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(54) **Title:** SYNTHETIC HLA BINDING PEPTIDE ANALOGUES OF MUTANT V617F JAK2 ENZYME AND USES THEREFOR

(57) **Abstract:** The present invention provides synthetic peptides comprising at least analogues of a mutant V617F JAK2 peptide that specifically bind to HLA class I or class II molecules on a cell characteristic of a myeloproliferative disorder or disease in a subject. Pharmaceutical compositions and immunogenic compositions comprising at least the peptide analogue segments or a DNA encoding the same are provided. Also provided are methods of using the synthetic peptides and immunogenic compositions to induce a heteroclitic immune response or to treat a myeloproliferative disorder or disease.

SYNTHETIC HLA BINDING PEPTIDE ANALOGUES OF MUTANT V617F JAK2 ENZYME AND USES THEREFOR

Federal Funding Legend

This invention was produced in part using funds obtained through grants PO1 CA 23766 and RO1 55349 from the National Institutes of Health. Consequently, the federal government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to the fields of immunology and therapies for neoplastic disorders. More specifically, this invention relates to the use of synthetic analogue peptides of mutant V617F JAK2 enzyme to induce heteroclitic human T cell responses against cells presenting mutant V617F JAK2 in subjects with myeloproliferative disorders.

Description of the Related Art

Classic chronic myeloproliferative disorders (MPD) which include polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (IMF) and chronic myelogenous leukemia (CML) are diagnosed in over 20,000 patients annually in the United States. These disorders are thought to arise from a single, multipotent hematopoietic progenitor or stem cell, resulting in the clonal proliferation of myeloid cells including erythrocytes, platelets and leukocytes, which come to dominate the marrow and blood. The cells appear to proliferate in the absence of any apparent stimulus and this hyperactive hematopoiesis often also extends to one or more extramedullary sites. Patients often display thrombosis and

hemorrhagic complications, bone marrow fibrosis, and leukemic transformation. In the last 20 years there has been much progress regarding the molecular pathogenesis of CML, resulting in new diagnostic methods and targeted therapies. However, the diagnosis of the bcr-abl negative MPD disorders is often difficult and their treatment usually has been a non-specific suppression of hematopoiesis.

CML is invariably associated with the Philadelphia chromosome, which is generated from the t(9,22) reciprocal chromosomal translocation. The resulting bcr-abl gene product leads to a disregulated Abl protein tyrosine kinase which has been shown to be the transforming event in CML. The bcr-abl fusion protein is now used as a molecular diagnostic tool and an effective target for various treatment and therapies. Recently, a number of independent groups have published studies defining an amino acid point mutation (V617F) in the JAK2 protein tyrosine kinase as the molecular basis for the enhanced myeloproliferation and clonal dominance that characterizes bcr-abl negative MPDs. This mutation as been described, on average, in 85% of patients with PV, 40% of patients with ET and 45% of those with IMF, yet not in any normal patients.

Endogenous erythroid colony formation is a characteristic in a significant proportion of patients with a chronic myeloproliferative disorder in which erythroid progenitor cells obtained from the marrow or peripheral blood of patients proliferate in semi-solid, serum-containing cultures in the absence of exogenous EPO. Presently, endogenous erythroid colony formation is the only relatively useful diagnostic test for myeloproliferative disorders. Unfortunately, the assay is technically demanding and is positive in only about 35-80% of patients with these diseases. The molecular basis of the erythropoietin negative endogenous erythroid colonies is likely to lie downstream of the EPO receptor.

The EPO receptor belongs to a unique class of receptors that contain no cytoplasmic tyrosine kinase domains or intracellular signaling motifs. These receptors, termed type I cytokine receptors must rely on intracellular signaling molecules, such as the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway. For the type I cytokine receptors, all signaling begins with the JAK molecules that are non-covalently bound to the "box1/2 motifs present in the juxtamembrane cytoplasmic region of the receptors.

Upon ligand binding, a large conformational shift of the receptor ensues, bringing the cytoplasmic domains of two receptors closer together. This close apposition of two prebound JAK molecules leads to their activation by trans-phosphorylation. In turn, the activated JAK kinase phosphorylates tyrosine residues on the initiating receptor, which can then serve as a docking site for src homology SH2 bearing secondary signaling molecules, such as nascent transcription factors (STATs), adaptor molecules (Shc, Gab/IRS), and kinase regulatory subunits (p85 phosphoinositol-3-kinase), amongst others.

The four members of the Janus family of kinases include JAK1, JAK2, JAK3 and TYK2. JAK2 is the primary tyrosine kinase associated with signaling from EPO, SCF, GM-CSF, IL-3, TPO and IGF-1 receptors. Hematopoietic progenitors in myeloproliferative disorders are hypersensitive to all of these growth factors. Molecular modeling of the JH1 and JH2 domains of JAK2, based on the dimeric structure of the fibroblast growth factor receptor kinase, indicates that a region of JH2 interacts with the activation loop of the kinase domain. Subsequent mutagenesis experiments have more directly shown that the region of amino acids 619-670 is required for JH2 induced inhibition of JH1 domain kinase activity. Herein lies the V617F mutation that has been characterized in the bcr-abl negative MPD. Based on the predicted crystal structure, it appears that this mutation prevents the auto-inhibition of JAK2, resulting in a constitutively active signaling molecule, which clearly provides a molecular basis for the observations in patients with bcr-abl negative MPDs.

Blood cells of patients with chronic myeloproliferative disorders display constitutive activation of STAT 3, upregulation of BclXL, and increased Akt activity. The autoinhibitory domains of 85 other kinases were screened in these patients, and no mutations were found, further implicating the role of JAK2. The majority of individuals with the V617F mutation carry one copy of the gene, although approximately one third were reported as homozygous in each of the studies, the result of a mitotic cross-over and clonal expansion.

The contribution of this mutation to the myeloproliferative state is virtually certain, based on several reported findings. First, in patients with mixed clonal and polyclonal progenitors, only erythroid progenitors carrying the mutation were able to grow in the absence of exogenous EPO. Second, a siRNA reagent that

reduces JAK2 expression by 90% greatly reduced EEC formation, a hallmark of these disorders. Third, expression of the mutant kinase in human kidney (293T) cells lead to its autophosphorylation (activation), and when expressed in a factor dependent cell line, BaF3/EPO, expression of the mutant, but not the wild type JAK2 kinase leads to EPO hypersensitivity and growth factor independent cell survival. Fourth, the occurrence of individuals homozygous for the mutation further suggests that the cellular cross-over events that lead to loss of heterozygousity result in a proliferative advantage, allowing their replacement of the clone of cells carrying a single mutant JAK2 locus. Fifth, expression of V617F JAK2, but not the wild type kinase, along with the EPO-R in a cell line lead to EPO-independent STAT5 activation. Finally, and most compelling, introduction of V617F JAK2 into murine marrow cells, but not the wild type kinase, leads to substantial erythrocytosis (Hct ~60%) following their transplantation into lethally irradiated recipients.

There is a recognized need in the art for peptide vaccines and vaccine therapies against myelogenous disorders. Thus, the prior art is deficient in the lack of synthetic analogue peptides of mutant V617F JAK2 enzyme that could generate an immune response that not only recognizes the immunizing epitopes, but that also cross reacts with the original mutant V617F JAK2 peptides. Specifically, the prior art is deficient in synthetic peptide analogs of mutant V617F JAK2 with both improved HLA binding and improved ability to elicit a greater immunogenic response against myeloproliferative disorders. The present invention fulfills this longstanding need and desire in the art.

SUMMARY OF THE INVENTION

The present invention is directed to a synthetic peptide. The synthetic peptide comprises a sequence of amino acids containing at least a segment that is an analogue of a mutant V617F JAK2 peptide that specifically binds to HLA class I or HLA class II molecules on a cell characteristic of a myeloproliferative disorder or disease in a subject. In a related invention the synthetic peptide further may comprise an immunogenic carrier linked thereto.

The present invention is directed to a related synthetic peptide that binds HLA A2 molecules. The synthetic peptide has an analogues segment with an

amino acid sequence of VLWYGVCFC (SEQ ID NO: 12), VLNYGVCFV (SEQ ID NO: 13), VLWYGVCFV (SEQ ID NO: 14), LNWGVCFCG (SEQ ID NO: 15), LMYGVCFCG (SEQ ID NO: 16), LLYGVCFCG (SEQ ID NO: 17), LNYGVCFCV (SEQ ID NO: 18), LNWGVCFCV (SEQ ID NO: 19), LMYGVCFCV (SEQ ID NO: 20), LLYGVCFCV (SEQ ID NO: 21), LMWGVCFCV (SEQ ID NO: 22), LLWGVCFCV (SEQ ID NO: 23), FMGDENILV (SEQ ID NO: 24), or FLGDENILV (SEQ ID NO: 25).

The present invention is directed to another related synthetic peptide that binds HLA A3 molecules. The synthetic peptide has an analogues segment with an amino acid sequence of VLYYGVCFC (SEQ ID NO: 26), VLFYGVCFC (SEQ ID NO: 27), VLNYGVCFK (SEQ ID NO: 28), VLWYGVCFK (SEQ ID NO: 29), VLFYGVCFK (SEQ ID NO: 30), LNMGVCFCG (SEQ ID NO: 31), LNLGVCFCG (SEQ ID NO: 32), LNYGVCFCK (SEQ ID NO: 33), LLYGVCFCK (SEQ ID NO: 34), LMYGVCFCK (SEQ ID NO: 35), LNMGVCFCGD (SEQ ID NO: 36), LNLGVCFCGD (SEQ ID NO: 37), LNYGVCFCGK (SEQ ID NO: 38), LLYGVCFCGK (SEQ ID NO: 39), LMYGVCFCGK (SEQ ID NO: 40), VCFCCGDENK (SEQ ID NO: 41), VMFCGDENI (SEQ ID NO: 42), VLFCGDENI (SEQ ID NO: 43), VLFCGDENK (SEQ ID NO: 44) or VMFCGDENK (SEQ ID NO: 45). The present invention is directed to yet another related synthetic peptide that binds HLA DRB molecules. The synthetic peptide has an analogues segment with an amino acid sequence of GVCFCGDENILVQEF (SEQ ID NO: 46).

The present invention also is directed to a pharmaceutical composition comprising a therapeutically effective amount of the synthetic peptides described herein or a DNA encoding the synthetic peptide and a suitable carrier. The present invention is directed further to an immunogenic composition comprising an immunogenically effective amount of the synthetic peptide described herein and a pharmaceutically acceptable carrier, adjuvant or diluent or a combination thereof.

The present invention is directed further yet to a method of inducing formation and proliferation of human cytotoxic T cells that produce a heteroclitic immune response against cells characteristic of a myeloproliferative disorder or disease. The method comprises contacting human immune cells with one or more of the synthetic peptides described herein to activate the immune cells. The formation and proliferation of the human cytotoxic T cells reactive against the activated cells

presenting at least the analogue segment of said synthetic peptide is thereby induced such that the proliferating T cells cross react with cells characteristic of a myeloproliferative disorder or disease presenting a mutant V617F peptide from which the analogue segment is derived. The human cytotoxic T cells thereby are capable of producing a heteroclitic immune response against the cells characteristic of a myeloproliferative disorder or disease.

The present invention is directed further still to a method of inducing a heteroclitic immune response in a subject. The method comprises administering to the subject an effective amount of the immunogenic composition described herein and activating human immune cells with the immunogenic composition. The formation and proliferation of human cytotoxic T cells are induced against the activated cells presenting at least the analogue segment of the synthetic peptide comprising the immunogenic composition. The human cytotoxic T cells will cross-react with a cell characteristic of a chronic myeloproliferative disorder or disease presenting a mutant V617F JAK2 peptide from which the analogue segment is derived, thereby inducing the heteroclitic immune response. In a related invention the method may further comprise isolating the cytotoxic T-cells from the subject and donating the isolated cytotoxic T-cells to another subject having a myeloproliferative disorder or disease.

Other and further aspects, features, benefits, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features, advantages and objects of the invention, as well as others which will become clear, are attained and can be understood in detail, more particular descriptions of the invention are briefly summarized. Details of the above may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted; however, that the appended drawings illustrate

preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figures 1A-1D show T2 stabilization assays using peptides predicted to bind to HLA A0201 and HLA A0301 molecules. The peptides are derived from mutant V617F JAK2 peptides of SEQ ID NO: 3 (**Figure 1A**) and SEQ ID NO: 7 (**Figure 1B**) for HLA A0301 molecules and peptides of SEQ ID NO: 3 (**Figure 1C**) and SEQ ID NO: 5 (**Figure 1D**) for HLA A0301 molecules. Fluorescence index represents the median fluorescence on the peptide tested divided by no peptide as a function of peptide concentration.

