Another unexpected finding was that methods using both EBV lytic antigens and EBV latent antigens in stimulations did not result in a substantial reduction of the frequency of immune cells specific for EBV latent antigens in the expanded population.

The inventors further demonstrate that populations of immune cells comprising immune cells specific for EBV lytic antigens and immune cells specific for EBV latent antigens are more effective for treating EBV-associated disease than populations of immune cells comprising immune cells specific for EBV latent antigens only.

In a first ~~aspect~~ example the present disclosure provides a method for generating or expanding a population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen, comprising stimulating immune cells specific for an EBV lytic antigen by contacting peripheral blood mononuclear cells (PBMCs) with: (i) one or more peptides corresponding to all or part of one or more EBV lytic antigens; or (ii) antigen presenting cells (APCs) presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens.

In some examples the method further comprises re-stimulating the immune cells specific for an EBV lytic antigen by contacting them with APCs presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens.

Also provided is a method for generating or expanding a population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen and immune cells specific for an EBV latent antigen, comprising stimulating immune cells specific for an EBV lytic antigen and immune cells specific for an EBV latent antigen by contacting peripheral blood mononuclear cells (PBMCs) with: (i) one or more peptides corresponding to all or part of one or more EBV lytic antigens, and one or more peptides corresponding to all or part of one or more EBV latent antigens; or (ii) antigen presenting cells (APCs) presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens, and one or more peptides corresponding to all or part of an EBV latent antigen.

In ~~another~~ one aspect, the present disclosure provides a method for generating or expanding a population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen and immune cells specific for an EBV latent antigen, comprising stimulating immune cells specific for an EBV lytic antigen and immune cells specific for an EBV latent antigen by contacting peripheral blood mononuclear cells (PBMCs) with: (i) ~~one or more~~ peptides corresponding to all or part of EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2~~one or more EBV lytic antigens, and one or more peptides corresponding to all or part of one or more EBV latent antigens~~; or (ii) antigen presenting cells (APCs) presenting ~~one or more~~ peptides corresponding to all or part of EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2~~one or more EBV lytic antigens, and one or more peptides corresponding to all or part of one or more EBV latent antigens~~.

In some examples the method further comprises re-stimulating the immune cells specific for an EBV lytic antigen and the immune cells specific for an EBV latent antigen by contacting them with APCs presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens, and one or more peptides corresponding to all or part of an EBV latent antigen.

In some examples in accordance with various aspects of the present disclosure the one or more EBV lytic antigens are selected from BZLF1, BRLF1, BMLF1, BMRF1, BXLF1, BALF1, BALF2, BGLF5, BHRF1, BNLF2A, BNLF2B, BHLF1, BLLF2, BKRF4, BMRF2, BALF4, BILF1, BILF2, BNFR1, BVRF2, BALF3, BALF5 and BDLF3. In some examples in accordance with various aspects of the present disclosure the one or more EBV lytic antigens are selected from the group consisting of BZLF1, BRLF1, BMLF1, BMRF1, BXLF1, BALF1, BALF2, BGLF5, BHRF1, BNLF2A, BNLF2B, BHLF1, BLLF2, BKRF4, BMRF2, BALF4, BILF1, BILF2, BNFR1, BVRF2, BALF3, BALF5, BDLF3, and a combination thereof.

In some examples the one or more EBV lytic antigens are selected from BZLF1, BRLF1, BMLF1, BMRF1, BALF2, BNLF2A, BNLF2B, BMRF2 and BDLF3. In some examples the one or more EBV lytic antigens are selected from the group consisting of BZLF1, BRLF1, BMLF1, BMRF1, BALF2, BNLF2A, BNLF2B, BMRF2, BDLF3, and a combination thereof.

In some examples the one or more EBV latent antigens are selected from EBNA1, EBNA-LP, EBNA2, EBNA3A, EBNA3B, EBNA3C, BARF1, LMP1, LMP2A and LMP2B. In some examples the one or more EBV latent antigens are selected from the group consisting of EBNA1, EBNA-LP, EBNA2, EBNA3A, EBNA3B, EBNA3C, BARF1, LMP1, LMP2A, LMP2B, and a combination thereof.

In some examples the one or more EBV latent antigens are selected from EBNA1, LMP1, LMP2A and LMP2B. In some examples the one or more EBV latent antigens are selected from the group consisting of EBNA1, LMP1, LMP2A, LMP2B, and a combination thereof.

In some examples the PBMCs are PBMCs depleted of CD45RA-positive cells.

In one aspect, the present disclosure also provides an isolated population of immune cells obtained or obtainable by the method according to the present disclosure.

Also provided is an isolated population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen.

Also provided is an isolated population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen and immune cells specific for an EBV latent antigen.

