durrbach A, Dausset J, Carosella ED, Rouas-Freiss N Alloreactive CD4+, and CD8+ T cells express the immunotolerant HLA-G molecule in mixed lymphocyte reactions: in vivo implications in transplanted patients. Eur J Immunol. 2004;34(3):649–660. DOI.1002/eji.200324266.

[31] LeMaoult J, Rouas-Freiss N, Carosella ED. HLA-G5 expression by trophoblast cells: the facts. Mol Hum Reprod. 2005;11(10):719–722. DOI: 10.1093/molehr/gah224

[32] Morales PJ, Pace JL, Platt JS, Phillips TA, Morgan K, Fazleabas AT, Hunt JS. Placental cell expression of HLA-G2 isoforms is limited to the invasive trophoblast phenotype. J Immunol. 2003;171(11):6215–6224. DOI: 10.4049/jimmunol.171.11.6215

[33] Carosella ED, Favier B, Rouas-Freiss N, Moreau P, Lemaoult J. Beyond the increasing complexity of the immunomodulatory HLA-G molecule. Blood, 2008;111(10):4862–4870. DOI: 10.1182/blood-2007-12-127662.

[34] Clements CS, Kjer-Nielsen L, Kostenko L, Hoare HL, Dunstone MA, Moses E, Freed K, Brooks AG, Rossjohn J, McCluskey J. Crystal structure of HLA-G: a nonclassical MHC class I molecule expressed at the fetal-maternal interface. Proc Natl Acad Sci U S A. 2005; 102(9):3360–3365. DOI: 10.1073/pnas.0409676102.

[35] Ravindranath MH, Terasaki PI, Pham T, Jucaud V. The Monospecificity of Novel Anti-HLA-E Monoclonal Antibodies Enables Reliable Immunodiagnosis, Immunomodulation of HLA-E, and Upregulation of CD8+ T Lymphocytes. Monoclon Antib Immunodiagn Immunother. 2015; 34 (3): 135–153. DOI: 10.1089/mab.2014.0096.

[36] Ravindranath MH, Taniguchi M, Chen CW, Ozawa M, Kaneku H, El-Awar N, Cai J, Terasaki PI. HLA-E monoclonal antibodies recognize shared peptide sequences on classical HLA class Ia: relevance to human natural HLA antibodies. Mol Immunol. 2010; 47(5): 1121–1131. DOI: 10.1016/j.molimm.2009.10.024.

[37] Ravindranath MH, Pham T, El-Awar N, Kaneku H, Terasaki PI. Anti-HLA-E mAb 3D12 mimics MEM-E/02 in binding to HLA-B and HLA-C alleles: Web-tools validate the immunogenic epitopes of HLA-E recognized by the antibodies. Mol Immunol. 2011; 48(4): 423–430. DOI: 10.1016/j.molimm.2010.09.011.

[38] Ravindranath MH, Kaneku H, El-Awar N, Morales-Buenrostro LE, Terasaki PI. Antibodies to HLA-E in nonalloimmunized males: pattern of HLA-Ia reactivity of anti-HLA-E-positive sera. J. Immunol.2010;185(3): 1935–1948. DOI: 10.4049/jimmunol.1000424.

[39] Ravindranath MH, Selvan SR, Terasaki PI. Augmentation of anti-HLA-E antibodies with concomitant HLA-Ia reactivity in IFNγ-treated autologous melanoma cell vaccine recipients. J

Immunotoxicol. 2012;9(3):282–291. DOI: 10.3109/1547691X.2011. 645582.

[40] Ben Yahia H, Babay W, Bortolotti D, Boujelbene N, Laaribi AB, Zidi N, Kehila M, Chelbi H, Boudabous A, Mrad K, Mezlini A, Di Luca D, Ouzari HI, Rizzo R, Zidi I. Increased plasmatic soluble HLA-G levels in endometrial cancer. Mol Immunol. 2018;99:82–86. DOI: 10.1016/j.molimm.2018.04.007.

[41] Rizzo R, Gabrielli L, Bortolotti D, Gentili V, Piccirilli G, Chiereghin A, Pavia C, Bolzani S, Guerra B, Simonazzi G, Cervi F, Capretti MG, Fainardi E, Luca DD, Landini MP, Lazzarotto T.J Study of Soluble HLA-G in Congenital Human Cytomegalovirus Infection. Immunol Res. 2016;2016:3890306. DOI: 10.1155/2016/3890306.

[42] Alegre E, Rizzo R, Bortolotti D, Fernandez-Landázuri S, Fainardi E, González A. Some basic aspects of HLA-G biology. J Immunol Res. 2014;2014: 657625. DOI: 10.1155/ 2014/657625.

