 doi:10.20944/preprints202105.0183.v1

REVIEW

Therapeutic potential of HLA-I polyreactive mAbs mimicking the HLA-I polyreactivity and immunoregulatory functions of IVIg

Mepur H. Ravindranath[1] Fatiha E. Hilali[2] Edward Filippone[3]

[1]Department. of Hematology and Oncology, Children's Hospital, Los Angeles, CA 90027, Formerly, Research Scientist at Terasaki Foundation Laboratory, Santa Monica, CA. *thiruranganath04@gmail.com,* Orcid No: 0000-0002-5731-41
[2]Fatiha El Hilali, 28 DB Dighoussi Zitoune Meknes, Morocco, *hfatiha@gmail.com*
[3]Division of Nephrology, Dept. of Medicine, Sidney Kimmel Medical College at Thomas Jefferson University. Philadelphia PA 19145. *kidneys@comcast.net* Orcid No: 0000 0002 3161 5820.

Corresponding Author: Mepur H. Ravindranath, *thiruranganath04@gmail.com,* Orcid No: 0000-0002-5731-41

Keywords:
Intravenous Immunoglobulin (IVIg); Human Leukocyte Antigen-I (HLA-1); Polyreactive mAbs; Monospecific mAbs; Shared epitopes; Immunosuppression; T-cells; B-memory cells; T-regulatory Cells; Blastogenesis, proliferation,
Antibody production

 doi:10.20944/preprints202105.0183.v1

ABSRACT

HLA class-I (HLA-I) polyreactive monoclonal antibodies (mAbs) reacting to all HLA-I alleles were developed by immunizing HLA-E monomeric α–heavy chain (αHC) (Open Conformers, OCs). Two of the mAbs (TFL-006 and TFL-007) bound to the αHC's coated on a solid matrix. The binding was inhibited by a peptide ^{117}AYDGKDY^{123}, present in all alleles of the six HLA-I isoforms but masked by β2-microglobulin (β2-m) in intact HLA-I trimers (Closed Conformers, CCs). Identical HLA-I polyreactivity is observed in IVIg administered to lower anti-HLA antibodies (Abs) in HLA-sensitized patients, but the mechanism is unknown. We hypothesized that the mAbs that mimic IVIg HLA-I polyreactivity might mimic the immunomodulatory functions of IVIg. We tested the relative binding affinity of the mAbs and IVIg for both OCs- and CCs and compared their effects on (a) the phytohemagglutinin (PHA)-activation T-cells, (b) the production of anti-HLA-II antibody (Ab) by B-memory cells, and anti-HLA-I Ab by immortalized B-cells, and (c) the upregulation of CD4+, CD25+, and Fox P^3+ T-regs. The mAbs bound only to OCs, whereas IVIg is bound to both CCs and OCs. The mAbs suppressed blastogenesis and proliferation of PHA-activated T-cells, anti-HLA Ab production by B-cells and expanded the T-regs, better than IVIg. We conclude that a humanized version of the TFL-mAbs could be an ideal therapeutic IVIg-mimetic. (words 208)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

1.0. INTRODUCTION

Personalized passive immunotherapy encompasses the transfer of monoclonal antibodies (mAbs) or polyclonal antibodies (Abs) or purified natural Abs, with a specific purpose of either upregulating or suppressing immune functions depending on the nature and status of the disease. A variety of mAbs capable of performing specific functions are available, including depletion of various cells, such as B-cells (Rituximab), CD52-bearing leukocytes (Alemtuzumab), and CD33 bearing cells (gemtuzumab). They can also inhibit various cytokine pathways such as the TNF-TNFR axis (Etanercept, Adalimumab, Infliximab) and the IL-6-IL-6R axis (Tocilizumab, Actemra). In contrast, therapeutic Intravenous Immunoglobulin (IVIg) represents purified natural Abs, prepared by pooling and purifying IgG from the plasma of 10 to 60 thousands of normal and healthy donors, after screening their blood for known pathogens. The United States Food and Drug Adminstration (FDA) has approved immunopropylaxis with IVIg therapy for chronic inflammatory demyelinating polyneuropathy (CIDP), immune thromocytopenic purpura (ITP), primary pmmunodeficiency (PI), secondary immunodeficiency in chronic lymphocytic leukemia, Pediatric human immunodeficiency virus (HIV) infection, Kawasaki disease, prevention of graft vs host disease in adult bone marrow transplant recipients, and organ transplantation [1].

IVIg is administered to both end stage organ disease patients pretransplantation and allograft recipients post-transplantation for reduction (desensitization) of the Abs formed against Human Leukocyte Antigens (HLA) (called sensitization), which may cause severe Ab-mediated rejection and early graft loss [2, 3]. The exact mechanism by which IVIg eliminates the HLA-Abs remains ambiguous. However, IVIg *per se* contains polyreactive natural IgGs against multiple targets, including endogenous and exogenous Abs, immunomodulating peptides, blood group antigens and various cytokines. Whereas few of the immunoregulatory mechanisms of action of IVIg are proven *in vitro* and in animal models, many proposed mechanisms still remain enigmatic, due to IVIg polyantigen-reactivity, polyclonality, and diversity in the preparations of IVIg.. Several mechanisms of action of IVIg have been proposed [4]. These include (a) Fc-receptor blockade; (b) neutralization of autoAbs by idiotypic and anti-idiotypic Abs; (c) blockade of the Fas apoptotic pathway by anti-Fas autoAbs; (d) regulation of complement components; (e) modulation of cytokine secretion; (f) hindrance of natural-killer cell activity; (g) inhibition of

 doi:10.20944/preprints202105.0183.v1

matrix metalloproteinase-9; (h) suppression of NF-kB activation and IkB degradation; (i) G1 cell cycle arrest; (j) prevention of tumor growth; (k) decrease in leukocyte recruitment; (l) attenuation of T-cell stimulation; (m) effects on Ab kinetics; and (n) effects on dendritic cells. These mechanisms of IVIg operate synergistically.

Additionally, more observations have accrued to document that normal healthy human sera contain diverse varieties of IgG Abs against HLA [5 – 9]. Hence, since IVIg may carry anti-HLA IgG Abs, the question then arises as to whether these anti-HLA Abs are directed against self-HLA antigens or against allogeneic HLA antigens.

Under the able guidance of Late Professor Paul Ichiro Terasaki, a wide variety of anti-HLA IgG Abs, their immunomodulatory roles, and their immunoregulatory capabilities in therapeutic IVIg preparations were unraveled. This research commenced with studying the structure of a non-classical HLA-I antigen, namely HLA-E, and its mAbs [10, 11]. It is essential to identify the unique and shared amino acid sequences of the diverse HLA-I antigens, and this to elucidate whether the mAbs generated by immunizing an HLA-I antigen recognize unique (monospecific, restricted to one HLA class I locus) or shared (polyreactive, expressed by multiple class I loci) amino acid sequences and their relative immunoregulatory capabilities.

2.0. HLA-E SHARES AMINO ACID SEQUENCES (EPITOPES) COMMON TO ALL OTHER HLA-I ISOFORMS.

HLA antigens,located on the surface of nucleated cells of the human body, are of two different major classes, HLA-I and HLA-II. The genes that encode HLA-I and HLA-II are closely associated with each other on the short arm of human chromosome 6. The entire complex of HLA encompasses four million base pairs of DNA and "is of a size comparable to the genome of *Escherichia coli"* [12].

The HLA-I molecule is a heterodimer with 45kDa α-heavy chain (αHC) anchored to the bilayered lipid membrane. It is complexed with a water-soluble 12kDa light chain, called β2-microlobulin (β2-m), encoded by a gene located on chromosome 15. The HLA-I loci consist of the classical HLA-Ia and non-classical HLA-Ib loci, each containing three loci: HLA-Ia (HLA-A, HLA-B and HLA-C), and HLA-Ib (HLA-E, HLA-F, and HLA-G). The αHC of HLA-I consists

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

of different domains encoded by different exons. First, the leader peptide, then the three extracellular domains (α1, α2, & α3), the transmembrane domain and two cytoplasmic domains. Each domain is encoded by different exons. The antigenic polymorphism of different isoforms is reflected in the amino acid sequences. The sequences or epitopes characteristic of each allele of each locus are referred to as its "*private epitopes*" or "specific sequences". However, all isoforms also share several common or shared sequences with other alleles of the same locus or other loci, referred to as "*public epitopes*".

2.1. *Private and Public epitopes of HLA-E*

Table 1 shows both HLA-E amino acid sequences that are specific and those that are shared with alleles of other loci of HLA-I. Some amino acid sequences or epitopes are specific for the HLA-E locus, since they are not found in other HLA-Ia or Ib loci. The most striking private epitopes or sequences are ^{65}RSARDTA^{70} and ^{143}SEGKSNDASE^{152}. **Table 2** summarizes the results of comparing HLA-E sequences with the hundreds of alleles of HLA-Ia and HLA-Ib loci. Some sequences of HLA-E are found in only one allele. The HLA-E sequence, PRAPWMEQE and EPPKTHVT are found in HLA-A*3306 and HLA-B*8201, respectively, whereas the following sequences of HLA-E, ^{117}AYDGKDY^{123} and ^{126}LNEDLRSWTA^{135} are shared with all other HLA loci (HLA-A, HLA-B, HLA-C, HLA-F, and HLA-G) that we have examined. These latter sequences perfectly befit the definition of "shared" or "public epitopes".

2.2. The most widely shared public epitopes are cryptic in HLA-I

The HLA-I molecule is a heterodimer since the α-HC is closely associated with β2-m (**Figure 1**). As a consequence not all sequences of HLA α-HC are exposed. **Figure 1A** shows that the specific sequences or the private epitopes of HLA-E (shown in yellow), ^{65}RSARDTA^{70}, and ^{143}SEGKSNDASE^{152}are exposed for direct binding of Abs. **Figure 1B** shows that the most commonly shared sequences or the public epitopes (shown in yellow) ^{117}AYDGKDY^{123} and ^{126}LNEDLRSWTA^{135} are cryptic as they lie in close proximity with β2-m. **Figure 2** illustrates the crypticity of the shared epitope, ^{117}AYDGKDY^{123}. The exposure of these shared epitopes depends on the nature of the HLA molecule. Most often the HLA molecules occur as heterodimers, also

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

designated as "closed conformers" or CCs. In CCs, the most prevalent shared epitopes ^{117}AYDGKDY^{123} and ^{126}LNEDLRSWTA^{135} are masked by β2-m. However, in β2m-free α-HC, designated as "open conformers" (OCs), they are exposed.

Thus, the HLA-I molecules not only occur as heterodimers complexed with β2m on the cell surface but also as β2m-free HC OCs. Schnabl et al [13] reported that T lymphocytes, activated *in vitro* or *in vivo*, but not resting, expressed many OCs. Using the mAb W6/32 that specifically recognized HLA-I CCs and the mAbs L45 and HC10 that bound specifically to OCs but not to CCs, they confirmed the existence of HLA-OCs. Immunoprecipitation and co-capping experiments showed that LA45 bound to HLA-I OCs at the cell surface. The mAb L45 bound to PHA-activated T-cells from a panel of 12 people with different HLA types, suggesting that LA45 may bind to epitopes shared by all HLA-I HCs. The HLA-I OCs expressed on the cell surface of activated T-cells-or EBV-transformed B-cells-are referred to as "peptide-binding empty HLA" [14, 15]. The presence of HLA-I OCs was confirmed on activated T-cells *in vitro* and *in vivo* and on B cell lines (RAJI, NALM6), EBV-transformed B-cells, and the myeloid cell line KG-1A [16, 17]. Interestingly, the expression of OCs on the cell surface in normal human T-cells upon activation and cell division correlated with the level of proliferation [18]. Indeed, the OCs on the cell surface were glycosylated. The inhibition of tyrosine phosphorylation with the Src-family tyrosine kinase inhibitor PP2 resulted in the enhanced release of HLA-I α-HC from the cell surface of activated T-cells. Further studies indicate that the OCs are regulators of ligand-receptor interactions and have potential implications for immune activation [18, 19, 20] and immune regulation [21]. The inflamed tissues in spondyloarthropathy showed increased levels of OCs on CD14b+ monocytes compared to other leukocyte subsets. The level of OCs also increased on activated dendritic cells of the extravillous trophoblast [22]. Interestingly, the OCs maintained the peptide-binding groove *in vitro* [23]. Khare et al [24] reported that β2m-free HC of HLA-B27 may induce arthritis in transgenic mice and β2m-free HC-specific Abs decreased the disease incidence in this model. OCs exist as dimers or in clusters at the cell surface *in vivo* [23, 25 – 27], profoundly impacting receptor engagement. Recognition of OCs as ligands by members of the killer Ig receptor family (KIR) and Ig-like transcript (ILT)/LIR/LILR family could influence their immunomodulatory function in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

inflammatory disease [28]. The shared sequences present in HLA-E OCs contribute to the generation of the HLA-I polyreactivity Abs.

Though the OCs are found on activated immune cells, the soluble forms.of HLA-I present in human occur as OCs. Demaria et al [29, 30] found that the levels of OCs in circulation are regulated by proteolytic cleavage. The release is mediated by a Zn(2+)-dependent, membrane-bound metalloprotease (MMP), Specific separation by the metalloprotease occurs at a site close to the papain cleavage site in the α3 domain of HLA-I HCs. This site is not accessible to MMP in CCs. During the shedding of HLA-I trimers under different pathological conditions, the exogenous peptide and β2-m dissociate from these CCs and subsequent cleavage of OCs **(Figure 3)** may be partially responsible for controlling the levels of OCs on the surface of activated cells. Since the shed OCs expose previously cryptic epitopes, they may be recognized by B-cells for antibody production.

2.3. Antigenicity Rank of the most widely shared public epitopes of HLA-E.

It is known that an Ab directed against a protein antigen can recognize either a linear or a discontinuous sequence (still closely aligned in space due to protein folding) in its native conformation. At the core of this recognition is the antigen's epitope, typically one to several amino acids, which, as noted above, may be continuous or discontinuous. The immunogenicity of an epitope is defined as its ability to actively induce an immune response, whereas the antigenicity of an epitope is defined as its passive ability to be recognized by the immune system. The antigenicity of a sequence or epitope in a polypeptide can be predicted using parameters such as hydrophilicity, flexibility, accessibility, beta turns, cell surface exposure, and polarity. Several methods are known for predicting the antigenicity of a continuous sequence. Chou and Fasman [31] developed a methodology to predict antigenicity based on α-turns in protein secondary structures. Kolaskar and Tongaokar [32] developed a semi-empirical method using physicochemical properties of amino acid residues and their frequency of occurrence in segmental epitopes. Karplus and Scholuz [33] predicted antigenicity based on three flexibility scales. Parker et al [34] have predicted the antigenicity with a novel hydrophilicity scale. In these methods, when computing the score for given residue *i*, the amino acids in an interval of the chosen length,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

centered around residue *i*, are considered. Further details are provided in the antibody epitope prediction web (*http://tools. immuneepitope.org/ tools/bcell/ iedb_input*). The methods were collectively employed to assess the antigenicity ranking of different sequences of HLA-E listed in **Table 2.** Among shared peptide sequences listed in the table, the most prevalent shared epitope ^{117}AYDGKDY^{123} ranked first in antigenicity. We have used these peptide sequences and the second-ranking epitope for peptide inhibition studies of anti-HLA-E mAbs and established that mAbs TFL-006 and TFL-007 are the most polyreactive mAbs [10, 11, 35, 36].

3.0. HLA-I POLYREACTIVITY OF HLA-E mAbs

3.1. Methodology-development and characterization of HLA-E mAbs.

3.1.1. Anti-HLA-E mAb production

The mAbs were produced following the recommendations of the National Research Council's Committee on Methods of Producing mAbs [37]. The recombinant α-HCs HLA-E^{R107}and HLA-E^{G107} (Source: Immune Monitoring Laboratory, Fred Hutchinson Cancer Research Center, Concentration:10 mg/mL in MES buffer) were used. Two mice were immunized with 50 mg of each antigen in PBS (pH 7.4, 100 mL) admixed with TiterMax (100 mL Sigma-Aldrich, St Louis, MO) adjuvant before injection into the footpad and intraperitoneally. Three immunizations were given at about 12-day intervals, with an additional immunization after 12 days for mice receiving HLA-E^{G107}.

The clones were cultured in a medium (RPMI 1640 w/L-glutamine and sodium bicarbonate, with 15% FBS Omega Scientific Inc, Tarzana, CA, USA; Cat No: FB-11, Lot: 810225 15%; Penicillin Streptomycin(corning) Mediatech Inc, Manassas, VA, USA Cat No. 30-002-cl. Lot. 30002312; L-Gluatamine Mediatech Inc Cat No. 25-005-cl Lot 25005245; Sodium pyruvate (Gibco) Thermo Fischer Cat No. 1136-070 Lot. 2028919). Hybridomas were cryopreserved in RPMI containing 10% DMSO and 20% FBS. All culture supernatants were screened for IgG reacting to HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G using single antigen microbeads on a Luminex platform. The mean fluorescent intensity (MFI) was determined for supernatants after diluting to 1/2. The MFI values were corrected against those obtained with negative control beads. Further details are provided elsewhere [35, 38].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

3.1.2. HLA-1 antigens coated Beadsets and the Luminex Platform for monitoring the reactivity and the density of these mAbs

The affinity of Abs to different HLA-I antigens are monitored on a Luminex Platform using HLA-I molecules coated as single antigens on multiplex fluorescinated microbeads. Three different kinds of microbeads are available for investigation as illustrated in **Figure 4**: (i) beads with an admixture of CCs and OCs (Regular LABScreen beads, Source: One Lambda Inc. Canoga Park, CA), (ii) beads restricted with CCs only (iBeads developed by trypsinization of regular LABScreen beads, and Immucore LIFECODES beads), (iii) beads restricted with OCs (alkali or acid denatured beads). The regular LABScreen microbeads are coated with HLA-A (31 different antigens), HLA-B (50 different antigens), and HLA-C (16 different antigens). 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.

The different kinds of microbeads were characterized using three different mAbs (**Figure 4**). The mAb W6/32 (IgG2a) bound to CCs but not with OCs [39] and bound to both peptide-associated and peptide-free CCs [40, 41]. The mAb HC-10, (IgG2a) recognized an epitope in HLA-I between amino acid positions 57 and 62, with arginine at position 62 (R62) is crucial for HC-10 binding [42]. HC-10 recognizes cell surface CCs devoid of a peptide, whereas the presence of a peptide reduced the HC-10 reactivity [43[. The mAb TFL-006 (IgG2a) bound to OCs of all HLA-I and was inhibited by peptides from the amino acid sequences shared by all HLA-I [35, 36].