In another aspect, the present disclosure also provides a pharmaceutical composition comprising the isolated population of immune cells according to the present disclosure.

Also provided is an isolated population of immune cells or a pharmaceutical composition according to the present disclosure, for use in a method for treating or preventing a disease or disorder.

~~In one aspect, the present disclosure provides the isolated population of immune cells according to the present disclosure, or the pharmaceutical composition according to the present disclosure, for use in the treatment or prevention of a disease or disorder.~~

Also provided is the use of an isolated population of immune cells or a pharmaceutical composition according to the present disclosure in the manufacture of a medicament for use in a method of treatment or prevention of a disease or disorder.

In another aspect, the present disclosure provides use of the isolated population of immune cells according to the present disclosure, or the pharmaceutical composition according to the present disclosure, in the manufacture of a medicament for the treatment or prevention of a disease or disorder associated with EBV infection.

Also provided is a method for treating or preventing a disease or disorder, comprising administering an isolated population of immune cells or a pharmaceutical composition according to the present disclosure to a subject.

Also provided is a method for killing a cancer cell, comprising contacting a cancer cell with an isolated population of immune cells or a pharmaceutical composition according to the present disclosure. The method may be an *in vitro* or an *in vivo* method.

In another aspect, the present disclosure provides a method for killing a cancer cell, wherein the cancer cell is infected with EBV, comprising contacting the cancer cell with the isolated population of immune cells according to the present disclosure, or the pharmaceutical composition according to the present disclosure, wherein the method is an *in vitro* method.

Also provided is the use of an isolated population of immune cells or a pharmaceutical composition according to the present disclosure to kill a cancer cell.

In some examples, the cancer cell is infected with EBV.

Description

The present disclosure is based on the unexpected finding that immune cells specific for EBV lytic antigens are capable of killing EBV-infected cells, and that populations of cells comprising immune cells specific for EBV latent antigens and EBV lytic antigens display improved ability to control EBV-positive malignancies as compared to populations of cells only comprising immune cells specific for EBV latent antigens.

Epstein-Barr Virus replication

Epstein-Barr Virus (EBV) virology is described e.g. in Stanfield and Luftiq, F1000Res. (2017) 6:386 and Odumade et al., Clin Microbiol Rev (2011) 24(1):193-209, both of which are hereby incorporated by reference in entirety.

EBV infects epithelial cells via binding of viral protein BMRF2 to β1 integrins, and binding of viral protein gH/gL with integrins avβ6 and avβ8. EBV infects B cells through interaction of viral glycoprotein gp350 with CD21 and/or CD35, followed by interaction of viral gp42 with MHC class II. These interactions trigger fusion of the viral envelope with the cell membrane, allowing the virus to enter the cell. Once inside, the viral capsid dissolves and the viral genome is transported to the nucleus.

EBV has two modes of replication; latent and lytic.

The latent cycle does not result in production of virions, and can take place in place B cells and epithelial cells. The EBV genome circular DNA resides in the cell nucleus as an episome and is copied by the host cell's DNA polymerase. In latency, only a fraction of EBV's genes are expressed, in one of three different patterns known as latency programs, which produce distinct sets of viral proteins and RNAs. The latent cycle is described e.g. in Amon and Farrell, Reviews in Medical Virology (2004) 15(3): 149–56, which is hereby incorporated by reference in entirety.

EBNA1 protein and non-coding RNA EBER are expressed in each of latency programs I-III. Latency programs II and III further involve expression of EBNALP, LMP1, LMP2A and LMP2B proteins, and latency program III further involves expression of EBNA2, EBNA3A, EBNA3B and EBNA3C.

Claims:

~~1. A method for generating or expanding a population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen, comprising stimulating immune cells specific for an EBV lytic antigen by contacting peripheral blood mononuclear cells (PBMCs) with: (i) one or more peptides corresponding to all or part of one or more EBV lytic antigens; or (ii) antigen presenting cells (APCs) presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens.~~

~~2. The method according to claim 1, wherein the method further comprises re-stimulating the immune cells specific for an EBV lytic antigen by contacting them with APCs presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens.~~

1~~3~~. A method for generating or expanding a population of immune cells comprising immune cells specific for an Epstein Barr Virus (EBV) lytic antigen and immune cells specific for an EBV latent antigen, comprising stimulating immune cells specific for an EBV lytic antigen and immune cells specific for an EBV latent antigen by contacting peripheral blood mononuclear cells (PBMCs) with: (i) ~~one or more~~ peptides corresponding to all or part of EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2~~one or more EBV lytic antigens, and one or more peptides corresponding to all or part of one or more EBV latent antigens~~; or (ii) antigen presenting cells (APCs) presenting ~~one or more~~ peptides corresponding to all or part of EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2~~one or more EBV lytic antigens, and one or more peptides corresponding to all or part of one or more EBV latent antigens~~.