Figures 2A-2E depict a gamma interferon ELISPOT assay using CD3+ T cells from normal HLA A0201 (**Figures 2A-2B**) and HLA A0301 (**Figures 2C-2D**) donors. CD3+ T cells were stimulated three times with JAK2 heteroclitic peptides and challenged with CD14+ cells pulsed with the stimulating heteroclitic peptide (black), CD14+ cells pulsed with the corresponding native JAK2 peptide (white), CE14+ cells pulsed with the corresponding V617F mutant peptide (hatched), CD14+ cells pulsed with an irrelevant A0201 peptide (grey). Unchallenged cells are represented by white bars. In Figure 2A CD3+ cells pulsed with J2.5 did not grow and were therefore not tested. *= <0.006 (Figure 2A), <0.002 (Figure 2B) and <0.004 (Figures 2C-2E)to all the controls (no peptide, native and negative).

Figure 3 demonstrates the effects of J2.2 peptide on SKLY (A0201+JAD2 V617F-) target cells. *= < 0.01 to all the controls (no peptide, native and negative), **= < 0.0001 to all the controls (no peptide, native and negative).

Figure 4 depicts a gamma interferon ELISPOT assay using CD3+ T cells from an HLA DRB1*701/11XX donor. CD3+ T cells were stimulated three times with JAK2 mutant and WT class II peptides and challenged with CD14+ cells pulsed with JAK2DR WT peptide (white), the JAK2DR V617F mutant peptide (hatched), CD14+ cells pulsed with an irrelevant class II peptide (grey). Unchallenged cells are represented by white bars and HEL (HLADRB1*701/1303) V617F+ cell line is represented by black bars. *= <0.05 (JAK2DR V617F compared to no peptide control) and **=<0.07 (JAK2DR V617F compared to JAK2DR WT).

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment of the present invention, there is provided a synthetic peptide, comprising a sequence of amino acids containing at least a segment that is an analogue of a mutant V617F JAK2 peptide that specifically binds to HLA class I or HLA class II molecules on a cell characteristic of a myeloproliferative disorder or disease in a subject. Further to this embodiment the method may comprise an immunogenic carrier linked thereto. In this further embodiment the immunogenic carrier may be a protein, a peptide or an antigen-presenting cell. Examples of a protein or of a peptide may be keyhole limpet hemocyanin, an albumin or a polyamino acid. An example of an antigen-presenting cell is a dendritic cell.

In both these embodiments a total number of amino acids in the analogue segment is about 70% to about 130% of a total number of amino acids in the mutant V617F JAK2 peptide. In one aspect the analogue segment may have about 8 to about 12 amino acids and may bind to HLA A2 or to HLA A3 molecules. An example of an HLA A2 molecule is HLA A0201 and an example of an HLA A3 molecule is HLA A0301. Also in both embodiments the myeloproliferative disorder or disease may be polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (IMF), chronic myelogenous leukemia, chronic myelomonocytic leukemia (CMML), chronic neutrophilic leukemia (CNL), hypereosinophilic syndrome (HES), systemic mastocytosis (SM), or myelodysplastic syndrome (MDS). Examples of the amino acid sequence of these analogue segments binding to HLA A2 molecules are VLWYGVCFC (SEQ ID NO: 12), VLNYGVCFV (SEQ ID NO: 13), VLWYGVCFV (SEQ ID NO: 14), LNWGVCFCG (SEQ ID NO: 15), LMYGVCFCG (SEQ ID NO: 16), LLYGVCFCG (SEQ ID NO: 17), LNYGVCFCV (SEQ ID NO: 18), LNWGVCFCV (SEQ ID NO: 19), LMYGVCFCV (SEQ ID NO: 20), LLYGVCFCV (SEQ ID NO: 21), LMWGVCFCV (SEQ ID NO: 22), LLWGVCFCV (SEQ ID NO: 23), FMGDENILV (SEQ ID NO: 24), or FLGDENILV (SEQ ID NO: 25).

Examples of the amino acid sequence of the analogue segments binding to HLA A3 molecules are VLYYGVCFC (SEQ ID NO: 26), VLFYGVCFC (SEQ ID NO: 27), VLNYGVCFK (SEQ ID NO: 28), VLWYGVCFK (SEQ ID NO: 29), VLFYGVCFK (SEQ ID NO: 30), LNMGVCFCG (SEQ ID NO: 31),

LNLGVCFCG (SEQ ID NO: 32), LNYGVCFCK (SEQ ID NO: 33), LLYGVCFCK (SEQ ID NO: 34), LMYGVCFCK (SEQ ID NO: 35), LNMGVCFCGD (SEQ ID NO: 36), LNLGVCFCGD (SEQ ID NO: 37), LNYGVCFCGK (SEQ ID NO: 38), LLYGVCFCGK (SEQ ID NO: 39), LMYGVCFCGK (SEQ ID NO: 40), VCFCCGDENK (SEQ ID NO: 41), VMFCGDENI (SEQ ID NO: 42), VLFCGDENI (SEQ ID NO: 43), VLFCGDENK (SEQ ID NO: 44) or VMFCGDENK (SEQ ID NO: 45). In another aspect of both embodiments the analogue segment may have about 14 to about 25 amino acids and may bind to HLA DRB molecules. An example of an HLA DRB molecule is HLA DRB 1501. The amino acid sequence of these analogue segments binding to HLA DRB molecules may be G V C F C G D E N I L V Q E F (SEQ ID NO: 46).

In a related embodiment there is provided a synthetic peptide with an amino acid sequence VLWYGVCFC (SEQ ID NO: 12), VLNYGVCFV (SEQ ID NO: 13), VLWYGVCFV (SEQ ID NO: 14), LNWGVCFCG (SEQ ID NO: 15), LMYGVCFCG (SEQ ID NO: 16), LLYGVCFCG (SEQ ID NO: 17), LNYGVCFCV (SEQ ID NO: 18), LNWGVCFCV (SEQ ID NO: 19), LMYGVCFCV (SEQ ID NO: 20), LLYGVCFCV (SEQ ID NO: 21), LMWGVCFCV (SEQ ID NO: 22), LLWGVCFCV (SEQ ID NO: 23), FMGDENILV (SEQ ID NO: 24), or FLGDENILV (SEQ ID NO: 25) or combinations thereof.

In another related embodiment there is provided a synthetic peptide with an amino acid sequence VLYYGVCFC (SEQ ID NO: 26), VLFYGVCFC (SEQ ID NO: 27), VLNYGVCFK (SEQ ID NO: 28), VLWYGVCFK (SEQ ID NO: 29), VLFYGVCFK (SEQ ID NO: 30), LNMGVCFCG (SEQ ID NO: 31), LNLGVCFCG (SEQ ID NO: 32), LNYGVCFCK (SEQ ID NO: 33), LLYGVCFCK (SEQ ID NO: 34), LMYGVCFCK (SEQ ID NO: 35), LNMGVCFCGD (SEQ ID NO: 36), LNLGVCFCGD (SEQ ID NO: 37), LNYGVCFCGK (SEQ ID NO: 38), LLYGVCFCGK (SEQ ID NO: 39), LMYGVCFCGK (SEQ ID NO: 40), VCFCCGDENK (SEQ ID NO: 41), VMFCGDENI (SEQ ID NO: 42), VLFCGDENI (SEQ ID NO: 43), VLFCGDENK (SEQ ID NO: 44) or VMFCGDENK (SEQ ID NO: 45) or combinations thereof.

In yet another related embodiment there is provided a synthetic peptide with an amino acid sequence GVCFCGDENILVQEF (SEQ ID NO: 46). In still

another related embodiment there is provided a pharmaceutical composition comprising a therapeutically effective amount of the synthetic peptide described *supra* or a DNA encoding the synthetic peptide; and a pharmaceutically acceptable carrier. In aspects of this embodiment where the pharmaceutical composition comprises a DNA encoding the synthetic peptide, the DNA may be inserted into a vector or into an antigen-presenting cell. An example of an antigen presenting cell is a dendritic cell.

In still another related embodiment of the present invention, there is provided an immunogenic composition comprising an immunogenically effective amount of the synthetic peptides described *supra* and a pharmaceutically acceptable carrier, adjuvant or diluent or a combination thereof. The carrier may be a protein, a peptide or an antigen-presenting cell linked to the synthetic peptide. Examples of a protein or peptide carrier are keyhole limpet hemocyanin, an albumin or a polyamino acid. An example of an antigen-presenting cell is a dendritic cell. The synthetic peptides and analogue segments comprising the synthetic peptides are as described *supra*. In this embodiment, the analogue segments may be derived from the mutant V617F JAK2 peptide described *supra*.

In another embodiment of the present invention, there is provided a method of inducing formation and proliferation of human cytotoxic T cells that produce a heteroclitic immune response against cells characteristic of a myeloproliferative disorder or disease, comprising contacting human immune cells with one or more of the synthetic peptides of described *supra* to activate the immune cells; and inducing formation and proliferation of the human cytotoxic T cells reactive against the activated cells presenting at least the analogue segment of the synthetic peptide, where the proliferating T cells will cross react with cells characteristic of a myeloproliferative disorder or disease presenting a mutant V617F peptide from which the analogue segment is derived, said human cytotoxic T cells thereby capable of producing a heteroclitic immune response against the cells characteristic of a myeloproliferative disorder or disease. Further to this embodiment the method comprises providing a DNA encoding the synthetic peptide and expressing the DNA. In this further embodiment the DNA may be inserted into a vector or into an antigen presenting cell.

In one aspect of both embodiments the human immune cells may be contacted *in vivo* in a subject having a myeloproliferative disorder or disease. In another aspect the human immune cells may be contacted *in vivo* in a donor where the method further comprises obtaining the cytotoxic T cells from the donor; and infusing the cytotoxic T cells into the subject having a chronic myeloproliferative disorder or disease. In yet another aspect the human immune cells are contacted *ex vivo* where the method further comprises obtaining human immune cells from a donor prior to the contacting step; and infusing the activated immune cells into a subject having a myeloproliferative disorder or disease prior to the inducing step. In still another aspect the human immune cells are contacted *ex vivo* where the method further comprises obtaining human immune cells from a donor prior to the contacting step, where, after the contacting step, formation and proliferation of the cytotoxic T-cells occurs *ex vivo*; and infusing the cytotoxic T-cells into a subject having a myeloproliferative disorder or disease.

In all aspects of these embodiments the human immune cells may be peripheral blood mononuclear cells, bone marrow cells, dendritic cells, or macrophages. Also, the cytotoxic T cells may be CD8+ or CD4+ or a combination thereof. Furthermore, the myeloproliferative disorder or disease may be polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (IMF), chronic myelogenous leukemia, chronic myelomonocytic leukemia (CMML), chronic neutrophilic leukemia (CNL), hypereosinophilic syndrome (HES), systemic mastocytosis (SM), or myelodysplastic syndrome (MDS).

In one example, the cytotoxic T cells may CD8+ T cells formed against one or more of the synthetic peptide analogue segments with an amino acid sequence of VLWYGVCFC (SEQ ID NO: 12), VLNYGVCFV (SEQ ID NO: 13), VLWYGVCFV (SEQ ID NO: 14), LNWGVCFCG (SEQ ID NO: 15), LMYGVCFCG (SEQ ID NO: 16), LLYGVCFCG (SEQ ID NO: 17), LNYGVCFCV (SEQ ID NO: 18), LNWGVCFCV (SEQ ID NO: 19), LMYGVCFCV (SEQ ID NO: 20), LLYGVCFCV (SEQ ID NO: 21), LMWGVCFCV (SEQ ID NO: 22), LLWGVCFCV (SEQ ID NO: 23), VLYYGVCFC (SEQ ID NO: 26), VLFYGVCFC (SEQ ID NO: 27), VLNYGVCFK (SEQ ID NO: 28), VLWYGVCFK (SEQ ID NO: 29), VLFYGVCFK (SEQ ID NO: 30), LNMGVCFCG (SEQ ID NO: 31), LNLGVCFCG (SEQ ID NO: 32), LNYGVCFCK (SEQ ID NO: 33), LLYGVCFCK

(SEQ ID NO: 34), LMYGVCFCK (SEQ ID NO: 35), LNMGVCFCGD (SEQ ID NO: 36), LNLGVCFCGD (SEQ ID NO: 37), LNYGVCFCGK (SEQ ID NO: 38), LLYGVCFCGK (SEQ ID NO: 39), LMYGVCFCGK (SEQ ID NO: 40), VCFCCGDENK (SEQ ID NO: 41), VMFCGDENI (SEQ ID NO: 42), VLFCGDENI (SEQ ID NO: 43), VLFCGDENK (SEQ ID NO: 44) or VMFCGDENK (SEQ ID NO: 45). In another example the cytotoxic T cells may be CD4+ T cells formed against the synthetic peptide analogue segments with an amino acid sequence of GVCFCGDENILVQEF (SEQ ID NO: 46).