[43] Felício LP, Porto IO, Mendes-Junior CT, Veiga-Castelli LC, Santos KE, Vianello-Brondani RP, Sabbagh A, Moreau P, Donadi EA, Castelli EC. Worldwide HLA-E nucleotide and haplotype variability reveals a conserved gene for coding and 3′ untranslated regions. Tissue Antigens. 2014;83(2):82–93. DOI:10.1111/ tan.12283.

[44] Grimsley C, Ober C. Population genetic studies of HLA-E: evidence for selection. Hum Immunol. 1997;52(1): 33–40. doi: 10.1016/S0198-8859(96) 00241-8.

[45] Grimsley C, Kawasaki A, Gassner C, Sageshima N, Nose Y, Hatake K, Geraghty DE, Ishitani A. Definitive high-resolution typing of HLA-E allelic polymorphisms: Identifying potential errors in existing allele data. Tissue Antigens. 2002;60(3):206–212. doi: 10.1034/j.1399-0039.2002.600302.x.

[46] Geraghty DE. HLA-E allelic variants. Correlating differential expression, peptide affinities, crystal structures, and thermal stabilities. J Biol Chem. 2003;278(7):5082–5090. DOI: 10.1074/jbc.M208268200.

[47] Kraemer T, Blasczyk R, Bade-Doeding C. HLA-E: a novel player for histocompatibility. J Immunol Res. 2014; 2014: 352160. DOI: 10.1155/2014/ 352160.

[48] Iwaszko M, Bogunia-Kubik K. Clinical significance of the HLA-E and CD94/NKG2 interaction. Arch Immunol Ther Exp (Warsz). 2011; 59(5): 353–367. DOI: 10.1007/s00005 011-0137-y.

[49] Kraemer T, Celik AA, Huyton T, Kunze-Schumacher H, Blasczyk R, Bade-Doding C. 2015. HLA-E: Presentation of a Broader Peptide Repertoire Impacts the Cellular Immune Response—Implications on HSCT Outcome. Stem Cells International. 2015, Article ID 346714, 12 pages. dx.doi.org/10.1155/2015/346714.

[50] Celik AA, Kraemer T, Huyton T, Blasczyk R, Bade-Döding C. The diversity of the HLA-E-restricted peptide repertoire explains the immunological impact of the Arg107Gly mismatch. Immunogenetics. 2016;68(1): 29–41. doi: 10.1007/s00251-015-0880-z.

[51] Goel R, Kabeerdoss J, Mohan H, Danda S, Jayaseelan V, Kumar TS, Jude J, Bacon P, Joseph G, Danda D.Soluble-HLA-E: A follow up biomarker in Takayasu arteritis, independent of HLA-E genotype. Int J Rheum Dis. 2017 Apr 19. DOI: 10.1111/1756-185X.13027.

[52] Wagner B, da Silva Nardi F, Schramm S, Kraemer T, Celik AA, Dürig J, Horn PA, Dührsen U, Nückel H, Rebmann V.HLA-E allelic genotype correlates with HLA-E plasma levels and predicts early progression in chronic lymphocytic leukemia. Cancer. 2017;123 (5):814–823. DOI: 10.1002/cncr.30427.

[53] Morandi F, Pozzi S, Carlini B, Amoroso L, Pistoia V, Corrias MV. Soluble HLA-G and HLA-E Levels in Bone Marrow Plasma Samples Are Related to Disease Stage in Neuroblastoma Patients. J Immunol Res. 2016;2016:7465741. DOI:10.1155/2016/7465741.

[54] Morandi F, Cangemi G, Barco S, Amoroso L, Giuliano M, Gigliotti AR, Pistoia V, Corrias MV. Plasma levels of soluble HLA-E and HLA-F at diagnosis may predict the overall survival of neuroblastoma patients. Biomed Res Int. 2013;2013:956878. DOI: 10.1155/2013/956878.

[55] Allard M, Oger R, Vignard V, Percier JM, Fregni G, Périer A, Caignard A, Charreau B, Bernardeau K, Khammari A, Dréno B, Gervois N. Serum soluble HLA-E in melanoma: a new potential immune-related marker in cancer. PLoS One. 2011;6(6):e21118. DOI: 10.1371/journal.pone.0021118.

[56] Coupel S, Moreau A, Hamidou M, Horejsi V, Soulillou JP, Charreau B. Expression and release of soluble HLA-E is an immunoregulatory feature of endothelial cell activation. Blood. 2007; 109(7): 2806–2814. DOI: 10.1182/blood-2006-06-030213.