3.2 The HLA-reactivity groups of mAbs generated by recombinant HLA-E OCs

More than 200 hybridomas were generated using OCs of HLA-E. The mAbs secreted by these hybridomas were both HLA-E monospecific and HLA-Ia and HLA-Ib polyreactive. These mAbs can be categorized into eight different groups as shown in **Table 3**. Group 1 correspond to monospecific mAbs reacting restrictively with HLA-E. Group 4 refers to HLA-Ib specific mAbs. Group 10 represents mAbs recognizing both HLA-Ia and HLA-Ib molecules (**Table 4**).

Table 4 compares the HLA-I reactivities of an HLA-E monospecific mAb TFL-033 with that of two HLA-I polyreactive mAbs, namely TFL-006 and TFL-007. TFL-006 shows reactivity with 32 alleles

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

of HLA-A, 48 alleles of HLA-B and 16 alleles of HLA-C, whereas TFL-007 reacts with 24 HLA-A, 44 HLA-B and 16 HLA-C alleles. TFL-006 did not react with B*4901 and B*5701 although the shared sequences or the public epitopes are present in them, suggesting that Both TFL-006 and TFL-007 react with all the three HLA-Ib isoforms as follows (HLA-E (+++) > HLA-G (++) > HLA-F (+). Among HLA-Ia isoforms the reactivities in general are as follows: HLA-C (+++) > HLA-B (++) > HLA-A (+). The MFI of the mAbs are the highest with A*1101 among HLA-A isoforms, with B*4006 the highest among HLA-B isoforms and C*1802 the highest among HLA-C isoforms. Of the seven HLA-polyreactive mAbs belonging to group 10, TFL-006 ranks first and TFL-007 ranks second. The reactivities of other mAbs of group 10 are presented elsewhere [44].

We have designed a set of experiments to determine the affinity of the mAbs TFL-006 and TFL-007 for shared sequences in the OCs. We have obtained synthetic peptides purified by reverse-Phase HPLC from GenScript Corporation (Piscataway, NJ) (Ravindranath et al. 2010). We have compared peptide inhibition using three commonly shared peptides listed in **Table-2**. They are DTAAQI, AYDGKDY and LNEDLRSWTA. These peptides were used separately to block the binding of the mAbs to the regular LABSCreen Beadsets in which the OCs (β2-m-free α-HC) are admixed with intact HLA trimers (CCs). Of the three peptides tested, AYDGKDY blocked more than 52% of the binding to the mAb TFL-006 compared to other two peptides. Similar results were obtained with mAb TFL-007.

To further ascertain that the mAbs recognize only the open conformers, we have compared binding of mAb TFL-006 on LABScreen beadsets (One Lambda Inc., Canoga Park, CA) which contain an admixture of OCs and CCs with LIFECODES beadsets (LSA Class I 03203F beads; Immucor, Norcross, GA), which are totally devoid of HLA-I OCs [45 -48]. The results presented in **Table 5** show that the mAb TFL-006 does not bind to any of the alleles of the isoforms of HLA-I on LIFECODE beadsets, confirming that the mAb TFL-006 recognizes only the OCs and did not bind to CCs on the LIFECODE beads. Earlier, we have shown that mAb TFL-006 binds well on acid-denatured LABScreen beads but not on iBeads, in which β2-m-free HCs (OCs) are selectively and enzymatically removed from the regular LABScreen beads [45, 46].

4.0. HLA-I REACTIVITY OF THE THERAPEUTIC PREPARATIONS OF IVIg

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

The FDA in 2004, approved the Cedars–Sinai IVIg desensitization protocol for minimizing allo-HLA Abs (Abs) in patients waiting for kidney trans transplantation, given the known ability of such antibodies to destroy an allograft. Removal, or significant reduction, of such antibodies would then allow transplantations to proceed that would have otherwise been contraindicated. Since that time, IVIg has emerged as a potential treatment strategy for desensitization protocols pre-transplantation, and is now used even post-transplantation, for treating Ab-mediated rejection (AMR) mediated by donor-specific anti-HLA Abs (DSA). Several clinical transplant centers adopted this strategy [2,3, 49 - 54]. However, subsequent studies document that IVIg preparations were often unable to reduce HLA Abs in sera of transplant patients [54 – 57]. We have examined five different theraeutic preparations of IVIg namely GamaSTAN S/D (Talecris Biotherapeutics, Inc, Research Triangle Park, NC), Sandoglobulin (6 gr, lot 4305800026; CSL Behring, Kankakee, IL), Octagam (6 gr, lot A913A8431; Octapharma Pharmazeutika, Switzerland); and IVIGlob EX (VHB Life Sciences Limited, India) [34, 35] and Immunoglobuline Normale (IV-LFB-CNTs LFB Biomedicaments. Courtaboeuf Cedex, France) [110].

HLA-I reactivities of the different dilutions (1/2 to 1/128) of IVIg preparations were tested on regular LABScreen beadsets, acid denatured LABScreen beadsets (OCs only), and on enzymatically treated regular LABScreen beadsets called iBeads (CCs only). The details of methodology were presented elsewhere [34]. **Figure 5** compares the Mean Fluorescent Intensity (MFI) signifying the antigen density of HLA-Ia and HLA-Ib IgG Abs on the three different beadsets for IVIg preparations from GamaSTAN (**Figure 5A**) and Octagram (**Figure 5B**). The data reveal that the strength of the Abs is much higher in denatured beadsets, which is predominant with β2-m-free HCs (OCs) than in intact β2-m-associated HCs (CCs) restricted to the iBeads. These findings confirm that the anti-HLA IgG Abs in the IVIg preparations recognize both CCs and OCs of HLA molecules but with a higher prevalence of Abs recognizing OCs. **Table 6** summarizes the HLA-I polyreactivity of commercial preparations of IVIg from different sources.

5.0. IMMUNOMODULATION BY IVIg COMPARED WITH POLYREACTIVE mAbs

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

5.1. Suppression of T-cell proliferation: IVIg vs HLA-I polyreactive mAbs.

5.1.1. Background and Hypothesis

Activation of T-cells involves both blastogenesis and proliferation. Activation can be accomplished by natural or recombinant cytokines and PHA (phytohemagglutnin). Activation induces transitory expression of several molecules both within the T-cell or on the cell surface. They include IL-2 receptor, Fc receptors for IgG, insulin receptors, α–fetoprotein and transferin receptors, MICA, HLA-II and β2-m-free HC (OCs) of HLA-I [58]. Several studies have documented that the commercial IVIg inhibited Phytohemagglutinin (PHA) or cytokine-induced T-cell activation and proliferation both *in vitro* and *in vivo* [59 – 73].

Kaveri et al [59] demonstrated that Abs to a conserved region of HLA-Ia present in pooled normal therapeutic IVIg are capable of modulating CD8 T cell-mediated function. Klaessn et al [60] showed that IgG and F(ab)2 fractions in IVIg are responsible for inhibitory function, whereas Miyagi et al (61) have attributed the inhibition of blastogenesis and proliferation of activated T-cells by IVIg to the Fc receptors for IgG, FcγRI (CD23), FcγRII (CD32), FcγRIII (CD16), FcγRIV (CD64) expressed on the immune cells upon activation. MacMillan et al [70] have documented further the IVIg-mediated suppression of T-cell proliferation, with or without CD28 co-stimulation. Sali et al (2015) demonstrated 2% of intact or Fab_2 fragments of IVIg can penetrate immune cells to modulate these activities. The penetrating fraction of IVIg inhibited he upregulation of the activation marker CD25 on CD4+ splenocytes. In a placebo-controlled trial, the administration of IVIg to patients with inflammatory myopathies was associated with a significant reduction of the number of T-lymphocytes *in vivo* [74].

Based on these observations, it is hypothesized that HLA-I polyreactive mAbs that mimic HLA-I reactivity of IVIg preparations may suppress activated T-cells similarly to IVIg. The hypothesis was tested by comparing dose-dependent effects on the suppression of PHA (PHA)-activated T-cells of three entities: IVIg and HLA-I polyreactive mAbs that mimic IVIg (TFL-006 and TFL-007) and mAbs that do not mimic IVIg (TFL-033 and TFL-037).

5.1.2. Hypothesis testing: Measurement of T-lymphocyte proliferation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

More details are presented elsewhere [58]. Briefly, we have labeled purified human T-lymphocytes (freshly collected from a healthy young adult male) with the intracellular fluorescent dye Carboxyfluorescein N-succinimidyl ester (CFSE), a cell-permeable dye with a long retention time. The dye gets covalently coupled to intracellular molecules utilizing its succinimidyl group. Once coupled, the dye is not transferred to adjacenT-cells but remains in the cell for several mitotic divisions. The technology used is illustrated in **Figure 6A**. Most importantly the cessation of the progress of mitotic activity can be monitored as successive two-fold reductions in the fluorescent intensity after 72 hrs of treatment, including addition of PHA and PHA plus IVIg or mAbs. More details are presented elsewhere [58].

5.1.3. The suppression of activated T-cells by IVIg vs mAbs.

A summary of the findings are presented in the **Figures 6B, 7 and 8**. The results established the differential effects on the suppression of PHA-activated T lymphocytes both by IVIg and by HLA-I polyreactive mAbs (TFL-006 and TFL-007). The mAbs (TFL-033 and TFL-037) that do not mimic the HLA-I reactivity of IVIg did not affect activation or proliferation of T-cells. The mAbs TFL-006 and TFL-007 appear to be more potent suppressors of blastogenesis and proliferation of activated CD4+ T lymphocytes than IVIg. The concentrations of the mAbs required for the suppression of T-cell proliferation were 50-fold lower than the required concentration of IVIg. The suppression of blastogenesis and proliferation of T-cells by both IVIg and the anti-HLA-E mAbs was dose-dependent, and the dose required with mAbs 50–150-fold lower than with IVIg. The mAb binding to the open conformer may signal T cell deactivation because the OCs have an elongated cytoplasmic tail with phosphorylation sites (tryosine320/ serine335). A summary of the findings are presented in the Figures 6B, 7 and 8.

A tentative model of PHA-mediated activation and HLA-polyreactive mAb-mediateddeactivation is proposed (**Figure 9**). It is known that PHA activation initiates phosphorylation of the cytoplasmic domain of CD3 and activation of transcription factors, leading to production of cell surface moleculs such as IL-Rα [74] and OCs of HLA-I [13 -22]. The binding of mAbs to the shared amino acid sequences or epitopes exposed on the OCs may dephosphorylate the tyrosyl and seryl residues on the elongated cytoplasmic tail of the HLA-I OCs [75 – 77]. This

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

may lead to transduction of signals to revert the PHA-activation of CD3 on T-cells. Upon dephosphorylation, T-cells are deactivated resulting in suppression of blastogenesis and proliferation. Based on the observations that the suppression of blastotogenesis and proliferation of PHA-activated CD4+ T-cells by HLA-I polyreactive anti-HLA-E mAbs (TFL-006 and TFL-007) but not by HLA-I non-reactive anti-HLA-E mAbs (TFL-033 and TFL-007), it is inferred that the IVIg mediated suppression of blastogenesis and proliferation of PHA-activated CD4+ T-cells could also due to binding of the HLA-I OCs-reactive IgG fraction in IVIg. Furthermore, when equal concentration of HLA-I polyreactive mAb TFL-007 and IVIg were compared the suppression by the mAbs is greater than that of the IVIg. Evidently, the admixture of other IgGs with HLA-polyreactive IgGs in IVIg may hinder T-cell suppressive efficacy of the IVIg.

5.2. Suppression of B-cell antibody production: IVIg vs HLA-I polyreactive mAbs.

5.2.1. Background and Hypothesis

Previous literature documented that the commercial IVIg not only inhibited Phytohemagglutinin (PHA) and cytokine-induced T-cell activation and proliferation and significantly reduced the number of T-lymphocytes *in vivo* in a placebo-controlled trial on patients with inflammatory myopathies but is also potentially capable of suppressing antibody production in patients under different disease conditions. Therefore, IVIg has become not only a substitution therapy for patients with immunodeficiencies [78], but also a therapeutic agent in autoimmune and systemic inflammatory diseases [79], as well as in organ and bone marrow transplantation [3, 80].

IVIg is extensively used in patients with end-stage organ disease and allograft recipients. High levels of HLA-antibodies are observed in patients with the end-stage organ disease, resulting from previous transplantation, pregnancy or blood transfusion. High levels of such allo-HLA Abs can more likely produce positive crossmatch results with potential organ donors and preclude transplantation. Consequently, antibody-positive patients may experience prolonged waiting time. Among renal transplant recipients alone, such sensitized patients constitute more than one third of those on the waiting list. For highly sensitized patients (with a panel reactive HLA-antibody (PRA) greater than 80%), the prospects of transplantation are grim.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

The formation of Abs against the allo-antigens depends on both T and B-cells. Therefore, aggressive suppressive strategies are developed to deplete both the T and B-cells, in order to suppress the development of allo-HLA Abs formed before and after transplantation. One such strategy is induction therapy with rabbit or horse anti-human thymocyte globulin, a polyreactive polyclonal mixture of non-specific cytotoxic Abs capable of killing the immune cells [81]. However, many clinical transplant centers worldwide have formulated alternate protocols to suppress the formation of anti-HLA Abs. These protocols may include plasmapheresis (PP), high-dose IVIg, or a combination of PP with low-dose IVIg [2, 49 – 54, 82 -87] or Rituximab, a monoclonal Ab (mAb) that depletes CD20+ B-cells [88 - 91]. Although several immunotherapeutic potentials are attributed to IVIg, its mechanism of action is far from clear. Possibly, it is due to the polyclonality and the mixture of several kinds of IgG Abs present, including Abs against all HLA class I loci and alleles as illustrated in **Table,** and **Figure 5A**, and **5B**.

A most interesting finding from this study [35] is that the HLA-Ia reactivity of IVIg is significantly abolished when antiHLA-E Abs are depleted specifically by passing IVIg through HLA-E heavy chain conjugated Affi-gel affinity columns. This suggests that IVIg's HLA-Ia reactivity could possibly due to the presence of HLA-E specific IgG Abs, and that there are anti-HLA-E mAbs (mAbs) that may simulate the HLA-reactivity of IVIg .

These observations led us to hypothesize that the anti-HLA-E mAbs that simulate the HLA-reactivity of IVIg can mimic IVIg in suppressing B-cells from producing Abs. We have compared the efficacy of IVIg versus that of a HLA-I a polyreactive anti-HLA-E mAb, TFL-007 (**Table 4**), in suppressing the B-cell blastogensis, proliferation and production of Abs.

5.2.2. Methodology to test the hypothesis

More details are presented elsewhere [92]. Two commercial IVIg preparations were used. They are. [1] IVIg-GamaSTAN™ (Lot 26NJ651, Talecris Biotherapeutics, Inc., Research Triangle Park, NC, USA) formulated as a 15–18% protein solution at a pH of 6·4–7·2 in 0·21–0·32 M glycine; [2] IVIgGamunex®-C (Lot 26NKLG1, Talecris) a solution at a pH of 6·4–7·2 in 0·16–0·24 M glycine, albumin < 20 μg/ml. The HLA-I polyreactive HLA-E mAbs used are

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

TFL-007s (culture supernatant) and TFL-007a (ascites). For suppressing antibody production by B-cells, two different experiments were carried out.

Experiment # 1 *on freshly purified B-cells from the peripheral blood lymphocytes of awoman alloimmunized with her husband's HLA DRB1*0101 antigens during her first pregnancy.*

The HLA typing of the woman, her husband and their two daughters (first 23 yrs, second 18 yrs) revealed that both the father and the first daughter carried DRB1*0101 allele, while the mother's sera showed the prevalence of high levels of allo-antibody against DRB1*0101. Periodic screening for the last 2 years indicated that the high MFI of the primary allo-HLA Abs persisted for the last two years. Most likely, the anti-DRB1*0101 IgG Abs with high MFI were formed as a consequence of alloimmunization during pregnancy that occurred 23 years before, which suggested the prevalence of long lived memory B-cells. Since this alloantibody is bound to the husband's primary allele, the antibody is designated as 'primary alloantibody'.

Therefore, her peripheral blood B-cells were isolated and purified, after obtaining her informed consent and Institutional (TFL) approval. The B-cells were purified from the PBMC by positive selection using CD19 Pan B Dynabeads® magnetic beads (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA) and detached using DETACHaBEAD® CD19 (Invitrogen). Purified B-cells (> 95% CD19+) were plated at 0·2 × 106 /200 µl/well in a sterile 96-well plate (Thermo Fisher Scientific, Inc.) and cultured in Iscove's modified Dulbecco's medium, containing HEPES, L-glutamine and sodium pyruvate (Gibco-Invitrogen) supplemented with AB-human serum [10%], recombinant human (rh) insulin[5 µg/ml], rh-transferrin [50 µg/ml], gentamycin [25 µg/ml] and 2-mercaptoethanol [50 µM]. The resting B-cells were activated with rh-IL-2 [50 ng/ml], rhIL-4 [100 ng/ml], rhIL-6 [100 ng/ml], rhIL-10 [50 ng/ml], and human CD40 antibody [1 µg/ml] [92]. The B cell population (CD19+) isolated from PBMC using positive selection on day 0, consisted of a major fraction including naive B-cells (CD20+ /CD27− /CD38+ / −) (74·47%), B-memory cells (CD20+ /CD27+ /CD38−) (8·47%) and plasma cells (CD20− /CD27++/CD38++) (0·26%) These cells, upon activation by the selected battery of cytokines IL-2/IL-4/IL-6/ IL-10/IL-21 (at a 1:4:4:2:2 ratio) and human CD40 antibody [1 µg/ml] for 7 days resulted in an increase in plasma cells from 0·26% on day 0

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

to 36·25% on day 7. On day 7, culture supernatant [10 µl] from each well were analyzed for anti-HLA class II IgG alloAbs. Cells from the positive wells were further harvested, washed (3X), seeded and activated again as above. On days 8 and 9, the culture supernatants were screened for the Abs. After ascertaining the consistency of the MFI of the Abs on days 8 and 9, the cells were pooled, washed (3X), and aliquoted. They were maintained in culture without any cytokine activators or anti-CD40 antibody for an additional 3 days. The culture supernatants [10 µl] from each well were analyzed for Abs at 0, 12, 24, 48 and 72 h.

