(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 February 2019 (14.02.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/028530 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *G01N 33/563* (2006.01)
A61P 31/22 (2006.01) *C12N 15/13* (2006.01)
C07K 16/08 (2006.01)

(21) International Application Number:
PCT/AU2018/050851

(22) International Filing Date:
10 August 2018 (10.08.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2017903197 10 August 2017 (10.08.2017) AU

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

(54) Title: ANTIBODY TO EPSTEIN BARR VIRUS AND USES THEREOF

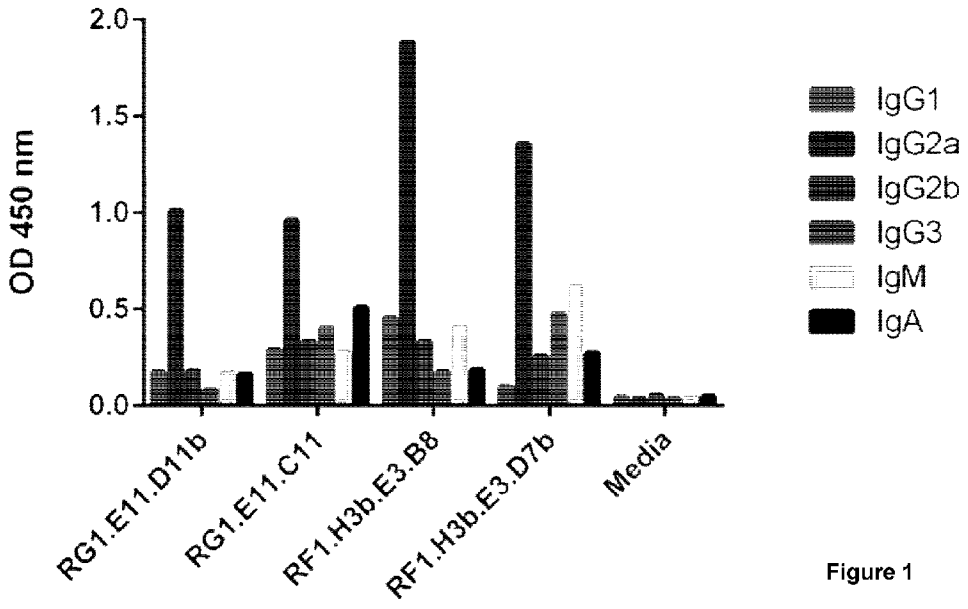

Figure 1

(57) Abstract: A recombinant, humanized antibody or antibody fragment that is capable of at least partly preventing or inhibiting Epstein Barr Virus gp350 binding to a human cell. The antibody may be useful for passively immunizing humans against Epstein Barr Virus and/or treating or preventing Epstein Barr Virus-associated diseases, disorders or conditions. The antibody or antibody fragment may also be used to detect Epstein Barr Virus.

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TITLE

ANTIBODY TO EPSTEIN BARR VIRUS AND USES THEREOF

TECHNICAL FIELD

THIS INVENTION relates to Epstein-Barr virus (EBV). More particularly, this invention relates to an antibody that at least partly blocks gp350-mediated entry of EBV into human cells.

BACKGROUND

Epstein-Barr virus (EBV) or Human Herpesvirus 4 (HHV-4) is a double stranded DNA virus that belongs to the γ-herpesvirus subfamily. It is a common human pathogen that predominantly infects hosts through epithelial cells and B cells, where it can then establish long-term latency in the human host. Primary infection of EBV causes over 90% of cases of infectious mononucleosis (IM) worldwide, infecting mainly children and young adults through the dramatic expansion of EBV infected B cells (Coghill and Hildesheim 2014). EBV has been associated with several cancers, including Burkitt and Hodgkins lymphomas, gastric and nasopharyngeal carcinomas, lymphomas in HIV-infected individuals and post-transplant lymphoproliferative disorder (PTLD). Recently, EBV has also been found to be implicated in autoimmune diseases, particularly multiple sclerosis (MS) (Coghill and Hildesheim 2014).

Epstein-Barr virus encoded gp350 is one of the major targets for anti-viral humoral immunity as it mediates attachment to B cells via complement receptor 2. Gp350 is the primary glycoprotein that elicits anti-viral neutralising antibody responses after natural EBV infection. Currently, there is no commercially available prophylactic or therapeutic treatment that can prevent or cure acute EBV infection. Clinical trials utilising vaccinia-vectored gp350 demonstrated potential immunogenicity and efficacy in young children (Gu, Huang et al. 1995). Subsequent studies by GlaxoSmithKline Biologicals based on a recombinant gp350/aluminium hydroxide and 3-O-desacyl-4′-monophosphoryl lipid A (AS04) candidate vaccine showed demonstrable efficacy (mean efficacy rate, 78.0% [95% confidence interval, 1.0%–96.0%]) in preventing the development of infectious mononucleosis induced by EBV infection, but it had no efficacy in preventing asymptomatic EBV infection (Moutschen, Leonard et al. 2007). Furthermore, Haque and colleagues have also assessed potential clinical application of a murine monoclonal antibody against gp350 (72A1) both *in vivo* and *in vitro* (Haque, Johannessen et al. 2006).

SUMMARY

The present invention is broadly directed to an anti-gp350 antibody that at least partly prevents or inhibits the binding of EBV gp350 to human cells. A particular form of the invention provides a human or humanized, recombinant anti-gp350 antibody.

In a first aspect the invention provides a recombinant, human or humanized antibody or antibody fragment that is capable of at least partly preventing or inhibiting Epstein Barr Virus (EBV) gp350 binding to a human cell.

In a particular embodiment, the human or humanized antibody or antibody fragment comprises, consists essentially of or consists of an amino acid sequence set forth in any one of SEQ ID NOS:1-156, FIG. 5 and/or Tables 1-12, or an amino acid sequence at least 70% identical thereto.

In a particular embodiment, the human or humanized antibody or antibody fragment comprises, consists essentially of or consists of heavy chain and/or light chain complementarity-determining region (CDR) regions that respectively comprise an amino acid sequence set forth in any one of SEQ ID NOS:1-156, FIG. 5 and/or Tables 1-12.

Preferred CDR amino acid sequences are set forth in SEQ ID NOS:1-144 and Tables 1-12, or an amino acid sequence at least 70% identical thereto.

In a particular embodiment, the human or humanized antibody or antibody fragment comprises, consists essentially of or consists of an amino acid sequence set forth in any one of SEQ ID NOS:145-156 and/or FIG. 5, or an amino acid sequence at least 70% identical thereto.

In a second aspect, the invention provides an antibody or antibody fragment that comprises, consists essentially of or consists of at least one complementarity-determining region (CDR) amino acid sequence according to any one of SEQ ID NOS:1-156, FIG. 5 and/or Tables 1-12, or an amino acid sequence at least 70% identical thereto.

In a particular embodiment, the antibody or antibody fragment comprises, consists essentially of or consists of heavy chain and/or light chain CDR regions that respectively comprise an amino acid sequence set forth in any one of SEQ ID NOS:1-156, FIG. 5 and/or Tables 1-12, or an amino acid sequence at least 70% identical thereto.

Preferred CDR amino acid sequences are set forth in SEQ ID NOS:1-144 and Tables 1-12, or an amino acid sequence at least 70% identical thereto.

In a particular embodiment, the antibody or antibody fragment is a humanized antibody or antibody fragment that comprises, consists essentially of or consists of an amino acid sequence set forth in any one of SEQ ID NOS:145-156 and/or FIG. 5.

Suitably, the antibody or antibody fragment of the second aspect is capable of at least partly preventing or inhibiting Epstein Barr virus (EBV) gp350 binding to a human cell.

Preferably, the antibody or antibody fragment of the first and second aspects is a neutralizing antibody.

In one embodiment, the antibody or antibody fragment of the first and/or second aspects is produced by phage display, wherein the phage comprise one or more nucleotide sequences of human or non-human origin encoding one or more amino acid sequences of the antibody or antibody fragment. The one or more amino acid sequences may be V_H, V_L, and/or CDR amino acid sequences of human origin such as set forth in SEQ ID NOS:1-156, FIG. 5 and/or Tables 1-12.

In a third aspect, the invention provides an isolated nucleic acid encoding the antibody or antibody fragment of the first or second aspects.

This aspect also includes genetic constructs comprising the isolated nucleic acid and/or host cells comprising the isolated nucleic acid and/or genetic construct.

In a fourth aspect, the invention provides a composition comprising the antibody or antibody fragment of the first aspect, the second aspect and/or the isolated nucleic acid of the third aspect and a pharmaceutically acceptable carrier, diluent or excipient

In a fifth aspect, the invention provides a method of treating or preventing an EBV infection in a human, said method including the step of administering the antibody of the first aspect, the second aspect and/or the composition of the fourth aspect to the human to thereby treat or prevent an EBV infection in the human.

In a sixth aspect, the invention provides a method of passively immunizing a human against an EBV infection, said method including the step of administering the antibody of the first aspect, the second aspect and/or the composition of the fourth aspect to the human to thereby passively immunize the human against an EBV infection.

In a seventh aspect, the invention provides a method of at least partly inhibiting or preventing EBV gp350 binding to a human cell, said method including the step of administering the antibody of the first aspect, the second aspect and/or the composition of the fourth aspect to the human to thereby at least partly inhibit or prevent EBV gp350 binding to a human cell.

In an eighth aspect, the invention provides a method or system for determining the efficacy of an anti-EBV antibody or antibody fragment, said method or system comprising infecting a mouse with EBV, the mouse comprising human B lymphocytes, and determining the efficacy of a candidate anti-EBV antibody or antibody fragment in the mouse.

Suitably, efficacy relates to or includes the ability of the candidate anti-EBV antibody or antibody fragment to prevent EBV gp350 binding to one or more of the B lymphocytes.

Suitably, the human B lymphocytes have been derived from human hematopoietic stem cells administered to the mouse. Preferably, the human hematopoietic stem cells are $CD34^+$.

In a ninth aspect, the invention provides a method of detecting EBVgp350 or a cell expressing EBVgp350, said method including the step of forming a complex between the antibody or antibody fragment of the first and/or second aspects and EBV gp350 to thereby detect EBVgp350 or the cell expressing EBVgp350.

Throughout this specification, unless the context requires otherwise, the words "comprise", "comprises" and "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

By "consists essentially of" in the context of an amino acid sequence means that the recited amino acid sequences includes an additional 1, 2, 3, 4, or 5 amino acids at an N- and/or C-terminus thereof.

As used herein, the indefinite articles '*a*' and '*an*' are used here to refer to or encompass singular or plural elements or features and should not be taken as meaning or defining "one" or a "single" element or feature.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Immunoglobulin isotype analysis of four murine monoclonal subclones (RG1.E11.D11b, RG1.E11.C11, RF1.H3b.E3.B8 and RF1.H3b.E3.D7b) specific for gp350 antigen.

Figure 2: A) Assessment of neutralizing capacity of gp350-specific murine monoclonal antibodies (RG1.E11.D11b, RG1.E11.C11, RF1.H3b.E3.B8 and RF1.H3b.E3.D7b). Serum from a seronegative donor (referred to as EBV-) and a seropositive donor (referred to as EBV+) were used as negative and positive controls respectively. B) Representative photomicrographs of outgrowth of EBV transformed B cells in the presence of seropositive (upper panel) and seronegative serum.

Figure 3: Schematic overview of antibody library production and selections using phage display. The phage antibody library repertoire is derived from the B cells of immune donors. Bio-panning represents selection of phage coated antibody binders.

Figure 4: Polyclonal ELISA to identify enriched binders specific to gp350. Round 4 of bio-panning shows significant enrichment for binders to gp350.