[57] Marín R, Ruiz-Cabello F, Pedrinaci S, Méndez R, Jiménez P, Geraghty DE, Garrido F. Analysis of HLA-E expression in human tumors. Immunogenetics. 2003;54(11):767–775. DOI: 10.1007/s00251-002-0526-9.

[58] Gonçalves AS, Oliveira JP, Oliveira CF, Silva TA, Mendonça EF, Wastowski IJ, Batista AC.Relevance of HLA-G, HLA-E and IL-10 expression in lip carcinogenesis. Hum Immunol. 2016;77 (9):785-790. doi: 10.1016/j. humimm.2015.12.001.

[59] Silva TG, Crispim JC, Miranda FA, Hassumi MK, de Mello JM, Simões RT, Souto F, Soares EG, Donadi EA, Soares CP. Expression of the nonclassical HLA-G and HLA-E molecules in laryngeal lesions as biomarkers of tumor Invasiveness. Histol Histopathol. 2011; 26(12):1487-97. doi: 10.14670/HH-26.1487.

[60] van Esch EM, Tummers B, Baartmans V, Osse EM, Ter Haar N, Trietsch MD, Hellebrekers BW, Holleboom CA, Nagel HT, Tan LT, Fleuren GJ, van Poelgeest MI, van der Burg SH, Jordanova ES. Alterations in classical and non-classical HLA expression in recurrent and progressive HPV-induced usual vulvar intraepithelial neoplasia and implications for immunotherapy. Int J Cancer. 2014;135(4):830–842. DOI: 10.1002/ijc.28713.

[61] Djajadiningrat RS, Horenblas S, Heideman DA, Sanders J, de Jong J, Jordanova ES. Classic and nonclassic HLA class I expression in penile cancer and relation to HPV status and clinical outcome. J Urol. 2015;193(4):1245-51. doi: 10.1016/j.juro.2014.11.057.

[62] Mittelbronn M, Simon P, Löffler C, Capper D, Bunz B, Harter P, Schlaszus H, Schleich A, Tabatabai G, Goeppert B, Meyermann R, Weller M, Wischhusen J. Elevated HLA-E levels in human glioblastomas but not in grade I to III astrocytomas correlate with infiltrating CD8+ cells. J Neuroimmunol. 2007;189 (1-2):50-8.

[63] Kren L, Muckova K, Lzicarova E, Sova M, Vybihal V, Svoboda T, Fadrus P, Smrcka M, Slaby O, Lakomy R, Vanhara P, Krenova Z, Michalek J. Production of immune-modulatory nonclassical molecules HLA-G and HLA-E by tumor infiltrating ameboid microglia/macrophages in glioblastomas: a role in innate immunity? J Neuro immunol. 2010;220(1-2):131-5. doi: 10.1016/j.jneuroim. 2010.01.014.

[64] Kren L, Slaby O, Muckova K, Lzicarova E, Sova M, Vybihal V,

Svoboda T, Fadrus P, Lakomy R, Vanhara P, Krenova Z, Sterba J, Smrcka M, Michalek J. Expression of immune-modulatory molecules HLA-G and HLA-E by tumor cells in glioblastomas: an unexpected prognostic significance? Neuropathology. 2011;31(2):129-34. doi: 10.1111/j.1440-1789.2010.01149.x.

[65] Wolpert F, Roth P, Lamszus K, Tabatabai G, Weller M, Eisele G. HLA-E contributes to an immune-inhibitory phenotype of glioblastoma stem-like cells. J Neuroimmunol. 2012; 50(1-2):27-34. doi: 10.1016/j.jneuroim.2012.05.010.

[66] Wischhusen J, Friese MA, Mittelbronn M, Meyermann R, Weller M. HLA-E protects glioma cells from NKG2D-mediated immune responses in vitro: implications for immune escape in vivo. J Neuropathol Exp Neurol. 2005; 64(6):523-8.

[67] Bossard C, Bézieau S, Matysiak-Budnik T, Volteau C, Laboisse CL, Jotereau F, Mosnier JF. HLA-E/β2 microglobulin overexpression in colorectal cancer is associated with the recruitment of inhibitory immune cells and tumor progression. Int J Cancer. 2012;131(4) :855–863. DOI: 10.1002/ijc.26453.

[68] Costa Arantes DA, Gonçalves AS, Jham BC, Duarte ECB, de Paula ÉC, de Paula HM, Mendonça EF, Batista AC. Evaluation of HLA-G, HLA-E, and PD-L1 proteins in oral osteosarcomas. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017 Jun;123(6):e188-e196. doi: 10.1016/j.oooo.2016.12.002.