On day 9, the cells were pooled, aliquoted, maintained without the cytokine combo or anti-CD40 mAb. These wells were exposed to medium or IVIg (1/100, 1·5 mg/ml) or mAb TFL-007s (1/100, 5 µg/ml) for 72 h. To study the effect of IVIg on secretion of allo-HLA IgG Abs, we used IVIg at a protein concentration 300-fold higher than that of purified culture supernatant of TFL-007s (5 µg/ml) used in treatment of B-cells in culture. The supernatants recovered from the respective wells were screened for the HLA-alloAbs.

Experiment # 2 *on the human hybridoma cell line HML16 generated from the resting B-cells of a multiparous woman.*

The human hybridoma cell line, HML16, produced anti-HLA class I alloAbs with differing MFIs: high against B*0702, B*8101, B*6701 and B*4201; and low against B*2708, B*2705, B*5501, B*5601 and B*8201. The cell line was cultured in RPMI-1640, as described earlier. The cells were seeded at 1000/100 µl/well in a Falcon 96-well, flat-bottomed plate, and divided into different treatment groups: Group 1-medium control; Group 2-mouse IgG control; Group 3- mAb TFL-007(ascites). Group 4- IVIg (GamaSTAN) and, Group 5-IVIg (Gamunex-C). The following sub-groups were established: mouse IgG control (100 and 50 µg/ml); GamaSTAN-IVIg subgroups were at dilutions 1:10 (15 mg/ ml), 1:20 (7·5 mg/ml) and 1:40 (3·75 mg/ml), Gamunex-IVIg subgroups were at dilutions 1:10 (10 mg/ml), 1:20 (5 mg/ml) and 1:40 (2·5 mg/ml), and mAb TFL-007a subgroups were at dilutions 1:10 (100 µg/ml), 1:20 (50 µg/ml), 1:40 (25 mg/ml) and 1:80 (12·5 µg/ml). Twenty µl of culture supernatant from each well were analyzed for allo-HLA Abs at 0 and 72 h.

The expression of CD19 was monitored using the fluorescein isothiocyanate (FITC)-labelled anti-human CD19 (mAb HIB19). On days 0 and 7, both resting and activated B-cells

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

were stained with phycoerythrin (PE) anti-human CD20 (mAb 2H7), peridinin chlorophyll (PerCP) antihuman CD27 (mAb 0323), and FITC antihuman CD38 (mAb HIT2) to examine the differential activation of B-cells. The source of the mAbs is from BioLegend. Prior to staining with Abs, Human TruStain FcX™ (BioLegend) was used to block FcR-involved unwanted staining.

5.2.3. TFL-007 suppressed HLA-antibody production better than that of IVIg.

In the First Experiment, both the serum of the woman and of those secreted by activated memory B-cells (culture supernatants) showed the presence of several alloAbs directed against DRB1*0102, DRB1*0404, DRB1*0405, DRB1*1402 and DRB1*0401, in addition to the primary alloantibody, anti-DRB1*0101 IgG. The secondary alloAbs were not directed against the husband's alleles. They may represent cross-reactive alloAbs in that they occurred in cultures containing the primary alloantibody, anti-DRB1*0101. These alleles shared amino acids or amino acid sequences with the primary allele, DRB1*0101. The details were presented elsewhere [92].

Figure 10 documents that the GamaSTAN IVIg suppressed the secretion of the primary alloantibody against DRB1*0101 at different time-points ($p^2 < 0{\cdot}01$). Similarly, the HLA-polyreactive mAb TFL-007s also significantly reduced the secretion of both primary alloantibody ($p^2 < 0{\cdot}0005$). However, the suppression of the secretion of primary alloantibody by the mAb TFL-007s is highly significant compared to the IVIg-induced suppression.

In the Second Experiment, the treatment effects of two preparations of IVIg (GamaSTAN and Gamunex) were tested on HM16 at different dilutions and protein concentrations and compared with the media control [92]. The IVIg concentration used for the hybridoma cell line HML16 is comparable to the high-dose IVIg used for desensitization and post-transplant therapy. Neither of the IVIg preparation showed any significant suppression of the secretion of allo-HLA-B IgG by the hybridoma cells. In contrast, the mAb TFL-007a had a strikingly significant suppressive effect on the secretion of both HLA-B*0702 and B*8101. More importantly, the mAb TFL-007a showed dosimetric suppression of allo-HLA Abs. When HML16 was treated with the highest concentration of TFL-007a (100 μg/ml), suppression was 33% for B*0702 and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

34% for B*8101 compared with the medium control group. When the dosage of TFL007a was decreased, the suppression effect declined. In short, in marked contrast to IVIg preparations, the HLA-I polyreactive mAb TFL-007a significantly suppressed the secretion of both allo-HLA-B Abs.

5.3. Expansion of Foxp^3+ Tregs *in vitro: IVIg versus HLA-I polyreactive mAbs.*

Human CD4+ CD25+ Foxp3+ regulatory T-cells are a naturally occurring population of regulatory T-cells (Tregs) in circulation [93, 94]. Their presence in liver allografts is attributed to tolerance of the transplanted organ [95- 101]. They suppress Ab production by downregulating B memory and plasma cells [102] and depleting both CD4+[103] and CD8+ [104-105] T-cells, and hence they play a major role in graft tolerance [101, 106, 107]. While performing skin graft experiments on human-CD52 transgenic CP1-CBA/Ca (H-2^k) mice, Garca et al (106) have observed that the "T cell suppression of graft rejection is an active process that operates beyond secondary lymphoid tissue, and involves the persistent presence of regulatory T-cells at the site of the tolerated transplant.(p.1641)"

The therapeutic mAb Tocilizumab given to patients with rheumatoid arthritis increases the T-regs and correlates with clinical response [108]. Uupregulation of T-regs by IVIg is considered as a critical factor in controlling experimental autoimmune encephalomyelitis IVIg is known to upregulate Tregs [109].

Since IVIg can upregulate T-regs, it is hypothesized that the HLA-I polyreactive mAbs TFL-006 and TFL-007 may also induce proliferation of T-regs. For testing this hypothesis CD4+CD25+foxp^3+ Tregs were obtained from the pheripheral blood of normal and healthy donors, after obtaining necessary consent and institutional IRB approval. A variety of cell surface markers, which include CD4, CD25 (IL-2R□), CD45RA and FoxP3, were monitored using their respective monoclonal Abs.

We compared the impact of.different commercial preparations of IVIg and HLA-I polyreactive mAb TFL-007 in triplicates on untreated T-regulatory cells (CD4+/ **CD25**+/ FoxP^3+) obtained from a TFL healthy volunteer, The mAb purified from ascites was used throughout. **Figure 11** illustrates that the different commercial preparations of IVIg at two

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

different dilutions (1/10 and 1/80) failed to upregulate the Tregs, while mAb TFL-007a showed a significant increase in the number of cells compared to controls.

6.0. DISCUSSION

IVIg is a mixture of polyclonal IgG Abs pooled and purified from thousands of normal individuals. The binding affinity of IVIg is multivarious, because the. IgG Abs in IVIg can bind to blood groups, MHC complex antigens, cytokines, chemokines, their receptors and several other antigens including human albumin. Most importantly, in the last three decades [2, 3, 49 -57, 82 - 94], IVIg is administered to HLA-sensitized patients who posses higher level of HLA allo-Abs with the intention to lower these Abs. Due the high level of these allo-HLA Abs, such patients are more likely to have a positive crossmatch result with potential organ donors, and they often languish for years on the waiting list. For highly sensitized patients, the prospects of transplantation get grim. Realizing the imminent need to lower the impact of HLA-sensitization, the US-FDA approved IVIg for therapeutic administration in HLA sensitized patients. However, due to lack of efficacy as monotherapy [55 – 57], the IVIg is often combined with other therapeutic agents [89 – 91], such as plasmapheresis and Rituximab, (a mAb that depletes CD20+ B-cells. Ccommercial therapeutic preparations of IVIg formulated in different countries possess allo-HLA IgG Abs against almost all alleles of all six loci of HLA-I antigens (35, 110). In this regard**,** HLA polyreactive mAb developed from HLA-E αHC mimics the allo-HLA antibody profile of IVIg [U.S patent 10,800,847;10/13/2020].

The structure of a single HLA -I antigen has two different profiles as illustrated in **Figure 2.** The most well-known profile is the cell surface heteromer, consisting of α-HC associated with β2-m (**Figure 1**). In the heteromeric profile many of the most commonly shared amino acid sequences [**Tables 1 & 2**] such as ^{117}AYDGKDY^{123} and ^{127}LNEDLRSWTA^{135} are cryptic, hidden by b2-m. The second less known profile of a single HLA-I antigen is a b2-m-free a-HC, i.e., an OC (**Figure 2)**. Although this structure is naturally occurring, it is often misconstrued to be identical to denatured HLA anitgens as found on alkali or acid treated Beadsets (111). In fact, the misconception that the monomeric versions of naturally occurring HLA molecules are “denatured” HLA is well documented in several reports (13 – 30). These reports clearly demonstrate several

 doi:10.20944/preprints202105.0183.v1

novel aspects of the monomeric versions. The shared amino acid sequences common to all the six loci of HLA are exposed for immune recognition and serves as receptor for signal transduction. Particularly the long cytoplasmic tails of the monomeric versions on cells with tyrosyl an seryl residues (**Figure 2**) are shown to be involved in signal transduction (**Figure 9**). Arosa et al (19, 112) have elucidated further the difference between the intact HLA antigens as CCs and the monomeric variant as OCs when expressed *in vivo* on the cell surface (**Figure 4**). One difference is that HLA molecules shed from the cell surface (117) occur as the monomeric variants or OCs in the circulation (**Figure 3**). Sincee they expose cryptic epitopes, they serve as better immunogen for allo-HLA Ab production. Indeed, the immunogenicity and antigenicity of the shared cryptic epitope ^{117}AYDGKDY^{123} are much greater than other epitopes (**Table 2**]

Visualizing the antibody recognition sites (epitopes of CCs and OCs would clarify and elucidate the HLA profile instrumental for generation of HLA-I polyreactive IgG mAbs and naturally occurring IgG Abs. Theoretically one can expect the presence of polyreactive IgG Abs recognizing OCs of HLA in IVIg. Indeed **Figure 5** confirms the prevalence of two commercial preparations of IVIg reacting to monomeric variants on the acid denatured beadsets. The density of IgG binding to monomeric variants is much higher than those recognizing the CCs on 'iBeads' (**Figure 5**). Kavery et al [59] pointed out "Abs to a conserved region (cryptic domain) of HLA class I molecules, capable of modulating CD8 T cell-mediated function, are present in pooled normal immunoglobulin for therapeutic use" (p865). Strikingly, HLA polyreactive IgG2a mAbs (TFL-006 and TFL-007) do not recognize CCs but recognize only the OCs on the beadsets (**Table 5**). Importantly, as noted above, these naturally occurring OCs existing on the cell surface in vivo should not be considered synonymous with chemical treatment (acid or alkali) on synthetic beads disrupting intact CCs to produce denatured αHCs in vitro.

The binding of the HLA-I polyreative mAbs to OCs is responsible for the suppression of blastogenesis and proliferation. It may involve the reversal of phases of activation of T lymphocytes, mediated by signal transduction. The elongation of the cytoplasmic tail of the HLA-I OCs exposes otherwise cryptic tyrosine-320 [76] and serine-335 [112] residues with a provision for phosphorylation (**Figure 9**). Although serine-335 is generally considered the primary site of phosphorylation in this tail, phosphorylation of tyrosine-320 has been indicated by others [76].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Thus the HLA-I OCs in activated T-cells are associated with tyrosine phosphorylation and are capable of enabling cis interactions with cell surface receptors or other signaling molecules [76, 77, 112 – 116]. The binding by TFL mAbs may result in dephosphorylation of the cytoplasmic tails of CD3 molecules by activating phosphatases. The result is to arrest transcription factors and inhibit synthesis of the proteins involved in blastogenesis and mitosis (**Figure 9**). These events suggest that suppression of activation of T-cells could be due to binding of HLA-I polyreactive mAbs, mimicking the HLA-I polyreactivity of IVIG, to the shared amino acid sequences exposed on the naturally occurring OCs of HLA-I molecules (**Figure 2**). This activation involve not one HLA-I locus but all alleles of the six loci (HLA-A, HLA-B, HLA-C, HLA-E, HLA-F and HLA-G) expressed on the surface of activated T-cells. The efficacy of the monoclonality of the HLA-I polyreactive mAbs and their F(ab′)2 binding is restricted to OCs.. Furthermore poor efficacy of IVIg is due to admixture of CC-binding Abs, other non-HLA Abs, anti-idiotypic Abs and antigens, in addition other chemicals such as sugars added to render stability to IVIg.

Possibly, the same or similar phosphorylation and dephosphorylation mechanisms may be involved in suppressing antibody production by B-cells. The HLA-I polyreactive mAb, but not IVIg, suppressd the allo-HLA-I and allo-HLA-II Abs production by B-memory cells and immortalized B-cells. During monitoring the efficacy of the HLA-I polyreactive mAbs, the cytokine combination and anti-CD40 antibody were both removed to precisely evaluate the impact of the antibody. Possibly, the HLA-I polyreactive mAbs may also help to suppress other Abs produced by B-cells, and alloAbs as well.

Also, the HLA-I polyreactive mAbs upregulate T-regs better than IVIg (**Figure 11**). T-regs are well known for their immunoregulatary properties. They suppress Ab production by downregulating B memory and plasma cells [102] and depleting CD4+[103] and CD8+ [104-105] T-cells. We believe that therapeutic IVIg administered in patients perform the immunosuppressive functions stated above both by binding to OCs of HLA, as does HLA-I polyreactive TFL mAbs but also by upregulation T-regs.

8.0. CONCLUSION

The observations reported in this review regarding the effects of IVIg point out conclusively that: (a) suppression of blastogenesis and proliferation of T-cells, (b) minimization of the HLA-II allo-antibody production by B-memory cells of a parous women and HLA-I antibody production of an immortalized cell line, and (3) expansion of T-regs. These effectshttps://susy.mdpi.com/user/manuscripts/upload/21fc80651caf941fe1a61cbf9e82d4b3 are all due to HLA polyreactive Abs present in IVIg. The finding is further strengthened by HLA-I polyreactive TFL mAbs (TFL-006 and FL-007). The mAbs, which recognize shared epitopes on naturally occurring OCs, with no ability to bind to the CCs, perform the same suppressive functions better than the different preparations of IVIg. Hence, HLA-I polyreactive TFL mAbs are capable of serving as IVIg-mimetics, perhaps more efficiently than IVIg itself. Clinical trials are clearly warranted. Possibly, humanized versions of these TFL-mAbs, either combined or alone, can be better therapeutic tools than IVIg to suppress HLA-sensitization and minimize antibody production post-transplantation.(words; 7300)

ACKNOWLEDGEMENT

The experiments reported in this review were carried out at Terasaki Foundation Laboratory, at Santa Monica, California with the guidance of Late Professor Paul Ichiro Terasaki, with the support of several dynamic research associates. Luminex Single Antigen Bead immunoassays for monitoring HLA-I reactivities of IVIg and the TFL mAbs and the experiments related to CD4+ T-cells were assisted by Mr. Tho Pham, Mr. Vadim Jucaud, and Mr. Satoru Kawakita, the experiments related to B-cells were carried out by Dr. Dong Zhu, Mr. T. Miyazaki,‡ Mr. Tho Pham and Mr. Vadim Jucaud and the experiments related to T-regs were performed by Dr. Michiko Taniguch. The current report is encouraged and financially supported by the funds from Professor Dr. Mark Terasaki, first son of Late Professor Paul Terasaki. We dedicate this review to Late Professor Paul Ichiro Terasaki, whose constant discussions both at his office and at weekly seminar molded the thought process for this review, besides he is a co-inventor of the patent involving the IVIg mimetic (U.S patent 10,800,847;10/13/2020).

REFERENCES

1. Darabi, K., O. Abdel-Wahab, O, and Dzik, W. H. (2006) Current usage of intravenous immune globulin and the rationale behind it: the Massachusetts General Hospital data and a review of the literature. Transfusion, 2006. 46(5): p. 741-53.doi: 10.1111/j.1537-2995.2006.00792.x.
2. Glotz, D., Haymann, J-P., Sansonetti, N., Francois A, Menoyo-Calonge V, Bariety J, and Druet P. (1993) Suppression of HLA-specific alloAbs by high-dose intravenous immunoglobulins (IVIg). Transplantation 1993, 56(2): 335-7.doi: 10.1097/00007890-199308000-00015.
3. Tyan, D. B., Li, V. A., Czer, L., Trento, A., and Jordan, S. C. (1994) Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. Transplantation 1994, 57:553–62.
4. Sapir, T., and Shoenfeld, Y. (2005) Facing the enigma of immunomodulatory effects of intravenous immunoglobulin. Clin. Rev. Aller.Immunol. 2005, 29(3):185-99.
5. Tongio, M. M., Falkenrodt, A,, Mitsuishi, Y, Urlacher, A., Bergerat, J. P., North, M. L., Mayer, S. (1985) Natural HLA Abs. Tissue Antigens. 1985, 26(5):271-285, doi: 10.1111/j.1399-0039.1985.tb02225.x.
6. Luscher, M. A., Choy, G., Embree, J. E, Nagelkerke, N. J., Bwayo, J. J., Nienga, S., Plummer, F. A., Barber, B. H., MacDonald, K. S. (1988) Anti-HLA alloAb is found in children but does not correlate with a lack of HIV type 1 transmission from infected mothers. AIDS Res Hum Retroviruses. 1998,14(2):99-107. doi: 10.1089/aid.1998.14.99.
7. Morales-Buenrostro, L. E., Terasaki, P. I., Marino-Vazquez, L. A., Lee, J-H., El-Awar, N., and Alberú, J. (2008) "Natural" human leukocyte antigen Abs found in nonalloimmunized healthy males. Transplantation. 2008, 86(8):1111-1115. doi: 10.1097/TP.0b013e 318186d87b.
8. Zhou, B., Saito, S., Nakazawa, Y., et al. (2008) Existence of an immunoglobulin G component of naturally occurring HLA class I Abs that are not directed against self-

antigens in human serum. Tissue Antigens. 2008, 72(2):98-104. doi: 10.1111/j.1399-0039.2008.01074.x.