In yet another embodiment of the present invention there is provided a method of treating a myelogenous disorder or disease in a subject, comprising administering the pharmaceutical composition described *supra* to the subject; and inducing a heteroclitic response by cytotoxic T-cells that recognize at least the analogue segment of the synthetic peptide against cells characteristic of a myelogenous disorder or disease presenting a mutant V617F JAK2 peptide from which the analogue segment is derived such that the cytotoxic T-cells recognize or kill the characteristic cells thereby treating the myelogenous disorder or disease.

In this embodiment the cytotoxic T cells may CD8+ or CD4+ or a combination thereof. These CD8+ and/or CD4+ cytotoxic T cells may recognize the synthetic peptide analogue segments with the amino acid sequences described *supra*. Also, the myeloproliferative disorder or disease is as described *supra*.

In yet another embodiment of the present invention there is provided a method of inducing a heteroclitic immune response in a subject, comprising administering to the subject an effective amount of the immunogenic composition described *supra*; activating human immune cells with the immunogenic composition; and inducing formation and proliferation of human cytotoxic T cells against the activated cells presenting at least the analogue segment of the synthetic peptide comprising the immunogenic composition, where the human cytotoxic T cells will cross-react with a cell characteristic of a myeloproliferative disorder or disease presenting a mutant V617F JAK2 peptide from which the analogue segment is derived, thereby inducing the heteroclitic immune response. Further to this embodiment the method may comprise isolating the cytotoxic T-cells from the subject and donating the isolated cytotoxic T-cells to another subject having a myeloproliferative disorder or disease. In both embodiments the cytotoxic T cells

may CD8+ or CD4+ or a combination thereof. These CD8+ and/or CD4+ cytotoxic T cells form against the synthetic peptide analogue segments with the amino acid sequences described *supra*. Also, the myeloproliferative disorder or disease is as described *supra*.

As used herein, the term, "a" or "an" refers to one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" refers to one or more than one. As used herein "another" or "other" refers to at least a second or more of the same or different claim element or components thereof. As used herein the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

As used herein, the term "heteroclitic response" refers to cross-reaction of cytotoxic T cells activated with an analogue peptide segment from mutant V617F JAK2 protein with mutant V617 JAK2 protein presented on antigen presenting cells without cross-reaction with native JAK2 protein by these cytotoxic T cells. As used herein, the term "contacting" in terms of activating target immune cells to elicit a subsequent immune response refers to any suitable delivery method of bringing an immunogenic agent into contact with the target cells. *In vitro* or *ex vivo* this is achieved by exposing the target cells to the agent in a suitable medium. For *in vivo* applications, any known method of administration is suitable as described herein. As used herein, the term "neoplastic disease" refers to a disease or disorder associated with or caused by a mass of tissue or cells or neoplasm characterized by, *inter alia*, abnormal cell proliferation. The abnormal cell proliferation results in growth of these tissues or cells that exceeds and is uncoordinated with that of the normal tissues or cells and persists in the same excessive manner after the stimuli which evoked the change ceases or is removed. Neoplastic tissues or cells show a lack of structural organization and coordination relative to normal tissues or cells which usually results in a mass of tissues or cells which can be either benign or malignant. As would be apparent to one of ordinary skill in the art, a neoplastic disease may be benign or malignant.

As used herein, the terms "treating" or "treatment" includes prophylactic treatment as well as alleviation of ongoing or intermittent symptoms

occurring in a neoplastic disease or disorder, such as, preferably, a myeloproliferative or myelodysplastic disease or disorder. As used herein, the terms "effective amount" or "therapeutically effective amount" are interchangeable and refer to an amount that results in an improvement or remediation of the symptoms of the disease or condition. Those of skill in the art understand that the effective amount may improve the patient's or subject's condition, but may not be a complete cure of the disease and/or condition. As used herein, the term "subject" refers to any target of an immunotherapeutic treatment, preferably a mammal, more preferably a human.

Provided herein are synthetic immunogenic peptides with an amino acid sequence containing at least a highly homologous analogue segment of a V617F JAK2 peptide that demonstrates improved binding over the mutant peptide to human class I, i.e., HLA-A2 and HLA-A3, and class II HLA, i.e., HLA-DRB, molecules. These synthetic peptides or analogue segments can stimulate T-cells to cross-react with the mutant V617F JAK2 peptide, thus eliciting a heteroclitic immune response that will recognize or kill cells presenting the mutant V617F JAK2 peptide. T cells stimulated or activated by the synthetic immunogenic peptides do not recognize the native, nonmutated JAK2 kinase.

Furthermore, beneficially the V617F mutation is not found in T lymphocytes, leaving them fully functional. It is contemplated that at least the analogue segments comprising the synthetic peptides will bind with more affinity to the HLA class I and class II molecules that are instrumental in presenting the analogue segments to the T-cells than the mutant V617F peptide itself. Such cells are characteristic of a neoplastic disease or neoplastic disorder, for example, a myeloproliferative or myelodysplastic disease or disorder, including chronic, acute and atypical leukemias.

Peptides were identified based on their predictive binding affinity to HLA molecules using a peptide library based algorithm which ranks the binding of peptides on a predicted half-time coefficient from common HLA class molecules. Any predictive algorithms available publicly or commercially may be utilized, for example, the online software BIMAS and/or SYFPEITHI, as more fully described in Example 1, may be used to predict binding scores to HLA A0201 and HLA A0301 molecules or to HLA DRB molecules. Such synthetic analog peptides are generated

by introducing amino acid point mutations into certain HLA anchor motifs, thereby enhancing the peptide/HLA binding affinities, while not interfering with the peptide/T-cell receptor (TCR) binding.

The synthetic peptide analogue segments are designed by making one or two amino acid substitutions in anchor or auxiliary residues. Although the mutated JAK2 peptides, from which the synthetic peptides binding to HLA class I molecules are designed, particularly described herein are nonamers or decamers encompassing the anchor or auxiliary residues, analogues may be designed having about 70% to about 130% of the amino acids in the mutated JAK2 peptides. In the instant invention preferably the synthetic peptide analogues binding to class I HLA A2 or HLA A3 molecules may have about 8-12 amino acids.

The present invention also provides a pharmaceutical composition of a therapeutic amount of the synthetic peptides or analogue segments or a genetic sequence or DNA encoding the same and a pharmaceutical carrier, as is known in the art. The pharmaceutical composition may be formulated with the pharmaceutical carrier for administration by any of the many techniques known to those of skill in the art. For example, the pharmaceutical composition may be administered parenterally, intravenously, subcutaneously, intradermally, intramucosally, topically, orally, or by inhalation.

Therefore, it is contemplated that the synthetic peptides or analogue segments or pharmaceutical compositions thereof may be used in the preparation of an immunogenic composition suitable to effect immunization of a subject. The immunogenic composition may comprise a carrier or a suitable adjuvant to boost immune response or a combination thereof, as are known in the art. The immunogenic composition further may comprise a diluent standard in the art as described herein. The immunogenic composition may comprise a vaccine.

A carrier may comprise one or more proteins or peptides. Examples of carriers are well known and may be, although not limited to keyhole limpet hemocyanin, an albumin, such as human serum albumin or a polyamino acid. Additionally, a carrier may comprise a live antigen-presenting cell, such as a dendritic cell, which presents the synthetic peptides described herein. A suitable adjuvant may be incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, alum, QS21, BCG BCG, montinide, and GMCSF. These compositions

further may comprise a physiologically acceptable diluent, e.g., water, phosphate buffered saline or saline.

Additionally, a genetic sequence encoding a synthetic peptide or an analogue segment thereof may be delivered as naked DNA to an individual via appropriate methods known in the art. Alternatively, the genetic sequence may be introduced or inserted into a suitable vector, such as for example, but not limited to, attenuated viral or bacterial vectors, as are standard in the art. Furthermore, the naked DNA or vectors comprising the genetic sequence or DNA may be transduced into an antigen-presenting cell, e.g., a dendritic cell. The genetic sequence, DNA, vector or transduced antigen-presenting cell may be introduced into an individual in need of the treatment or into a healthy donor whereupon the DNA encoding the genetic sequence expresses the synthetic peptide to elicit a cytotoxic T-cell response. Donor T-cells may then be infused into a patient in need thereof.

The pharmaceutical or immunogenic compositions may be used to treat a neoplastic disease or a neoplastic disorder such as a myeloproliferative disease or disorder, including chronic and atypical myeloproliferative disorders and acute, chronic and atypical leukemias. For example, it is contemplated that the pharmaceutical or immunogenic compositions have a therapeutic or immunotherapeutic effect against polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (IMF), or chronic or acute leukemias, such as, but not limited to, chronic myelogenous leukemia and atypical leukemias, chronic myelomonocytic leukemia (CMML) and chronic neutrophilic leukemia (CNL), and against other atypical myeloproliferative disorders, such as, hypereosinophilic syndrome (HES), systemic mastocytosis (SM) and myelodysplastic syndrome (MDS). Administration of the synthetic peptides or analogue segments comprising the pharmaceutical compositions induces a heteroclitic response against the mutant V617F JAK2 peptide expressed on the neoplastic cells characteristic of the disease or disorder thereby effecting a therapeutic result.

It is contemplated that the synthetic peptides or synthetic analogue segments thereof or genetic sequences encoding the same or the pharmaceutical or the immunogenic compositions thereof can induce human cytotoxic T cells to produce a heteroclitic immune response against cells presenting the V167F JAK2 mutant peptide, but not the native, non-mutated JAK2 peptide. Contacting human

immune cells with at least the analogue segment that is or comprises the synthetic peptides activates the immune cells to induce formation and proliferation of human cytotoxic T cells that will recognize or react against a cell presenting the synthetic peptide. Such cytotoxic T cells cross react with human cells presenting the mutant JAK2 peptide from which the analogue segment is derived thereby producing a heteroclitic response.

Alternatively, a synthetic peptide or synthetic analog segment or genetic sequences encoding the same or the pharmaceutical or the immunogenic compositions thereof that binds to HLA DRB molecules may be used to induce a CD4+ T cell response. Such a CD4+ T cell response could be a heteroclitic response or could be adjunctive to or enhance the heteroclitic CD8+ T cell response generated against cells presenting the V167F JAK2 mutant peptide. It is contemplated that synthetic peptide analogues binding to class II HLA DRB1 molecules may have about 14-25 amino acids.

Thus, the synthetic peptides or analogue segments thereof described herein may be used to activate T-cells *ex vivo* or *in vivo*. *In vivo*, the synthetic peptides or analogue segments thereof or DNA encoding the same may be administered to a patient or to a healthy donor to induce cytotoxic T-cells. If administered to a healthy donor these cytotoxic T-cells are obtained from the donor and infused into an individual in need of them, such as an individual with an active cancer, in remission from a cancer or at risk for developing a cancer.

Ex vivo, the T cells are obtained from a patient or from a healthy donor and are incubated in the presence of antigen presenting cells and a synthetic peptide or at least an analogue segment thereof to activate the T-cells. The activated T-cells subsequently are infused back into the patient where they will recognize and/or destroy cells presenting the V617F mutant JAK2 peptide. Alternatively, human immune cells may be incubated with the synthetic peptide or at least an analogue segment thereof whereupon the activated immune cells are infused back into the patient to induce T-cell production against both the activated cells and cell presenting the native peptide. Examples of immune cells may be peripheral blood mononuclear monocytic cells, bone marrow cells, dendritic cells, or macrophages.

It is contemplated further that administration of the synthetic peptide or at least an analogue segment thereof or pharmaceutical or immunological

compositions thereof induces an immune response in a subject, preferably, although not limited to, a CD4+/HLA DRB class II response and/or a CD8+ HLA A class I immune response. As such, the synthetic peptides or at least an analogue segment thereof may be used in a method of immunizing a subject against a neoplastic disease or disorder presenting HLA molecules, e.g., a myeloproliferative or myelodysplastic disease or disorder, including, but not limited to, chronic and acute leukemias, such as chronic myelogenous leukemia. As used herein, immunizing or immunization of a subject encompasses full and partial immunization whereby the subject becomes fully immune to the condition or partially immune to the condition. The subject may be a mammal, preferably a human.

The subject may have a neoplastic disease or disorder, preferably a myeloproliferative or myelodysplastic disease or disorder, including chronic and acute leukemias, more preferably a chronic myeloproliferative disease or disorder, which may be active or in remission, prior to immunization. Alternatively, if at risk for developing the neoplastic disease or disorder, e.g., chronic myelogenous leukemia, the subject may be immunized prior to such development. One of ordinary skill in the art would be able to assess the risk factors, such as environmental risk factors or personal risk factors, such as family history, genetic makeup or behavior, to make a determination of risk in the subject.