Figure 5: Alignment of reformatted IgG1 heavy chain (HC; Panel A) and light chain (LC; Panel B) amino acid sequences of six gp350-specific clones. Amino acid changes in the original CDR regions are shown in **bold** text. Signal sequence for extracellular secretion of immunoglobulin is <u>underlined</u>. Clone B8 HC = SEQ ID NO: 145; Clone A11 HC = SEQ ID NO: 146; Clone E10 HC = SEQ ID NO: 147; Clone B7 HC = SEQ ID NO: 148; Clone D7 HC = SEQ ID NO: 149; Clone C10 HC = SEQ ID NO: 150; Clone B8 LC = SEQ ID NO: 151; Clone A11 LC = SEQ ID NO: 152; Clone E10 LC = SEQ ID NO: 153; Clone B7 LC = SEQ ID NO: 154; Clone D7 LC = SEQ ID NO: 155; Clone C10 LC = SEQ ID NO: 156.

Figure 6: SDS-PAGE and Western blot analysis (A) of gp350-sepcific clone B8 recombinant human antibody expressed in Expi293F cells grown in serum-free Expi293FTM expression medium. The recombinant plasmids encoding B8 heavy and light chain were transiently co-transfected into suspension Expi293F cell cultures. The cell culture supernatants collected on day 6 were used for purification. Lane M1: Protein Marker, Lane M2: Protein Marker, Lane 1: Reducing conditions, Lane 2: Non-reducing conditions, Lane P: Human IgG_1, Kappa (as positive control). The purified protein was also analyzed by SEC-HPLC analysis (B) for molecular weight and purity measurements.

Figure 7: SDS-PAGE and Western blot analysis of gp350-specific clone B7 recombinant human antibody expressed in Expi293F cells grown in serum-free

Expi293FTM expression medium. The recombinant plasmids encoding B8 heavy and light chain were transiently co-transfected into suspension Expi293F cell cultures. The cell culture supernatants collected on day 6 were used for purification. Lane M1: Protein Marker, Lane M2: Protein Marker, Lane 1: Reducing conditions, Lane 2: Non-reducing conditions, Lane P: Human IgG_1, Kappa (as positive control). The purified protein was also analyzed by SEC-HPLC analysis (B) for molecular weight and purity measurements.

Figure 8: Assessment of EBV neutralizing capacity of human monoclonal antibody clones. Purified EBV was initially incubated with serially diluted purified human monoclonal antibodies specific for gp350 or human serum from seronegative or seropositive donors for 1 h. Following pre-treatment with these antibodies, EBV was exposed to human PBMC pre-labelled with CTV. After 7 days these cells were assessed for EBV-driven proliferation.

Figure 9: Therapeutic assessment approach of humanized mice infected with EBV. Mice were given 100µg antibody treatment or PBS in each group after 5 and 10 days post EBV injections, n=18.

Figure 10: Interactions of gp350 (immobilised on CM5 sensor chip) with increasing concentrations of humanized monoclonal antibodies A11, B7, B8, C10, D7 and E10 via single cycle kinetics titrations.

Figure 11: Affinity steady-state plot of humanized monoclonal antibodies against gp350, fitted using 1:1 Langmuir model of interaction.

Figure 12: (**A**) Viral loads of B7, B8 and PBS treatment groups in humanized mice over the course of 28 days post virus injection. B7 and B8 show significant differences in viral loads 28 days post EBV injection ($p<0.001$). (**B**) Histochemical stain analysis of spleen tissue of humanized mice for each treatment group, B7, B8 and PBS. Green represents the presence of EBER in spleen tissues and blue represents normal spleen tissue. (**C**) Representation of normal spleen seen in B7 treatment group (pictured left) compared to splenomegaly with the presence of tumours (pictured right) which is representative of PBS treatment group.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1	CloneB8 Heavy Chain CDR1
SEQ ID NO:2	CloneB8 Heavy Chain CDR2
SEQ ID NO:3	CloneB8 Heavy Chain CDR3

SEQ ID NO:4	CloneA11 Heavy Chain CDR1
SEQ ID NO:5	CloneA11 Heavy Chain CDR2
SEQ ID NO:6	CloneA11 Heavy Chain CDR3
SEQ ID NO:7	CloneE10 Heavy Chain CDR1
SEQ ID NO:8	CloneE10 Heavy Chain CDR2
SEQ ID NO:9	CloneE10 Heavy Chain CDR3
SEQ ID NO:10	CloneB7 Heavy Chain CDR1
SEQ ID NO:11	CloneB7 Heavy Chain CDR2
SEQ ID NO:12	CloneB7 Heavy Chain CDR3
SEQ ID NO:13	CloneD7 Heavy Chain CDR1
SEQ ID NO:14	CloneD7 Heavy Chain CDR2
SEQ ID NO:15	CloneD7 Heavy Chain CDR3
SEQ ID NO:16	CloneC10 Heavy Chain CDR1
SEQ ID NO:17	CloneC10 Heavy Chain CDR2
SEQ ID NO:18	CloneC10 Heavy Chain CDR3
SEQ ID NO:19	CloneB8 Light Chain CDR1
SEQ ID NO:20	CloneB8 Light Chain CDR2
SEQ ID NO:21	CloneB8 Light Chain CDR3
SEQ ID NO:22	CloneA11 Light Chain CDR1
SEQ ID NO:23	CloneA11 Light Chain CDR2
SEQ ID NO:24	CloneA11 Light Chain CDR3
SEQ ID NO:25	CloneE10 Light Chain CDR1
SEQ ID NO:26	CloneE10 Light Chain CDR2
SEQ ID NO:27	CloneE10 Light Chain CDR3
SEQ ID NO:28	CloneB7 Light Chain CDR1
SEQ ID NO:29	CloneB7 Light Chain CDR2
SEQ ID NO:30	CloneB7 Light Chain CDR3
SEQ ID NO:31	CloneD7 Light Chain CDR1
SEQ ID NO:32	CloneD7 Light Chain CDR2
SEQ ID NO:33	CloneD7 Light Chain CDR3
SEQ ID NO:34	CloneC10 Light Chain CDR1
SEQ ID NO:35	CloneC10 Light Chain CDR2
SEQ ID NO:36	CloneC10 Light Chain CDR3
SEQ ID NO:37	CloneB8 Heavy Chain CDR1

SEQ ID NO:38	CloneB8 Heavy Chain CDR2
SEQ ID NO:39	CloneB8 Heavy Chain CDR3
SEQ ID NO:40	CloneA11 Heavy Chain CDR1
SEQ ID NO:41	CloneA11 Heavy Chain CDR2
SEQ ID NO:42	CloneA11 Heavy Chain CDR3
SEQ ID NO:43	CloneE10 Heavy Chain CDR1
SEQ ID NO:44	CloneE10 Heavy Chain CDR2
SEQ ID NO:45	CloneE10 Heavy Chain CDR3
SEQ ID NO:46	CloneB7 Heavy Chain CDR1
SEQ ID NO:47	CloneB7 Heavy Chain CDR2
SEQ ID NO:48	CloneB7 Heavy Chain CDR3
SEQ ID NO:49	CloneD7 Heavy Chain CDR1
SEQ ID NO:50	CloneD7 Heavy Chain CDR2
SEQ ID NO:51	CloneD7 Heavy Chain CDR3
SEQ ID NO:52	CloneC10 Heavy Chain CDR1
SEQ ID NO:53	CloneC10 Heavy Chain CDR2
SEQ ID NO:54	CloneC10 Heavy Chain CDR3
SEQ ID NO:55	CloneB8 Light Chain CDR1
SEQ ID NO:56	CloneB8 Light Chain CDR2
SEQ ID NO:57	CloneB8 Light Chain CDR3
SEQ ID NO:58	CloneA11 Light Chain CDR1
SEQ ID NO:59	CloneA11 Light Chain CDR2
SEQ ID NO:60	CloneA11 Light Chain CDR3
SEQ ID NO:61	CloneE10 Light Chain CDR1
SEQ ID NO:62	CloneE10 Light Chain CDR2
SEQ ID NO:63	CloneE10 Light Chain CDR3
SEQ ID NO:64	CloneB7 Light Chain CDR1
SEQ ID NO:65	CloneB7 Light Chain CDR2
SEQ ID NO:66	CloneB7 Light Chain CDR3
SEQ ID NO:67	CloneD7 Light Chain CDR1
SEQ ID NO:68	CloneD7 Light Chain CDR2
SEQ ID NO:69	CloneD7 Light Chain CDR3
SEQ ID NO:70	CloneC10 Light Chain CDR1
SEQ ID NO:71	CloneC10 Light Chain CDR2

SEQ ID NO:72	CloneC10 Light Chain CDR3
SEQ ID NO:73	CloneB8 Heavy Chain CDR1
SEQ ID NO:74	CloneB8 Heavy Chain CDR2
SEQ ID NO:75	CloneB8 Heavy Chain CDR3
SEQ ID NO:76	CloneA11 Heavy Chain CDR1
SEQ ID NO:77	CloneA11 Heavy Chain CDR2
SEQ ID NO:78	CloneA11 Heavy Chain CDR3
SEQ ID NO:79	CloneE10 Heavy Chain CDR1
SEQ ID NO:80	CloneE10 Heavy Chain CDR2
SEQ ID NO:81	CloneE10 Heavy Chain CDR3
SEQ ID NO:82	CloneB7 Heavy Chain CDR1
SEQ ID NO:83	CloneB7 Heavy Chain CDR2
SEQ ID NO:84	CloneB7 Heavy Chain CDR3
SEQ ID NO:85	CloneD7 Heavy Chain CDR1
SEQ ID NO:86	CloneD7 Heavy Chain CDR2
SEQ ID NO:87	CloneD7 Heavy Chain CDR3
SEQ ID NO:88	CloneC10 Heavy Chain CDR1
SEQ ID NO:89	CloneC10 Heavy Chain CDR2
SEQ ID NO:90	CloneC10 Heavy Chain CDR3
SEQ ID NO:91	CloneB8 Light Chain CDR1
SEQ ID NO:92	CloneB8 Light Chain CDR2
SEQ ID NO:93	CloneB8 Light Chain CDR3
SEQ ID NO:94	CloneA11 Light Chain CDR1
SEQ ID NO:95	CloneA11 Light Chain CDR2
SEQ ID NO:96	CloneA11 Light Chain CDR3
SEQ ID NO:97	CloneE10 Light Chain CDR1
SEQ ID NO:98	CloneE10 Light Chain CDR2
SEQ ID NO:99	CloneE10 Light Chain CDR3
SEQ ID NO:100	CloneB7 Light Chain CDR1
SEQ ID NO:101	CloneB7 Light Chain CDR2
SEQ ID NO:102	CloneB7 Light Chain CDR3
SEQ ID NO:103	CloneD7 Light Chain CDR1
SEQ ID NO:104	CloneD7 Light Chain CDR2
SEQ ID NO:105	CloneD7 Light Chain CDR3

SEQ ID NO:106	CloneC10 Light Chain CDR1
SEQ ID NO:107	CloneC10 Light Chain CDR2
SEQ ID NO:108	CloneC10 Light Chain CDR3
SEQ ID NO:109	CloneB8 Heavy Chain CDR1
SEQ ID NO:110	CloneB8 Heavy Chain CDR2
SEQ ID NO:111	CloneB8 Heavy Chain CDR3
SEQ ID NO:112	CloneA11 Heavy Chain CDR1
SEQ ID NO:113	CloneA11 Heavy Chain CDR2
SEQ ID NO:114	CloneA11 Heavy Chain CDR3
SEQ ID NO:115	CloneE10 Heavy Chain CDR1
SEQ ID NO:116	CloneE10 Heavy Chain CDR2
SEQ ID NO:117	CloneE10 Heavy Chain CDR3
SEQ ID NO:118	CloneB7 Heavy Chain CDR1
SEQ ID NO:119	CloneB7 Heavy Chain CDR2
SEQ ID NO:120	CloneB7 Heavy Chain CDR3
SEQ ID NO:121	CloneD7 Heavy Chain CDR1
SEQ ID NO:122	CloneD7 Heavy Chain CDR2
SEQ ID NO:123	CloneD7 Heavy Chain CDR3
SEQ ID NO:124	CloneC10 Heavy Chain CDR1
SEQ ID NO:125	CloneC10 Heavy Chain CDR2
SEQ ID NO:126	CloneC10 Heavy Chain CDR3
SEQ ID NO:127	CloneB8 Light Chain CDR1
SEQ ID NO:128	CloneB8 Light Chain CDR2
SEQ ID NO:129	CloneB8 Light Chain CDR3
SEQ ID NO:130	CloneA11 Light Chain CDR1
SEQ ID NO:131	CloneA11 Light Chain CDR2
SEQ ID NO:132	CloneA11 Light Chain CDR3
SEQ ID NO:133	CloneE10 Light Chain CDR1
SEQ ID NO:134	CloneE10 Light Chain CDR2
SEQ ID NO:135	CloneE10 Light Chain CDR3
SEQ ID NO:136	CloneB7 Light Chain CDR1
SEQ ID NO:137	CloneB7 Light Chain CDR2
SEQ ID NO:138	CloneB7 Light Chain CDR3
SEQ ID NO:139	CloneD7 Light Chain CDR1