9. Ravindranath, M. H., Kaneku, H., El-Awar, N., Morales-Buenrostro, L. E., Terasaki, P. I. (2010) Abs to HLA-E in alloimmunized males: pattern of HLA-Ia reactivity of anti-HLA-E-positive sera. J Immunol. 2010, 185(3):1935-1948. doi: 10.4049/ jimmunol.1000424.
10. Ravindranath, M. H., Taniguchi, M., Chen, C. W., Ozawa, M., Kaneku, H., El-Awar, N., Cai, J., Terasaki, P. I. (2010) HLA-E mAbs recognize shared peptide sequences on classical HLA class Ia:relevance to human natural HLA Abs. Mol Immunol. 2010, 47(5):1121-31. doi:10.1016/j.molimm.2009.10.024.
11. Ravindranath, M. H., Pham, T., El-Awar, N., Kaneku, H., Terasaki, P. I. (2011) 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 Abs. Mol Immunol. 2011, 48(4):423-30. doi:10.1016/j.molimm.2010.09.011.
12. Marsh, S. G, E., Parham, P., and Barber, L. D. (2000) The HLA. Facts Book. Academic Press. San Diego, 2000, 400p.
13. Schnabl, E., Stockinger, H., Majdic, O., Gaugitsch, H., Lindley, I. J., Maurer, D., et al., (1990) Activated human T lymphocytes express MHC class I heavy chains not associated with beta 2m. J Exp Med. 1990, 171(5):1431-1442. doi: 10.1084/jem. 171.5.1431.
14. Schumacher, T. N., Heemels, M. T., Neefjes, J. J., Kast, W. M., Melief, C. J, Ploegh, H. L. (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
15. Benjamin, R. J., Madrigal, J. A., Parham, P. (1991) Peptide binding to empty HLA-B27 molecules of viable human cells. Nature, 1991, 351(6321): 74-77. doi: 10. 1038/351074a0.
16. Majdic, O., Schnabl, E., Stockinger, H., Gadd, S., Maurer, D., Radaszkiewics, T. et al. (1989) LA45, an activation-induced human lymphocyte antigen with strong homology to MHC class I molecules. In Leukocyte Typing IV. Oxford University Press, 1989, 8p. 511.
17. Madrigal, J. A., Belich, M. P., Benjamin, R. J., Little, A. M., Hildebrand, W. H., Mann, D. L., et al., (1991) 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.

18. Santos, S. G., Simon, J. Powis, S. J., Arosa, F. A. (2004) Misfolding of Major Histo-compatibility 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
19. Arosa, F. A., Santos, S. G., Powis, S. J. (2007) OCs: the hidden face of MHC-I molecules. Trends Immunol, 2007, 28(3):115-123. doi: 10.1016/j.it. 2007 01.002.
20. Demaria, S., Schwab, R., and Bushkin, Y. (1992) The origin and fate of beta 2m-free MHC class I molecules induced on activated T-cells. Cell Immunol. 1992, 142(1):103-113. doi: 10.1016/0008-8749(92)90272-q.
21. Demaria, S., and Bushkin, Y. (1993) CD8 and beta-2 m-free MHC class I molecules in T cell immunoregulation. Int J Clin Lab Res. 1993, 23(2):61-69. doi: 10.1007/BF02592285..
22. Raine, T., Brown, D., Bowness, P., Hill Gaston, J. S., Moffett, A., Trowsdale, J., et al. (2006) Consistent patterns of expression of HLA class I free heavy chains in healthy individuals and raiAllensed expression in spondyloarthropathy patients point to physiological and pathological roles. Rheumatology (Oxford), 2006, 45(11):1338-1344. doi: 10.1093/ rheumatology/ kel305
23. Allen, R. L, O'Callaghan, C. A., McMichael, A. J, and Bowness, P. (1999) Cutting edge: HLA-B27 can form a novel beta2-m-free H chain homodimer structure. J. Immunol. 1999, 162:5045-5048.
24. Khare, S. D., Hansen, J., Luthra, H. S., and David, C. S. (1996) HLA-B27 heavy chains contribute to spontaneous inflammatory disease in B27/human beta2m (beta2m) double transgenic mice with disrupted mouse beta2m. J Clin Invest. 1996, 98(12):2746-2755.
25. Chakrabarti, A., Matko, J., Rahman, N. A., Barisas, B. G., and Edidin, M. (1992) Self-association of class I major histocompatibility complex molecules in liposome and cell surface membranes. Biochemistry. 1992, 31(31):7182-9, doi: 10.1021/bi00146a022.

26. Capps, G. G., Robinson, B. E, Lewis, K. D., and Zúñiga, M. C. (1993) In vivo dimeric association of class I MHC heavy chains. Possible relationship to class I MHC heavy chain-beta 2m dissociation. J Immunol. 1993, 151(1):159-169.
27. Matko, J., Bushkin, Y., Wei, T., and Edidin, M. (1994) Clustering of class I HLA molecules on the surfaces of activated and transformed human cells. J Immunol. 1994, 152(7):3353-3360.
28. Allen, R. L, Raine, T., Haude, A., Trowsdale, J., and Wilson, M. J. (2001) Leukocyte receptor complex-encoded immunomodulatory receptors show differing specificity for alternative HLA-B27 structures. J Immunol. 2001, 167(10):5543-5547. doi: 10.4049/jimmunol.167.10.5543.
29. Demaria, S., DeVito-Haynes, L. D., Salter, R. D., Burlingham, W. J, and Bushkin, Y. (1999) Peptide-conformed beta2m-free class I heavy chains are intermediates in generation of soluble HLA by the membrane-bound metalloproteinase. Hum Immunol. 1999, 60(12):1216-1226,.doi: 10.1016/s0198-8859(99)00113-5.
30. Demaria, S., Schwab, R., Gottesman, S. R., and Bushkin Y. (1994) Soluble beta 2-m-free class I heavy chains are released from the surface of activated and leukemia cells by a metalloprotease. J Biol Chem. 1994, 269(9):6689-6694.
31. Chou, P.Y., and Fasman, G.D. (1978) Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. Relat. Areas Mol. Biol. 1978, 47:45–148, doi: 10.1002/9780470122921.ch2..
32. Kolaskar, A.S., and Tongaonkar, P.C. (1990). A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett. 1990, 276(1-2):172-4.doi: 10.1016/0014-5793(90)80535-q.
33. Karplus, P.A., and Schulz, G.E. (1985). Prediction of chain flexibility in proteins – a tool for the selection of peptide antigens. Naturwissenschaft. 1985, 72: 212–213.
34. Parker, J.M., Guo, D., and Hodges, R.S. (1986). New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. Biochemistry, 1986, 25(19):5425-32, doi: 10.1021/bi00367a013.

35. Ravindranath, M. H., Terasaki, P.I., Pham, T., Jucaud, V., Kawakita, S. (2013) Therapeutic preparations of IVIg contain naturally occurring anti-HLA-E Abs that react with HLA-Ia (HLA-A/-B/-Cw) alleles. Blood. 2013, 121(11):2013-28. doi: 10.1182/blood-2012-08-447771.

36. Ravindranath, M. H., Zhu, D., Pham, T., Jucaud, V., Hopfield, J., Kawakita, S., Terasaki P. I. (2013) Anti-HLA-E mAbs reacting with HLA-la and lb alleles like IVIg as potential IVIg-immunomimetics: an evolving therapeutic concept. Clin Transpl. 2013, 293-305.

37. Ward, P. A., Adams J, Faustman D, et al. (1999) Monoclonal Antibody Production A Report of the.Washington, DC: Committee on Methods of Producing MAbs Institute for Laboratory Animal Research National Research Council National Academy Press; 1999, 1-47.

38. Sasaki, T., Ravindranath, M. H., Terasaki, P. I., Freitas, M. C., Kawakita, S., and Jucaud, V. (2014) Gastric cancer progression may involve a shift in HLA-E profile from an intact heterodimer to β2-m-free monomer. Int J Cancer. 2014, 134(7):1558-70. doi: 10.1002/ijc.28484.

39. Parham, P., Barnstable, C. J., and Bodmer, W. F. (1979) Use of a monoclonal antibody (W6/32) in structural studies of HLA-A,B,C, antigens. *J Immunol* 1979, 123: 342-9.

40. Smith, K. D., Mace, B. E., Valenzuela, A., Vigna, J. L., McCuthcheon J. A., Barbosa, J. A., Huczko, E., Engelhard, V. H. and Lutz, C. T. (1996) Probing HLA-B7 conformational shifts induced by peptide-binding groove mutations and bound peptide with anti-HLA mAbs. J Immunol. 1996, 157: 2470 - 8.

41. Vigna, J. L, Smith, K. D., and Lutz, C. T. (1996) Invariant chain association with MHC class I: preference for HLA class I/beta 2-m heterodimers, specificity, and influence of the MHC peptide-binding groove. J Immunol. 1996, 157: 4503-10.

42. Perosa, F., Luccarelli, G., Prete, M., Favoino, E., Ferrone, S., and Dammacco, F. (2003) Beta 2-m-free HLA class I heavy chain epitope mimicry by monoclonal antibody HC-10-specific peptide. J Immunol 2003, 171:1918-26, doi: 10.4049/jimmunol.171.4.1918.

43. Carreno, B. M., and Hansen, T. H. (1994) Exogenous peptide ligand influences the expression and half-life of free HLA class I heavy chains ubiquitously detected at the cell surface. Eur J Immunol 1994, 24:1285-92, doi: 10.1002/eji.1830240607..

44. Ravindranath, M. H., and El Hilali, F. (2021) Monospecific and Polyreactive MAbs against Human Leukocyte Antigen-E: Diagnostic and Therapeutic Relevance, Chapter in "MAbs" 38p. Ed. Nima Resaei, 2021, Intech Open. United Kingdom, ISBN: 978-1-83968-370-1.

45. Jucaud, V., Ravindranath, M. H, Terasaki, P. I. (2017) Conformational Variants of the Individual HLA-I Antigens on Luminex Single Antigen Beads Used in Monitoring HLA Abs: Problems and Solutions. Transplantation. 2017, 101(4):764-777. doi: 10.1097/ TP.0000000000001 420. PMID: 27495776

46. Ravindranath, M. H., and Jucaud, V. (2017) Conformational variants of HLA-I antigens on Luminex Single Antigen Beads for monitoring Abs. Transplantation, 2017, 101(4):e153-e154. doi:0.1097/TP.0000000000001629. PMID: 28323776.

47. Ravindranath, M. H., Jucaud, V., and Ferrone, S. (2017) Monitoring native HLA-I trimer specific Abs 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. 2017.07.016. Corrigendum to Monitoring native HLA-I trimer specific Abs 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.

48. Ravindranath, M. H. (2017) HLA Class Ia and Ib Polyreactive Anti-HLA-E IgG2a MAbs (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, 2017:3475926.doi: 10.1155/2017/3475926. PMID:28634589

49. Jordan, S. C., Quartel, A. W., Czer, L. S, Admon, D.,, Chen, G., Fishbein, M C et al., (1998) 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, 66:800-805, doi: 10.1097/00007890-199809270-00017.

50. Jordan, S. C., Vo, A., Bunnapradist, S., Toyoda, M., Peng, A., Puliyanda, D., Kamil, E., and Tyan D., (2003) Intravenous immune globulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients. Transplantation 2003, 76(4):631-6. doi:10.1097/01.TP. 0000080685.31697.FC.

51. Perez, E. E,, Orange, J. S., Bonilla, F., Chinen, J., Chinn, I. K., Dorsey, M., El-Gamal, Y., Harville, T. O., Hossny, E., Mazer, B., Nelson, R. , Secord, E,, **Jordan, S. C.** , Stiehm, E. R., Vo, A. A., Ballow, M. (2017) Update on the use of immunoglobulin in human disease: A review of evidence.J Allergy Clin Immunol. 2017 Mar;139(3S):S1-S46. doi: 10.1016/j.jaci.2016.09.023.

52. Jordan, S. C., Toyoda, M., Vo, A. A. (2009) Intravenous immunoglobulin a natural regulator of immunity and inflammation. Transplantation 2009, 88(1):1-6. doi: 10.1097/ TP.0b013e3181a9e89a.

53. Montgomery, R. A., Zachary, A. A., Racusen, L. C., Leffell, M. S., King, K. E., Burdick, J., Maley, W. R., and Ratner L. E. (2000) Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. Transplantation. 2000, 70(6):887-895. doi: 10.1097/00007890-200009270-00006.

54. Zachary, A. A., Montgomery, R. A., Ratner, L. E., Samaniego-Picota, M., Haas, M., Kopchaliiska, D., and Leffell, M.S.. (2003) Specific and durable elimination of antibody to donor HLA antigens in renal-transplant patients. Transplantation 2003, 76: 1519. doi: 10.1097/01.TP.0000090868.88895.E0.

55. Nair, V., Sawinski, D., Akalin, E., Friedlandeer, R., Ebcioglu, Z., Sehgal, V., Dinayahi, R., et al. (2012) Effect of high-dose intravenous immunoglobulin on anti-HLA Abs in sensitized kidney transplant candidates. Clin Transplant. 2012, 26(3):E261-E268. doi: 10.1111/j.1399-0012.2012.01657.x.

56. Alachkar, N., Lonze, B. E., Zachary, A. A.,Holechek, M.J., Schillinger, K., Cameron A. M., Desai, N. M., et al. (2012) Infusion of high-dose intravenous immunoglobulin fails to

lower the strength of human leukocyte antigen Abs in highly sensitized patients. Transplantation. 2012, 94(2):165-171. doi: 10.1097/TP.0b013e318253f7b6.

57. Marfo, K., Ling, M., Bao, Y., Calder B., Ye, B., Hayde, N., GreensteinS., et al. (2012) Lack of effect in desensitization with intravenous immunoglobulin and rituximab in highly sensitized patients. Transplantation. 2012, 94(4):345-351. doi: 10.1097/TP. 0b0133 e182590d2e.

58. Ravindranath, M. H., Terasaki, P. I., Pham, T., Jucaud, V., and Kawakita, S. (2014) Suppression of blastogenesis and proliferation of activated CD4(+) T-cells: intravenous immunoglobulin (IVIg) versus novel anti-human leucocyte antigen (HLA)-E mAbs mimicking HLA-I reactivity of IVIg. Clin Exp Immunol. 2014, 178(1):154-77. doi: 10.1111/cei.12391. MID: 24889882.

59. Kaveri, S., Vassilev, T., Hurez, V., Lengagne, R., Lefranc, C., Cot, S., Pouletty, P.,Glotz, D., and Kazatchkine, M. D. (1996) Antibodies to a conserved region of HLA class I molecules, capable of modulating CD8 T cell-mediated function, are present in pooled normal immunoglobulin for therapeutic use. J Clin Invest 1996, 97:865–9, doi: 10.1172/JCI118488.

60. Klaesson, S., Ringdén, O., Markling, L., Remberger, M., and Lundkvist I. (1993) Immune modulatory effects of immunoglobulns on cellmediated immune responses in vitro. Scand J Immunol 1993, 38:477–84. doi: 10.1111/j.1365-3083.1993.tb02591.x.

61. Miyagi, F., Horiuchi, H., Nagata, I., S Kitahara, S., Kiyoki, M., Komoriya, K., Yuki, N. (1997) Fc portion of intravenous immunoglobulins suppresses the induction of experimental allergic neuritis. J Neuroimmunol. 1997, 78:127–31, doi: 10.1016/s0165-5728(97)00090-8.

62. Sali A. D., Karakasiliotis, I., Evangelidou, M., Avarameas, S., Lymberi, P. (2015) Immunological evidence and regulatory potential for cell-penetrating antibodies in intravenous immunoglobulin. Clin Transl Immunol. 2015, 4(10):e42. doi: 10.1038/cti. 2015.18.

63. van Schaik, I. N., Lundkvist, I., Vermeulen, M., and Brand, A. (1992) Polyvalent immunoglobulin for intravenous use interferes with cell proliferation in vitro. J Clin Immunol. 1992, 12:325–34, doi: 10.1007/BF00920789.

64. Sbrana, S., Ruocco, L., Vanacore, R., Azzarà, A., and Ambrogi, F. (1993) In vitro effects of an immunoglobulin preparation for intravenous use (IVIG) on T-cells activation. Allerg Immunol (Paris) 1993, 25:35–7.

65. Hurez, V., Kaveri, S. V., Mouhoub, A., Dietrich, G., Mani, J. C., Klatzmann, D., and Kazatchkine M. D. (1994) Anti-CD4 activity of normal human immunoglobulin G for therapeutic use (intravenous immunoglobulin, IVIg). Ther Immunol 1994, 1:269–77.

66. Amran, D., Renz, H., Lack, G., Bradley, K., and Gelfand, E. W. (1994) Suppression of cytokine-dependent human T-cell proliferation by intravenous immunoglobulin. Clin Immunol Immunopathol. 1994, 73:180–6. doi: 10.1006/clin.1994.1186.

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

68. Prasad, N. K., Papoff, G., Zeuner, A., Bonnin, E., Kazatchkine, M. D., Ruberti, G., and Kaveri, S. V. (1998) Therapeutic preparations of normal polyspecific IgG (IVIg) induce apoptosis in human lymphocytes and monocytes: a novel mechanism of action of IVIg involving the Fas apoptotic pathway. J Immunol 1998, 161:3781–90.

69. Aktas, O., Waiczies, S., Grieger, U., Wendling, U., Zschenderlein, R., and Zipp, F. (2001) Polyspecific immunoglobulins (IVIg) suppress proliferation of human (auto)antigen-specific T-cells without inducing apoptosis. J Neuroimmunol. 2001, 114:160–7. doi: 10.1016/s0165-5728(01)00243-0.

70. MacMillan, H. F., Lee, T., and Issekutz, A. C. (2009) Intravenous immunoglobulin G-mediated inhibition of T-cell proliferation reflects an endogenous mechanism by which IgG modulates T-cell activation. Clin. Immunol. 2009, 132:222–33. doi: 10.1016/ j.clim.2009.04.002.

71. Aubin, E., Lemieux, R., and Bazin, R. (2010) Indirect inhibition of in vivo and in vitro T-cell responses by intravenous immunoglobulins due to impaired antigen presentation. Blood 2010, 115:1727–34. doi: 10.1182/blood-2009-06-225417.

72. Tawfik, D. S., Cowan, K. R., Walsh, A. M., Hamilton, W. S, and Goldman, F. D. (2012) Exogenous immunoglobulin downregulates T-cell receptor signaling and cytokine production. Pediatr Allergy Immunol 2012, 23:88–95. doi: 10.1111/j.1399-3038.2010.01129.x.