The pharmaceutical compositions and immunogenic compositions may be administered one or more times to achieve a therapeutic or an immunogenic effect. It is well within the skill of an artisan to determine dosage or whether a suitable dosage comprises a single administered dose or multiple administered doses. As is well known in the art, a specific dose level for any particular patient depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, and the severity, e.g., progression or remission, of the particular disease undergoing therapy. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

Peptide Sequence Analysis: Amino acid sequences and predicted binding of putative CD8+ epitopes to HLA-A0201 or HLA-A0301 molecules were identified using the predictive algorithms of the Bioinformatics & Molecular Analysis Section (BIMAS; National Institutes of Health, Washington, DC) which ranks 9-mer or 10-mer peptides on a predicted half-time dissociation coefficient from HLA class I molecules (1). Amino acid sequences and predicted binding of putative CTL epitope peptides epitopes were identified using the predictive software SYFPEITHI which scores each amino acid based on a database of published motifs, with 36 being the maximal score for HLA-A0201 (2) and the RANKPEP database (Dana Farber Cancer Institute, Cambridge, MA) which predicts peptide binding to Class I and Class II MHC molecules (3). These databases are readily available online.

Synthetic peptides: Peptides were selected from the larger population of all JAK2 peptides spanning 10 amino acids upstream and 10 amino acids downstream of the V617F mutation (amino acids 607-627) by scanning for peptides found in myeloproliferative disorders with a potential binding capacity for HLA-A0201 (about 40% of the Caucasian population) and HLA-A0301 (about 20% of the Caucasian population) using the computer algorithms. Analog sequences with positive scores were selected first, and the corresponding native sequences were included for comparison. Each of the peptides used in this study were purchased and synthesized by Genemed Synthesis Inc, (San Francisco, CA) using fluorenylmethoxycarbonyl chemistry and solid phase synthesis and purified by high-pressure liquid chromatography. The quality of the peptides was assessed by high-performance liquid chromatography analysis, and the expected molecular weight was observed using matrix-assisted laser desorption mass spectrometry. Peptides were sterile and 70-90% pure. The peptides were dissolved in DMSO and diluted in phosphate-buffered saline (PBS; pH 7.4) or saline at 5mg/ml and were stored at -80°C. Irrelevant control peptides used for *in vitro* experiments were: A0201 class I peptides HIV pol (ILKEPVHGV; SEQ ID NO: 48), CML F (YLKALQRPY; SEQ ID NO: 48) or HBV (FLPSDYFPSV; SEQ ID NO: 50); A0301 class I peptides HIV or CML; and RAS (TEYKLVVVGAPGVGKSALTIQ; SEQ ID NO: 51) or CML b2a2 (VHSIPLTINKEEALQRPVASDFE; SEQ ID NO: 52) for Class II.

Cell lines: Cell lines were cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS), penicillin, streptomycin, 2mM glutamine and 2-mercaptoethanol at 37°C/5% CO_2. T2, an A0201+, TAP-deficient human cell line that cannot present endogenous peptides to the surface of the cells was obtained from the ATCC. Two erythroleukemia cell lines, HEL, an A0301+ V617F+ (4) was obtained from the ATCC and AP217, an A0301+, V617F- was obtained from Dr. Nimer at MSKCC. All cells were HLA typed by the Department of Cellular Immunology at MSKCC.

293T cells were transfected with letivirus plasmids including: the packaging construct, envelope-coding plasmid and the transfer vector containing GFP and encoding either the WT JAK2, the mutated V617F JAK2 or an empty vector. 293T cells were incubated overnight and viral supernatants were collected and concentrated to 1-5 10^3 titer/ml. 5 x 10^5 SKLY-16, an A0201+ human B cell lymphoma obtained from the ATCC were then infected with 1 ml of lentivirus along with 8 ug/ml of Polybrene. Cells were grown, and infection was repeated at 6-12 hour intervals. Target cells were then sorted by FACs for GFP expression.

T2 stabilization assay: 1 x 10^6 cells T2 cells/ml were incubated overnight at 37°C in FCS-free RPMI medium supplemented with 10 μg/ml human β2-microglobulin (β2m; Sigma, St Louis, MO) in the absence (negative control) or presence of either control HBV peptide or JAK2 test peptides at various final concentrations ranging from 100-1 μg/ml. Cells were then incubated with 5 ug/ml of brefeldin-A (Sigma) for 2 hours at 37°C. T2 cells were then washed twice with FACS buffer, and incubated for 30 min at 4°C with a saturating amount of FITC labeled anti-human HLA-A2 antibody, (BB7.2. BD PharmingenTM). Fluorescence was assayed on a CytomicsTM FC 500, (Beckman Coulter) and analyzed using the FlowJo program. Each concentration of peptide (1, 10, 50 and 100 mg/ml) was assayed in triplicate wells, and each binding assay was repeated three times. The mean intensity of fluorescence (MIF) for each peptide is calculated by dividing the mean fluorescence of T2 + test peptide by T2 without peptide. Peptides were considered to show positive in vitro binding if the MIF (averaged over 10,000 events) was at least 50% of the binding of the control HBV peptide (SEQ ID NO: 50). All experiments are normalized relative to control HBV peptide known to have high affinity for HLA-

A201, and expressed as a Fluorescence Index. The half time of complexes is an estimate of the time required for a 50% reduction of the MIF value at time = 0.

In vitro immunization and human T cell cultures: After informed consent on MSKCC IRB protocols, peripheral blood mononuclear cells (PBMCs) from HLA-typed healthy donors were obtained by Ficoll-density centrifugation. Monocyte derived dendritic cells (DCs) were generated from PBMCs using a plastic adherence technique. The adherent cells were cultured for 7 days in RPMI 1640/1-5% autologous plasma, 500 U/mL recombinant human IL-4 (R&D Systems), and 1000 U/mL recombinant human GMCSF (Immunex, Seattle).

On days 2 and 4 of incubation, fresh medium with IL-4 and GM-CSF was added. For CD4+ stimulation assays only, 10 ug/ml peptide was added to immature DCs on day 5. On day 6, maturation cytokine cocktail was added (IL4, GMCSF, 400 IU/ml IL1-beta (R&D Systems), 1000 IU/ml IL-6 (R&D Systems), 10 ng/ml TNF-alpha (R&D Systems) and 1 ug/ml PGE2 (Sigma)). On day 8, CD3+ T lymphocytes were isolated from the same donors using negative selection by depletion with an anti-CD11b, anti-CD56 and CD19 MoAb (Miltenyi, CA), and stimulated at a 10:1 effector:target (E:T) ratio with the monocyte-derived DCs. The mature DCs expressed dendritic cell-associated antigens, such as CD80, CD83, CD86, and HLA class I and class II on their cell surfaces (data not shown).

CD3+ cells were stimulated for 7 days in the presence of RPMI 1640/5% autologous plasma, 10 ug/ml JAK2 synthetic peptides, 1 ug/ml b2 microglobulin (Sigma, St. Louis) and 10 ng/ml IL-15 (R&D Systems). On day 8, CD3+ cells were re-stimulated with either a 5:1 E:T ratio of freshly isolated CD14+ cells, or a 50:1 E:T ratio of monocyte derived DCs. In some cases, cells were re-stimulated after another 6-7 days, in the same manner. After the second or third stimulation, gamma-IFN secretion of these cells was examined by ELISPOT. In some cases, T cells were again stimulated for 7 days with autologous CD14+ cells and used to test cytotoxicity in a standard ^{51}Cr-release assay.

Gamma interferon ELISPOT: HA-Multiscreen plates (Millipore, Burlington, MA) were coated with 100 µl of mouse-anti-human IFN-gamma antibody (10 µg/ml; clone 1-D1K, Mabtech, Sweden) in PBS, incubated overnight at 4°C, washed with

PBS to remove unbound antibody and blocked with RPMI/10% autologous plasma. Peptide stimulated CD3+ T cells were challenged with either autologous CD14+ (5:1 E:T ratio) or T2 cells (10:1 E:T ratio) in the presence of 10μg/ml ß2-microglobulin (Sigma, St. Louis) and 50 ug/ml of various test peptides. Negative control wells contained APCs with or without T cells or T cells with peptides alone. Positive control wells contained T cells + APC+ 10 ug/ml PHA (Sigma). All conditions were done in triplicate. After incubation for 20 h at 37°C, plates were extensively washed with PBS/0.05% Tween and 100 μl/well biotinylated detection antibody against human IFN-g (2 μg/ml; clone 7-B6-1, Mabtech, Sweden) was added. Plates were incubated for an additional 2 h at 37°C and spot development was performed as described (Herr W). Spot numbers were automatically determined with the use of a computer-assisted video image analyzer with KS ELISPOT 4.0 software (Carl Zeiss Vision, Germany).

Chromium 51 cytotoxicity assay: The presence of specific CTLs was measured in a standard 4 hour ^{51}Chromium release assay as described. Briefly, target cells are pulsed with 10 ug/ml of synthetic peptides overnight at 37°C, after which they are labeled with 300μCi of $Na_2{}^{51}CrO_4$ (NEN Life Science Products, Inc., Boston, MA). After extensive washing, target cells are incubated with T cells at an E:T ratio ranging from 100:1 to 10:1. All conditions were performed in triplicate. Plates were incubated for 4 hours at 37°C in 5% CO_2. Supernatant fluids were harvested and radioactivity was measured in a gamma counter. Percent specific lysis was determined from the following formula: 100 x [(experimental release minus spontaneous release)/(maximum release minus spontaneous release)]. Maximum release was determined by lysis of radiolabeled targets in 2.5% Triton X-100.

EXAMPLE 2

Identification of peptides with high predictive binding to HLA A0201 and A0301

Preferred synthetic peptides or analogue segments effective to induce a cytotoxic CD8+ T cell response are shown in Tables 1 and 2. Table 1 and Table 2 show the amino acid sequences and binding predictions of native and mutated JAK2 peptides and synthetic analogues of mutated JAK2 peptide to human class I molecules HLA A2 and HLA A3, particularly A0201 and A0301. The amino acid

residues in bold represent modifications from the native and mutated JAK2 sequence, particularly the sequence from about position 609 to about position 625 of SEQ ID NO: 1 (603 K L S H K H L V L N Y G V C V C G D E N I L V Q E F V).

TABLE 1

Class 1 JAK2 AO201 peptides

JAK2	Sequence	Predicted Half Life	Score	RANK	MHC (%Ref)	SEQ ID NO
J2A	VLNYGVCVC	7.9	17	37/32.46	18	2
J2A1	VLNYGVC**F**C	107	17	32/28.07	18	3
J2.1	VL**W**YGVCFC	887	17	27/23.68	63	12
J2.2	VLNYGVCF**V**	1496	27	59/51.75	90	13
J2.3	VL**W**YGVCF**V**	12414	27	54/47.37	121	14
J2B	LNYGVCVCG	0.0	ND			4
J2B1	LNYGVC**F**CG	0.02	ND			5
J2B2	LN**W**GVCFCG	0.01	ND			15
J2B3	L**M**YGVCFCG	1.7	ND			16
J2B4	L**L**YGVCFCG	2.35	ND			17
J2B5	LNYGVCFC**V**	14	ND			18
J2B6	LN**W**GVCFC**V**	37.2	ND			19
J2B7	L**M**YGVCFC**V**	1585	ND			20
J2B8	L**L**YGVCFC**V**	2194	ND			21
J2B9	L**M**W**W**GVCFC**V**	4110	ND			22
J2B10	L**L**W**W**GVCFC**V**	5691	ND			23
J2C	VCGDENILV	3.2	12	31/27.19	46	6
J2C1	**F**CGDENILV	8.6	12	28/24.56	41	7
J2.4	FMGDENILV	947	20	55/48.25	74	24
J2.5	F**L**GDENILV	1312	22	65/57.02	87	25

Two A0201 specific peptides containing the V617F mutation were identified based on the presence of preferred primary anchor motifs at position two and nine (Table 1). J2A1 contained a leucine (L) at position two and had a relatively high predicted binding affinity. J2C1 contained a valine (V) at position nine, but had a much lower predicted binding affinity. Their native counterparts without the V617F mutation, J2A and J2C had very low binding scores.