SEQ ID NO:140	CloneD7 Light Chain CDR2
SEQ ID NO:141	CloneD7 Light Chain CDR3
SEQ ID NO:142	CloneC10 Light Chain CDR1
SEQ ID NO:143	CloneC10 Light Chain CDR2
SEQ ID NO:144	CloneC10 Light Chain CDR3
SEQ ID NO:145	Clone B8 Heavy Chain IgG_1 reformatted antibody fragment
SEQ ID NO:146	CloneA11 Heavy Chain IgG_1 reformatted antibody fragment
SEQ ID NO:147	CloneE10 Heavy Chain IgG_1 reformatted antibody fragment
SEQ ID NO:148	CloneB7 Heavy Chain IgG_1 reformatted antibody fragment
SEQ ID NO:149	CloneD7 Heavy Chain IgG_1 reformatted antibody fragment
SEQ ID NO:150	CloneC10 Heavy Chain IgG_1 reformatted antibody fragment
SEQ ID NO:151	Clone B8 Light Chain IgG_1 reformatted antibody fragment
SEQ ID NO:152	CloneA11 Light Chain IgG_1 reformatted antibody fragment
SEQ ID NO:153	CloneE10 Light Chain IgG_1 reformatted antibody fragment
SEQ ID NO:154	CloneB7 Light Chain IgG_1 reformatted antibody fragment
SEQ ID NO:155	CloneD7 Light Chain IgG_1 reformatted antibody fragment
SEQ ID NO:156	CloneC10 Light Chain IgG_1 reformatted antibody fragment
SEQ ID NO: 157	Human igG1 constant region amino acid sequence

DETAILED DESCRIPTION

The present invention is at least partly based on the creation of a humanized, recombinant antibodies directed to EBV anti-gp350 that at least partly prevent or

inhibit the binding of EBV gp350 to thereby block EBV entry into human cells. This humanized, recombinant antibody may be particularly suitable for administration to humans to passively immunize against EBV infection.

In a particular aspect, the invention provides a recombinant, human or humanized antibody or antibody fragment that is capable of at least partly preventing or inhibiting Epstein Barr Virus (EBV) gp350 binding to a human cell, to thereby block EBV entry into the human cell.

In another particular aspect, the invention provides an antibody or antibody fragment comprising at least one CDR amino acid sequence according to any one of SEQ ID NOS:1-144 or present in any one of SEQ ID NOS:145-156, that is preferably capable of at least partly preventing or inhibiting Epstein Barr Virus (EBV) gp350 binding to a human cell, to thereby block EBV entry into the human cell.

Preferably, the antibody or antibody fragment of these particular aspects is a neutralizing antibody.

In one embodiment, the antibody or antibody fragment of these aspects is produced by phage display.

As used herein, by *"isolated"* is meant material that has been removed from its natural state or otherwise been subjected to human manipulation. Isolated material may be substantially or essentially free from components that normally accompany it in its natural state, or may be manipulated so as to be in an artificial state together with components that normally accompany it in its natural state. Isolated material may be in recombinant, chemical synthetic, enriched, purified or partially purified form.

As used herein a "*protein*" is an amino acid polymer, wherein the amino acids may include D- amino acids, L-amino acids, natural and/or non-natural amino acids. As typically used herein, a "*peptide*" is a protein comprising no more than sixty (60) contiguous amino acids. As typically used herein, a "*polypeptide*" is a protein comprising more than sixty (60) contiguous amino acids. The term "*protein*" should also be understood to encompass protein-containing molecules such as glycoproteins and lipoproteins, although without limitation thereto.

As used herein, an "*antibody*" is or comprises an immunoglobulin protein. The term "*immunoglobulin*" includes any antigen-binding protein product of a mammalian immunoglobulin gene complex, including immunoglobulin isotypes IgA, IgD, IgM, IgG and IgE and antigen-binding fragments thereof. Included in the term "*immunoglobulin*" are immunoglobulins that are recombinant, chimeric or humanized

or otherwise comprise altered or variant amino acid residues, sequences and/or glycosylation, whether naturally occurring or produced by human intervention (*e.g.* by recombinant DNA technology).

Generally, antibodies and antibody fragments may be polyclonal or monoclonal. It will also be appreciated that antibodies may be produced as recombinant synthetic antibodies or antibody fragments by expressing a nucleic acid encoding the antibody or antibody fragment in an appropriate host cell. Non-limiting examples of recombinant antibody expression and selection techniques, inclusive of phage display methods, are provided in Chapter 17 of Coligan *et al.*, CURRENT PROTOCOLS IN IMMUNOLOGY and Zuberbuhler *et al.*, 2009, Protein Engineering, Design & Selection **22** 169. A non-limiting example of a phage display system for selecting anti-gp350 antibodies is provided hereinafter in the Examples.

Typically, an antibody comprises: respective light chain (V_L) and heavy chain (V_H) variable regions that each comprise complementarity determining region (CDR) 1, 2 and 3 amino acid sequences; and respective light chain (C_L) and heavy chain (CH_1, CH_2, CH_3) constant regions. CDR identification and numbering may be according to any known CDR numbering system inclusive of Kabat, Chothia, AbM and Contact. Non-limiting examples of CDR amino acid sequences are set forth in SEQ ID NOS:1-144 and shown in Tables 1-12. CDR identification and numbering was performed using abYsis version 2.7.3 and IMGT/V-QUEST. SEQ ID NOS:1-36 use Chothia numbering; SEQ ID NOS:37-72 use AbM numbering; SEQ ID NOS:73-108 use Kabat numbering; and SEQ ID NOS:109-144 use Contact numbering. Antibodies according to the invention may comprise 1, 2 or 3 V_L CDR amino acid sequences (*e.g* CDR1, CDR2 and/or CDR3) and/or 1, 2, or 3 V_H CDR amino acid sequences (*e.g* CDR1, CDR2 and/or CDR3), such as preferably set forth in SEQ ID NOS:1-144.

Antibody fragments include Fab and Fab'2 fragments, diabodies, triabodies, bi-specific antibodies and single chain antibody fragments (*e.g.* ScFvs), although without limitation thereto. In some embodiments, an antibody fragment may comprise at least a portion of a CDR1, 2 and/or 3 amino acid sequence, such as set forth in SEQ ID NOS:1-36. A preferred antibody fragment comprises at least one entire light chain variable region CDR and/or at least one entire heavy chain variable region CDR.

As broadly used herein, "*humanized*" antibodies may include antibodies entirely or at least partly of human origin, inclusive of modified antibodies or antibody fragments obtained from a non-human "foreign" species. In some embodiments,

antibodies and antibody fragments may be modified so as to be administrable to one species having being produced in, or originating from, the same or another "foreign" species without eliciting a deleterious immune response to the "foreign" antibody. Human or non-human antibody fragments such as comprising complementarity determining regions (CDRs) or variable regions (*i.e* V_H and V_L domains) may be "grafted" onto a human antibody scaffold or backbone to produce a "humanized" antibody or antibody fragment. In a particular embodiment, human or non-human CDRs or V_L and V_L domains are recombinantly grafted with a human antibody constant region, preferably a human IgG_1 constant region.

As disclosed herein in more detail in the Examples, human antibody V_H and V_L fragment-encoding nucleotide sequences were isolated by phage display and recombinantly grafted onto a hman IgG_1 constant region "backbone".

A non-limiting example of a human IgG_1 constant region comprises the amino acid sequence TVSSASTKGPSVFP (SEQ ID NO:157), as originally described in Jones *et al.*, 2010, J. Immunol. Methods **354** 85.

Preferably, the antibody or antibody fragment is a neutralizing antibody or antibody fragment. By this is meant an antibody or antibody fragment that at least partly blocks, inhibits or reduces one or more infective or pathogenic properties of EBV. In a particular embodiment, the antibody or antibody fragment at least partly blocks, inhibits or reduces gp350-mediated entry of EBV into a human cell.

Suitably, the antibody or antibody fragment binds an epitope of an EBV gp350 protein. As generally used herein, an "*epitope*" is an antigenic protein fragment that comprises a continuous or discontinuous sequence of amino acids of a protein, wherein the epitope can be recognized or bound by an element of the immune system, such as an antibody or other antigen receptor.

The invention also includes variants of the the antibody or antibody fragment disclosed herein, such as a CDR variant.

Suitably, an antibody or antibody fragment comprising at least one variant is capable of preventing Epstein Barr Virus (EBV) gp350 binding to a human cell.

In particular embodiments, a variant has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with the amino acid sequence set forth in any one of SEQ ID NOS:1-156. The protein "*variant*" disclosed herein may have one or more amino acids deleted or substituted by different

amino acids. It is well understood in the art that some amino acids may be substituted or deleted without changing biological activity of the peptide (conservative substitutions).

In one embodiment, the variant is an antibody or antibody fragment comprising an amino acid sequence at least 70% identical to any one of SEQ ID NOS:1-144, referred to herein as a CDR "variant". By way of example, CDR amino acid sequences may be altered to improve recognition and/or binding to EBV gp350.

In some embodiments, variants may be produced by recombinant mutagenesis techniques.

Terms used generally herein to describe sequence relationships between respective proteins and nucleic acids include "*comparison window*", "*sequence identity*", "*percentage of sequence identity*" and "*substantial identity*". Because respective nucleic acids/proteins may each comprise (1) only one or more portions of a complete nucleic acid/protein sequence that are shared by the nucleic acids/proteins, and (2) one or more portions which are divergent between the nucleic acids/proteins, sequence comparisons are typically performed by comparing sequences over a "comparison window" to identify and compare local regions of sequence similarity. A "*comparison window*" refers to a conceptual segment of typically 6, 9 or 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence for optimal alignment of the respective sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerised implementations of algorithms (Geneworks program by Intelligenetics; GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA, incorporated herein by reference) or by inspection and the best alignment (*i.e.* resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.*, 1997, Nucl. Acids Res. **25** 3389, which is incorporated herein by reference. A detailed discussion of sequence analysis can be found in Unit 19.3 of CURRENT PROTOCOLS IN MOLECULAR BIOLOGY Eds. Ausubel *et al.* (John Wiley & Sons Inc NY, 1995-2015).

The term "*sequence identity*" is used herein in its broadest sense to include the number of exact nucleotide or amino acid matches having regard to an appropriate

alignment using a standard algorithm, having regard to the extent that sequences are identical over a window of comparison. Thus, a "*percentage of sequence identity*" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For example, "*sequence identity*" may be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA).

Derivatives of the antibody, antibody fragments or variants thereof disclosed herein are also provided.

As used herein, "*derivative*" antibodies, antibody fragments or variants thereof have been altered, for example by conjugation or complexing with other chemical moieties, by post-translational modification (*e.g.* phosphorylation, ubiquitination, glycosylation), chemical modification (*e.g.* cross-linking, acetylation, biotinylation, oxidation or reduction and the like), conjugation with labels (*e.g.* fluorophores, enzymes, radioactive isotopes) and/or inclusion of additional amino acid sequences as would be understood in the art.