73. Koffman, B. M., and Dalakas, M. C. (1997) Effect of high-dose intravenous immunoglobulin on serum chemistry, hematology, and lymphocyte subpopulations: assessments based on controlled treatment trials in patients with neurological diseases. Muscle Nerve, 1997, 20(9):1102-7. doi: 10.1002/(sici)1097-4598(199709)20:9<1102: aid-mus2>3.0.co;2-c.

74. Rodriguez, M. A., De Sanctis, J. B., Blasini, A., Khare, S. D, Hansen, J., Luthra, H. S., and David, C. S. (1996) HLA-B27 heavy chains contribute to spontaneous inflammatory disease in B27/human beta2m (beta2m) double transgenic mice with disrupted mouse beta2m. J Clin Invest. 1996, 98(12):2746-2755, doi: 10.1172/JCI119100.

75. Cohen, G. B., Gandhi, R. T., Davis, D. M.,Mandelboim, O., Chen, B. K., Strominger, J. L. and Baltimore, D/. (1999) The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. Immunity 1999, 10:661–71. doi: 10.1016/s1074-7613(00)80065-5.

76. Santos, S. G., Antoniou, A. N., Sampaio, P., Powis, S. J., and Arosa, F. A. (2006) Lack of tyrosine 320 impairs spontaneous endocytosis and enhances release of HLA-B27 molecules. J Immunol. 2006, 176:2942–9. doi: 10.4049/jimmunol.176.5.2942.

77. Guild, B. C., and Strominger, J. L. (1984) Human and murine class I MHC antigens share conserved serine 335, the site of HLA phosphorylation in vivo. J Biol Chem. 1984, 259: 9235–40

78. Buckley, R. H., and Schiff, R. I. (1991) The use of intravenous immune globulin in immuno-deficiency diseases. N Engl J Med 1991, 325:110–7. doi: 10.1056/ NEJM 1991 07113250207.

79. Kaveri, S. V., Dietrich, G., Hurez, V., and Kazatchkine, M. D. (1991) Intravenous immunoglobulins (IVIg) in the treatment of autoimmune diseases. Clin Exp Immunol. 1991, 86:192–8 doi: 10.1111/j.1365-2249.1991.tb05794.x.

80. McIntyre, J. A., Higgins, N., Britton, R., Faucett, S., Johnson, S.,Beckman, D., Hormuth, D., Fehrenbacher, J., Halbrook, H. (1996) Utilization of intravenous immunoglobulin to ameliorate all-Abs in a highly sensitized patient with a cardiac assist device awaiting heart transplantation. Fluorescence-activated cell sorter analysis. Transplantation. 1996 62(5):691-3, doi: 10.1097/00007890-199609150-00027.

81. Ippoliti, F., De Santis, W., Volterrani, A., Canitano, N., Frattolillo, D., Lucarelli, S., Frediani, S., Frediani, T. (2006) Psychological stress affects response to sublingual immunotherapy in asthmatic children allergic to house dust mite.Pediatr Allergy Immunol. 2006,17(5):337-45. doi: 10.1111/j.1399-3038.2006.00417.x.

82. Glotz, D., Antoine, C., Julia, P., Suberbielle, C, Samir, B., Rabah, B., et al., (2002) Desensitization and subsequent kidney transplantation of patients using intravenous immunoglobulins (IVIg). Am J Transplant. 2002, 2(8): 758-60. doi: 10.1034/j.1600-6143.2002.20809.x.

83. Sethi. S., Choi, J., Toyoda, M., Vo, A. Peng, A., and Jordan, S. C. (2017) Desensitization: Overcoming the Immunologic Barriers to Transplantation. J. Immunol. Res. 2017, Article ID 6804678, 11 pages.doi.org/10.1155/2017/6804678.

84. Jordan, S. C., Tyan, D., Stablein, D., McIntosh, M., Rose, S., Vo, A. Toyoda, M.,et al., (2004) Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. J Am Soc Nephrol. 2004, 15(12): 3256-62. doi: 10.1097/01.ASN .0000145878 .92906.9F.

85. Ross, C. N., Gaskin, G., Gregor-Macgregor, S., Patel, A. A., Davey, N. J., Lehler, R. I., et al. (1993) Renal transplantation following immunoadsorption in highly sensitized recipients. Transplantation, 1993. 5(4):785-9. doi: 10.1097/00007890-199304000-00019.

86. larabi, A., Backman, U., Wikstrom, B, Sjoberg, O., and Tufveson, G. (1995) Pretransplantation plasmapheresis in HLA-sensitized patients: five years experience. Transplant Proc 1995, 27: 3448.

87. Stegall, M. D., Gloor, J., Winters, J. L., Moore, S. B., and Degoey, S. (2006) A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantib ody. Am J Transplant. 2006, 6(2): 346. doi: 10.1111/j.1600-6143.2005.01178.x

88. Onrust, S. V., Lamb, H. M., and Balfour, J. A. (1999) Rituximab Drugs.1999,58(1):79-88. doi: 10.2165/00003495-199958010-00009.

89. Vo, A. A., Lukovsky, M., Toyoda, M., Wang, J. Reinsmoen, N.L., Lai, C.-H., Peng, A., et al. (2008) Rituximab and intravenous immune globulin for desensitization during real transplantation. N Engl J Med 2008, 359(3): 242-51. doi: 10.1056/NEJMoa0707894

90. Vo, A. A., Peng, A, Toyoda, M., Kawalii, J., Cao, K., Lai, C-H., REinsmoen, N. et al. (2010) Use of intravenous immune globulin and rituximab for desensitization of highly HLA-sensitized patients awaiting kidney transplantation. Transplantation 2010, 89(9): 1095 - 1102. doi. 10.1097/TP.0b013e3181d21e7f

91. Vo, A. A., Choi, J., Cisneros, K., Reinsmoen, N,, Haas, M., Ge, S., Toyoda M., et al., (2014) Benefits of rituximab combine with intravenous immunoglobulin for desensitization of kidney transplant recipients. Transplantation, 2014. 98(3): 312-319. doi: 10.1097/TP.0000000000000064

92. Zhu, D., Ravindranath, M. H., Terasaki, P. I., Miyazaki, T., Pham, T., Jucaud, V. (2014) Suppression of allo-human leucocyteantigen (HLA) Abs 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): 464-77. doi: 10.1111/cei.12307.

93. Baecher-Allan, C., Brown, J.. Freeman, G. et al. (2001) CD4+CD25+ high regulatory cells in human peripheral blood. J. Immunol. 2001, 167:1245–1253. doi: 10.4049/jimmunol.167.3.1245.

94. Ng, W. F., Duggan, P. J., Ponchel, F.. Matarese, G., Lombardi, G., Edwards A. D., Isaacs, J. D., and Lechler, R, I. (2001) Human CD4+CD25+ cells: a naturally occurring population of regulatory T-cells. Blood, 2001, 98 (9):2736–2744. doi:10.1182/blood. v98.9.2736.

95. Li, Y., Koshiba, T., Yoshizawa, A., Yonekawa, Y., Masuda, K., Ito, A. et al. (2004) Analyses of peripheral blood mononuclear cells in operational tolerance after pediatric living donor liver Transplantation. Am J Transplant. 2004, 4:2118–25. doi: 10.1111/j. 1600-6143.200 4.00611.x

96. Pons, J. A., Revilla-Nuin, B., Baroja-Mazo, A., Ramirez, P., Martinez-Alarcon, L., Sanchez-Bueno, F. et al., (2008) FoxP3 in peripheral blood is associated with operational tolerance in liver transplant patients during immunosuppression withdrawal. Transplantation. 2008, 86:1370–8. doi: 10.1097/TP.0b013e318188d3e6

97. Baroja-Mazo, A., Revilla-Nuin, B., Parrilla, P., Martinez-Alarcon, L., Ramirez, P., Pons, J. A. (2016) Tolerance in liver Transplantation: Biomarkers and clinical relevance. World J Gastroenterol. 22:7676–91. doi: 10.3748/wjg.v22.i34.7676

98. Baroja-Mazo, A., Revilla-Nuin, B., de Bejar, A., Martinez-Alarcon, L., Herrero, J. I., El-Tayeb, A. et al. (2019) Extracellular adenosine reversibly inhibits the activation of human regulatory T-cells and negatively influences the achievement of the operational tolerance in liver Transplantation. Am J Transplant. 2019, 9:48–61. doi: 10.1111/ajt.15023

99. Jhun, J., Lee, S. H., Lee, S. K., Kim, H. Y., Jung, E. S., Kim, D. G, et al. (2018) Serial monitoring of immune markers being represented regulatory T cell/T helper 17 cell ratio: indicating tolerance for tapering immunosuppression after liver Transplantation. Front Immunol. 2018, 9:352. doi: 10.3389/fimmu.2018.00352

100. Sablik, K. A., Jordanova, E. S., Pocorni, N., Clahsen-van Groningen, M. C., and Betjes, M. G. H. (2019) Immune Cell Infiltrate in Chronic-Active Antibody-Mediated Rejection. Front Immunol. 2019, 10: 3106.doi: 10.3389/fimmu.2019.03106

101. Dai, H., Zheng, Y., Thomson, A. W., and Rogers, N. M. (2020) Transplant Tolerance Induction: Insights from the Liver. Frontiers Immunol. 2020, 11:1044. doi: 10.3389/ fimmu. 2020.01044.

102. Hara, M., Kingsley, C. I., Niimi M, et al. (2001) IL-10 is required for regulatory T-cells to mediate tolerance to alloantigens *in vivo*. J. Immunol. 2001, 166 (6): 3789–3796. doi: 10.4049/jimmunol.166.6.3789.

103. Levings, M., Sangregorio, R., and Roncarolo, M. G. (2001) Human CD25+CD4+ T regulatory cells suppress naive and memory T-cell proliferation and can be expanded *in vitro* without loss of function. J. Exp.Med..2001, 193:1295–1301. doi: 10.1084/jem. 193.11.1295.

104. van Maurik, K., Wood, J., and Jones, N. (2002) Cutting edge: CD4+CD25+ alloantigen-specific immunoregulatory cells that can prevent CD8+ T-cell-mediated graft rejection: implications for anti-CD154 immunotherapy. J. Immunol. 2002, 169: 5401–5404. doi: 10.4049/jimmunol.169.10.5401.

105. Lin, C.–Y. Graca, L. Cobbold, S. P., and Waldmann, H. (2002) Dominant transplantation tolerance impairs CD8+ T-cell function but not expansion. Nature Immunol. 2002, 3: 1208–1213. doi: 10.1038/ni853

106. Graca, L., Cobbold, S. P.. and Waldmann, H. (2002) Identification of regulatory T-cells in tolerated allografts .J. Exp.Med. 2002, 195:1641–1646. doi: 10.1084/jem.20012097

107. Cobbold, S. P., and Waldmann H. (2013) Regulatory cells and transplantation tolerance Cold Spring Harbor Perspect.Med. 2013, 3(6): 1-17 (pii: a015545).

108. Kikuchi, J., Hashizume, M., Kaneko, Y., Yoshimoto, K., Nishina, N., and Takeuchi, T. (2015) Peripheral blood CD4(+) CD25(+) CD127 (low) regulatory T-cells are significantly increased by tocilizumab treatment in patients with rheumatoid arthritis: increase in regulatory T-cells correlates with clinical response. Arthritis Res Ther. 2015, 17(1): 1-10. doi: 10.1186/s13075-015-0526-4.

109. Ephrem, A., Chamat, S., Miquel, C., Fisson, S., Mouthon, L., Caligiro, G., et al., (2008) Expansion of CD4+CD25+regulatory T-cells by intravenous immunoglobulin: a critical factor in controlling experimental autoimmune encephalomyelitis. Blood. 2008, 111: 715–722. doi: 10.1182/blood-2007-03-079947.

110. EL Hilali, F., Jucaud, V., EL Hilali, H., Bhuiyan, M. H., Mancuso, A., LiuSullivan, N., Elidrissi, A., and Mazouz, H. (2017) Characterization of the Anti-HLA Class I and II

IgG Abs 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

111. Ravindranath, M. H, and Jucaud, V. (2917) Conformational variants of HLA-I antigens on Luminex Single Antigen Beads for monitoring Abs. Transplantation. 2017, 101(4): e153-e154. doi:0.1097/TP.0000000000001629.

112. Arosa, F. A., Esgalhado, A. J., Padrão, C. A, and Cardoso, E. M. (2017) Divide, Conquer, and Sense: CD8+CD28- T-cells in Perspective. Front Immunol 2017, 7:665.doi: 10.3389/ fimmu. 2016.00665.

113. Arosa, F. A., de Jesus, O., Porto, G., Carmo, A. M., and de Sousa, M. (1999) Calreticulin is expressed on the cell surface of activated human peripheral blood T lymphocytes in J. Biol Chem. 1999, 274:16917–22. doi: 10.1074/jbc.274.24.16917.

114. Allen, R. L., and Trowsdale, J. (2004) Recognition of classical and heavy chain forms of HLA-B27 by leukocyte receptors. Curr Mol Med. 2004, 4:59–65. doi: 10.2174/1566524 043479329.

115. Jones, D. C., and Kosmoliaptsis, V., Apps, R., et al. (2011) HLA class I allelic sequence and conformation regulate leukocyte Ig-like receptor binding. J Immunol. 2011, 186: 2990–7. doi.org/10.4049/jimmunol.1003078

116. Tamir, I., and Cambier, J. C. (1988) Antigen receptor signaling: integration of protein tyrosine kinase functions. Oncogene, 1988; 17:1353–64. doi: 10.1038/sj.onc.1202187.

117. Angulo, R., and Fulcher, D. A. (1998) Measurement of Candida-specific blastogenesis: comparison of carboxyfluorescein succinimidyl ester labelling of T-cells, thymidine incorporation, and CD69 expression. Cytometry 1998; 34:143–51.

118. Mustelin, T., Vang, T., and Bottini, T. N. Protein tyrosine phosphatases and the immunearesponse. Nat Rev Immunol 2005; 5:43–57. doi: 10.1038/nri1530.

119. Nissen, M. H., Bjerrum, O J., Plesner, T., Wilken, M., Rørth, M. (1987) Modification of beta-2-microglobulin in sera from patients with small cell lung cancer: evidence for involvement of a serine protease. Clin Exp Immunol. 1987, 67(2):425-32.

120. Nissen, M. H., and Claësson , M H. (1987) Proteolytically modified human beta 2-microglobulin augments the specific cytotoxic activity in murine mixed lymphocyte culture, J.Immunol.1987,139(4):1022-9.

121. Nissen, M. H. (1993) Proteolytic modification of beta 2-microglobulin in human serum. Dan Med Bull.1993,40(1):56-64.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Figure Legends

Figure 1. Structure of HLA-E showing specific amino acid sequences or private epitopes and shared amino acid sequences or public epitopes. A. Private epitopes recognized by anti-HLA-E monospecific mAb, TFL-033 [44], B. Public epitopes recognized by anti-HLA-E mAbs, TFL-006 and TFL-007 [44]. Both private and public epitopes are shown in yellow.

Figure 2. Diagrammatic illustration of six isoforms of HLA class I molecules. Upper row shows the structures of intact trimeric closed conformers (CCs) of HLA. Note the shortened cytoplasmic tail. Lower row shows β2-m-free aHC monomeric open conforms (OCs) of HLA. OCs expose epitopes masked by β2-m. These epitopes, particularly, ^{117}AYDGKDY^{123}and ^{126}LNEDLRSWTA^{135} are found in almost all alleles of the six isoforms of HLA. (modified from [92].

Figure 3. Shedding of membrane-bound CCs. Shedding results in dissociation of HLA α-HC from □2-m. The membrane bound α-HC is cleaved by Zn^{2+}-dependant membrane metalloproteinase [119 -121]. The shed α-HC and □2-m are further degraded. Immune recognition of shed β2-m and α-HC can occur at any time.

Figure 4. Three different kinds of microbeads used: (i) beads admixed with losed and OCs, (ii) beads with OCs only, (iii) beads restricted with CCs. The microbeads were characterized using W6/32 (IgG2a) bound to peptide-associated and peptide-free CCs but not with OCs. HC-10, (IgG2a) recognized CCs devoid of a peptide. HLA-I polyreactive mAb TFL-006 (IgG2a) bound to OCs only and was inhibited by the shared peptides (^{117}AYDGKDY^{123}and ^{126}LNEDLRSWTA^{135}) found in all HLA-I isoforms. (modified from 45).

Figure 5. HLA-I polyreactivity of IVIg. IVIg is tested on three kinds of beadsets (i) Regular LABScreen beadset coated with an admixture of CCs and OCs. (ii) The regular LABScreen beadset is treated with mild acid to convert OCs to CCs. The beadset is loaded with OCs. (iii) "iBeads" generated from regular LABScreen beadset to enzymatically eliminate OCs, and therefore contains only CCs. The Luminex immunoassay with IVIg from GammaSTAN (A) and OctaGram (B) confirms the HLA-I polyreactivity of IVIg. Importantly IVIG reacts with the monomeric variants on the acid denatured beadsets better than that with iBeads. The density of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

IgG binding is illustrated by Mean Fluroescent Intensity (MFI) as shown in different colors. The binding affinity of IVIg to monomeric variants is much higher than those recognizing the CCs on 'iBeads'.

Figure 6A. The proliferation assay is based on labelling the purified lymphocytecs during PHA activation with the intracellular fluorescent dye carboxyfluorescein succinimidyl ester (CFSE: C25H15NO9; mol. mass: 473·39 g/mol) and, using flow cytometry, measuring mitotic activity by the successive twofold reductions in fluorescent intensity of the T-cells placed in culture for 72 h [117]. CFSE is cell-permeable, and is retained for long periods within cells by covalently coupling by means of its succinimidyl group to intracellular molecules. Due to this stable linkage, once incorporated within cells, CFSE is not transferred to adjacent T-cells, but remains in the cell even after several mitotic divisions.

Figure 6B. Suppression of blastogenesis and proliferation of CD4+ T-cells by IVIg (Globex) and HLA-I polyreactive mAb TFL-007 at similar protein concentrations. CFSC profile illustrates suppression as indicated by asterisks.

Figure 7. Suppression of PHA-activated CD4+ T-cells by HLA-polyreactive monoclonals TFL-006 and TFL-007 at 1/10 dilutions. Control mAb TFL-037 fails to suppress PHA activated proliferation, whereas, the HLA-I polyreactive mAbs suppresses proliferation significantly. Dosimetric suppression of TFL-007 is shown.