Each mutant peptide was then modified at one amino acid to improve the predicted binding. Two different modifications of the J2A1 peptide were made. The introduction of an aromatic amino acid residue in the first or third position greatly increases the binding affinity. Therefore, in J2.1, the third amino acid was changed to a tryptophan (W). Peptide J2.2 contained a substitution of the valine (V) anchor residue at position nine. These changes improved the predicted half-life of the peptides up to ten times that of the original V617F mutated sequence, and close to a hundred fold greater than the JAK2 native sequence in the BIMAS algorithm. When both amino acid changes were incorporated, a synergistic effect was seen, and peptide J2.3 has a predicted affinity over a thousand times greater than the J2A native sequence. The SYFPEITHI predictions were not at sensitive to these changes, although the addition of the V anchor motif did effect the predicted binding affinity.

The second group of A0201 specific peptides J2.4 and J2.5 derived from the J2C1 mutated peptides, and were designed by introducing a hydrophobic methionine (M) or leucine (L) in the second position. Since these peptides already contained the valine (V) anchor motif in position nine, a single amino acid change at position two increased the predicted affinity to HLA A0201 close to one hundred fold. These peptides were examined despite the low affinity of the parental V617F sequence, since it is possible that the reactive T cells may not have been deleted from the peripheral TCR repertoire.

TABLE 2

Class 1 JAK2 AO301 peptides

JAK2	Sequence	Predicted Half Life	Score	RANK	MHC (%Ref)	SEQ ID NO
J3A	VLNYGVCVC	0.6	16	ND	ND	2
J3A1	VLNYGVC**F**C	0.6	12			3
J3A2	VL**Y/F**YGVCFC	3	ND			26/27
J3A3	VLNYGVCF**K**	30	ND			28
J3.1	VL**W**YGVCF**K**	300	26			29
J3.2	VL**F**YGVCF**K**	300	22			30
J3B	LNYGVCVCG	0	11			4
J3B1	LNYGVC**F**CG	0	7			5
J3B2	LN**M/L**GVCFCG	0.7	ND			31/32
J3B3	LNYGVCFC**K**	1.3	ND			33
J3.3	L**L**YGVCFC**K**	675	27			34
J3.4	L**M**YGVCFC**K**	675	17			35
J3C	LNYGVCVCGD	0.0	6			8
J3C1	LNYGVC**F**CGD	0	6			9
J3C2	LN**M/L**GVCFCGD	0.45	ND			36/37
J3C3	LNYGVCFCG**K**	0.9	ND			38
J3.5	L**L**YGVCFCG**K**	450	26			39
J3.6	L**M**YGVCFCG**K**	450	16			40
J3D	VCVCGDENI	0	ND			10
J3D1	VC**F**CGDENI	0.3	ND			11
J3D2	VCFCCGDEN**K**	1	ND			41
J3D3	V**M/L**FCGDENI	3	ND			42/43
J3.7	V**L**FCGDEN**K**	100	ND			44
J3.8	V**M**FCGDEN**K**	100	ND			45

Three A0301 peptides from the JAK2 V617F mutated sequence J3A1, J3B1 and J3C1 were identified based on the position of preferred primary or secondary anchor residues. Despite the presence of either a hydrophobic leucine (L) at position two, or a tyrosine (Y) at position 3, neither these, nor their native counterparts J3A, J3B or J3C had any predicted affinity to the HLA A0301 molecule. Only once two mutations were introduced, a lysine at position nine or ten and an aromatic at position three or a hydrophobic residue at position two, was there an increase in the predicted affinity of the analog peptides (Table 2). These mutations reflect the necessity of having both anchor residues present for increased binding predictions to HLA A0301.

The synthetic peptides J3.1 and J3.2 and the synthetic peptides J3.3 and J3.4 were generated from mutant V617F peptides J3A1 and J3B1, respectively, that demonstrate very low or no binding to HLA A0301 (Table 2). The synthetic peptides bound well to HLA A0301. J3.1 and J3.2 have a tyrosine or phenylalanine (F) substitution in position 3 and a lysine substitution in position 9. J3.3 and J3.4 have a leucine or methionine substitution in position 3 and a lysine substitution in position 9.

EXAMPLE 3

Identification of peptides with high predictive binding to HLA DRB1

The induction and maintenance of the CD8+ CTL response requires CD4+ T cell help. CD4+ T cells recognize peptides bound to the HLA class II molecule on APC. Once activated, CD4+ cells enhance immunity by licensing dendritic cells thereby sustaining the activation and survival of the cytotoxic T cells. {Rammensee, 1999 #124} (Table 1). The V617F amino acid change in the long CD4+ epitope appears to have an impact on the binding to some of the HLA-DRB1 alleles, but not to others.

A CD4+ epitope spanning the JAK2 V617F region was identified using a predictive algorithm based on binding motifs through the SYFPEITHI Database as described in Example 1. Long peptides comprising about 14 to about 25 amino acids from mutated V617F JAK2 that have a high predicted affinity to HLA class II molecules, e.g., HLA DRB, were identified. Table 3 lists the binding predictions to various HLA DRB molecules for the JAK2DR WT (SEQ ID NO: 46)

V617F mutant (SEQ ID NO: 47) peptide sequences from about position 614 to about position 628 of SEQ ID NO: 1. The amino acid residues in bold represent modifications from the native and mutated JAK2 sequence.

TABLE 3

Class II DRB1 peptides

Name	Sequence	Score 101	301	401	701	1101	1501
JAK2DR V617F	GVC**F**CGDENILVQEF (SEQ ID NO: 46)	17	28	26	22	7	24
JAK2DR WT	GVCVCGDENILVQEF (SEQ ID NO: 47)	19	26	28	24	11	22
Freq. Caucasian Population		18.5	17.7	23.6	26.2	17.0	19.9

EXAMPLE 4

Peptide binding and stabilization of HLA-A0201

The immunogenicity of MHC class I-restricted peptides requires the capacity to bind and stabilize MHC class I molecules on the live cell surface. The computer prediction models used to identify the potential CD8+ epitopes have only 60-80% predictive accuracy, so direct measurement of the strength of the interaction between the peptides and the HLA-A0201 molecules using a conventional binding and stabilization assay that uses the antigen-transporting deficient (TAP2 negative) HLA-A0201 human T2 cells was used. T2 cells lack TAP function and consequently are defective in properly loading class I molecules with antigenic peptides generated in the cytosol. The association of exogenously added peptides with thermolabile, empty HLA-A0201 molecules stabilizes them and results in an increase in the level of surface HLA-A0201 recognizable by specific anti- HLA A0201 mAb such as BB7.2.

The two sets of A0201 peptides (Figs. 1A-1B) and two sets of A0301 peptides (Figs. 1C-1D) were analyzed, and their in vitro binding appears to correlate with the predictive binding. However, MHC binding affinity and/or stability of MHC-peptide complexes for class I epitopes does not necessarily correlate with antigenicity. Furthermore, the lack of available TAP2 deficient cells that express A0301 to test the in vitro binding of the A0301 specific peptides requires in vitro stimulation assays to confirm that the peptides described above are immunogenic and can stimulate CD8+ T cells that recognize the mutant V617F JAK2 kinase and not the native JAK2 kinase.

EXAMPLE 5

Induction of CD8 immune response against heteroclitic peptides

CD3+ cells from seven healthy HLA-A0201 donors were stimulated in vitro two or three times with autologous DCs in the presence of the A0201 JAK2 heteroclitic peptides or the V617F mutated parent peptide. Consistently, J2.2 was able to generate T cells that secreted IFN-gamma when challenged with A0201 target cells pulsed with either J2.2, the immunizing peptide or J2A1, the V617F mutant parent (Figs. 2A-2B). These T cells did not recognize target cells pulsed with the J2A, the native JAK2 sequence. J2.1 and J2.3 were able to generate T cells that recognized target cells pulsed with the immunizing peptide, but they never induced a heteroclitic response that could recognize target cells pulsed with peptide J2A1 or J2A. The V617F mutated peptides J2A1 and J2C1, and peptides J2.4 and J2.5 were not immunogenic, and were unable to generate a significant T cell response.

CD3+ cells from four healthy HLA-A0301 donors were stimulated in the same manner in the presence of the A0301 JAK2 heteroclitic peptides. The results varied between donors, with some T cells responding to target cells pulsed with the immunizing peptide, and some T cells not responding at all (Figs. 2A-2E). However, in two out of the four donors tested, a heteroclitic response was generated by analog peptides J3.3 and J3.6 (Figs. 2D-2E). In both cases, T cells stimulated with these analog peptides secreted IFN-gamma in the presence of autologous APCs pulsed with either the immunizing peptide or the V617F mutant peptide, but not in the presence of the native A0301 JAK2 peptide.

EXAMPLE 6

J2.2 peptide kills SKLY target cells

CD3+ T cells from an A0201 donor were stimulated five times with JAK2 heteroclitic peptide J2.2 as described in Example 1. Stimulated T cells were used as effector cells in a 51 Cr release assay. T cells were incubated at a 60:1 E:T ratio with radiolabeled SKLY (A0201+ JAK2 V617F-) target cells pulsed with various peptides (Fig. 3)

EXAMPLE 7

In vitro stimulation of CD4+ T cells from normal HLA DRB1 donors

CD3+ cells from healthy donors were stimulated with either the native JAK2DR peptide, or the V617F JAK2DR peptide as described in Example 1. Donors expressing HLA DRB1*701/1202; HLA DRB1*407/1302 and HLA DRB1*701/1303 induced a peptide specific response to the WT and mutant JAK2DR peptide. Only in the setting of HLA DRB1*701/11XX was it possible to discriminate between the WT and mutant JAK2DR peptide (Figure 4). It is contemplated that the long peptide is a better vaccine candidate since the DR expression varies greatly in the population.

The following references are cited herein.

1. Parker et al. J Immunol, 152:163-175, 1994.
2. Rammensee et al. Immunogenetics, 50:213-219, 1999.
3. Reche et al. Human Immunology, 63:701-709 2002.
4. Quentmeier et al. Leukaemia, 20:471-476, 2006.

Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. Further, these patents and publications are incorporated by reference herein to the same extent as if each individual publication was incorporated specifically and individually by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary, and are

not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A synthetic peptide, comprising:

a sequence of amino acids containing at least a segment that is an analogue of a mutant V617F JAK2 peptide that specifically binds to HLA class I or HLA class II molecules on a cell characteristic of a myeloproliferative disorder or disease in a subject.

2. The synthetic peptide of claim 1, further comprising:

an immunogenic carrier linked thereto.

3. The synthetic peptide of claim 2, wherein said immunogenic carrier is a protein, a peptide or an antigen-presenting cell.

4. The synthetic peptide of claim 3, wherein said protein or peptide is keyhole limpet hemocyanin, an albumin or a polyamino acid.

5. The synthetic peptide of claim 3, wherein said antigen presenting cell is a dendritic cell.

6. The synthetic peptide of claim 1, wherein a total number of amino acids in said analogue segment is about 70% to about 130% of a total number of amino acids in said mutant V617F JAK2 peptide.

7. The synthetic peptide of claim 1, wherein said analogue segment is derived from a mutant V617F JAK2 peptide with the sequence shown in SEQ ID NO: 1.

8. The synthetic peptide of claim 7, wherein said analogue segment has about 8 to about 12 amino acids.

9. The synthetic peptide of claim 8, wherein said analogue segment binds to HLA A2 molecules.

10. The synthetic peptide of claim 9, wherein said HLA A2 molecule is HLA 0201.

11. The synthetic peptide of claim 10, wherein an amino acid sequence of said analogue segment is VLWYGVCFC (SEQ ID NO: 12), VLNYGVCFV (SEQ ID NO: 13), VLWYGVCFV (SEQ ID NO: 14), LNWGVCFCG (SEQ ID NO: 15), LMYGVCFCG (SEQ ID NO: 16), LLYGVCFCG (SEQ ID NO: 17), LNYGVCFCV (SEQ ID NO: 18), LNWGVCFCV (SEQ ID NO: 19), LMYGVCFCV (SEQ ID NO: 20), LLYGVCFCV (SEQ ID NO: 21), LMWGVCFCV (SEQ ID NO: 22), LLWGVCFCV (SEQ ID NO: 23), FMGDENILV (SEQ ID NO: 24), or FLGDENILV (SEQ ID NO: 25).