[69] Mosconi C, Arantes DAC, Gonçalves AS, Alencar RCG, Oliveira JC, Silva TA, Mendonça EF, Batista AC. Immunohistochemical investigations on the expression of programmed cell death ligand 1, human leukocyte antigens G and E, and granzyme B in intraoral mucoepidermoid carcinoma. Arch Oral Biol. 2017 Nov;83:55-62. doi: 10.1016/j.archoralbio.2017.07.004.

[70] Reimer MS, Engels CC, Putter H, Morreau H, Liefers GJ, van de Velde CJ, Kuppen PJ. Prognostic value of HLA class I, HLA-E, HLA-G and Tregs in rectal cancer: a retrospective cohort study. BMC Cancer. 2014 Jul 5;14:486. doi: 10.1186/1471-2407-14-486.

[71] Levy EM, Bianchini M, Von Euw EM, Barrio MM, Bravo AI, Furman D, Domenichini E, Macagno C, Pinsky V, Zucchini C, Valvassori L, Mordoh J. Human leukocyte antigen-E protein is overexpressed in primary human colorectal cancer. Int J Oncol. 2008;32 (3):633–641.

[72] Levy EM, Sycz G, Arriaga JM, Barrio MM, von Euw EM, Morales SB, González M, Mordoh J, Bianchini M. Cetuximab-mediated cellular cytotoxicity is inhibited by HLA-E membrane expression in colon cancer cells. Innate Immun. 2009 Apr;15(2):91-100. doi: 10.1177/175342 5908101404.

[73] Benevolo M, Mottolese M, Tremante E, Rollo F, Diodoro MG, Ercolani C, Sperduti I, Lo Monaco E, Cosimelli M, Giacomini P. High expression of HLA-E in colorectal carcinoma is associated with a favorable prognosis. J Transl Med. 2011 Oct 27;9:184. doi: 10.1186/1479-5876-9-184.

[74] Zhen ZJ, Ling JY, Cai Y, Luo WB, He YJ. Impact of HLA-E gene polymorphism on HLA-E expression in tumor cells and prognosis in patients with stage III colorectal cancer. Med Oncol. 2013 Mar;30(1):482. doi: 10.1007/s12032-013-0482-2.

[75] Zeestraten EC, Reimers MS, Saadatmand S, Goossens-Beumer IJ, Dekker JW, Liefers GJ, van den Elsen PJ, van de Velde CJ, Kuppen PJ. Combined analysis of HLA class I, HLA-E and HLA-G predicts prognosis in colon cancer patients. Br J Cancer. 2014 Jan 21; 110(2):459-68. doi: 10.1038/bjc.2013.696.

[76] Guo ZY, Lv YG, Wang L, Shi SJ, Yang F, Zheng GX, Wen WH, Yang AG. Predictive value of HLA-G and HLA-E in the prognosis of colorectal cancer patients. Cell Immunol. 2015 Jan;293(1): 10-6. doi: 10.1016/j.cellimm.2014. 10.003.

[77] Özgül Özdemir RB, Özdemir AT, Oltulu F, Kurt K, Yiğittürk G, Kırmaz C. A comparison of cancer stem cell markers and nonclassical major histocompatibility complex antigens in colorectal tumor and noncancerous tissues. Ann Diagn Pathol. 2016 Dec;25: 60-63. doi: 10.1016/j.anndiagpath. 2016.09.012.

[78] Huang R, Zhang D, Li F, Xiao Z, Wu M, Shi D, Xiang P, Bao Z. Loss of Fas expression and high expression of HLA-E promoting the immune escape of early colorectal cancer cells. Oncol Lett. 2017 May;13(5):3379-3386. doi: 10.3892/ ol.2017.5891.

[79] Stangl S, Gross C, Pockley AG, Asea AA, Multhoff G. Influence of Hsp70 and HLA-E on the killing of leukemic blasts by cytokine/Hsp70 peptide-activated human natural killer (NK) cells. Cell Stress Chaperones. 2008 Summer;13(2): 221-30. doi: 10.1007/s12192-007-0008-y.

[80] Chen A, Shen Y, Xia M, Xu L, Pan N, Yin Y, Miao F, Shen C, Xie W, Zhang J. Expression of the nonclassical HLA class I and MICA/B molecules in human hepatocellular carcinoma. Neoplasma. 2011;58(5):371-6.

[81] Talebian Yazdi M, van Riet S, van Schadewijk A, Fiocco M, van Hall T, Taube C, Hiemstra PS, van der Burg SH. The positive prognostic effect of stromal CD8+ tumor-infiltrating T cells is restrained by the expression of HLA-E in non-small cell lung carcinoma. Oncotarget. 2016 Jan 19;7(3):3477-88. doi: 10.18632/oncotarget.6506.