Figure 8. Suppression of proliferation of the PHA-activated CD4+ T-cells by purified culture supernatant of anti-HLA-E mAb TFL-006s (at 5 μg/ml and at 0.5 μg/ml) but not by the control mAb TFL-037 (at 5 μg/ml)

Figure 9. A model illustrating the possible mechanism underlying phytohaemagglutinin (PHA)-activation of T-cells and the suppression of activated T-cells mediated by HLA-I polyreactive mAbs TFL-006 and TLF-007) and possibly by IVIg. The model is based on a model proposed by Mustelin, Vang and Bottini [118] for T cell activation. The structure of CD3/T cell receptor (TCR)/CD4 on the lipid raft (pink zone) of the bi-layered lipid membrane on the non-phosphorylated non-activated CD4+ T-cells is illustrated. Lymphocyte-specific protein tyrosine kinase induces phosphorylation of tyrosine-based activation in the cytoplasmic domain of CD3, which leads to activation of transcription factors and transcription of cell surface

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

molecules such as interleukin (IL)-2Rα and open conformers of HLA class I. SH-1, SH-2 and SH-3 represent family members of Src homology; they are involved in mediating the cytoplasmic domain of CD3. Further activation of the tyrosyl-phosphorylated motifs then interaction with SH-1 domains within the protein kinase LCK, are leading to further signaling function [118]. Importantly, the exposure of shared amino acid sequences of all the HLA open conformers is indicated by a blue circle. It is this site that is recognized by TFL-006 and TFL-007. Possible interaction and consequences of recognition of the shared peptide sequences by the HLA-I poldyreactive IgG mAbs are illustrated in three steps: first, the exposure of the shared peptide sequence on the open conformer; secondly, recognition of the shared epitopes on the open conformer by the mAbs; thirdly, possible phosphorylation of the elongated cytoplasmic tail of open conformers. That elongation results in exposure of cryptic tyrosine (Tyr320) and serine (Ser355) residues in the cytoplasmic tail. It may be the binding of the mAbs to the shared peptide sequences that initiates the phosphorylation leading to signal transduction. A final step involves initiation of dephosphorylation of the cytoplasmic domain of CD3, resulting in arrest of activation or suppression. That seems plausible, as the phosphorylation is known to be reversible.

Figure 10. The impact of IVIg (GamaSTAN) and HLA-I polyreactive mAb (TFL-007) on the primary alloantibody DRB1*0101 (IgG) secreted by activated B lymphocytes obtained from the alloimmunized woman. GamaSTAN S/D IVIg was used at 1:100 dilution; 1·5 mg protein/ml. At the time when IVIg was added, cytokine combo and anti-CD40 antibody were not added. In all panels, the mean fluorescent intensity (MFI) of alloantibody secretion is compared between medium control and treatment with IVIg and HLA-I polyreactive IgG2a mAb TFL-007. IVIg inhibited the secretion of the primary alloantibody at a significant level ($p^2 = 0{\cdot}01$). HLA-I polyreactive mAb inhibited the secretion of the primary alloantibody at a higher significant level ($p^2=0.0005$).

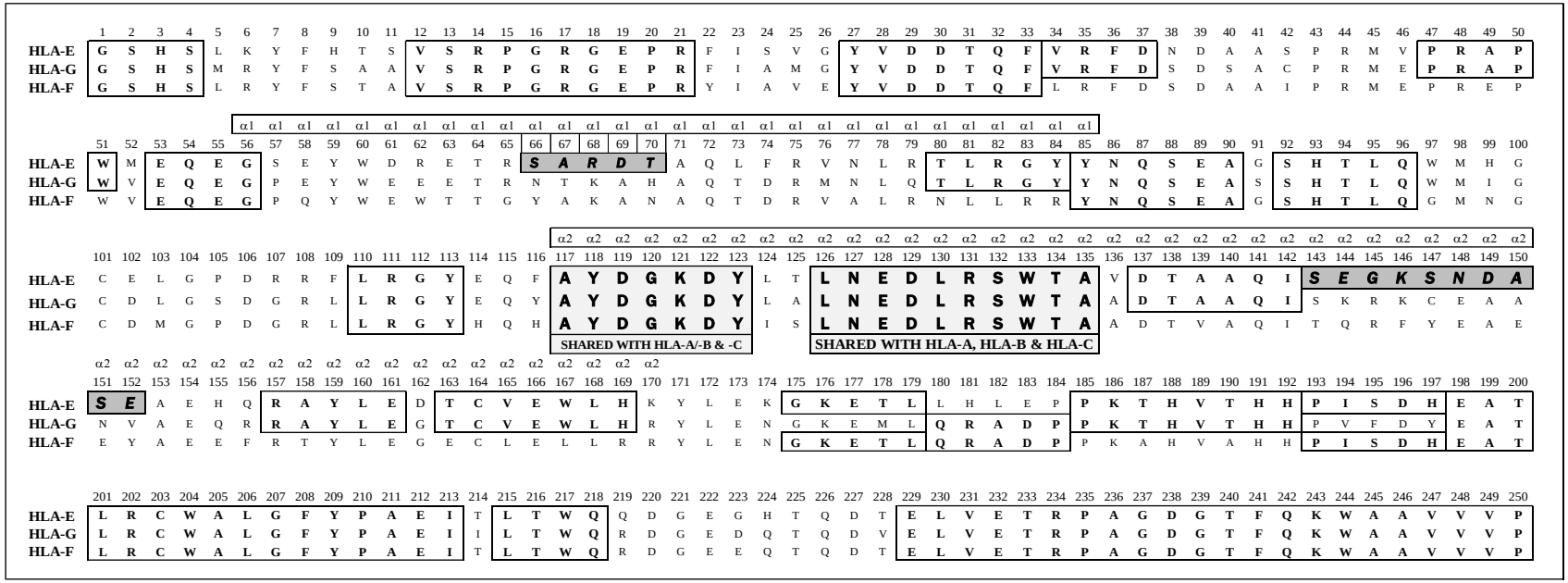
Figure 11. Effects of different commercial preparations of IVIg which include GamaSTAN™ S/D (15-18 gm%, Lot 26NHCVI; Telacris Biotherapeutics, Inc,) at dilutions 1/10 (Conc. 15 mg/ml) and 1/80 (Conc. 1.2 mg/ml), Octagam® (6 gm%, Lot A913A8431; Octapharma Pharmazeutika) at dilutions 1/20 (Conc. 3 mg/ml) and 1/80 (Conc. 0.75 mg/ml), and Gamunex®-C (10 gm%, Lots 26NKLG1 and 26NKLK1, Telacris) at dilutions 1/10 (Conc. 10

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

mg/ml and 1/80 (Conc. 8 mg/ml) and mAb TFL-007a (at dilution 1/10, conc. 62.7 μg/ml; 1/80, conc. 7.84 μg/ml) on PHA-untreated cells were compared with the effect of medium alone on the proliferation of Treg cells, defined as CD4+/CD25+/Foxp^3+. Note that IVIg preparations used in this study failed to upregulate Tregs in contrast to TFL-007a which significantly upregulates Treg cells at dilution 1/10. p^2 (two-tailed p value).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Table 1. Amino acid sequences of HLA-E compared with HLA-F and HLA-G. There are several sequences restricted to HLA-E, as illustrated by a short sequence ^{66}SARDT^{70} and a long sequence ^{143}SEGKSNDASE^{152}. HLA-E shares several sequences with HLA-F and HLA-G. In addition, HLA-E may selectively share several sequences with HLA-G and a few sequences (^{175}GKETL^{179} and ^{193}PISDH^{197}) with HLA-F. Above all, HLA-E shares ^{117}AYDGKDY^{123}and ^{126}LNEDLRSWTA^{135}with all other HLA isoforms (HLA-A, HLA-B, HLA-C, HLA-F, and HLA-G).



 doi:10.20944/preprints202105.0183.v1

Table 2. Comparison of amino acid sequence of HLA-E with sequences of other five HLA isoforms. Several peptide sequences of HLA-E are shared with the alleles of other HLA-I isoforms. Note amino acid sequence AYDGKDY is shared with maximum number of alleles of all isoforms of HLA-I, while sequences PRAPWMEQE, and EPPKTHVT are shared with one alleles of HLA-A (A*3306) and one allele of HLA-B (B*8201). The bioinformatics analysis was carried out by using the Immune Epitope Database (IEDB) to predict antigenicity rank of epitopes. Chou and Fasman beta turn, Kolaskar and Tongaonkar antigenicity, Karplus and Schulz flexibility and Parker hydrophilicity prediction methods in IEDB were employed. The methods predict the probability of specific sequences in HLA-E that bind to Abs being in a beta turn region, being antigenic, being flexible, and being in a hydrophilic region. Antigenicity rank is calculated by pooling the probability values.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

HLA-E peptide sequences [total number of amino acids]	HLA alleles: Classical HLA-Ib A	B	Cw	Non-Classical HLA-Ib F	G	Specificity	Method 1 Beta-Turn Chou & Fasman (1978)	Method 2 Antigencity Kolaskar & Tangaonkar (1990)	Method 3 Flexibility Karplus & Schulz (1985)	Method 4 Hydrophilicity Parker (1986)	Rank of Antigenicity
							Prediction SCORES				
^{47}PRAPWMEQE^{55} [9]	1	0	0	0	0	**A*3306**	0.993	0.948	0.969	0.586/1.143/1.657	
^{58}EYWDRETR^{65} [8]	5	0	0	0	0	A restricted	0.993	0.915	1.024	3.301/2.786	**10**
^{90}AGSHTLQW^{97} [8]	1	10	48	0	0	Polyspecific	1.019	1.033	0.989	2.629/0.901	**6**
^{108}RFLRGYE^{114} [7]	24	0	0	0	0	A restricted	0.933	0.996	0.996	0.229	**8**
^{115}QFAYDGKDY^{123} [9]	1	104	75	0	0	Polyspecific	1.059	1.001	0.993	2.629/3.201	**5**
^{117}AYDGKDY^{123} [7]	**491**	**831**	**271**	**21**	**30**	**Polyspecific**	**1.204**	**0.989**	**1.061**	**4.243**	**1**
^{126}LNEDLRSWTA^{135} [10]	239	219	261	21	30	Polyspecific	1.046	0.983	1.039	2.443/2.329	**2**
^{137}DTAAQI^{142} [6]	0	824	248	0	30	Polyspecific	0.813	1.065	0.978	1.957	**3**
^{137}DTAAQIS^{143} [7]	0	52	4	0	30	Polyspecific	0.946	1.012	0.97	3.414	**7**
^{157}RAYLED^{162} [6]	0	1	0	0	0	**B*8201**	0.929	0.996	0.969	2.601	
^{163}TCVEWL^{168} [6]	282	206	200	0	30	Polyspecific	0.841	1.115	0.929	-0.914	**4**
^{183}EPPKTHVT^{190} [8]	0	0	19	0	0	C restricted	1.029	1.044	1.042	3.043	**9**
^{65}RSARDTA^{71} [7]	0	0	0	0	0	**E restricted**	1.011	0.952	1.038	4.901	**2**
^{143}SEQKSNDASE^{152} [10]	0	0	0	0	0	**E restricted**	1.231	0.923	1.222	7.071/ 6.443/ 6.257/ 6.514	**1**

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Table 3. The HLA-1 signatures of the mAbs generated after immunizing recombinant Heavy chains of HLA-E. Group 10 is truly befit the definition of Polyreactive mAb category.

IMMUNOGEN HLA-E^{R107} OR HLA-E^{G107}							
Groups	**Number**	**HLA-CLASS Ib**			**HLA-CLASS IC**		
of mAbs	**of mAbs**	**HLA-E**	**HLA-F**	**HLA-G**	**HLA-A**	**HLA-B**	**HLA-C**
Group 1	24	+	-	-	-	-	-
Group 2	1	+	+	-	-	-	-
Group 3	1	+	-	+			
Group 4	8	+	+	+	-	-	-
Group 5	4	+				+	
Group 6	31	+				+	+
Group 7	109	+	-	-	+	+	+
Group 8	11	+	+	-	+	+	+
Group 9	18	+	-	+	+	+	+
Group 10	7	+	+	+	+	+	+

 doi:10.20944/preprints202105.0183.v1

Table 4. HLA-I allele reactivities of the polyreactive mAbs, compared with monospecific mAbs. The values represent Mean Fluorescent intensitities (MFI) of the mAbs corrected against background values. The mAbs were generated using HLA-E recombinant Heavy chain. The number of HLA antigens showing positive reactivity with the mAbs are shown in bold letters.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

mAbs	*Monospecific*	*Polyreactive*	
	TFL-033 *IgG1*	TFL-006 *IgG2a*	TFL--007 *IgG2a*
Neg	3	15	7
Pos	71	88	85
HLA-E	24411	22522	21618
HLA-F		12650	11035
HLA-G		7193	2670
A* alleles			
A*0101		2395	1037
A*0201		856	
A*0203		1095	
A*0206		1494	843
A*0301		818	
A*1101		10190	8476
A*1102		860	
A*2301		614	
A*2402		3133	2011
A*2403		3151	1967
A*2501		1230	692
A*2601		3368	1638
A*2901		3194	2256
A*2902		2235	1136
A*3001		2229	1237
A*3002		3353	2211
A*3101		858	
A*3201		2237	1508
A*3301		2791	1627
A*3303		4212	2961
A*3401		6268	3968
A*3402		1399	893
A*3601		5806	3826
A*4301		4420	2364
A*6601		3644	1526
A*6602		1395	789
A*6801		1314	859
A*6802		2078	1276
A*6901		1964	917
A*7401		723	
A*8001		2841	1430
A alleles*	*0*	*32*	*24*

mAbs	*Monospecific*	*Polyreactive*	
	TFL-033 *IgG1*	TFL-006 *IgG2a*	TFL--007 *IgG2a*
B* alleles			
B*0702		1331	841
B*0801		2092	1033
B*1301		5654	3979
B*1302		2237	1426
B*1401		11319	8767
B*1402		4414	2558
B*1501		1097	
B*1502		6256	4497
B*1503		2831	1926
B*1510		2616	1470
B*1511		9041	5902
B*1512		1624	996
B*1513		5326	3365
B*1516		5614	3443
B*1801		6990	4890
B*2705		2591	1576
B*2708		4437	2671
B*3501		10205	8594
B*3701		6472	4338
B*3801		3844	1820
B*3901		7093	5304
B*4001		5743	3758
B*4002		6118	4675
B*4006		15643	13758
B*4101		7191	5277
B*4201		636	
B*4402		7062	4059
B*4403		7256	5638
B*4501		9535	7646
B*4601		6491	4130
B*4701		6528	3895
B*4801		4365	2716
B*4901			
B*5001		741	
B*5101		6205	3724
B*5102		5251	3579
B*5201		4524	2728
B*5301		8807	7323
B*5401		5556	4153
B*5501		2829	1887
B*5601		1386	777
B*5701			
B*5703		1229	600
B*5801		10160	8047
B*5901		5646	3001
B*6701		675	
B*7301		3347	2171
B*7801		6089	4597
B*8101		1352	729
B*8201		4367	3069
B alleles*	*0*	*48*	*44*

mAbs	*Monospecific*	*Polyreactive*	
	TFL-033 *IgG1*	TFL-006 *IgG2a*	TFL--007 *IgG2a*
C* alleles			
C*0102		7242	3268
C*0202		10690	6084
C*0302		5917	3062
C*0303		7114	4250
C*0304		6584	3891
C*0401		2843	1272
C*0501		16131	13096
C*0602		9396	4274
C*0702		12251	6919
C*0801		13456	10733
C*1203		5055	2102
C*1402		8727	4936
C*1502		6030	3225
C*1601		8462	4364
C*1701		13521	9069
C*1802		17918	15207
C alleles*	*0*	*16*	*16*

 doi:10.20944/preprints202105.0183.v1

Table 5. Evidence to show that the HLA-I polyreactive mAb TFL-006 binds to OCs (β2-m-free αHLA HC) but not to CCs (b2-m-associated aHLA heavy chains or intact HLA molecules). TFL-006 binds only with LABScreen beadsets (contains both open and CCs) but not with LIFECODES which contains only CCs as established in previous reports [45, 47].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

TFL-006 (20ug/mL)

HLA-A

	NC	PC	A*01:01	A*02:01	A*02:03	A*03:01	A*11:01	A*11:02	A*23:01	A*24:02	A*24:03	A*25:01	A*26:01	A*29:01	A*29:02	A*30:01	A*31:01	A*32:01	A*33:01	A*33:03	A*34:02	A*36:01	A*43:01	A*66:01	A*66:02	A*68:01	A*68:02	A*69:01	A*74:01	A*80:01
REGULAR LABSCreen BEADSET (Lot: LS1A04-lot 10) [*Closted Conformers admixed with Open Conformers*]	0	12	933	339	1018	193	4782	537	133	716	2516	194	2221	1017	778	1496	396	515	1038	554	1535	1353	2479	1886	1454	713	1185	3128	652	3132
LIFECODES BEADSET (Lot # 3005619) (Closed Conformers only)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

HLA-B

	B*07:02	B*08:01	B*13:02	B*14:01	B*14:02	B*15:01	B*15:02	B*15:03	B*15:12	B*15:13	B*15:16	B*18:01	B*27:05	B*27:08	B*35:01	B*37:01	B*38:01	B*39:01	B*40:01	B*40:02	B*41:01	B*42:01	B*44:02	B*44:03	B*45:01	B*46:01	B*47:01	B*48:01	B*49:01	B*50:01	B*51:01	B*52:01	B*53:01	B*54:01	B*55:01	B*56:01	B*57:01	B*58:01	B*59:01	B*67:01	B*73:01	B*78:01	B*81:01
REGULAR LABSCreen BEADSET (Lot: LS1A04-lot 10) [*Closted Conformers admixed with Open Conformers*]	862	1226	2514	7805	1831	335	1935	1822	770	3135	3076	3096	634	1659	6128	2650	2521	704	3429	2697	3739	347	3650	1829	1736	3572	2152	3262	1554	1799	2461	2146	5442	1662	2519	3662	2089	5268	3553	406	1423	2996	1525
LIFECODES BEADSET (Lot # 3005619) (Closed Conformers only)	0	0	12	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	8	0	2	0	0	0	0	0	1	0	2	0	0

HLA-C

	C*01:02	C*02:02	C*03:03	C*03:04	C*04:01	C*05:01	C*06:02	C*07:02	C*08:01	C*14:02	C*15:02	C*16:01	C*17:01
REGULAR LABSCreen BEADSET (Lot: LS1A04-lot 10) [*Closted Conformers admixed with Open Conformers*]	4066	7446	2458	4504	3337	9124	5644	8702	6090	3937	4465	4648	8296
LIFECODES BEADSET (Lot # 3005619) (Closed Conformers only)	1	20	0	0	8	24	4	97	1	0	4	0	10

 doi:10.20944/preprints202105.0183.v1

Table 6. HLA-I reactivity of different therapeutic preparations of IVIg.