12. The synthetic peptide of claim 8, wherein said analogue segment binds to HLA A3 molecules.

13. The synthetic peptide of claim 12, wherein said HLA A3 molecule is HLA 0301.

14. The synthetic peptide of claim 13, wherein an amino acid sequence of said analogue segment is VLYYGVCFC (SEQ ID NO: 26), VLFYGVCFC (SEQ ID NO: 27), VLNYGVCFK (SEQ ID NO: 28), VLWYGVCFK (SEQ ID NO: 29), VLFYGVCFK (SEQ ID NO: 30), LNMGVCFCG (SEQ ID NO: 31), LNLGVCFCG (SEQ ID NO: 32), LNYGVCFCK (SEQ ID NO: 33), LLYGVCFCK (SEQ ID NO: 34), LMYGVCFCK (SEQ ID NO: 35), LNMGVCFCGD (SEQ ID NO: 36), LNLGVCFCGD (SEQ ID NO: 37), LNYGVCFCGK (SEQ ID NO: 38), LLYGVCFCGK (SEQ ID NO: 39), LMYGVCFCGK (SEQ ID NO: 40), VCFCCGDENK (SEQ ID NO: 41), VMFCGDENI (SEQ ID NO: 42), VLFCGDENI (SEQ ID NO: 43), VLFCGDENK (SEQ ID NO: 44) or VMFCGDENK (SEQ ID NO: 45).

15. The synthetic peptide of claim 7, wherein said analogue segment has about 14 to about 25 amino acids.

16. The synthetic peptide of claim 15, wherein said analogue segment binds to HLA DrB molecules.

17. The synthetic peptide of claim 16, wherein said HLA DRB molecule is HLA DRB 1501.

18. The synthetic peptide of claim 17, wherein an amino acid sequence of said analogue segment is GVCFCGDENILVQEF (SEQ ID NO: 46).

19. The synthetic peptide of claim 1, wherein said myeloproliferative disorder or disease is polycythemia vera (PV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (IMF), chronic myelogenous leukemia, chronic myelomonocytic leukemia (CMML), chronic neutrophilic leukemia (CNL), hypereosinophilic syndrome (HES), systemic mastocytosis (SM), or myelodysplastic syndrome (MDS).

20. A method of inducing formation and proliferation of human cytotoxic T cells that produce a heteroclitic immune response against cells characteristic of a myeloproliferative disorder or disease, comprising:

contacting human immune cells with one or more of the synthetic peptides of claim 1 to activate the immune cells; and

inducing formation and proliferation of said human cytotoxic T cells reactive against the activated cells presenting at least the analogue segment of the synthetic peptide, wherein said proliferating T cells will cross react with cells characteristic of a myeloproliferative disorder or disease presenting a mutant V617F peptide from which said analogue segment is derived, said human cytotoxic T cells thereby capable of producing a heteroclitic immune response against the cells characteristic of a myeloproliferative disorder or disease.

21. The method of claim 20, wherein said human immune cells are contacted in vivo in a subject having a myeloproliferative disorder or disease.

22. The method of claim 20, wherein said human immune cells are contacted in vivo in a donor, the method further comprising:

obtaining said cytotoxic T cells from the donor; and

infusing said cytotoxic T cells into the subject having a myeloproliferative disorder or disease.

23. The method of claim 20, wherein said human immune cells are contacted ex vivo, the method further comprising:

obtaining human immune cells from a donor prior to said contacting step; and

infusing said activated immune cells into a subject having a myeloproliferative disorder or disease prior to said inducing step.

24. The method of claim 20, wherein said human immune cells are contacted ex vivo, the method further comprising:

obtaining human immune cells from a donor prior to said contacting step, wherein after said contacting step formation and proliferation of the cytotoxic T-cells occurs ex vivo; and

infusing said cytotoxic T-cells into a subject having a myeloproliferative disorder or disease.

25. The method of claim 20, further comprising:

providing a DNA encoding the synthetic peptide; and

expressing said DNA.

26. The method of claim 25, wherein said DNA is inserted into a vector or into an antigen presenting cell.

27. The method of claim 20, wherein the human immune cells are peripheral blood mononuclear cells, bone marrow cells, dendritic cells, or macrophages.

28. The method of claim 20, wherein the cytotoxic T cells are CD8+ or CD4+ or a combination thereof.

29. The method of claim 28, wherein the cytotoxic T cells are CD8+ T cells formed against one or more of the synthetic peptide analogue segments with an amino acid sequence of VLWYGVCFC (SEQ ID NO: 12), VLNYGVCFV (SEQ ID NO: 13), VLWYGVCFV (SEQ ID NO: 14), LNWGVCFCG (SEQ ID NO: 15), LMYGVCFCG (SEQ ID NO: 16), LLYGVCFCG (SEQ ID NO: 17), LNYGVCFCV (SEQ ID NO: 18), LNWGVCFCV (SEQ ID NO: 19), LMYGVCFCV (SEQ ID NO: 20), LLYGVCFCV (SEQ ID NO: 21), LMWGVCFCV (SEQ ID NO: 22), LLWGVCFCV (SEQ ID NO: 23), VLYYGVCFC (SEQ ID NO: 26), VLFYGVCFC (SEQ ID NO: 27), VLNYGVCFK (SEQ ID NO: 28), VLWYGVCFK (SEQ ID NO: 29), VLFYGVCFK (SEQ ID NO: 30), LNMGVCFCG (SEQ ID NO: 31), LNLGVCFCG (SEQ ID NO: 32), LNYGVCFCK (SEQ ID NO: 33), LLYGVCFCK (SEQ ID NO: 34), LMYGVCFCK (SEQ ID NO: 35), LNMGVCFCGD (SEQ ID NO: 36), LNLGVCFCGD (SEQ ID NO: 37), LNYGVCFCGK (SEQ ID NO: 38), LLYGVCFCGK (SEQ ID NO: 39), LMYGVCFCGK (SEQ ID NO: 40), VCFCCGDENK (SEQ ID NO: 41), VMFCGDENI (SEQ ID NO: 42), VLFCGDENI (SEQ ID NO: 43), VLFCGDENK (SEQ ID NO: 44) or VMFCGDENK (SEQ ID NO: 45).

30. The method of claim 28, wherein the cytotoxic T cells are CD4+ T cells formed against the synthetic peptide analogue segment with an amino acid sequence of GVCFCGDENILVQEF (SEQ ID NO: 46).

31. The method of claim 20, wherein said myeloproliferative disorder or disease is polycythemia vera, essential thrombocythemia, chronic idiopathic myelofibrosis, chronic myelogenous leukemia, chronic myelomonocytic leukemia, chronic neutrophilic leukemia, hypereosinophilic syndrome, systemic mastocytosis, or myelodysplastic syndrome.

32. A pharmaceutical composition, comprising:

a therapeutically effective amount of the synthetic peptide of claim 1 or a DNA encoding said synthetic peptide; and

a pharmaceutically acceptable carrier.

33. The pharmaceutical composition of claim 32, wherein said DNA is inserted into a vector or into an antigen presenting cell.

34. A method of treating a myelogenous disorder or disease in a subject, comprising:

administering the pharmaceutical composition of claim 32 to said subject; and

inducing a heteroclitic response by cytotoxic T-cells that recognize at least the analogue segment of said synthetic peptide against cells characteristic of a myelogenous disorder or disease presenting a mutant V617F JAK2 peptide from which said analogue segment is derived, said cytotoxic T-cells recognizing or killing said characteristic cells thereby treating the myelogenous disorder or disease.

35. The method of claim 34, wherein the cytotoxic T cells are CD8+ or CD4+ or a combination thereof.

36. The method of claim 35, wherein the cytotoxic T cells are CD8+ T cells, said cytotoxic CD8+ T cells recognizing one or more of the synthetic peptide analogue segments with an amino acid sequence of VLWYGVCFC (SEQ ID NO: 12), VLNYGVCFV (SEQ ID NO: 13), VLWYGVCFV (SEQ ID NO: 14), LNWGVCFCG (SEQ ID NO: 15), LMYGVCFCG (SEQ ID NO: 16), LLYGVCFCG (SEQ ID NO: 17), LNYGVCFCV (SEQ ID NO: 18), LNWGVCFCV (SEQ ID NO: 19), LMYGVCFCV (SEQ ID NO: 20), LLYGVCFCV (SEQ ID NO: 21), LMWGVCFCV (SEQ ID NO: 22), LLWGVCFCV (SEQ ID NO: 23), VLYYGVCFC (SEQ ID NO: 26), VLFYGVCFC (SEQ ID NO: 27), VLNYGVCFK (SEQ ID NO: 28), VLWYGVCFK (SEQ ID NO: 29), VLFYGVCFK (SEQ ID NO: 30), LNMGVCFCG (SEQ ID NO: 31), LNLGVCFCG (SEQ ID NO: 32), LNYGVCFCK (SEQ ID NO: 33), LLYGVCFCK (SEQ ID NO: 34), LMYGVCFCK (SEQ ID NO: 35), LNMGVCFCGD (SEQ ID NO: 36), LNLGVCFCGD (SEQ ID NO: 37),

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37. The method of claim 35, wherein the cytotoxic T cells are CD4+ T cells, said cytotoxic CD4+ T cells recognizing the synthetic peptide analogue segment with an amino acid sequence of GVCFCGDENILVQEF (SEQ ID NO: 46).

38. The method of claim 34, wherein said myeloproliferative disorder or disease is polycythemia vera, essential thrombocythemia, chronic idiopathic myelofibrosis, or chronic myelogenous leukemia.

39. An immunogenic composition comprising an immunogenically effective amount of the synthetic peptide of claim 1 and a pharmaceutically acceptable carrier, adjuvant or diluent or a combination thereof.

40. The immunogenic composition of claim 39, wherein said immunogenic carrier is a protein, a peptide or an antigen presenting cell linked to said synthetic peptide.

41. The immunogenic composition of claim 40, wherein said protein or peptide is keyhole limpet hemocyanin, an albumin or a polyamino acid.

42. The immunogenic composition of claim 40, wherein said antigen presenting cell is a dendritic cell.

43. A method of inducing a heteroclitic immune response in a subject, comprising:

administering to said subject an effective amount of the immunogenic composition of claim 39;

activating human immune cells with said immunogenic composition; and

inducing formation and proliferation of human cytotoxic T cells against the activated cells presenting at least the analogue segment of the synthetic peptide comprising said immunogenic composition,

wherein said human cytotoxic T cells will cross-react with a cell characteristic of a myeloproliferative disorder or disease presenting a mutant V617F JAK2 peptide from which said analogue segment is derived, thereby inducing said heteroclitic immune response.

44. The method of claim 43, further comprising:

isolating said cytotoxic T-cells from the subject; and

donating said isolated cytotoxic T-cells to another subject having a myeloproliferative disorder or disease.

45. The method of claim 43, wherein the human immune cells are peripheral blood mononuclear cells, bone marrow cells, dendritic cells, or macrophages.

46. The method of claim 43, wherein the cytotoxic T cells are CD8+ or CD4+ or a combination thereof.

47. The method of claim 46, wherein the cytotoxic T cells are CD8+ T cells formed against one or more of the synthetic peptide analogue segments with an amino acid sequence of VLWYGVCFC (SEQ ID NO: 12), VLNYGVCFV (SEQ ID NO: 13), VLWYGVCFV (SEQ ID NO: 14), LNWGVCFCG (SEQ ID NO: 15), LMYGVCFCG (SEQ ID NO: 16), LLYGVCFCG (SEQ ID NO: 17), LNYGVCFCV (SEQ ID NO: 18), LNWGVCFCV (SEQ ID NO: 19), LMYGVCFCV (SEQ ID NO: 20), LLYGVCFCV (SEQ ID NO: 21), LMWGVCFCV (SEQ ID NO: 22), LLWGVCFCV (SEQ ID NO: 23), VLYYGVCFC (SEQ ID NO: 26), VLFYGVCFC (SEQ ID NO: 27), VLNYGVCFK (SEQ ID NO: 28), VLWYGVCFK (SEQ ID NO: 29), VLFYGVCFK (SEQ ID NO: 30), LNMGVCFCG (SEQ ID NO: 31), LNLGVCFCG (SEQ ID NO: 32), LNYGVCFCK (SEQ ID NO: 33), LLYGVCFCK (SEQ ID NO: 34), LMYGVCFCK (SEQ ID NO: 35), LNMGVCFCGD (SEQ ID NO: 36), LNLGVCFCGD (SEQ ID NO: 37),

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48. The method of claim 46, wherein the cytotoxic T cells are CD4+ T cells formed against the synthetic peptide analogue segment with an amino acid sequence of GVCFCGDENILVQEF (SEQ ID NO: 46).

49. The method of claim 43, wherein said myeloproliferative disorder or disease is polycythemia vera, essential thrombocythemia, chronic idiopathic myelofibrosis, chronic myelogenous leukemia, chronic myelomonocytic leukemia, chronic neutrophilic leukemia, hypereosinophilic syndrome, systemic mastocytosis, or myelodysplastic syndrome.