2. The method according to claim 1, wherein the method comprises contacting PBMCs with peptides corresponding to all or part of EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2.

3. The method according to claim 1 or claim 2, wherein the method comprises contacting PBMCs with EBNA1 pepmix, LMP1 pepmix, LMP2 pepmix, BZLF1 pepmix, BRLF1 pepmix, BMRF1 pepmix, BMLF1 pepmix, BALF2 pepmix, BNLF2A pepmix, BNLF2B pepmix and BMRF2 pepmix.

~~4. The method according to claim 3, wherein the method further comprises re-stimulating the immune cells specific for an EBV lytic antigen and the immune cells specific for an EBV latent antigen by contacting them with APCs presenting one or more peptides corresponding to all or part of one or more EBV lytic antigens, and one or more peptides corresponding to all or part of an EBV latent antigen.~~

~~5. The method according to any one of claims 1 to 4, wherein the one or more EBV lytic antigens are selected from BZLF1, BRLF1, BMLF1, BMRF1, BXLF1, BALF1, BALF2, BGLF5, BHRF1, BNLF2A, BNLF2B, BHLF1, BLLF2, BKRF4, BMRF2, BALF4, BILF1, BILF2, BNFR1, BVRF2, BALF3, BALF5 and BDLF3.~~

~~6. The method according to any one of claims 1 to 5, wherein the one or more EBV lytic antigens are selected from BZLF1, BRLF1, BMLF1, BMRF1, BALF2, BNLF2A, BNLF2B, BMRF2 and BDLF3.~~

~~7. The method according to any one of claims 3 to 6, wherein the one or more EBV latent antigens are selected from EBNA1, EBNA-LP, EBNA2, EBNA3A, EBNA3B, EBNA3C, BARF1, LMP1, LMP2A and LMP2B.~~

~~8. The method according to any one of claims 3 to 7, wherein the one or more EBV latent antigens are selected from EBNA1, LMP1, LMP2A and LMP2B.~~

4~~9~~. The method according to any one of claims 1 to 3~~8~~, wherein the PBMCs are PBMCs depleted of CD45RA-positive cells.

5. The method according to any one of claims 1 to 4, wherein the method further comprises re-stimulating the immune cells specific for an EBV lytic antigen and the immune cells specific for an EBV latent antigen by contacting them with APCs presenting peptides corresponding to all or part of EBNA1, LMP1, LMP2A, LMP2B, BZLF1, BRLF1, BMRF1, BMLF1, BALF2, BNLF2A, BNLF2B and BMRF2.

6~~10~~. An isolated population of immune cells obtained or obtainable by the method according to any one of claims 1 to 5~~9~~.

7~~11~~. A pharmaceutical composition comprising the isolated population of immune cells according to claim 6~~10~~.

~~12. The isolated population of immune cells according to claim 10, or the pharmaceutical composition according to claim 11, for use in the treatment or prevention of a disease or disorder.~~

8~~13~~. Use of the isolated population of immune cells according to claim 6~~10~~, or the pharmaceutical composition according to claim 7~~11~~, in the manufacture of a medicament for the treatment or prevention of a disease or disorder associated with EBV infection.

~~14. The isolated population of immune cells or pharmaceutical composition for use according to claim 12, or the use according to claim 13, wherein the disease or disorder is a disease or disorder associated with EBV infection.~~

9~~15~~. The ~~isolated population of immune cells or pharmaceutical composition for use according to claim 12, or the~~ use according to claim 8~~13~~, wherein the disease or disorder associated with EBV infection is an EBV-associated cancer.

~~16. The isolated population of immune cells or pharmaceutical composition for use, or the use according to claim 14, wherein the disease or disorder associated with EBV infection is an EBV-associated cancer.~~

~~17. The isolated population of immune cells or pharmaceutical composition for use, or the use according to claim 15, wherein the cancer is an EBV-associated cancer.~~

10~~18~~. The ~~isolated population of immune cells or pharmaceutical composition for use, or the~~ use according to claim ~~16 or claim 17~~9, wherein the EBV-associated cancer is selected from EBV-positive lymphoma, EBV-positive nasopharyngeal carcinoma, and EBV-positive gastric carcinoma.

11~~19~~. A method for killing a cell infected with EBV, comprising contacting the cell infected with EBV with the isolated population of immune cells according to claim 6~~10~~, or the pharmaceutical composition according to claim 7~~11~~, wherein the method is an *in vitro* method.

12~~20~~. A method for killing a cancer cell, wherein the cancer cell is infected with EBV, comprising contacting the cancer cell with the isolated population of immune cells according to claim 6~~10~~, or the pharmaceutical composition according to claim 7~~11~~, wherein the method is an *in vitro* method.

~~21. The method according to claim 20, wherein the cancer cell is infected with EBV.~~