Therapeutic Preparations of IVIg	**Reactivity of different HLA Class I antigens**					
	Classical HLA-Ia alleles			Non-classical HLA-Ib		
	A	**B**	**Cw**	**E**	**F**	**G**
IVIg (GammaStan, USA)	31	50	16	*Positive*	*Positive*	*Positive*
IVIg (Octogram, Mexico)	30	47	16	*Positive*	*Positive*	*Positive*
IVIg (Sandaglobulin, Euro)	30	47	16	*Positive*	*Positive*	*Positive*
IVIg (GlobEx, India)	20	39	16	*Positive*	*Positive*	*Positive*
IVIg (IV-LFB-CNTs LFB (France)	31	50	16	*Positive*	*Positive*	*Positive*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Table 7. Comparison of nature and functional characteristics of IVIg and HLA-I polyreactive mAbs

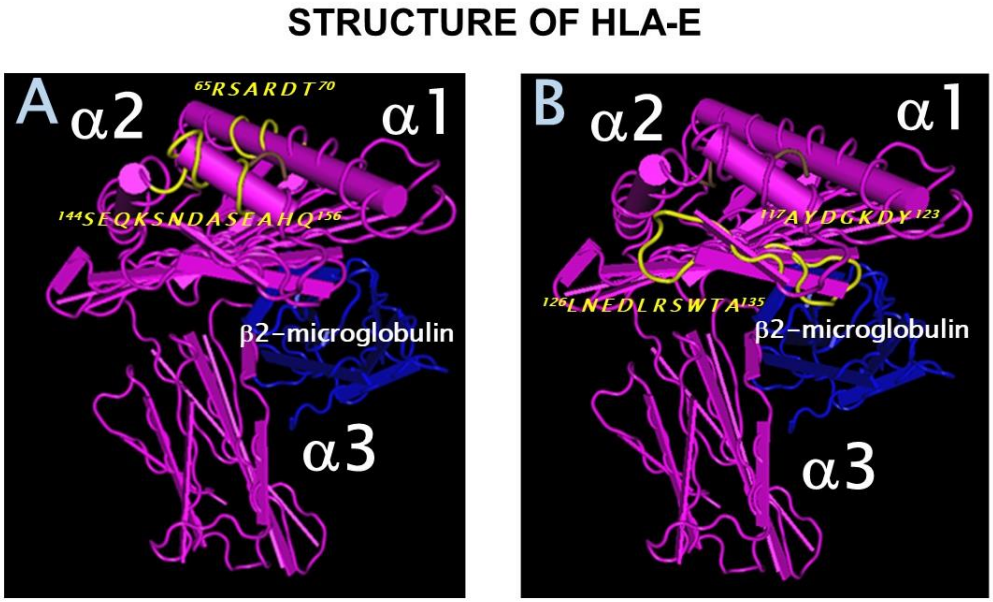
Source, Nature and Functions	Intravenous Immunoglobulin (IVIg)	HLA-I Polyreactive mAbs, TFL-006 & TFL-007
Manufacturer	Several pharmaceutical firms	Terasaki Foundation Laboratory, US Patent No.10,800.847. [10/13/2020]
Source	purified from Pooled plasma of 10,000 blood donors	Immunized in mice with αHeavy chain of HLA-E^{R107}
Nature of antibody	Human, Polyclonal IgG with trace level of IgA	murine, ascites purified monoclonal IgG.
Subclass of IgG antibodies	IgG1, IgG2a, IgG3, IgG4	IgG2a
Purity	contains soluble HLA antigens and other non-IgG proteins Cytokines, chemokines,	100% purified protein of IgG2a [44, 45]
antibody reactivity	HLA-A, HLA-B, HLA-Cw, HLA-E, HLA-F, HLA-G HLA-DR, HLA-DQA/DQB, HLA-DPA/DPB Fc-receptors: FcgI, FcgII, FcgIII, FcgIV (tested) [96] Blood groups A, B, Rh *Escherichia coli* bacterial antigens ranging from antigens by different preparations of IVIg Human albumin Phospholipids	HLA-A, HLA-B, HLA-Cw, HLA-E, HLA-F, HLA-G None FcgII (anticipated) No No No No No
Binding site	binds to both closed and open conformers	binds only to open conformers
Stabilizer	many including sucrose in some preparations	none
Protein concentration	highly variable from 2 to 12%	Protein concentration adjusted to requirement
CD4+ T-cell suppression	PHA or cytokine activated T cells by apoptosis & Necrosis	PHA-activated T cells
CD8+ T-cell proliferation	PHA-activatedT- cells	PHA-activated T cells
B cell proliferation	may induce differentiation	None
Anti-HLA antibody suppression	PRA-antibody reduction suppress selected HLA-II antibody produciton promote selected HLA-II antibody production	Suppress production of anti-HLA-I and anti-HLA-II IgG
Expansion of Tregs	Yes	Yes
Special application	Not applicable	To monitor the presence of Open conformerfs admixed wsith Closed conformers on the beadsets. (e.g LABScreen vs LIFECODE Beadsets used in monitorning HLA antibodies

 doi:10.20944/preprints202105.0183.v1

FIGURES

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Figure 1

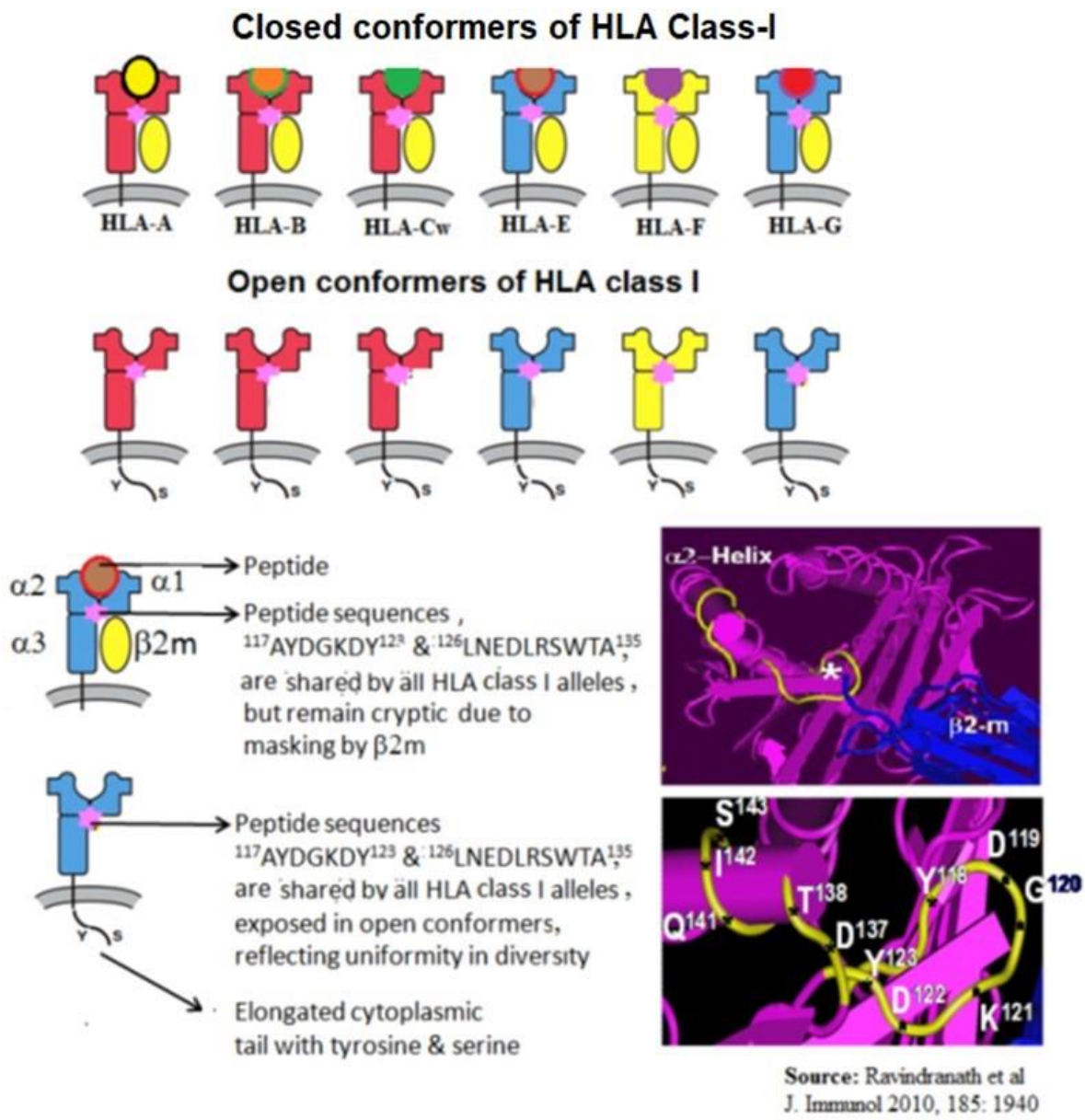


HLA-E Specific amino acid Sequences (private epitopes)

Amino acid Sequences shared with other HLA isoforms (public epitopes)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

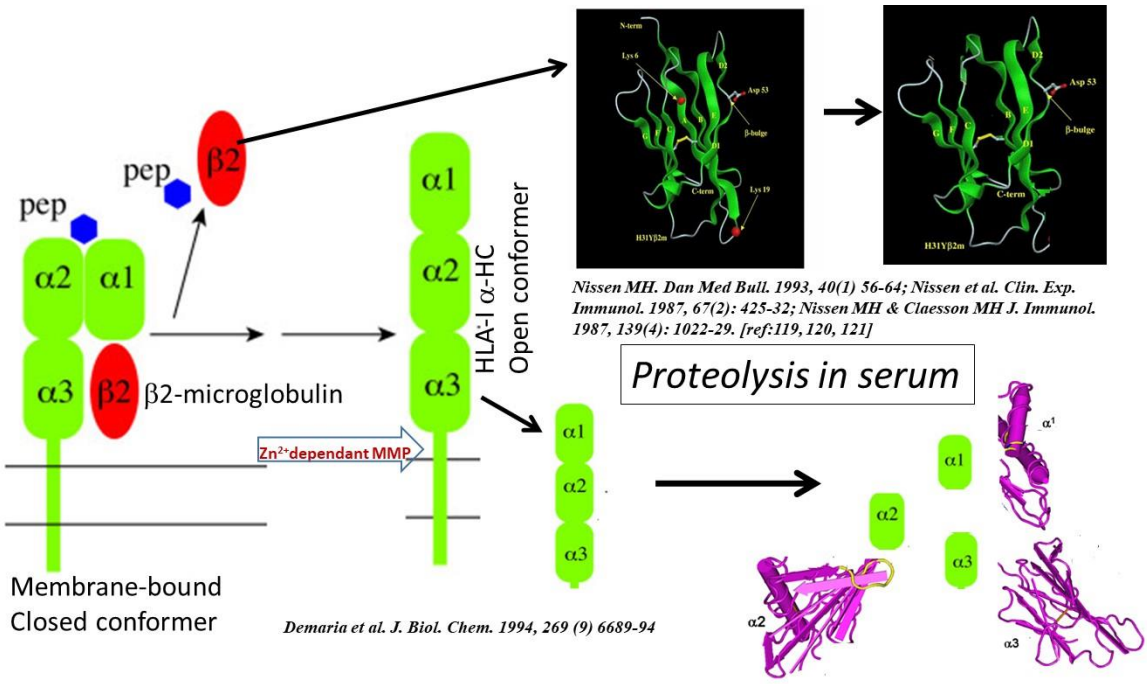
Figure 2



Source: Ravindranath et al
J. Immunol 2010, 185: 1940

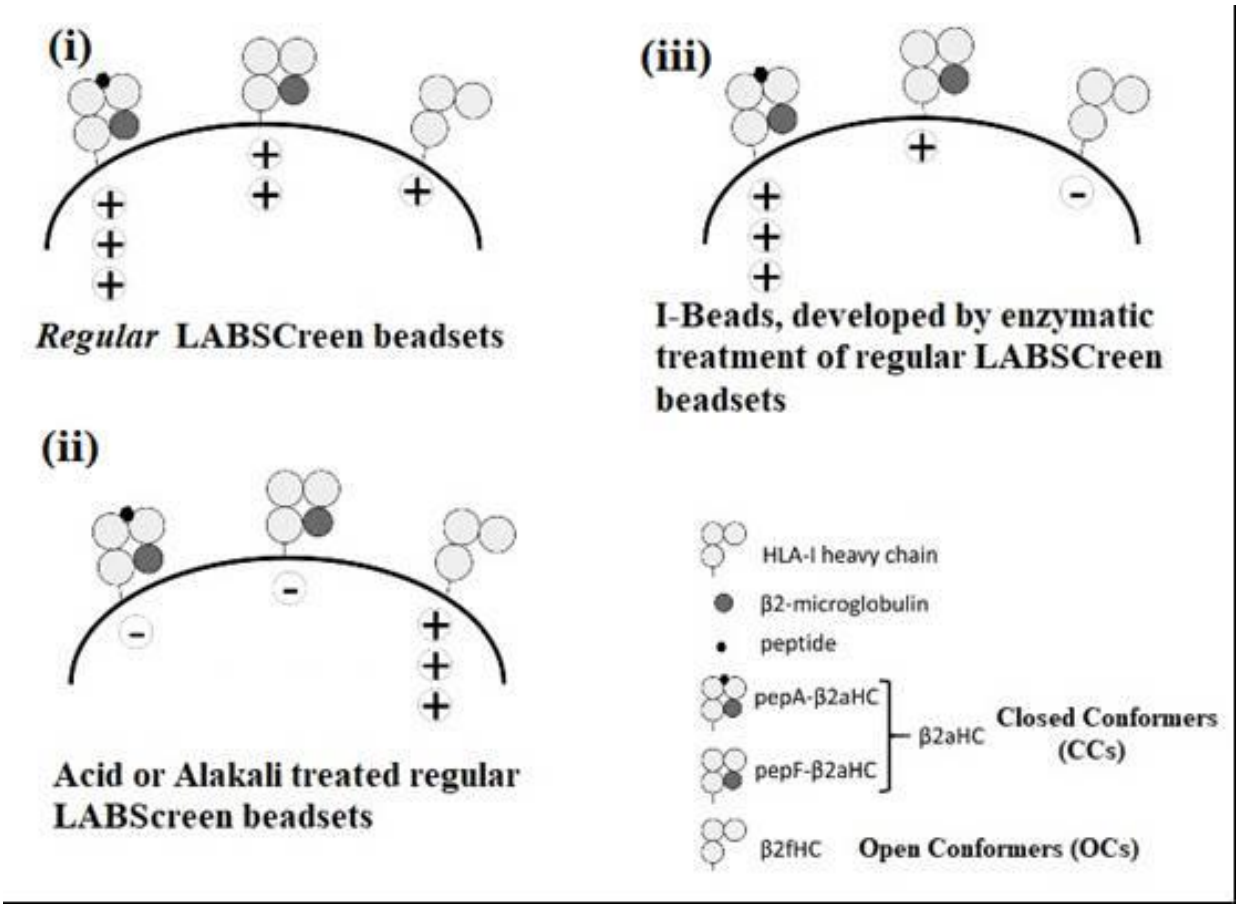
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Figure 3