50. A synthetic peptide with an amino acid sequence VLWYGVCFC (SEQ ID NO: 12), VLNYGVCFV (SEQ ID NO: 13), VLWYGVCFV (SEQ ID NO: 14), LNWGVCFCG (SEQ ID NO: 15), LMYGVCFCG (SEQ ID NO: 16), LLYGVCFCG (SEQ ID NO: 17), LNYGVCFCV (SEQ ID NO: 18), LNWGVCFCV (SEQ ID NO: 19), LMYGVCFCV (SEQ ID NO: 20), LLYGVCFCV (SEQ ID NO: 21), LMWGVCFCV (SEQ ID NO: 22), LLWGVCFCV (SEQ ID NO: 23), FMGDENILV (SEQ ID NO: 24), or FLGDENILV (SEQ ID NO: 25) or combinations thereof.

51. A synthetic peptide with an amino acid sequence VLYYGVCFC (SEQ ID NO: 26), VLFYGVCFC (SEQ ID NO: 27), VLNYGVCFK (SEQ ID NO: 28), VLWYGVCFK (SEQ ID NO: 29), VLFYGVCFK (SEQ ID NO: 30), LNMGVCFCG (SEQ ID NO: 31), LNLGVCFCG (SEQ ID NO: 32), LNYGVCFCK (SEQ ID NO: 33), LLYGVCFCK (SEQ ID NO: 34), LMYGVCFCK (SEQ ID NO: 35), LNMGVCFCGD (SEQ ID NO: 36), LNLGVCFCGD (SEQ ID NO: 37), LNYGVCFCGK (SEQ ID NO: 38), LLYGVCFCGK (SEQ ID NO: 39), LMYGVCFCGK (SEQ ID NO: 40), VCFCCGDENK (SEQ ID NO: 41),

VMFCGDENI (SEQ ID NO: 42), VLFCGDENI (SEQ ID NO: 43), VLFCGDENK (SEQ ID NO: 44) or VMFCGDENK (SEQ ID NO: 45) or combinations thereof.

52. A synthetic peptide with an amino acid sequence GVCFCGDENILVQEF (SEQ ID NO: 46).

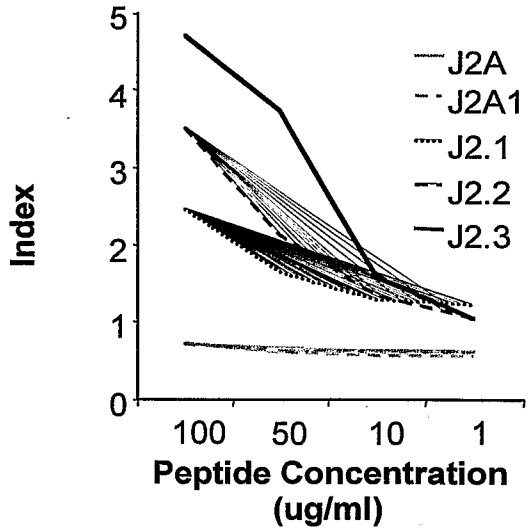
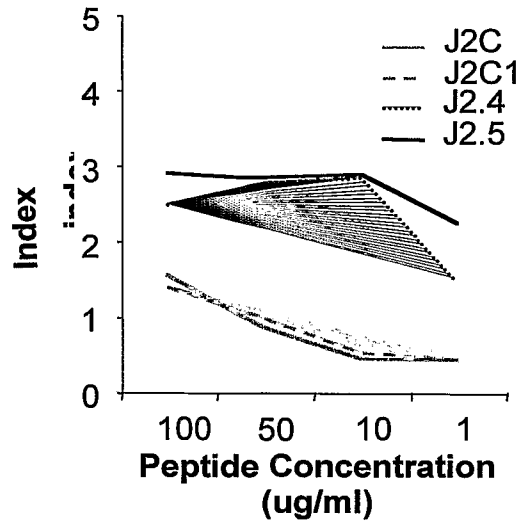


Fig. 1A



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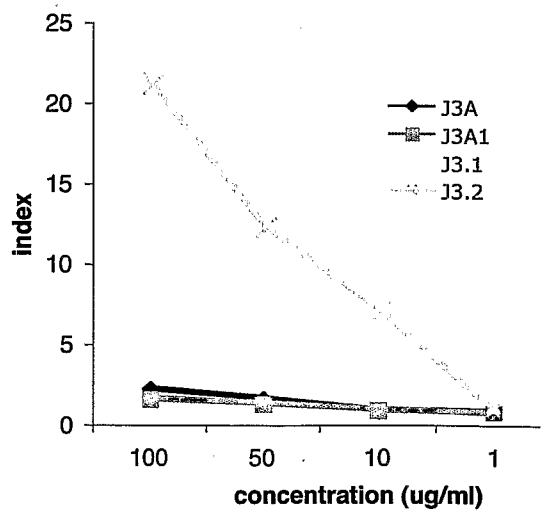

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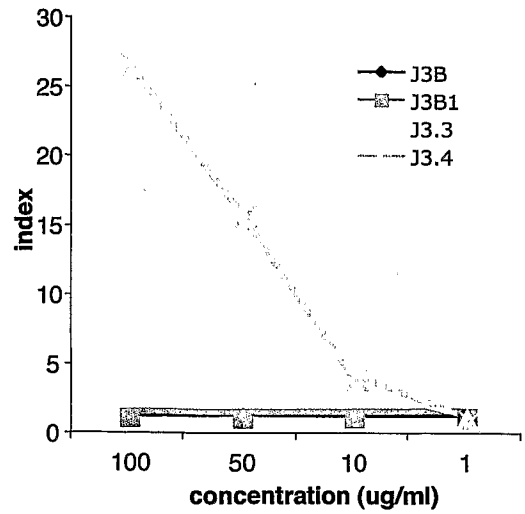

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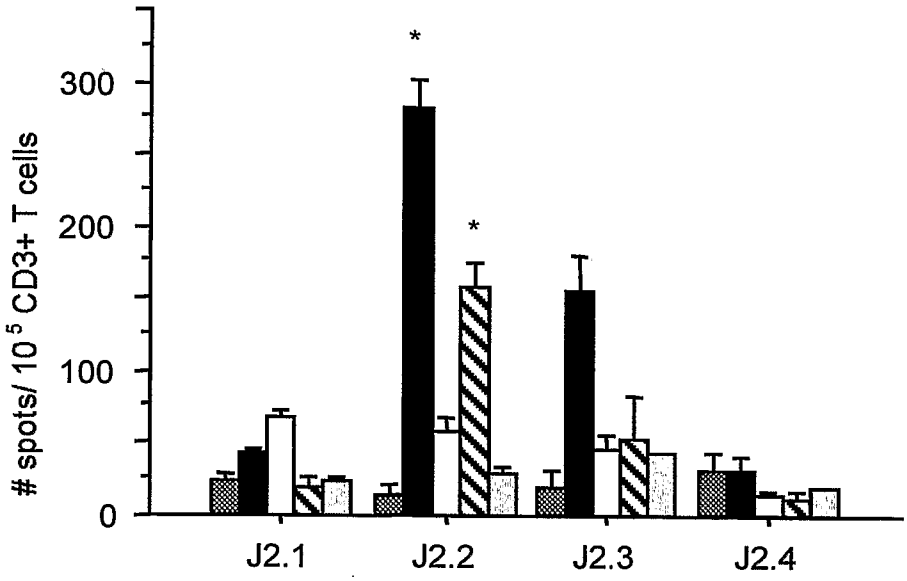
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Fig. 2A

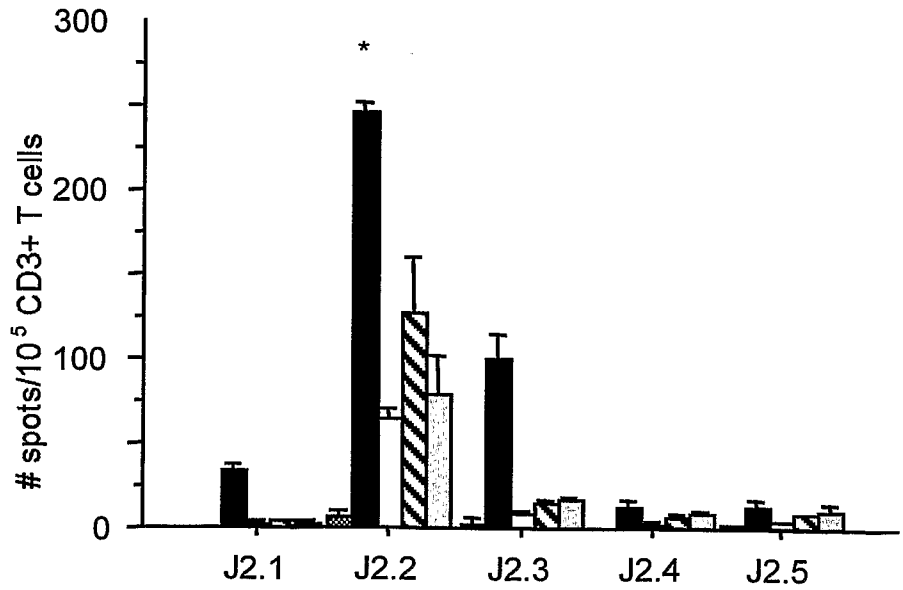
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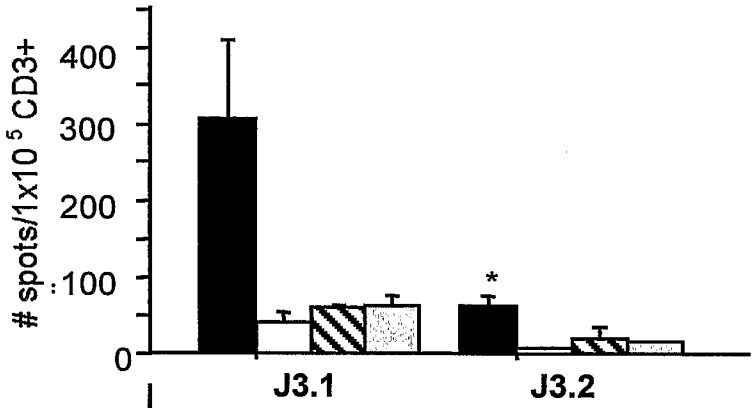
Fig. 2B



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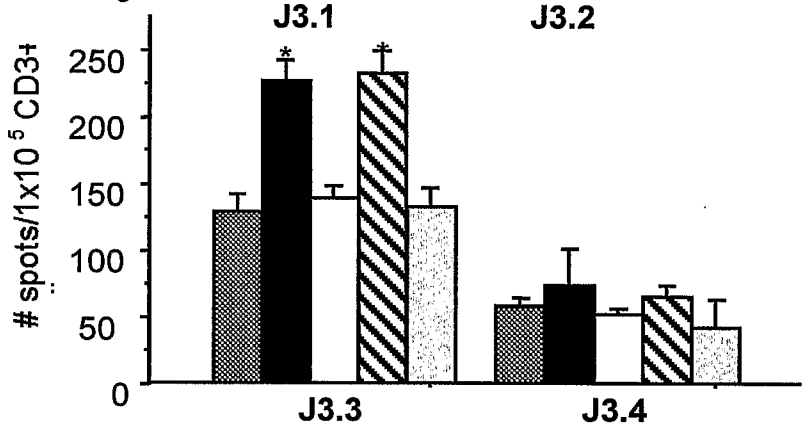

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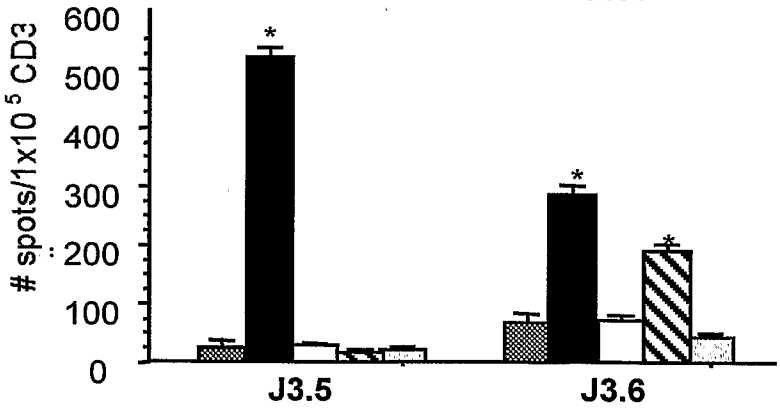