[82] de Kruijf EM, Sajet A, van Nes JG, Natanov R, Putter H, Smit VT, Liefers GJ, van den Elsen PJ, van de Velde CJ, Kuppen PJ. HLA-E and HLA-G expression in classical HLA class I-negative tumors are of prognostic value for the clinical outcome of early breast cancer patients. J Immunol. 2010;185 (12):7452–7459. DOI: 10.4049/ jimmunol.1002629.

[83] da Silva GB, Silva TG, Duarte RA, Neto NL, Carrara HH, Donadi EA, Gonçalves MA, Soares EG, Soares CP. Expression of the Classical and Nonclassical HLA Molecules in Breast Cancer. Int J Breast Cancer. 2013;2013: 250435. doi: 10.1155/2013/250435.

[84] Gooden M, Lampen M, Jordanova ES, Leffers N, Trimbos JB, van der Burg SH, Nijman H, van Hall T. HLA-E expression by gynecological cancers restrains tumor-infiltrating CD8 T lymphocytes. Proc Natl Acad Sci U S A. 2011;108(26):10656-61. doi: 10.1073/ pnas.1100354108.

[85] Gonçalves MA, Le Discorde M, Simões RT, Rabreau M, Soares EG, Donadi EA, Carosella ED. Classical and non-classical HLA molecules and p16 (INK4a) expression in precursors lesions and invasive cervical cancer. Eur J Obstet Gynecol Reprod Biol. 2008 Nov;141(1):70-4. doi: 10.1016/j. ejogrb.2008.06.010.

[86] Spaans VM, Peters AA, Fleuren GJ, Jordanova ES. HLA-E expression in cervical adenocarcinomas: association with improved long-term survival. J Transl Med. 2012 Sep 4;10:184. doi: 10.1186/1479-5876-10-184.

[87] Ferns DM, Heeren AM, Samuels S, Bleeker MCG, de Gruijl TD, Kenter GG, Jordanova ES. Classical and non-classical HLA class I aberrations in primary cervical squamous- and adenocarcinomas and paired lymph node metastases. J Immunother Cancer. 2016 Nov 15;4:78. doi: 10.1186/s40425-016-0184-3.

[88] Andersson E, Poschke I, Villabona L, Carlson JW, Lundqvist A, Kiessling R,

Seliger B, Masucci GV. Non-classical HLA-class I expression in serous ovarian carcinoma: Correlation with the HLA-genotype, tumor infiltrating immune cells and prognosis. Oncoimmunology. 2015 Jul 25;5(1):e1052213

[89] Zheng H, Lu R, Xie S, Wen X, Wang H, Gao X, Guo L. Human leukocyte antigen-E alleles and expression in patients with serous ovarian cancer. Cancer Sci. 2015 May;106(5):522-8. doi: 10.1111/cas.12641.

[90] Hanak L, Slaby O, Lauerova L, Kren L, Nenutil R, Michalek J. Expression pattern of HLA class I antigens in renal cell carcinoma and primary cell line cultures: methodological implications for immunotherapy. Med Sci Monit. 2009 Dec;15(12):CR638-43.

[91] Kren L, Valkovsky I, Dolezel J, Capak I, Pacik D, Poprach A, Lakomy R, Redova M, Fabian P, Krenova Z, Slaby O. HLA-G, and HLA-E specific mRNAs connote opposite prognostic significance in renal cell carcinoma. Diagn Pathol. 2012;29;7:58. DOI: 10.1186/1746-1596-7-58. PMID: 22640987.

[92] Zanetti BR, Carvalho-Galano DF, Feitosa NL, Hassumi-Fukasawa MK, Miranda-Camargo FA, Maciel LM, Ribeiro-Silva A, Soares EG. Differential expression of immune-modulatory molecule HLA-E in non-neoplastic and neoplastic lesions of the thyroid. Int J Immunopathol Pharmacol. 2013 Oct-Dec;26(4):889-96.

[93] Kren L, Fabian P, Slaby O, Janikova A, Soucek O, Sterba J, Krenova Z, Michalek J, Kral Z. Multifunctional immune-modulatory protein HLA-E identified in classical Hodgkin lymphoma: possible implications. Pathol Res Pract. 2012 Jan 15;208(1):45-9. doi: 10.1016/j.prp.2011.11.004.

[94] McWilliams EM, Mele JM, Cheney C, Timmerman EA, Fiazuddin F, Strattan EJ, Mo X, Byrd JC, Muthusamy N, Awan FT. Therapeutic CD94/NKG2A blockade improves natural killer cell dysfunction in chronic lymphocytic leukemia. Oncoimmunology. 2016 Sep 9;5(10): e1226720.