In this regard, the skilled person is referred to Chapter 15 of CURRENT PROTOCOLS IN PROTEIN SCIENCE, Eds. Coligan *et al.* (John Wiley & Sons NY 1995-2015) for more extensive methodology relating to chemical modification of proteins.

Additional amino acid sequences may include fusion partner amino acid sequences which create a fusion protein. By way of example, fusion partner amino acid sequences may assist in detection and/or purification of the isolated fusion protein. Non-limiting examples include metal-binding (*e.g.* polyhistidine) fusion partners, maltose binding protein (MBP), Protein A, glutathione S-transferase (GST), fluorescent protein sequences (*e.g.* GFP), epitope tags such as myc, FLAG and haemagglutinin tags.

Another aspect of the invention provides an isolated nucleic acid encoding an antibody, antibody fragment or variant thereof disclosed herein.

As generally used herein a "*nucleic acid*" designates single- or double-stranded DNA and RNA. DNA includes genomic DNA and cDNA. RNA includes mRNA, RNA, RNAi, siRNA, cRNA and autocatalytic RNA. Nucleic acids may also be DNA-RNA hybrids. A nucleic acid comprises a nucleotide sequence which typically includes nucleotides that comprise an A, G, C, T or U base. However, nucleotide sequences may include other bases such as inosine, methylycytosine, methylinosine, methyladenosine and/or thiouridine, although without limitation thereto.

In a preferred form, the nucleotide sequence may be codon-optimized, by which is meant that the nucleotide sequence is a synthetic or engineered sequence, rather than the original "source" nucleotide sequence, modified for optimal expression in a particular host cell type by taking advantage of codon sequence variations or redundancies that occur across different cell types and/or species.

In some embodiments, the nucleic acid may be in a genetic construct that facilitates delivery and expression of the nucleic acid. Broadly, the genetic construct may be in the form of, or comprise genetic components of, a plasmid, a transposon, a bacteriophage, a virus, a cosmid, a yeast or bacterial artificial chromosome as are well understood in the art. Suitably, the nucleic acid is operably linked or connected to one or more elements of the construct that facilitate propagation, manipulation and/or expression of the genetic construct and/or nucleic acid. The one or more elements may include promoters, enhancers, polyadenylation sequences, splice donor/acceptor sites, multiple cloning sites, bacterial origins of replication, selection markers for bacterial, mammalian or other host cells, translation initiation and stop sequences, as are well known in the art. Promoters may be constitutive or inducible/repressible promoters as are well known in the art.

The choice of said one or more elements may be at least partly dependent on the host cell type used for expression, particularly according to the origin of the host cell (*e.g.* mammalian or other vertebrates, plant, bacterial, insect or yeast cells such as *E. coli*, CHO, COS, Vero, HeLa, HEK-293, *Sf9* and *Pichia pastoris* host cells, although without limitation thereto).

It will be appreciated from the foregoing that an aspect of the invention provides a method of treating or preventing an EBV infection in a human, said method including the step of administering the antibody of the first aspect, the second aspect and/or the composition of the fourth aspect to the human to thereby treat or prevent an EBV infection in the human.

Another aspect of the invention provides a method of passively immunizing a human against an EBV infection, said method including the step of administering the antibody of the first aspect, the second aspect and/or the composition of the fourth aspect to the human to thereby passively immunizing the human against an EBV infection.

As generally used herein the terms "*immunize*", "*vaccinate*" and "*vaccine*" refer to antibodies, antibody fragments, methods and/or compositions that elicit or provide a protective immune response against EBV. Administration of said antibody or antibody fragment to human may be referred to as "*passive*" immunization which provides the human with at least temporary antibody-mediated immunity to an existing or potential EBV infection.

In certain embodiments the immune response may be suitable for preventing, treating or passively immunizing the human against EBV.

As used herein, "*treating*", "*treat*" or "*treatment*" refers to a therapeutic intervention that at least partly ameliorates, eliminates or reduces a symptom or pathological sign of an EBV infection after it has begun to develop. Treatment need not be absolute to be beneficial to the subject.

As used herein, "*preventing*", "*prevent*" or "*prevention*" refers to a course of action initiated prior to infection by, or exposure to, EBV or molecular components thereof and/or before the onset of a symptom or pathological sign of an EBV infection, so as to at least partly prevent infection and/or reduce the symptom or pathological sign. It is to be understood that such prevention need not be absolute or complete to be beneficial to a subject.

EBV is a common human pathogen and may cause, or be associated with, one or more diseases, disorders or conditions in humans. Thus, certain embodiments of the aforementioned methods relate to passively immunization, preventing and/or treating one or more diseases, disorders or conditions caused by, or associated with, an EBV infection in humans. EBV predominantly infects human hosts through epithelial cells and B lymphocytes where it can then establish long-term latency in the human host. Primary infection of EBV causes over 90% of cases of infectious mononucleosis (IM) worldwide, infecting mainly children and young adults through the expansion of EBV infected B cells. EBV has been associated with several cancers, including Burkitt and Hodgkin's lymphomas, gastric and nasopharyngeal carcinomas, lymphomas in HIV-infected individuals and post-transplant lymphoproliferative disorder (PTLD). EBV

has also been found to be implicated in autoimmune diseases, particularly multiple sclerosis.

In this context, another aspect, the invention provides at least partly inhibiting or preventing EBV gp350 binding to a human cell, said method including the step of administering the antibody of the first aspect, the second aspect and/or the composition of the fourth aspect to the human to thereby at least partly inhibit or prevent EBV gp350 binding to a human cell. Suitably the human cell is a B lymphocyte.

In some embodiments, the isolated antibodies, fragments and/or variants, or combinations of these, may be administered to a mammal in the form of a composition comprising a pharmaceutically acceptable carrier, diluent or excipient.

It will be appreciated that pharmaceutically acceptable carriers, diluents and/or excipients may include solid, semi-solid, gel or liquid fillers, diluents or encapsulating substances that may be safely used in systemic administration. Depending upon the particular route of administration, carriers, diluents and/or excipients may be selected from a group including sugars, starches, cellulose and its derivatives, malt, gelatine, talc, calcium sulfate, vegetable oils, synthetic oils, polyols, alginic acid, isotonic saline, pyrogen-free water, wetting or emulsifying agents, bulking agents, glidants, coatings (*e.g.* enteric coatings), emollients, binders, fillers, disintegrants, lubricants, pH buffering agents (*e.g.* phosphate buffers) and/or flavouring agents, although without limitation thereto. The composition may be administered to a human in any one or more dosage forms that include tablets, dispersions, suspensions, injectable solutions, syrups, troches, capsules, suppositories, aerosols, transdermal patches and the like.

Administration of the antibody, antibody fragment or variant thereof, or an encoding nucleic acid, or a composition comprising same may be by any known parenteral, topical or enteral route inclusive of intravenous, intramuscular, intraperitoneal, intracranial, transdermal, oral, intranasal, anal and intra-ocular, although without limitation thereto.

The composition may further include one or more additional agents inclusive of adjuvants or other immunostimulants.

By "*adjuvant*" is meant an agent which assists, augments or otherwise facilitates the elicitation of an immune response. Non-limiting examples of adjuvants include Freund's adjuvant, aluminium hydroxide (alum), aluminium phosphate,

squalene, IL-12, CpG-oligonucleotide, Montanide ISA720, imiquimod, SBAS2, SBAS4, MF59, MPL, Quil A, QS21 and ISCOMs.

For the purposes of methods of immunization, prevention and/or treatment of EBV infections, the recipient human may be referred to as a "subject" or "patient", which terms are used interchangeably.

As described herein, a preferred method of producing anti-gp350 antibodies or antibody fragments is by phage display, although other methods of antibody production may be utilized, as are well known in the art.

In one embodiment, "candidate" anti-gp350 antibodies or antibody fragments may subseqeuntly be tested and selected according to their ability to bind gp350 and preferably prevent EBV gp350 binding to one or more of the B lymphocytes and thereby block entry of EBV into the human cell.

Accordingly, a further aspect of the invention provides method or system for determining the efficacy of an anti-EBV gp350 antibody or antibody fragment, said method or system comprising infecting a mouse with EBV, the mouse comprising human B lymphocytes, and determining the efficacy of a candidate anti-EBV agent in the mouse.

Suitably, the efficacy relates to, or includes, the ability of the candidate anti-EBV antibody or antibody fragment to prevent EBV gp350 binding to one or more of the B lymphocytes and thereby block entry of EBV into the human cell.

Suitably, the human B lymphocytes have been derived from human hematopoietic stem cells administered to the mouse. Preferably, the human hematopoietic stem cells are $CD34^+$. Typically, the mice are immunocompromised or immunodeficient mice. A non-limiting example is irradiated adult NOD scid gamma (NSG) mice.

Thus, it will be appreciated that the method or system provides a means whereby a mouse which has been engineered to adopt certain characteristics of the human immune system can thereby a model for testing the potential efficacy of an anti-EBV gp350 antibody to block EBV infection in humans.

In a particular embodiment, the method or system includes:

(i) administering $CD34^+$ human pluripotential stem cells to an immunocompromised or immunodeficient mouse;

(ii) allowing human B lymphocytes to develop from the $CD34^+$ human pluripotential stem cells;

(iii) infecting the mouse with EBV; and

(iv) determining the ability of a candidate anti-EBV gp350 antibody or antibody fragment administered to the mouse to block EBV entry into the human B lymphocytes.

The antibodies or antibody fragments may be polyclonal, monoclonal, native or recombinant as hereinbefore described.

In another aspect, the invention provides a method of detecting EBVgp350 or a cell expressing EBVgp350, said method including the step of forming a complex between the antibody or antibody fragment of the first and/or second aspects and EBV gp350 to thereby detect EBVgp350 or the cell expressing EBVgp350.

This aspect relates to detection of gp350, such as by an EBV-infected cell.

It will therefore be understood that an antibody or antibody fragment disclosed herein may be used to assist medical diagnosis of EBV infection. Suitably, the method includes detecting gp350, such as when expressed by EBV-infected cells present in, or obtained from, a biological sample. In certain embodiments, the biological sample may be a pathology sample that comprises one or more fluids, cells, tissues, organs or organ samples obtained from a human. Non-limiting examples include blood, plasma, saliva, serum, lymphocytes, urine, faeces, amniotic fluid, cervical samples, cerebrospinal fluid, tissue biopsies, bone marrow and skin, although without limitation thereto.

In some embodiments, the antibody or antibody fragment is labeled.

The label may be selected from a group including biotin, avidin, digoxigenin, an enzyme (*e.g* alkaline phosphatase or horseradish peroxidase), a fluorophore (*e.g.* FITC, Texas Red, Coumarin), a radioisotope (*e.g.* ^{125}I, ^{131}I, ^{67}Ga, ^{111}In) and/or a direct visual label (*e.g.* a gold particle), although without limitation thereto.

Suitably, detection of gp350 includes the step of forming a detectable complex between an antibody or antibody fragment and gp350 or a cell expressing gp350. The complex so formed may be detected by any technique, assay or means known in the art including immunoblotting, immunohistochemistry, immunocytochemistry, immunoprecipitation, ELISA, flow cytometry, magnetic bead separation, biosensor-based detection systems such as surface plasmon resonance and imaging such as PET imaging, although without limitation thereto.

To facilitate detection the antibody may be directly labeled as hereinbefore described or a labeled secondary antibody may be used. The labels may be as hereinbefore described.

In some embodiments, a detection kit may be provided which comprises an antibody or antibody fragment disclosed herein together with one or more detection reagents such as enzymes, enzyme substrates (*e.g* Luminol, AMPPD, NBT), secondary antibodies and/or magnetic beads although without limitation thereto.

So that preferred embodiments may be described in detail and put into practical effect, reference is made to the following non-limiting Examples.