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

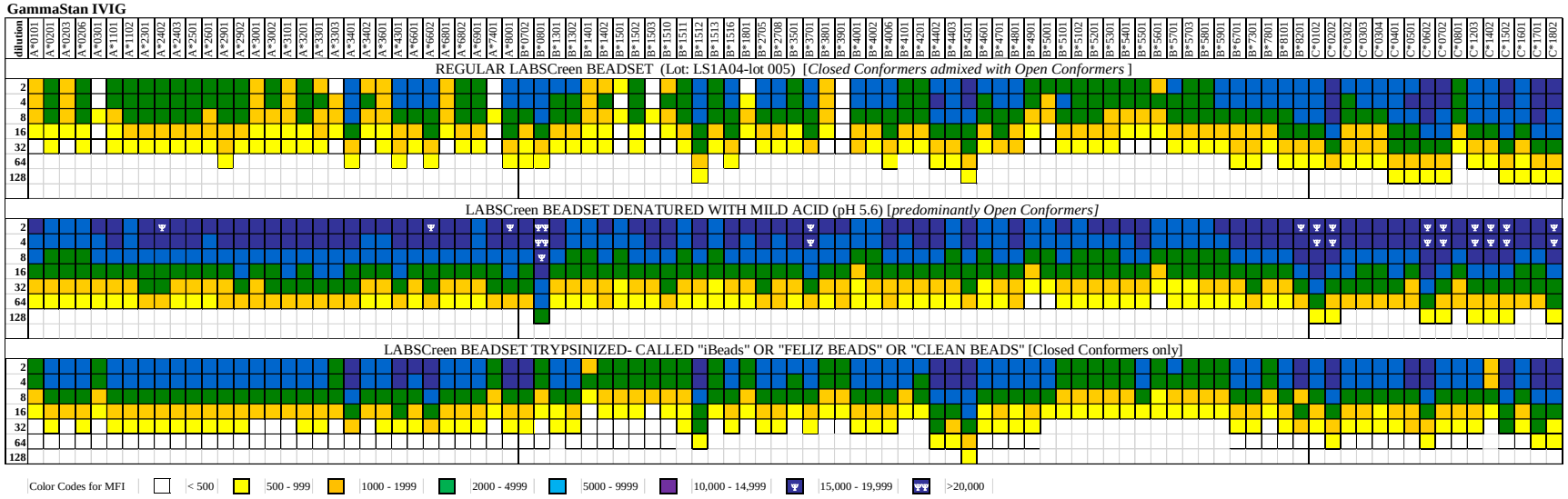
Figure 4



 doi:10.20944/preprints202105.0183.v1

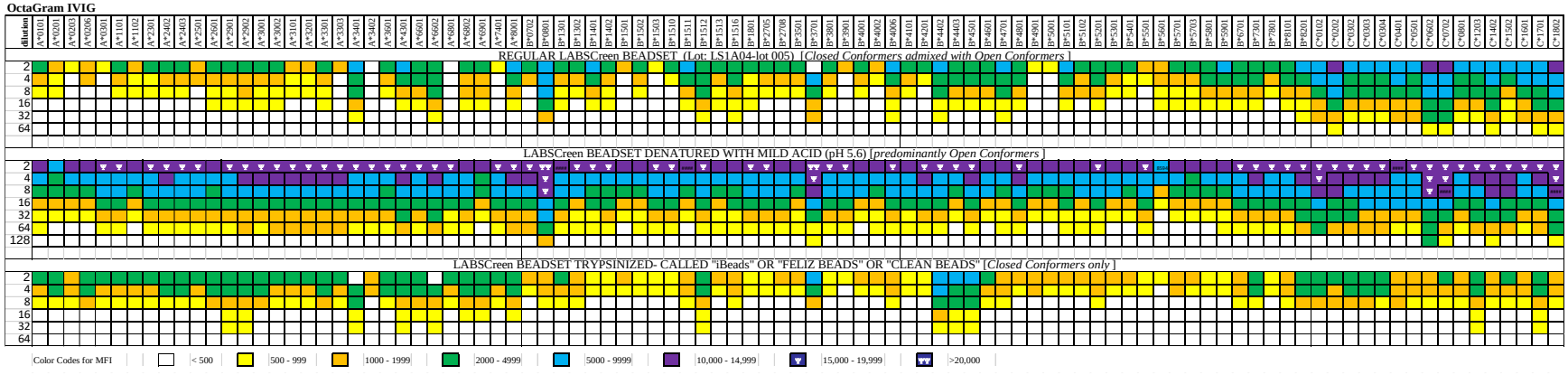
Figure 5

A



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

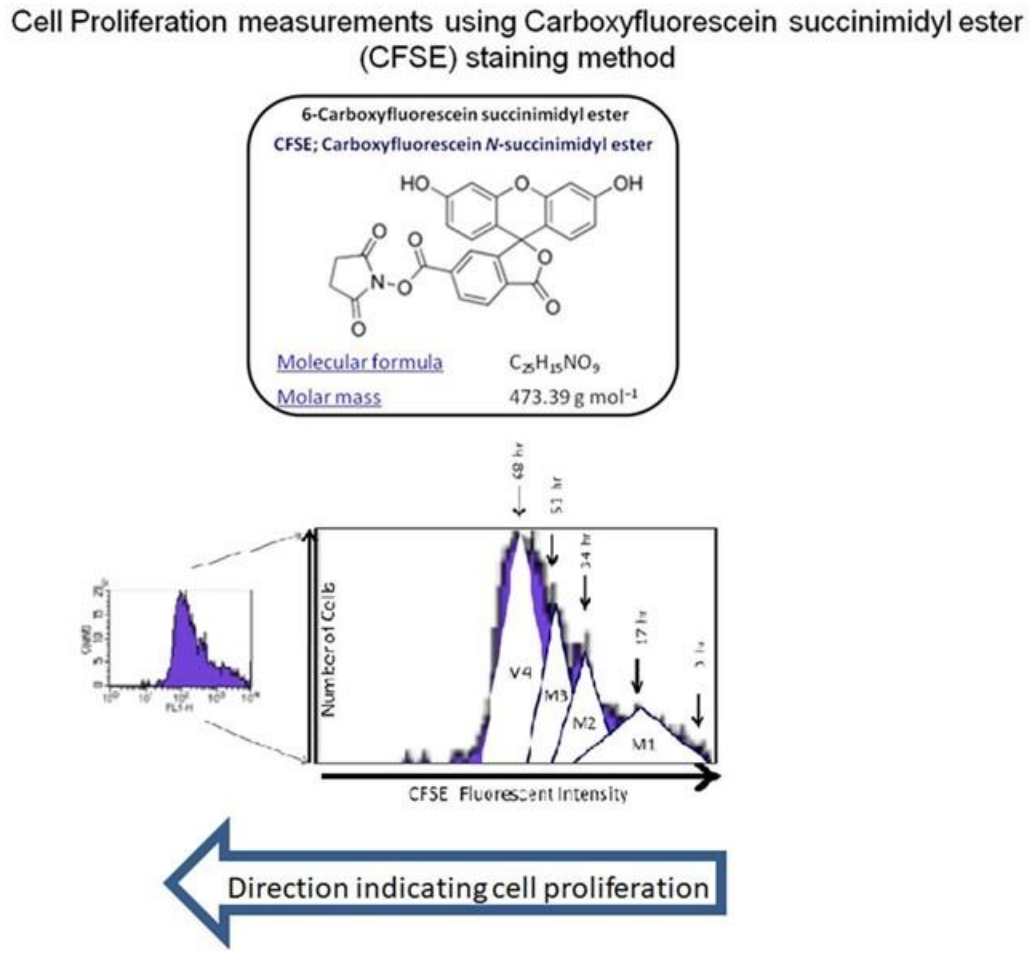
B



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

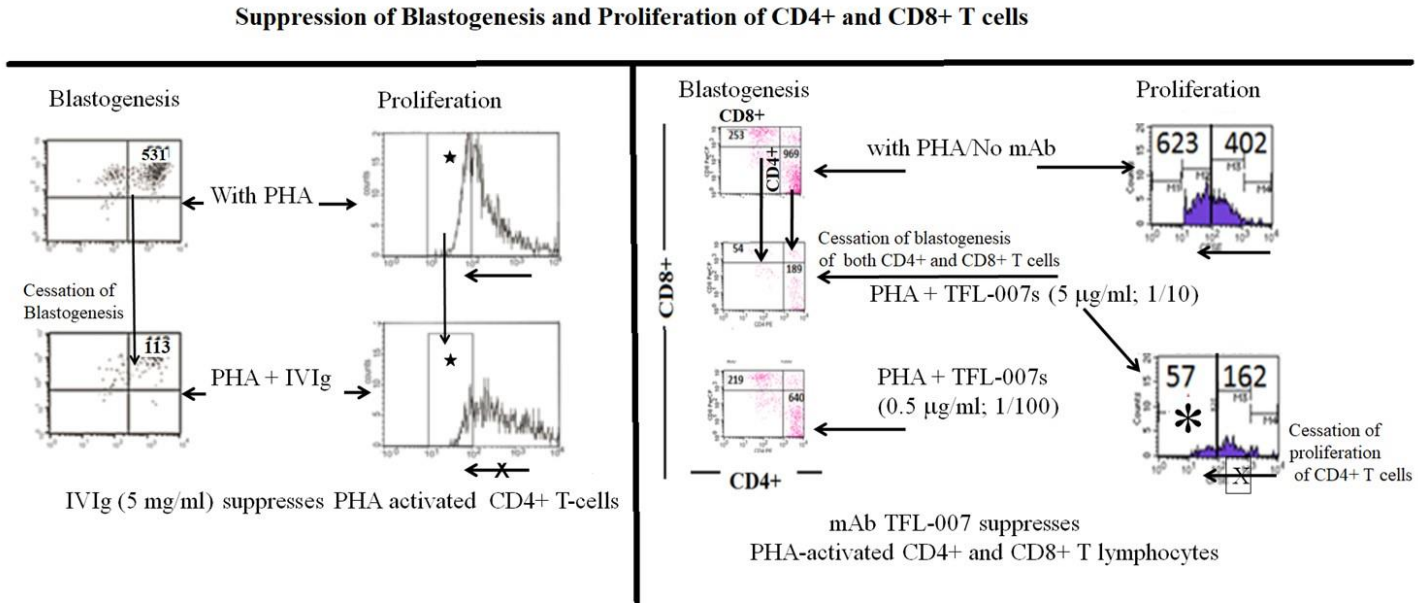
Figure 6

A



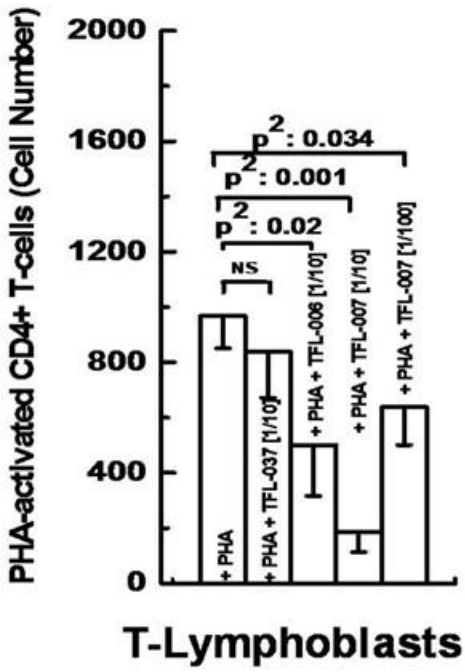
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

B



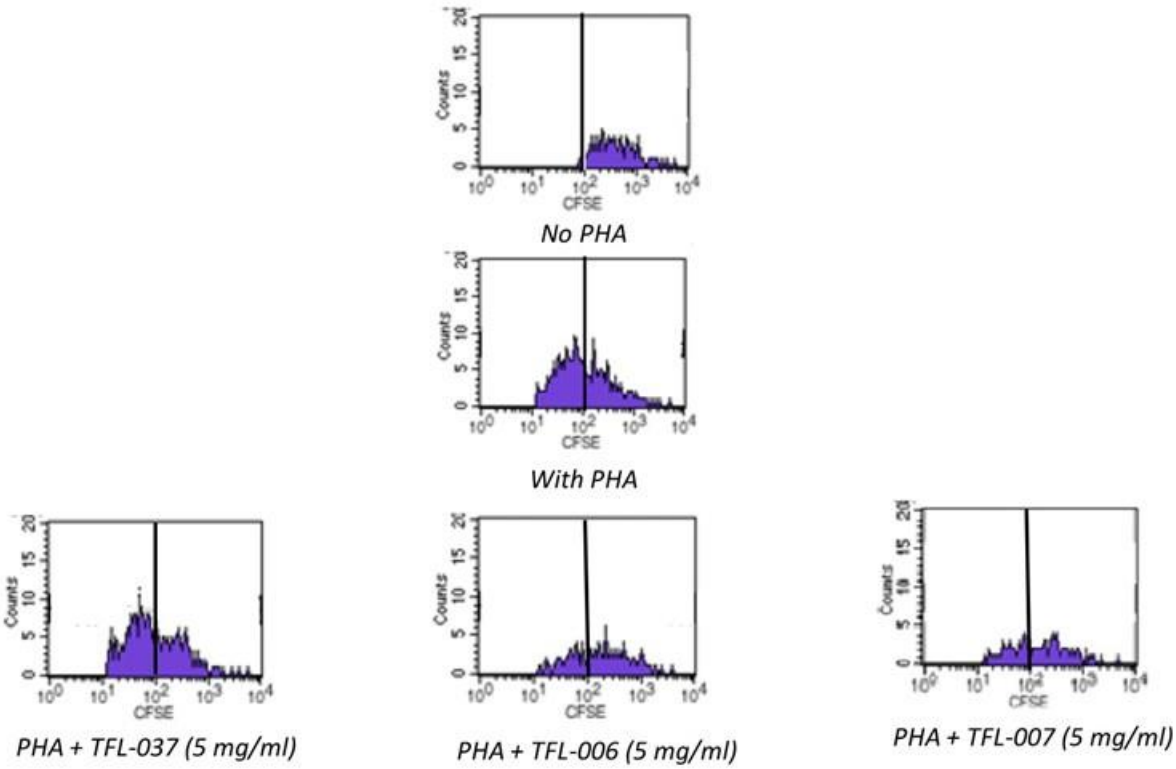
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Figure 7



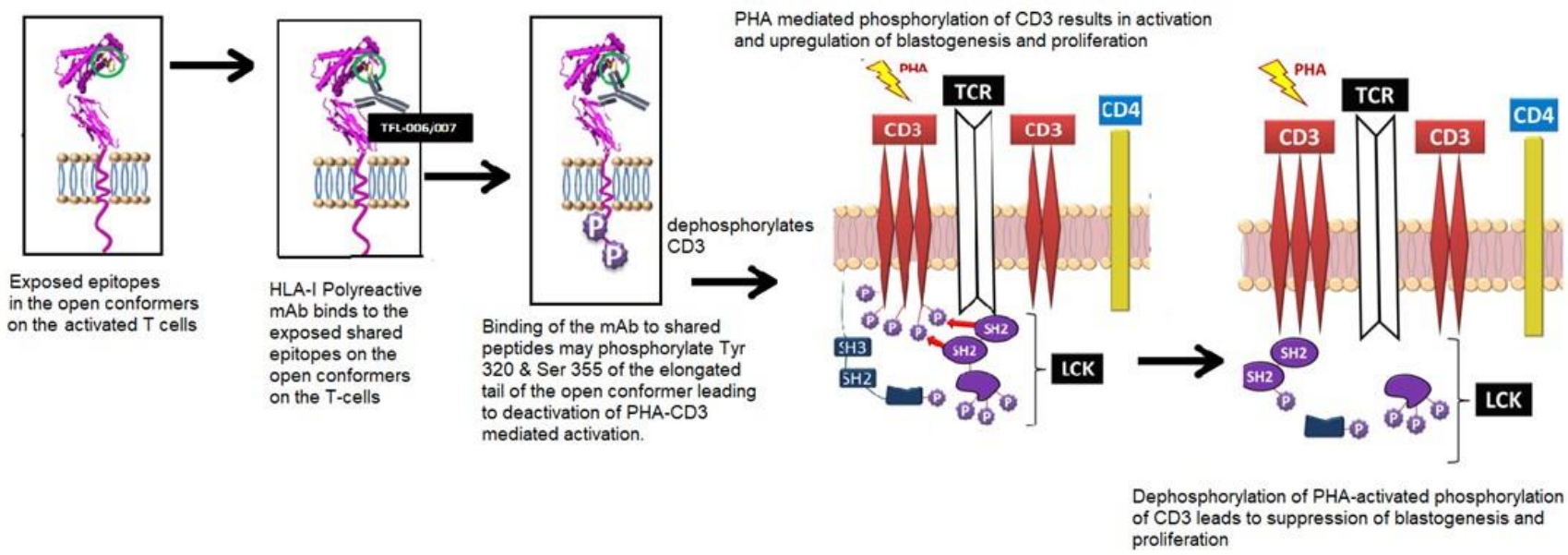
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Figure 8



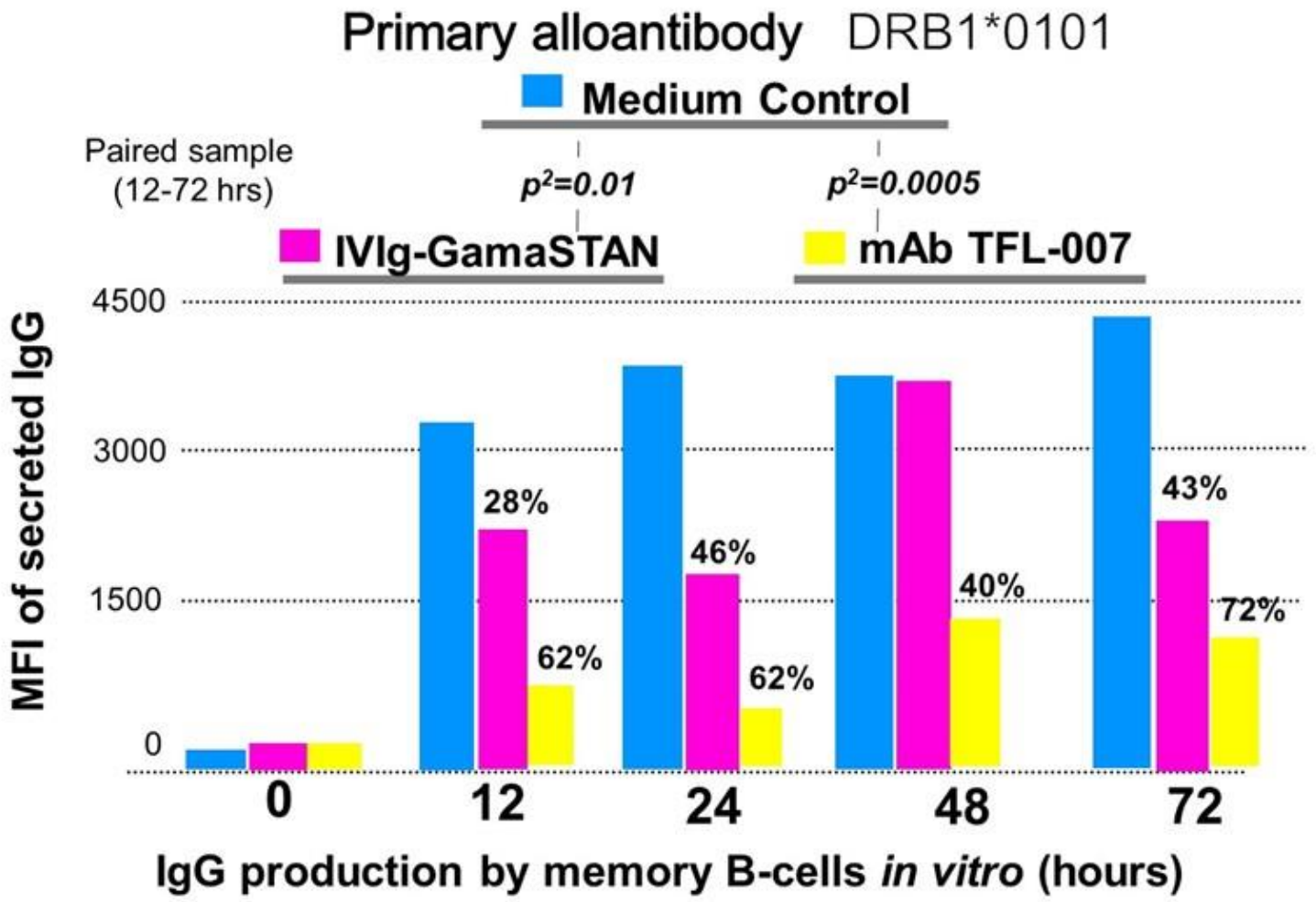
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Figure 9.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Figure 10



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 May 2021 doi:10.20944/preprints202105.0183.v1

Figure 11