[95] Sensi M, Pietra G, Molla A, Nicolini G, Vegetti C, Bersani I, Millo E, Weiss E, Moretta L, Mingari MC, Anichini A. Peptides with dual binding specificity for HLA-A2 and HLA-E are encoded by alternatively spliced isoforms of the antioxidant enzyme peroxiredoxin 5. Int Immunol. 2009 Mar;21(3):257-68. doi: 10.1093/intimm

[96] Ravindranath, M.H., Jucaud, V., Ferrone, S. (2017) Monitoring native HLA-I trimer specific antibodies in Luminex multiplex single antigen bead assay: Evaluation of beadsets from different manufacturers. J Immunol Methods. 2017, 450:73–80. DOI: 10.1016/j.jim 201707. 016 PMID: 28782523. Corrigendum to Monitoring native HLA-I trimer specific antibodies in Luminex multiplex single antigen bead assay: Evaluation of beadsets from different manufacturers J. Immunol. Methods, 2018, 460:125. DOI:10.1016/j. jim. 2018. 07. 008.

[97] Ravindranath MH, Hopfield J, Ferrone S. HLA-E restricted monoclonal antibodies: Therapeutic potential as a double-edged sword against tumor progression. Internal Rev. Med. 2017;3 (12):1–49.

[98] Sasaki T, Ravindranath MH, Terasaki PI, Freitas MC, Kawakita S, Jucaud V. Gastric cancer progression may involve a shift in HLA-E profile from an intact heterodimer to β2m-free monomer. Int J Cancer. 2014;134(7): 1558–1570. DOI: 10.1002/ijc.28484.

[99] Ravindranath, M.H., Flippone, E. J., Mahowald, G., Callender, C, Babu, A., Saidman, S., Ferrone, S. (2018) Significance of the intraindividual

variability of HLA IgG antibodies in renal disease patients observed with different beadsets monitored with two different secondary antibodies on a Luminex platform. Immunologic Res. 66: 584–604. DOI: 10.1007/s12026-018-9027-2.

[100] Ravindranath, MH, Zhu D, Pham T, Jucaud V, Hopfield J, Kawakita S, Terasaki PI. Anti-HLA-E monoclonal antibodies reacting with HLA-la and lb alleles like IVIg as potential IVIg-immunomimetics: an evolving therapeutic concept. Clin Transpl.2013: 293–305.

[101] Ravindranath, MH. HLA Class Ia and Ib Polyreactive Anti-HLA-E IgG2a Monoclonal Antibodies (TFL-006 and TFL-007) Suppress Anti-HLA IgG Production by CD19+ B Cells and Proliferation of CD4+ T Cells While Upregulating Tregs. J Immunol Res. 2017:3475926. DOI: 10.1155/ 2017/ 3475926.

[102] Hurez V, Kaveri SV, Mouhoub A, Dietrich G, Mani JC, Klatzmann D, Kazatchkine MD. Anti-CD4 activity of normal human immunoglobulin G for therapeutic use. (Intravenous immunoglobulin, IVIg). Ther Immunol. 1994;1(5):269–277.

[103] Amran D, Renz H, Lack G, Bradley K, Gelfand EW. Suppression of cytokine-dependent human T-cell proliferation by intravenous immunoglobulin. Clin Immunol Immunopathol. 1994 Nov;73(2):180–186. DOI: 10.1006/clin.1994.1186.

[104] Andersson J, Skansén-Saphir U, Sparrelid E, Andersson U. Intravenous immune globulin affects cytokine production in T lymphocytes and monocytes/macrophages. Clin Exp Immunol. 1996;104 Suppl 1:10–104 Suppl 1:20.

[105] Aktas O, Waiczies S, Grieger U, Wendling U, Zschenderlein R, Zipp F. Polyspecific immunoglobulins (IVIg) suppress proliferation of human (auto) antigen-specific T cells without inducing apoptosis. J Neuroimmunol. 2001 Mar 1;114(1–2):160–167. DOI: 10.1016/ s0165-5728(01)00243-0.

[106] MacMillan HF, Lee T, Issekutz AC. Intravenous immunoglobulin G-mediated inhibition of T-cell proliferation reflects an endogenous mechanism by which IgG modulates T-cell activation. Clin Immunol. 2009;132 (2):222–233. DOI: 10.1016/j. clim.2009.04.002.

[107] Aubin E, Lemieux R, Bazin R. Indirect inhibition of in vivo and in vitro T-cell responses by intravenous immunoglobulins due to impaired antigen presentation. Blood. 2010; 115 (9):1727–1734. DOI: 10.1182/blood-2009-06-225417.