EXAMPLES

The generation of EBV-specific gp350 neutralising monoclonal antibodies

To explore the ability to produce neutralising anti-gp350 antibodies and determine their effect against gp350, the aim was to first generate a panel of EBV-specific gp350 monoclonal antibodies. Monoclonal antibodies were generated using two different approaches. The first approach generated gp350-specific mouse hybridomas. The second approach generated recombinant anti-gp350 antibodies through a phage display system.

(i) Murine monoclonal antibodies via hybridomas

To generate gp350-specific mouse hybridomas, mice were immunized with a combination of gp350 antigen and an immune adjuvant (Sigma-Aldrich cat# S6322) and methylated CpG. Serum samples were collected from the immunized mice and reactivity to the antigen was tested by ELISA at a dilution of 1:250 and 1:1250 and compared to a pre-immunization sample. Animals with the highest anti-gp350 antibody titre were selected for the generation of hybridomas.

To generate hybridoma cells the mouse spleen was excised, dissociated into a single cell suspension and fused to SP2/0-Ag14 myeloma cells using polyethylene glycol. ELISA analysis was used to screen nine hybridoma supernatants for their reactivity to gp350. Of the nine that were screened, two clones with the highest absorbance reading F1 (OD 450nm: 21) and G1 (OD 450nm: 15.1) were selected for further subcloning rounds to generate murine monoclonal antibodies.

After subsequent subcloning rounds, an ELISA which tested the supernatants of each subclone at 2-fold dilutions revealed four subclones with the

highest reactivity against gp350. These subclones were selected and expanded into larger volumes for purification in order to generate monoclonal antibodies.

To generate purified monoclonal antibodies, the isotypes of the four subclones were determined by performing a mouse antibody isotype ELISA where each subclone was tested against six mouse immunoglobulin isotype-specific monoclonal antibodies. Analysis shows that all subclones were positively reactive to IgG2a (Figure 1), which enabled purification of these subclone supernatants using a protein G column.

A protein G column (Sigma-Aldrich cat# ge17-0405-01) was used for the purification, in which 200 mL of supernatant from each subclone was passed through the column and eluted to generate purified gp350-specific murine monoclonal antibodies. ELISA analysis revealed that subclone RG1.E11.D11b generated 521µg/mL of purified monoclonal antibody, RG1.E11.C11 eluted 111 µg/mL, RF1.H3b.E3.B8 generated 479 µg/mL and RF1.H3b.E3.D7b contained 374 µg/mL. We then assessed whether both approaches post production of murine and humanized monoclonal antibodies are capable of neutralising EBV infection. A preliminary test of the gp350-specific monoclonal antibodies for their EBV neutralizing ability was performed. Monoclonal antibodies and serum obtained from a sero-positive donor were tested at 2-fold dilutions with PBMCs (Peripheral Blood Mononuclear Cells) at $2x10^5$cells per well, from a sera-negative donor against B95-8 (infectious mononucleosis-derived isolate of EBV) in a 96-well plate format. The neutralization assay was performed in quadruplicate, and the plate was left to incubate for a duration of 6 weeks. Neutralization was determined by analyzing whether each antibody neutralized B cell transformation by EBV infection. It was found that none of the murine monoclonal antibodies were able to neutralize EBV and thus failed to block transformation of B cells (Figure 2A&B).

(ii) Generation of human gp350-specific antibodies using Antibody Phage Display (ADP) platform technology

This second approach was aimed to generate fully functional recombinant human monoclonal antibodies through antibody phage display which is based on genetic engineering of bacteriophages and repeated rounds of antigen-guided selection and phage propagation. In this procedure, a library of purified phage having DNA inserts from peripheral blood B lymphocytes obtained from healthy human donors was

exposed to gp350 in order to isolate a pool of phage particles that bind specifically to glycoprotein gp350. Phage particles remaining after washing away non-binders are then harvested and used to infect *E.coli* bacteria for amplification (Figure 3). This procedure was repeated 4 times to enrich the pool with specific binders to gp350. A polyclonal ELISA was then performed to determine the round with enriched binders specific to gp350 (Figure 4). The ELISA involves immobilizing gp350 onto microtiter plates, followed by the addition of various dilutions of the phage pool from each round, and then detection of bound phage using an anti-M13 phage HRP antibody.

As the objective was to obtain monoclonal antibodies, individual clones from the fourth round of bio-panning was isolated from cell glycerol stocks. This procedure involved growing single colonies from the cell glycerol stocks onto 4x 150mm 2YT-Ampicillin Glucose (2%) plates overnight, after which single cell colonies were individually plated onto a 96-well plate. Helper phage was added to each well of the 96-well plate to induce the production of phage particles. Phage particles from each clone were then tested via monoclonal phage ELISA for specific binding to gp350. Of the 96 clones, it was found that 44 were positive (absorbance >1) to gp350. The CDR sequences of all 44 positive clones were analyzed and six (6) clones were found to be uniquely positive to gp350.

PCR amplification products encoding V_H and V_L fragments were produced from the original six (6) phage clones. The translated CDR sequences shown in Tables 1-12 (SEQ ID NOS:1-144) were identified and numbered using abYsis version 2.7.3 and IMGT/V-QUEST. These unique six (6) clones were used to generate fully functional human monoclonal antibodies by antibody reformatting with a human IgG_1 backbone (Figure 5A&B; SEQ ID NOS:145-156). The human IgG constant region comprises the amino acid sequence TVSSASTKGPSVFP (SEQ ID NO:157), as originally described in Jones *et al.*, 2010, J. Immunol. Methods, *supra*

These recombinant human monoclonal antibodies were then expressed using a mammalian expression system. Target DNA sequence encoding each of full-length antibody sequences were optimized, synthesized and sub-cloned into pcDNA3.4 vector. The recombinant plasmids encoding target antibody were transiently co-transfected into suspension Expi293F cell cultures. The cell culture supernatants collected on day 6 were used for purification. Cell culture supernatants were centrifuged and followed by filtration. These filtered cell culture supernatants were loaded onto affinity purification column at an appropriate flowrate. After washing and

elution with appropriate buffer, the eluted fractions were pooled and buffer exchanged to final formulation buffer. The purified protein was analyzed by SDS-PAGE, Western blotting and SEC-HPLC analysis for molecular weight and purity measurements.

Representative data for Clone B8 and Clone B7 are presented Figure 6 and 7. These antibodies were tested for their capacity to neutralise EBV and block proliferation of EBV-infected B cells. Data presented in Figure 8 shows that all antibody clones E10, A11, B7, D10 and D7 showed strong inhibition of EBV infection while B8 clone showed no neutralization.

Biophysical analysis of humanized monoclonal antibodies bound to gp350 via surface plasmon resonance

Surface Plasmon Resonance (SPR) analysis of gp350 glycoprotein and humanized monoclonal antibodies was performed using the Biacore T100 system (GE Healthcare). CM5 sensor chips were used with 1x PBS as the running buffer, at a flow rate of 30µL/min. Glycoprotein gp350 was immobilised onto the flow cells of the sensor chip with a capture level of 1243RU with a flow rate of 10µL/min, a flow cell of the sensor chip was left blank for referencing of the sensograms. Single cycle kinetics was then used to determine the dissociation constant (K_d) for each humanized monoclonal antibody against gp350, with concentrations ranging from 25ug/mL to 1.2mg/mL with 1:2 dilution series. A flow rate of 40µL/min was used for the duration of the experiment, with contact time of 120 seconds and 600 seconds for the dissociation time per antibody for each of their concentrations. The regeneration of the surface was achieved using 10mM glycine buffer pH 2.1 with a 30 second injection at 30µL/min.

Single cycle kinetic binding curves (Figure 10) show the dissociation constants of each antibody against gp350. Antibody A11 with the lowest K_D value of 0.723nM has the best dissociation constant, with the ability to stay bound to gp350 during contact time, it also has the best affinity constant of $3.090E^{-7}M$ (Figure 11). Antibody B7 had a low dissociation value of 4.06754nM and an affinity value of $7.683E^{-7}M$, although it is not the lowest dissociation and affinity constant values in comparison to A11, D7 (3.862nM, $4.651E^{-7}M$) and E10 (2.2293nM, $6.635E^{-7}M$), it is important to outline previous data which shows that B7 had the best gp350 neutralising capacity *in vitro* compared to C10, D7 and E10. B7 is also capable of maintaining low levels of

dissociation throughout constant exposure to harsh surface regeneration buffers known to denature antibodies and proteins. Interestingly, antibody B8 which had the highest dissociation constant of 33.3209nM, as well as a high affinity constant of $8.286E^{-7}$M did not show any neutralising ability *in vitro* and can be observed to quickly dissociate once bound to gp350.

Assessing the therapeutic efficacy of humanized antibodies against EBV infected humanized mice

Referring to the schematic shown in Figure 9, the therapeutic efficacy of the humanized antibodies against humanized mice infected with EBV was assessed by twice irradiating adult (6-10 weeks) NOD SCID Gamma (NSG) mice and intravenously injecting them with human hematopoietic stem cells ($CD34^+$) to reconstitute human immunity. These mice were assessed every 4 weeks for the percentage of lymphocytes present via flow cytometry and maintained for 12 weeks post injection. The humanized mice were then intraperitoneally injected with EBV, where viral loads for each mouse was monitored weekly using quantitative PCR for up to 4 weeks post EBV injection. Three treatment groups were assessed by utilising EBV gp350 specific neutralising antibody (B7), EBV gp350 specific non-neutralising antibody (B8) and a control PBS group. Each group which consisted of 6 mice (n=18) received 100µg intravenously of the appropriate antibody 5 and 10 days post EBV injection (Figure 9). Mice were sacrificed 4 weeks post EBV injects and their spleens were harvested for histochemical staining via EBER.

Virus loads for each treatment group was monitored using quantitative PCR, which shows B7 (0.052 copies/mL) and B8 (0.047 copies/mL) with significantly decreased viral loads compared to PBS (97.196 copies/mL) treated mice group on day 28, $p<0.0001$ (Figure 12A). This correlates with histochemical stains where spleens were harvested and stained for the presence EBER (EBV-encoded RNA) in tissues (Figure 12B). Remarkably, mice that were given the B7 EBV neutralising antibody treatment showed almost no signs of EBER in the spleen tissues compared to mice treated with B8 EBV non-neutralising antibody and PBS (Figure 12B).

Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. It will therefore be appreciated by those of skill in the art that, in light of the instant disclosure, various modifications and changes can

be made in the particular embodiments exemplified without departing from the scope of the present invention.

All computer programs, algorithms, patent and scientific literature referred to herein is incorporated herein by reference.

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Table 1: CloneB8 Heavy Chain CDR1, 2 and 3 amino acid sequences.