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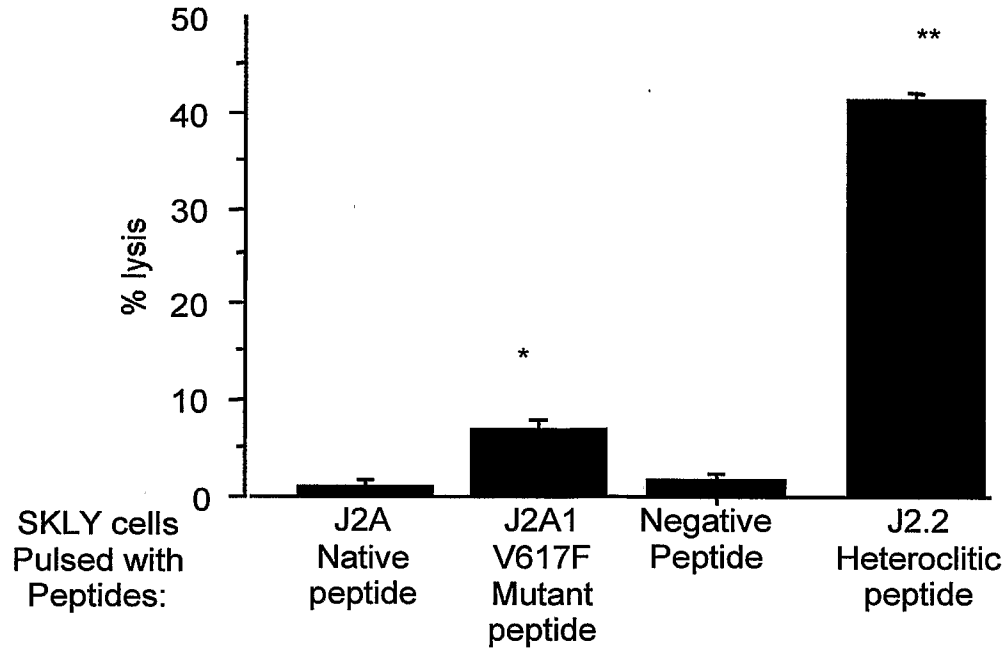
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J2.2 Heteroclitic peptide

Fig. 3

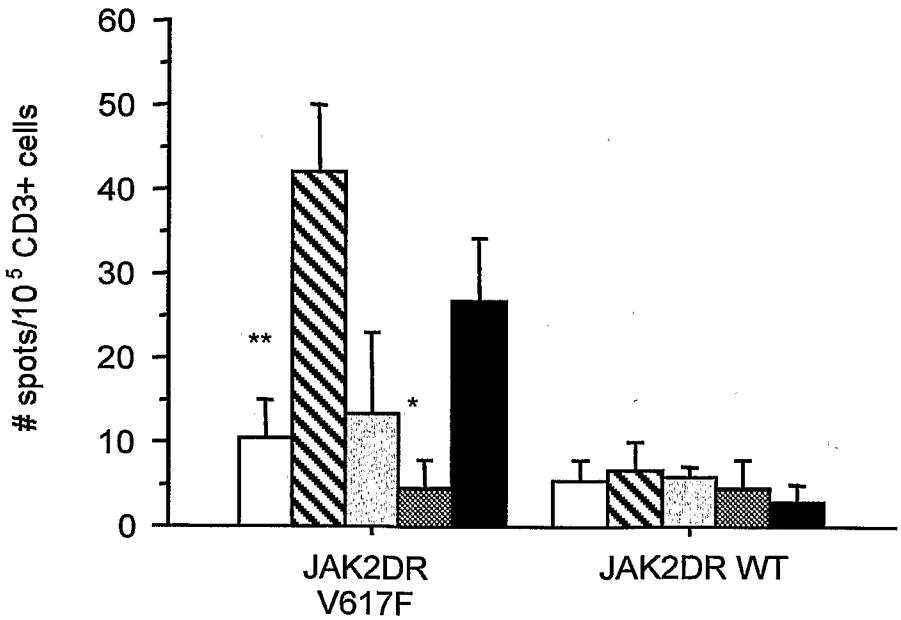
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Fig.4

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May, Rena J.

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<223> J2.2 heteroclitic JAK2 V617F peptide with C618V substitution

<400> 13
Val Leu Asn Tyr Gly Val Cys Phe Val
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<210> 14
<211> 9
<212> PRT
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<220>
<222> 610..618
<223> J2.3 heteroclitic JAK2 V617F peptide with N612W and C618V substitutions

<400> 14
Val Leu Trp Tyr Gly Val Cys Phe Val
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<210> 15
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 611..619
<223> J2B2 heteroclitic JAK2 V617F peptide with Y613W substitution

<400> 15
Leu Asn Trp Gly Val Cys Phe Cys Gly
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<210> 16
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 611..619
<223> J2B3 heteroclitic JAK2 V617F peptide with N612M substitution

<400> 16
Leu Met Tyr Gly Val Cys Phe Cys Gly
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<210> 17
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 611..619
<223> J2B4 heteroclitic JAK2 V617F peptide with N612L substitution

<400> 17
Leu Leu Tyr Gly Val Cys Phe Cys Gly
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<210> 18
<211> 9
<212> PRT
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<220>
<222> 611..619

<223> J2B5 heteroclitic JAK2 V617F peptide with G619V substitution

<400> 18
Leu Asn Tyr Gly Val Cys Phe Cys Val
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<210> 19
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 611..619
<223> J2B6 heteroclitic JAK2 V617F peptide with Y613W and G619V substitutions

<400> 19
Leu Asn Trp Gly Val Cys Phe Cys Val
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<210> 20
<211> 9
<212> PRT
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<220>
<222> 611..619
<223> J2B7 heteroclitic JAK2 V617F peptide with N612M and G619V substitutions

<400> 20
Leu Met Tyr Gly Val Cys Phe Cys Val
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<210> 21
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 611..619
<223> J2N8 heteroclitic JAK2 V617F peptide with N612L and G619V substitutions

<400> 21
Leu Leu Tyr Gly Val Cys Phe Cys Val
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<210> 22
<211> 9

<212> PRT
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<220>
<222> 611..619
<223> J2B9 heteroclitic JAK2 V617F peptide with N612M, Y613W and G619V substitutions

<400> 22
Leu Met Trp Gly Val Cys Phe Cys Val
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<210> 23
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 611..619
<223> J2B10 heteroclitic JAK2 V617F peptide with N612L, Y613W and G619V substitutions

<400> 23
Leu Leu Trp Gly Val Cys Phe Cys Val
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<210> 24
<211> 9
<212> PRT
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<220>
<222> 617..625
<223> J2.4 heteroclitic JAK2 V617F peptide with C618M substitution

<400> 24
Phe Met Gly Asp Glu Asn Ile Leu Val
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<210> 25
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 617..625
<223> J2.5 heteroclitic JAK2 V617F peptide with C618L substitution

<400> 25
Phe Leu Gly Asp Glu Asn Ile Leu Val
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<210> 26
<211> 9
<212> PRT
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<220>
<222> 610..618
<223> J3A2 heteroclitic JAK2 V617F peptide with N612Y substitution

<400> 26
Val Leu Tyr Tyr Gly Val Cys Phe Cys
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<210> 27
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 610..618
<223> J3A2 heteroclitic JAK2 V617F peptide with N612F substitution

<400> 27
Val Leu Phe Tyr Gly Val Cys Phe Cys
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<210> 28
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<220>
<222> 610..618
<223> J3A3 heteroclitic JAK2 V617F peptide with C618K substitution

<400> 28
Val Leu Asn Tyr Gly Val Cys Phe Lys
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<210> 29
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<212> PRT
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<220>
<222> 610..618
<223> J3.1 heteroclitic JAK2 V617F peptide with N612W and C618K substitutions

<400> 29
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<210> 30
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<212> PRT
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<220>
<222> 610..618
<223> J3.2 heteroclitic JAK2 V617F peptide with N612F and C618K substitutions

<400> 30
Val Leu Phe Tyr Gly Val Cys Phe Lys
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<210> 31
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<212> PRT
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<220>
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<223> J3B2 heteroclitic JAK2 V617F peptide with Y613M substitution

<400> 31
Leu Asn Met Gly Val Cys Phe Cys Gly
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<210> 32
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<212> PRT
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<220>
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<223> K3B2 heteroclitic JAK2 V617F peptide with Y613L substitution

<400> 32
Leu Asn Leu Gly Val Cys Phe Cys Gly
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<210> 33
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<220>
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<223> J3B3 heteroclitic JAK2 V617F peptide with G619K substitution

<400> 33
Leu Asn Tyr Gly Val Cys Phe Cys Lys
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<210> 34
<211> 9
<212> PRT
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<220>
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<223> J3.3 heteroclitic JAK2 V617F peptide with L612L and G619K substitutions

<400> 34
Leu Leu Tyr Gly Val Cys Phe Cys Lys
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<210> 35
<211> 9
<212> PRT
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<220>
<222> 611..619
<223> J3.4 heteroclitic JAK2 V617F peptide with L612M and G619K substitutions

<400> 35
Leu Met Tyr Gly Val Cys Phe Cys Lys
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<210> 36
<211> 10
<212> PRT
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<220>
<222> 611..620

<223> J3C2 heteroclitic JAK2 V617F peptide with Y613M substitution

<400> 36
Leu Asn Met Gly Val Cys Phe Cys Gly Asp
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<210> 37
<211> 10
<212> PRT
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<220>
<222> 611..620
<223> J3C2 heteroclitic JAK2 V617F peptide with Y613L substitution

<400> 37
Leu Asn Leu Gly Val Cys Phe Cys Gly Asp
5 10

<210> 38
<211> 10
<212> PRT
<213> artificial sequence

<220>
<222> 611..620
<223> J3C3 heteroclitic JAK2 V617F peptide with D620K substitution

<400> 38
Leu Asn Tyr Gly Val Cys Phe Cys Gly Lys
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<210> 39
<211> 10
<212> PRT
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<220>
<222> 611..620
<223> J3.5 heteroclitic JAK2 V617F peptide with N612L and D620K substitutions

<400> 39
Leu Leu Tyr Gly Val Cys Phe Cys Gly Lys
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<210> 40
<211> 10

<212> PRT
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<220>
<222> 611..620
<223> J3.6 heteroclitic JAK2 V617F peptide with N612M and D620K substitutions

<400> 40
Leu Met Tyr Gly Val Cys Phe Cys Gly Lys
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<210> 41
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 615..623
<223> J3D2 heteroclitic JAK2 V617F peptide with I623K substitution

<400> 41
Val Cys Phe Cys Gly Asp Glu Asn Lys
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<210> 42
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 615..623
<223> J3D2 heteroclitic JAK2 V617F peptide with C616M substitution

<400> 42
Val Met Phe Cys Gly Asp Glu Asn Ile
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<210> 43
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 615..623
<223> J3D2 heteroclitic JAK2 V617F peptide with C616L substitution

<400> 43
Val Leu Phe Cys Gly Asp Glu Asn Ile
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<210> 44
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 615..623
<223> J3.7 heteroclitic JAK2 V617F peptide with C616L and I623K substitutions

<400> 44
Val Leu Phe Cys Gly Asp Glu Asn Lys
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<210> 45
<211> 9
<212> PRT
<213> artificial sequence

<220>
<222> 615..623
<223> J3.8 heteroclitic JAK2 V617F peptide with C616M and I623K substitutions

<400> 45
Val Met Phe Cys Gly Asp Glu Asn Lys
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<210> 46
<211> 15
<212> PRT
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<220>
<222> 614..628
<223> JAK2DR mutant V617F peptide

<400> 46
Gly Val Cys Phe Cys Gly Asp Glu Asn Ile Leu Val Gln Glu Phe
5 10 15

<210> 47
<211> 15
<212> PRT
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<220>
<222> 614..628
<223> JAK2WT wildtype V617F peptide

<400> 47
Gly Val Cys Val Cys Gly Asp Glu Asn Ile Leu Val Gln Glu Phe
5 10 15

<210> 48
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> HIV pol HLA A0201 class I peptide

<400> 48
Ile Leu Lys Glu Pro Val His Gly Val
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<210> 49
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> CML F HLA A0201 class I peptide

<400> 49
Tyr Leu Lys Ala Leu Gln Arg Pro Tyr
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<210> 50
<211> 10
<212> PRT
<213> artificial sequence

<220>
<223> HBV pol HLA A0201 class I peptide

<400> 50
Phe Leu Pro Ser Asp Tyr Phe Pro Ser Val
5 10

<210> 51
<211> 21
<212> PRT
<213> artificial sequence

<220>
<223> RAS HLA DRB class II peptide

<400> 51
Thr Glu Tyr Lys Leu Val Val Val Gly Ala Pro Gly Val Gly Lys
5 10 15
Ser Ala Leu Thr Ile Gln
20

<210> 52
<211> 23
<212> PRT
<213> artificial sequence

<220>
<223> CML b2a2 HLA DRB class II peptide

<400> 52
Val His Ser Ile Pro Leu Thr Ile Asn Lys Glu Glu Ala Leu Gln
5 10 15
Arg Pro Val Ala Ser Asp Phe Glu
20