[108] Kondo N, Ozawa T, Mushiake K, Motoyoshi F, Kameyama T, Kasahara K, Kaneko H, Yamashina M, Kato Y, Orii T. J Suppression of immunoglobulin production of lymphocytes by intravenous immunoglobulin. Clin Immunol. 1991;11(3):152–158. DOI: 10.1007/BF00918683.

[109] Kondo N, Kasahara K, Kameyama T, Suzuki Y, Shimozawa N, Tomatsu S, Nakashima Y, Hori T, Yamagishi A, Ogawa T, et al. Intravenous immunoglobulins suppress immunoglobulin productions by suppressing Ca(2+)-dependent signal transduction through Fc gamma receptors in B lymphocytes. Scand J Immunol. 1994 Jul;40(1):37–42. DOI: 10.1111/j.1365-3083.1994.tb03430.x.

[110] Glotz D, Haymann JP, Sansonetti N, Francois A, Menoyo-Calonge V, Bariety J, Druet P.Suppression of HLA-specific alloantibodies by high-dose intravenous immunoglobulins (IVIg). A potential tool for transplantation of immunized patients. Transplantation.

1993;56(2):335–337. DOI: 10.1097/00007890-199308000-00015.

[111] Glotz D, Antoine C, Haymann JP, Julia P, Duboust A, Bariéty J. Intravenous immunoglobulins and kidney transplantation in patients with anti-HLA antibodies. Adv Nephrol Necker Hosp. 2000;30:221–233.

[112] Tyan DB, Li VA, Czer L, Trento A, Jordan SC. Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. Transplantation. 1994;57(4):553–562.

[113] Jordan SC, Quartel AW, Czer LS, Admon D, Chen G, Fishbein MC, Schwieger J, Steiner RW, Davis C, Tyan DB.Posttransplant therapy using high-dose human immunoglobulin (intravenous gammaglobulin) to control acute humoral rejection in renal and cardiac allograft recipients and potential mechanism of action. Transplantation. 1998;66(6):800–805. DOI: 10.1097/00007890-199809270-00017.

[114] Okuda S, Kamei S, Sasaki T. Immunoglobulin G Enhances Generation of Inducible T Regulatory Cells and Increases Their Regulatory Function. Biol Pharm Bull. 2018;41(12): 1830–1836. DOI: 10.1248/bpb.b18-00548.

[115] Ephrem A, Chamat S, Miquel C, Fisson S, Mouthon L, Caligiuri G, Delignat S, Elluru S, Bayry J, Lacroix-Desmazes S, Cohen JL, Salomon BL, Kazatchkine MD, Kaveri SV, Misra N. Expansion of CD4+CD25+ regulatory T cells by intravenous immunoglobulin: a critical factor in controlling experimental autoimmune encephalomyelitis. Blood. 2008; 111(2): 715–722. DOI: 10.1182/blood-2007-03-079947.

[116] Zhu D, Ravindranath MH, Terasaki PI, Miyazaki T, Pham T, Jucaud V. Suppression of allo-human leucocyte antigen (HLA) antibodies secreted by B memory cells in vitro: intravenous immunoglobulin (IVIg) versus a monoclonal anti-HLA-E IgG that mimics HLA-I reactivities of IVIg. Clin Exp Immunol. 2014;177(2):464–477. DOI: 10.1111/cei.12307.

[117] Ravindranath MH, Terasaki PI, Pham T, Jucaud V, Kawakita S. Suppression of blastogenesis and proliferation of activated CD4(+) T cells: intravenous immunoglobulin (IVIg) versus novel anti-human leucocyte antigen (HLA)-E monoclonal antibodies mimicking HLA-I reactivity of IVIg. Clin Exp Immunol. 2014;178(1): 154–177. DOI: 10.1111/cei.12391.

[118] Ravindranath MH, Zhu D, Pham T, Jucaud V, Hopfield J, Kawakita S, Terasaki PI. Anti-HLA-E monoclonal antibodies reacting with HLA-la and lb alleles like IVIg as potential IVIg-immunomimetics: an evolving therapeutic concept. Clin Transpl. 2013: 293–305

[119] Hicklin DJ, Marincola FM, Ferrone S. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. Mol Med Today. 1999;5(4):178–186. DOI: 10.1016/s1357-4310(99)01451-3.

[120] Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. Adv Immunol. 2000;74: 181–273. DOI: 10.1016/s0065-2776(08)60911-6.

[121] Campoli M, Chang CC, Oldford SA, Edgecombe AD, Drover S, Ferrone S. HLA antigen changes in malignant tumors of mammary epithelial origin: molecular mechanisms and clinical implications. Breast Dis. 2004;20:105–125. DOI: 10.3233/bd-2004-20112.