Region	Definition	Sequence Fragment	Residues	Length
HFR1	Chothia	EVQLVESAGEVQKPGESLRISCKAS-----	1 - 25	25
	AbM	EVQLVESAGEVQKPGESLRISCKAS-----	1 - 25	25
	Kabat	EVQLVESAGEVQKPGESLRISCKASGYTFS	1 - 30	30
	Contact	EVQLVESAGEVQKPGESLRISCKASGYTF-	1 - 29	29
CDR-H1	Chothia	GYTFSHY---	26 - 32	7
	AbM	GYTFSHYWIG	26 - 35	10
	Kabat	-----HYWIG	31 - 35	5
	Contact	----SHYWIG	30 - 35	6
HFR2	Chothia	WIGWVRQLPGKGLEWMGII	33 - 51	19
	AbM	---WVRQLPGKGLEWMG--	36 - 49	14
	Kabat	---WVRQLPGKGLEWMG--	36 - 49	14
	Contact	---WVRQLPGKGLE-----	36 - 46	11
CDR-H2	Chothia	-----YPDDSD---------	52 - 57	6
	AbM	---IIYPDDSDSR-------	50 - 59	10
	Kabat	---IIYPDDSDSRYSPSFQG	50 - 66	17
	Contact	WMGIIYPDDSDSR-------	47 - 59	13
HFR3	Chothia	SRYSPSFQGQVTMSVDKSINTAYLQWNSLKVSDTATYYCVR	58 - 98	41
	AbM	--YSPSFQGQVTMSVDKSINTAYLQWNSLKVSDTATYYCVR	60 - 98	39
	Kabat	---------QVTMSVDKSINTAYLQWNSLKVSDTATYYCVR	67 - 98	32
	Contact	--YSPSFQGQVTMSVDKSINTAYLQWNSLKVSDTATYYC--	60 - 96	37
CDR-H3	Chothia	--HWLKRGSNFGFGFDP	99 - 113	15
	AbM	--HWLKRGSNFGFGFDP	99 - 113	15
	Kabat	--HWLKRGSNFGFGFDP	99 - 113	15
	Contact	VRHWLKRGSNFGFGFD-	97 - 112	16
HFR4	Chothia	-WGQGTTLTVSS	114 - 124	11
	AbM	-WGQGTTLTVSS	114 - 124	11
	Kabat	-WGQGTTLTVSS	114 - 124	11
	Contact	PWGQGTTLTVSS	113 - 124	12

Table 2: CloneB8 Light Chain CDR1, 2 and 3 amino acid sequences

Region	Definition	Sequence Fragment	Residues	Length
LFR1	Chothia	ESALTQPASVSGSPGQSITISC------	1 - 22	22
	AbM	ESALTQPASVSGSPGQSITISC------	1 - 22	22
	Kabat	ESALTQPASVSGSPGQSITISC------	1 - 22	22
	Contact	ESALTQPASVSGSPGQSITISCTGTSSD	1 - 28	28
CDR-L1	Chothia	TGTSSDVGGYNYVS--	23 - 36	14
	AbM	TGTSSDVGGYNYVS--	23 - 36	14
	Kabat	TGTSSDVGGYNYVS--	23 - 36	14
	Contact	------VGGYNYVSWY	29 - 38	10
LFR2	Chothia	WYQQHPGKAPKLMIY	37 - 51	15
	AbM	WYQQHPGKAPKLMIY	37 - 51	15
	Kabat	WYQQHPGKAPKLMIY	37 - 51	15
	Contact	--QQHPGKAPK----	39 - 47	9
CDR-L2	Chothia	----DVSNRPS	52 - 58	7
	AbM	----DVSNRPS	52 - 58	7
	Kabat	----DVSNRPS	52 - 58	7
	Contact	LMIYDVSNRP-	48 - 57	10
LFR3	Chothia	-GVSNRFSGSKSGNTASLTISGLQAEDEADYYC	59 - 90	32
	AbM	-GVSNRFSGSKSGNTASLTISGLQAEDEADYYC	59 - 90	32
	Kabat	-GVSNRFSGSKSGNTASLTISGLQAEDEADYYC	59 - 90	32
	Contact	SGVSNRFSGSKSGNTASLTISGLQAEDEADYYC	58 - 90	33
CDR-L3	Chothia	SSYTSSSTLV	91 - 100	10
	AbM	SSYTSSSTLV	91 - 100	10
	Kabat	SSYTSSSTLV	91 - 100	10
	Contact	SSYTSSSTL-	91 - 99	9
LFR4	Chothia	-FGGGTKLTVLG	101 - 111	11
	AbM	-FGGGTKLTVLG	101 - 111	11
	Kabat	-FGGGTKLTVLG	101 - 111	11
	Contact	VFGGGTKLTVLG	100 - 111	12

Table 3: CloneA11 Heavy Chain CDR1, 2 and 3 amino acid sequences.

Region	Definition	Sequence Fragment	Residues	Length
HFR1	Chothia	MQLVQSGAEVKKPGASVKVSCKTS-----	1 - 24	24
	AbM	MQLVQSGAEVKKPGASVKVSCKTS-----	1 - 24	24
	Kabat	MQLVQSGAEVKKPGASVKVSCKTSGYNFN	1 - 29	29
	Contact	MQLVQSGAEVKKPGASVKVSCKTSGYNF-	1 - 28	28
CDR-H1	Chothia	GYNFNHQ---	25 - 31	7
	AbM	GYNFNHQGIS	25 - 34	10
	Kabat	-----HQGIS	30 - 34	5
	Contact	----NHQGIS	29 - 34	6
HFR2	Chothia	GISWLRQAPGQGLEWMGWI	32 - 50	19
	AbM	---WLRQAPGQGLEWMG--	35 - 48	14
	Kabat	---WLRQAPGQGLEWMG--	35 - 48	14
	Contact	---WLRQAPGQGLE-----	35 - 45	11
CDR-H2	Chothia	-----SGFNGK---------	51 - 56	6
	AbM	---WISGFNGKTN-------	49 - 58	10
	Kabat	---WISGFNGKTNYAQKFQG	49 - 65	17
	Contact	WMGWISGFNGKTN-------	46 - 58	13
HFR3	Chothia	TNYAQKFQGRVTMTADRSTTTAYMELRSLRSDDTAVYYCAS	57 - 97	41
	AbM	--YAQKFQGRVTMTADRSTTTAYMELRSLRSDDTAVYYCAS	59 - 97	39
	Kabat	---------RVTMTADRSTTTAYMELRSLRSDDTAVYYCAS	66 - 97	32
	Contact	--YAQKFQGRVTMTADRSTTTAYMELRSLRSDDTAVYYC--	59 - 95	37
CDR-H3	Chothia	--GGEQWLVQNFVH	98 - 109	12
	AbM	--GGEQWLVQNFVH	98 - 109	12
	Kabat	--GGEQWLVQNFVH	98 - 109	12
	Contact	ASGGEQWLVQNFV-	96 - 108	13
HFR4	Chothia	-WGQGTLLTVSS	110 - 120	11
	AbM	-WGQGTLLTVSS	110 - 120	11
	Kabat	-WGQGTLLTVSS	110 - 120	11
	Contact	HWGQGTLLTVSS	109 - 120	12

Table 4: CloneA11 Light Chain CDR1, 2 and 3 amino acid sequences

Region	Definition	Sequence Fragment	Residues	Length
LFR1	Chothia	VLTQEPSLTVSPGGTVTLTC------	1 - 20	20
	AbM	VLTQEPSLTVSPGGTVTLTC------	1 - 20	20
	Kabat	VLTQEPSLTVSPGGTVTLTC------	1 - 20	20
	Contact	VLTQEPSLTVSPGGTVTLTCASSTGA	1 - 26	26
CDR-L1	Chothia	ASSTGAVTSGYYPN--	21 - 34	14
	AbM	ASSTGAVTSGYYPN--	21 - 34	14
	Kabat	ASSTGAVTSGYYPN--	21 - 34	14
	Contact	------VTSGYYPNWF	27 - 36	10
LFR2	Chothia	WFQQKPGQAPRALIY	35 - 49	15
	AbM	WFQQKPGQAPRALIY	35 - 49	15
	Kabat	WFQQKPGQAPRALIY	35 - 49	15
	Contact	--QQKPGQAPR----	37 - 45	9
CDR-L2	Chothia	----STSNKHS	50 - 56	7
	AbM	----STSNKHS	50 - 56	7
	Kabat	----STSNKHS	50 - 56	7
	Contact	ALIYSTSNKH-	46 - 55	10
LFR3	Chothia	-WTPARFSGSLLGGKAALTLSGVQPEDEAEYYC	57 - 88	32
	AbM	-WTPARFSGSLLGGKAALTLSGVQPEDEAEYYC	57 - 88	32
	Kabat	-WTPARFSGSLLGGKAALTLSGVQPEDEAEYYC	57 - 88	32
	Contact	SWTPARFSGSLLGGKAALTLSGVQPEDEAEYYC	56 - 88	33
CDR-L3	Chothia	LLYYGGAWV	89 - 97	9
	AbM	LLYYGGAWV	89 - 97	9
	Kabat	LLYYGGAWV	89 - 97	9
	Contact	LLYYGGAW-	89 - 96	8
LFR4	Chothia	-FGGGTKLTVL	98 - 107	10
	AbM	-FGGGTKLTVL	98 - 107	10
	Kabat	-FGGGTKLTVL	98 - 107	10
	Contact	VFGGGTKLTVL	97 - 107	11

Table 5: CloneE10 Heavy Chain CDR1, 2 and 3 amino acid sequences

Region	Definition	Sequence Fragment	Residues	Length
HFR1	Chothia	EVQLVQSGAEVKKPGASVEVSCKAS-----	1 - 25	25
	AbM	EVQLVQSGAEVKKPGASVEVSCKAS-----	1 - 25	25
	Kabat	EVQLVQSGAEVKKPGASVEVSCKASGYTFN	1 - 30	30
	Contact	EVQLVQSGAEVKKPGASVEVSCKASGYTF-	1 - 29	29
CDR-H1	Chothia	GYTFNAY---	26 - 32	7
	AbM	GYTFNAYYIH	26 - 35	10
	Kabat	-----AYYIH	31 - 35	5
	Contact	----NAYYIH	30 - 35	6
HFR2	Chothia	YIHWVRQAPGQGLDWMGWI	33 - 51	19
	AbM	---WVRQAPGQGLDWMG--	36 - 49	14
	Kabat	---WVRQAPGQGLDWMG--	36 - 49	14
	Contact	---WVRQAPGQGLD-----	36 - 46	11
CDR-H2	Chothia	-----NPNSGG---------	52 - 57	6
	AbM	---WINPNSGGTN-------	50 - 59	10
	Kabat	---WINPNSGGTNYAQNFKG	50 - 66	17
	Contact	WMGWINPNSGGTN-------	47 - 59	13
HFR3	Chothia	TNYAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	58 - 98	41
	AbM	--YAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	60 - 98	39
	Kabat	---------RVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	67 - 98	32
	Contact	--YAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFC--	60 - 96	37
CDR-H3	Chothia	--ERGYTSSFRRDAFDK	99 - 113	15
	AbM	--ERGYTSSFRRDAFDK	99 - 113	15
	Kabat	--ERGYTSSFRRDAFDK	99 - 113	15
	Contact	ATERGYTSSFRRDAFD-	97 - 112	16
HFR4	Chothia	-WGQGTLLTVSS	114 - 124	11
	AbM	-WGQGTLLTVSS	114 - 124	11
	Kabat	-WGQGTLLTVSS	114 - 124	11
	Contact	KWGQGTLLTVSS	113 - 124	12

Table 6: CloneE10 Light Chain CDR1, 2 and 3 amino acid sequences.

Region	Definition	Sequence Fragment	Residues	Length
LFR1	Chothia	ESVLTQPPSISAAPGQRVTIPC------	1 - 22	22
	AbM	ESVLTQPPSISAAPGQRVTIPC------	1 - 22	22
	Kabat	ESVLTQPPSISAAPGQRVTIPC------	1 - 22	22
	Contact	ESVLTQPPSISAAPGQRVTIPCSGSSSD	1 - 28	28
CDR-L1	Chothia	SGSSSDIGNHYVS--	23 - 35	13
	AbM	SGSSSDIGNHYVS--	23 - 35	13
	Kabat	SGSSSDIGNHYVS--	23 - 35	13
	Contact	------IGNHYVSWY	29 - 37	9
LFR2	Chothia	WYQQLPGAAPKLLIY	36 - 50	15
	AbM	WYQQLPGAAPKLLIY	36 - 50	15
	Kabat	WYQQLPGAAPKLLIY	36 - 50	15
	Contact	--QQLPGAAPK----	38 - 46	9
CDR-L2	Chothia	----EDNKRPS	51 - 57	7
	AbM	----EDNKRPS	51 - 57	7
	Kabat	----EDNKRPS	51 - 57	7
	Contact	LLIYEDNKRP-	47 - 56	10
LFR3	Chothia	-GIPDRFSGSKSGTSASLGITGLQTGDEADYYC	58 - 89	32
	AbM	-GIPDRFSGSKSGTSASLGITGLQTGDEADYYC	58 - 89	32
	Kabat	-GIPDRFSGSKSGTSASLGITGLQTGDEADYYC	58 - 89	32
	Contact	SGIPDRFSGSKSGTSASLGITGLQTGDEADYYC	57 - 89	33
CDR-L3	Chothia	GTWDNSLRSGF	90 - 100	11
	AbM	GTWDNSLRSGF	90 - 100	11
	Kabat	GTWDNSLRSGF	90 - 100	11
	Contact	GTWDNSLRSG-	90 - 99	10
LFR4	Chothia	-FGGGTKVTVLG	101 - 111	11
	AbM	-FGGGTKVTVLG	101 - 111	11
	Kabat	-FGGGTKVTVLG	101 - 111	11
	Contact	FFGGGTKVTVLG	100 - 111	12

Table 7: CloneB7 Heavy Chain CDR1, 2 and 3 amino acid sequences.