[122] Campoli M, Chang CC, Ferrone S. HLA class I antigen loss, tumor immune

escape, and immune selection. Vaccine. 2002;20 Suppl 4:A40–A45. DOI: 10.1016/s0264-410x(02)00386-9.

[123] Chang CC, Campoli M, Ferrone S. Classical and nonclassical HLA class I antigen and NK Cell-activating ligand changes in malignant cells: current challenges and future directions. Adv Cancer Res. 2005;93:189–234. DOI: 10.1016/S0065-230X(05)93006-6.

[124] Algarra I, García-Lora A, Cabrera T, Ruiz-Cabello F, Garrido F. The selection of tumor variants with altered expression of classical and nonclassical MHC class I molecules: implications for tumor immune escape. Cancer Immunol Immunother. 2004;53(10):904–10. DOI: 10.1007/s00262-004-0517-9.

[125] Cabrera T, López-Nevot MA, Gaforio JJ, Ruiz-Cabello F, Garrido F. Analysis of HLA expression in human tumor tissues. Cancer Immunol Immunother. 2003;52(1):1–9. DOI: 10.1007/s00262-002-0332-0.

[126] Seliger B. The non-classical antigens of HLA-G and HLA-E as diagnostic and prognostic biomarkers and as therapeutic targets in transplantation and tumors. Clin Transpl. 2013:465–472.

[127] Seliger B, Abken H, Ferrone S. HLA-G, MIC expression in tumors, and their role in anti-tumor immunity. Trends Immunol. 2003;24(2):82–87. DOI: 10.1016/s1471-4906(02) 00039-x.

[128] Bukur J, Jasinski S, Seliger B. The role of classical and non-classical HLA class I antigens in human tumors. Semin Cancer Biol. 2012;22(4):350–358. DOI: 10.1016/ j.semcancer.2012.03.003.

[129] Dutta N, Gupta A, Mazumder DN, Banerjee S. Down-regulation of locus-specific human lymphocyte antigen class I expression in Epstein-Barr virus-associated gastric cancer: implication for viral-induced immune evasion. Cancer. 2006;106(8):1685–1693. DOI: 10.1002/ cncr.21784.

[130] Dutta N, Majumder D, Gupta A, Mazumder DN, Banerjee S. Analysis of human lymphocyte antigen class I expression in gastric cancer by a reverse transcriptase-polymerase chain reaction. Hum Immunol. 2005;66(2): 164–169. DOI: 10.1016/j.humimm. 2004.10.010.

[131] Huang Z, Hyodo H, Fujii T, Nagamatsu T, Matsumoto J, Kawana K, Yamashita T, Yasugi T, Kozuma S, Taketani Y. Effect of progesterone on HLA-E gene expression in JEG-3 choriocarcinoma cell line. Am J Reprod Immunol. 2009;61(3):221–226. DOI: 10.1111/j.1600-0897.2008.00684.x.

[132] Bianchini M, Levy E, Zucchini C, Pinski V, Macagno C, De Sanctis P, Valvassori L, Carinci P, Mordoh J. Comparative study of gene expression by cDNA microarray in human colorectal cancer tissues and normal mucosa. Int J Oncol. 2006;29(1):83–94.

[133] Tremante E, Ginebri A, Lo Monaco E, Benassi B, Frascione P, Grammatico P, Cappellacci S, Catricalà C, Arcelli D, Natali PG, Di Filippo F, Mottolese M, Visca P, Benevolo M, Giacomini P. A melanoma immune response signature including Human Leukocyte Antigen-E. Pigment Cell Melanoma Res. 2014;27(1): 103–112. DOI: 10.1111/ pcmr.12164.

[134] Zhang X, Lin A, Zhang JG, Bao WG, Xu DP, Ruan YY, Yan WH. Alteration of HLA-F and HLA I antigen expression in the tumor is associated with survival in patients with esophageal squamous cell carcinoma. Int J Cancer. 2013;132(1):82–89. DOI: 10.1002/ ijc.27621.

[135] Petrie EJ, Clements CS, Lin J, Sullivan LC, Johnson D, Huyton T, Heroux A, Hoare HL, Beddoe T, Reid HH, Wilce MC, Brooks AG, Rossjohn J. CD94-NKG2A recognition of human

leukocyte antigen (HLA)-E bound to an HLA class I leader sequence. J Exp Med. 2008;205(3):725–735. DOI: 10.1084/jem.20072525.

[136] Braud VM, Allan DS, O'Callaghan CA, Söderström K, D'Andrea A, Ogg GS, Lazetic S, Young NT, Bell JI, Phillips JH, Lanier LL, McMichael AJ. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. Nature. 1998; 391(6669):795–799. DOI: 10.1038/35869.