Region	Definition	Sequence Fragment	Residues	Length
HFR1	Chothia	EVQLVQSGAEVKKPGASVEVSCKAS-----	1 - 25	25
	AbM	EVQLVQSGAEVKKPGASVEVSCKAS-----	1 - 25	25
	Kabat	EVQLVQSGAEVKKPGASVEVSCKASGYTFN	1 - 30	30
	Contact	EVQLVQSGAEVKKPGASVEVSCKASGYTF-	1 - 29	29
CDR-H1	Chothia	GYTFNAY---	26 - 32	7
	AbM	GYTFNAYYIH	26 - 35	10
	Kabat	-----AYYIH	31 - 35	5
	Contact	----NAYYIH	30 - 35	6
HFR2	Chothia	YIHWVRQAPGQGLDWMGWI	33 - 51	19
	AbM	---WVRQAPGQGLDWMG--	36 - 49	14
	Kabat	---WVRQAPGQGLDWMG--	36 - 49	14
	Contact	---WVRQAPGQGLD-----	36 - 46	11
CDR-H2	Chothia	-----NPNSGG---------	52 - 57	6
	AbM	---WINPNSGGTN-------	50 - 59	10
	Kabat	---WINPNSGGTNYAQNFKG	50 - 66	17
	Contact	WMGWINPNSGGTN-------	47 - 59	13
HFR3	Chothia	TNYAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	58 - 98	41
	AbM	--YAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	60 - 98	39
	Kabat	---------RVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	67 - 98	32
	Contact	--YAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFC--	60 - 96	37
CDR-H3	Chothia	--ERGYTSSFRRDAFDK	99 - 113	15
	AbM	--ERGYTSSFRRDAFDK	99 - 113	15
	Kabat	--ERGYTSSFRRDAFDK	99 - 113	15
	Contact	ATERGYTSSFRRDAFD-	97 - 112	16
HFR4	Chothia	-WGQGTMLTVSS	114 - 124	11
	AbM	-WGQGTMLTVSS	114 - 124	11
	Kabat	-WGQGTMLTVSS	114 - 124	11
	Contact	KWGQGTMLTVSS	113 - 124	12

Table 8: CloneB7 Light Chain CDR1, 2 and 3 amino acid sequences

Region	Definition	Sequence Fragment	Residues	Length
LFR1	Chothia	ESVLTQPPSVSAAPGQKVTISC------	1 - 22	22
	AbM	ESVLTQPPSVSAAPGQKVTISC------	1 - 22	22
	Kabat	ESVLTQPPSVSAAPGQKVTISC------	1 - 22	22
	Contact	ESVLTQPPSVSAAPGQKVTISCSGSSSN	1 - 28	28
CDR-L1	Chothia	SGSSSNIGNNYVS--	23 - 35	13
	AbM	SGSSSNIGNNYVS--	23 - 35	13
	Kabat	SGSSSNIGNNYVS--	23 - 35	13
	Contact	------IGNNYVSWY	29 - 37	9
LFR2	Chothia	WYQQLPGTAPKLLIY	36 - 50	15
	AbM	WYQQLPGTAPKLLIY	36 - 50	15
	Kabat	WYQQLPGTAPKLLIY	36 - 50	15
	Contact	--QQLPGTAPK----	38 - 46	9
CDR-L2	Chothia	----ENNKRPS	51 - 57	7
	AbM	----ENNKRPS	51 - 57	7
	Kabat	----ENNKRPS	51 - 57	7
	Contact	LLIYENNKRP-	47 - 56	10
LFR3	Chothia	-GIPDRFSGSKSGTSATLGITGLQTGDEAGYYC	58 - 89	32
	AbM	-GIPDRFSGSKSGTSATLGITGLQTGDEAGYYC	58 - 89	32
	Kabat	-GIPDRFSGSKSGTSATLGITGLQTGDEAGYYC	58 - 89	32
	Contact	SGIPDRFSGSKSGTSATLGITGLQTGDEAGYYC	57 - 89	33
CDR-L3	Chothia	GTWDSSLSAVV	90 - 100	11
	AbM	GTWDSSLSAVV	90 - 100	11
	Kabat	GTWDSSLSAVV	90 - 100	11
	Contact	GTWDSSLSAV-	90 - 99	10
LFR4	Chothia	-FGGGTKLTVLG	101 - 111	11
	AbM	-FGGGTKLTVLG	101 - 111	11
	Kabat	-FGGGTKLTVLG	101 - 111	11
	Contact	VFGGGTKLTVLG	100 - 111	12

Table 9: CloneD7 Heavy Chain CDR1, 2 and 3 amino acid sequences.

Region	Definition	Sequence Fragment	Residues	Length
HFR1	Chothia	EVQLVQSGAEVKKPGASVEVSCKAS-----	1 - 25	25
	AbM	EVQLVQSGAEVKKPGASVEVSCKAS-----	1 - 25	25
	Kabat	EVQLVQSGAEVKKPGASVEVSCKASGYTFN	1 - 30	30
	Contact	EVQLVQSGAEVKKPGASVEVSCKASGYTF-	1 - 29	29
CDR-H1	Chothia	GYTFNAY---	26 - 32	7
	AbM	GYTFNAYYIH	26 - 35	10
	Kabat	-----AYYIH	31 - 35	5
	Contact	----NAYYIH	30 - 35	6
HFR2	Chothia	YIHWVRQAPGQGLDWMGWI	33 - 51	19
	AbM	---WVRQAPGQGLDWMG--	36 - 49	14
	Kabat	---WVRQAPGQGLDWMG--	36 - 49	14
	Contact	---WVRQAPGQGLD-----	36 - 46	11
CDR-H2	Chothia	-----NPNSGG---------	52 - 57	6
	AbM	---WINPNSGGTN-------	50 - 59	10
	Kabat	---WINPNSGGTNYAQNFKG	50 - 66	17
	Contact	WMGWINPNSGGTN-------	47 - 59	13
HFR3	Chothia	TNYAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	58 - 98	41
	AbM	--YAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	60 - 98	39
	Kabat	---------RVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	67 - 98	32
	Contact	--YAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFC--	60 - 96	37
CDR-H3	Chothia	--ERGYTSSFRRDAFDK	99 - 113	15
	AbM	--ERGYTSSFRRDAFDK	99 - 113	15
	Kabat	--ERGYTSSFRRDAFDK	99 - 113	15
	Contact	ATERGYTSSFRRDAFD-	97 - 112	16
HFR4	Chothia	-WGQGTMLTVSS	114 - 124	11
	AbM	-WGQGTMLTVSS	114 - 124	11
	Kabat	-WGQGTMLTVSS	114 - 124	11
	Contact	KWGQGTMLTVSS	113 - 124	12

Table 10: CloneD7 Light Chain CDR1, 2 and 3 amino acid sequences.

Region	Definition	Sequence Fragment	Residues	Length
LFR1	Chothia	ESVLTQPPSVSAAPGQKVTMSC------	1 - 22	22
	AbM	ESVLTQPPSVSAAPGQKVTMSC------	1 - 22	22
	Kabat	ESVLTQPPSVSAAPGQKVTMSC------	1 - 22	22
	Contact	ESVLTQPPSVSAAPGQKVTMSCSGSTSN	1 - 28	28
CDR-L1	Chothia	SGSTSNIGSNSVS--	23 - 35	13
	AbM	SGSTSNIGSNSVS--	23 - 35	13
	Kabat	SGSTSNIGSNSVS--	23 - 35	13
	Contact	------IGSNSVSWY	29 - 37	9
LFR2	Chothia	WYQHLPGTAPKLLLF	36 - 50	15
	AbM	WYQHLPGTAPKLLLF	36 - 50	15
	Kabat	WYQHLPGTAPKLLLF	36 - 50	15
	Contact	--QHLPGTAPK----	38 - 46	9
CDR-L2	Chothia	----DNAKRPS	51 - 57	7
	AbM	----DNAKRPS	51 - 57	7
	Kabat	----DNAKRPS	51 - 57	7
	Contact	LLLFDNAKRP-	47 - 56	10
LFR3	Chothia	-GIPDRFSGSKSGTSATLGITGLQTGDEADYYC	58 - 89	32
	AbM	-GIPDRFSGSKSGTSATLGITGLQTGDEADYYC	58 - 89	32
	Kabat	-GIPDRFSGSKSGTSATLGITGLQTGDEADYYC	58 - 89	32
	Contact	SGIPDRFSGSKSGTSATLGITGLQTGDEADYYC	57 - 89	33
CDR-L3	Chothia	GTWDSGLSVMV	90 - 100	11
	AbM	GTWDSGLSVMV	90 - 100	11
	Kabat	GTWDSGLSVMV	90 - 100	11
	Contact	GTWDSGLSVM-	90 - 99	10
LFR4	Chothia	-FGGGTKLTVLG	101 - 111	11
	AbM	-FGGGTKLTVLG	101 - 111	11
	Kabat	-FGGGTKLTVLG	101 - 111	11
	Contact	VFGGGTKLTVLG	100 - 111	12

Table 11: CloneC10 Heavy Chain CDR1, 2 and 3 amino acid sequences.

Region	Definition	Sequence Fragment	Residues	Length
HFR1	Chothia	EVQLVQSGAEVKKPGASVKVSCKAS-----	1 - 25	25
	AbM	EVQLVQSGAEVKKPGASVKVSCKAS-----	1 - 25	25
	Kabat	EVQLVQSGAEVKKPGASVKVSCKASGYTFN	1 - 30	30
	Contact	EVQLVQSGAEVKKPGASVKVSCKASGYTF-	1 - 29	29
CDR-H1	Chothia	GYTFNAY---	26 - 32	7
	AbM	GYTFNAYYIH	26 - 35	10
	Kabat	-----AYYIH	31 - 35	5
	Contact	----NAYYIH	30 - 35	6
HFR2	Chothia	YIHWVRQAPGQGLDWMGWI	33 - 51	19
	AbM	---WVRQAPGQGLDWMG--	36 - 49	14
	Kabat	---WVRQAPGQGLDWMG--	36 - 49	14
	Contact	---WVRQAPGQGLD-----	36 - 46	11
CDR-H2	Chothia	-----NPNSGG---------	52 - 57	6
	AbM	---WINPNSGGTN-------	50 - 59	10
	Kabat	---WINPNSGGTNYAQNFKG	50 - 66	17
	Contact	WMGWINPNSGGTN-------	47 - 59	13
HFR3	Chothia	TNYAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	58 - 98	41
	AbM	--YAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	60 - 98	39
	Kabat	---------RVTMTRDTSISTAYLELRSLTSDDTAVYFCAT	67 - 98	32
	Contact	--YAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFC--	60 - 96	37
CDR-H3	Chothia	--ERGYTSSFRRDAFDK	99 - 113	15
	AbM	--ERGYTSSFRRDAFDK	99 - 113	15
	Kabat	--ERGYTSSFRRDAFDK	99 - 113	15
	Contact	ATERGYTSSFRRDAFD-	97 - 112	16
HFR4	Chothia	-WGQGTMLTVSS	114 - 124	11
	AbM	-WGQGTMLTVSS	114 - 124	11
	Kabat	-WGQGTMLTVSS	114 - 124	11
	Contact	KWGQGTMLTVSS	113 - 124	12

Table 12: CloneC10 Light Chain CDR1, 2 and 3 amino acid sequences.

Region	Definition	Sequence Fragment	Residues	Length
LFR1	Chothia	EAVVTQPPSASGTPGQGVTISC------	1 - 22	22
	AbM	EAVVTQPPSASGTPGQGVTISC------	1 - 22	22
	Kabat	EAVVTQPPSASGTPGQGVTISC------	1 - 22	22
	Contact	EAVVTQPPSASGTPGQGVTISCSGSSSN	1 - 28	28
CDR-L1	Chothia	SGSSSNIGSNFVY--	23 - 35	13
	AbM	SGSSSNIGSNFVY--	23 - 35	13
	Kabat	SGSSSNIGSNFVY--	23 - 35	13
	Contact	------IGSNFVYWY	29 - 37	9
LFR2	Chothia	WYQQLPGTAPKLLIY	36 - 50	15
	AbM	WYQQLPGTAPKLLIY	36 - 50	15
	Kabat	WYQQLPGTAPKLLIY	36 - 50	15
	Contact	--QQLPGTAPK----	38 - 46	9
CDR-L2	Chothia	----RNNQRPS	51 - 57	7
	AbM	----RNNQRPS	51 - 57	7
	Kabat	----RNNQRPS	51 - 57	7
	Contact	LLIYRNNQRP-	47 - 56	10
LFR3	Chothia	-GVPDRFSGSKSATSASLAISGLRSEDEADYYC	58 - 89	32
	AbM	-GVPDRFSGSKSATSASLAISGLRSEDEADYYC	58 - 89	32
	Kabat	-GVPDRFSGSKSATSASLAISGLRSEDEADYYC	58 - 89	32
	Contact	SGVPDRFSGSKSATSASLAISGLRSEDEADYYC	57 - 89	33
CDR-L3	Chothia	ATWDDSLSGYV	90 - 100	11
	AbM	ATWDDSLSGYV	90 - 100	11
	Kabat	ATWDDSLSGYV	90 - 100	11
	Contact	ATWDDSLSGY-	90 - 99	10
LFR4	Chothia	-FGTGTKLTVLG	101 - 111	11
	AbM	-FGTGTKLTVLG	101 - 111	11
	Kabat	-FGTGTKLTVLG	101 - 111	11
	Contact	VFGTGTKLTVLG	100 - 111	12

CLAIMS

1. A recombinant, human or humanized antibody or antibody fragment that is capable of at least partly preventing or inhibiting Epstein Barr Virus (EBV) gp350 binding to a human cell.

2. The recombinant human or humanized antibody or antibody fragment of Claim 1, produced by phage display wherein the phage comprise one or more nucleotide sequences of human origin encoding one or more amino acid sequences of the human or humanized antibody or antibody fragment.

3. The recombinant human or humanized antibody or antibody fragment of Claim 1 or Claim 2 which comprises, consists essentially of or consists of at least one complementarity determining region (CDR) amino acid sequence according to any one of SEQ ID NOS:1-156, FIG. 5 and/or Tables 1-12 or an amino acid sequence at least 70% identical thereto.

4. The recombinant human or humanized antibody or antibody fragment of Claim 1, Claim 2 or Claim 3, which comprises, consists essentially of or consists of an amino acid sequence set forth in any one of SEQ ID NOS:145-156, FIG. 5 and/or an amino acid sequence at least 70% identical thereto.

5. The recombinant, human or humanized antibody or antibody fragment of any one of Claims 1-4, further comprising a human IgG1 constant region amino acid sequence.

6. An antibody or antibody fragment comprising at least one CDR amino acid sequence according to any one of SEQ ID NOS:1-156, FIG. 5 and/or Tables 1-12 or an amino acid sequence at least 70% identical thereto.

7. The antibody or antibody fragment of Claim 6, which comprises, consists essentially of or consists of an amino acid sequence set forth in any one of SEQ ID NOS:145-156 and/or FIG. 5.

8. The antibody or antibody fragment of Claim 6 or Claim 7, produced by phage display wherein the phage comprise one or more nucleotide sequences of human origin encoding one or more amino acid sequences of the antibody or antibody fragment.

9. A recombinant, human or humanized antibody or antibody fragment according to any one of Claim 1-5, or an antibody or antibody fragment according to Claim 6 or Claim 7 or Claim 8, which is capable of at least partly preventing or inhibiting Epstein Barr Virus (EBV) gp350 binding to a human cell.

10. A recombinant, human or humanized antibody or antibody fragment according to any one of Claim 1-5 or 9, or an antibody or antibody fragment according to any one of Claims 6-9, which is capable of at least partly blocking entry of EBV into a human cell.

11. An isolated nucleic acid encoding a recombinant, human or humanized antibody or antibody fragment according to any one of Claim 1-5, 9 or 10, or an antibody or antibody fragment according to any one of Claims 6-10.

12. A genetic construct comprising the isolated nucleic acid of Claim 11.

13. A host cell comprising the genetic construct of Claim 12.

14. A composition comprising a recombinant, human or humanized antibody or antibody fragment according to any one of Claim 1-5, 9 or 10, or an antibody or antibody fragment according to any one of Claims 6-10, and a pharmaceutically acceptable carrier diluent or excipient.

15. A method of treating or preventing an EBV infection in a human, said method including the step of administering a recombinant, human or humanized antibody or antibody fragment according to any one of Claim 1-5, 9 or 10, or an antibody or antibody fragment according to any one of Claims 6-10, or the composition of Claim 14 to the human to thereby treat or prevent an EBV infection in the human.

16. A method of passively immunizing a human against an EBV infection, said method including the step of administering a recombinant, human or humanized antibody or antibody fragment according to any one of Claim 1-5, 9 or 10, or an antibody or antibody fragment according to any one of Claims 6-10, or the composition of Claim 14 to the human, thereby passively immunizing the human against an EBV infection.

17. A method of at least partly inhibiting or preventing EBV gp350 binding to a human cell, said method including the step of administering a recombinant, human humanized antibody or antibody fragment according to any one of Claim 1-5, 9 or 10, or an antibody or antibody fragment according to any one of Claims 6-10, or the composition of Claim 14 to the human to thereby at least partly inhibit or prevent EBV gp350 binding to a human cell.

18. The method of Claim 17, which at least partly blocks EBV entry into the human cell.

19. A method or system for determining the efficacy of an anti-EBV antibody or antibody fragment, said method or system comprising infecting a mouse with EBV, the mouse comprising human B lymphocytes, and determining the efficacy of a candidate anti-EBV antibody or antibody fragment in the mouse.

20. The method or system of Claim 19, wherein efficacy relates to, or includes, the ability of the candidate anti-EBV antibody or antibody fragment to prevent EBV gp350 binding to one or more of the B lymphocytes.

21. The method or system of Claim 20, wherein the human B lymphocytes have been derived from human hematopoietic stem cells administered to the mouse.

22. The method or system of Claim 21, wherein the human hematopoietic stem cells are $CD34^+$.

23. The method or system of any one of Claims 18-21, wherein the mouse is an irradiated adult NOD scid mouse.

24. A method of detecting EBVgp350 or a cell expressing EBVgp350, said method including the step of forming a complex between a recombinant, human or humanized antibody or antibody fragment according to any one of Claim 1-5, 9 or 10, or an antibody or antibody fragment according to any one of Claims 6-10 and EBV gp350 to thereby detect EBVgp350 or the cell expressing EBVgp350.

25. An isolated protein comprising an amino acid sequence set forth in any one of SEQ ID NOS:1-156, Tables 1-12 and/or FIG. 5.

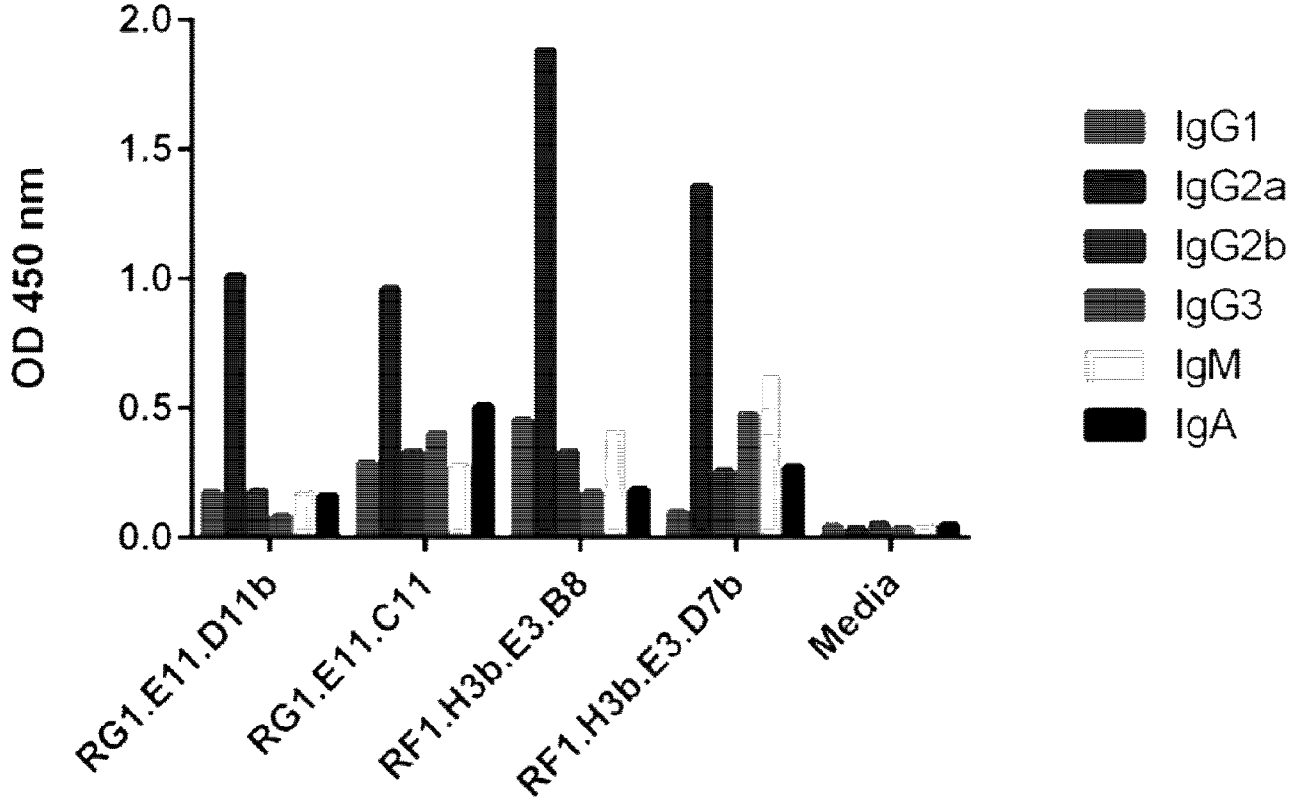
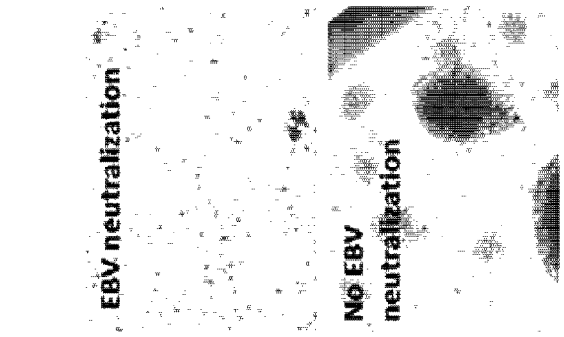

OD 450 nm
2.0
1.5
1.0
0.5
0.0
RG1.E11.D11b
RG1.E11.C11
RF1.H3b.E3.B8
RF1.H3b.E3.D7b
Media
IgG1
IgG2a
IgG2b
IgG3
IgM
IgA

Figure 1



10ug/mL each

Neat	100	0	75	75	75	100
1/2	100	0	100	100	100	100
1/4	100	0	100	100	100	100
1/8	100	0	100	100	100	100
1/16	100	0	100	100	100	100
1/32	100	50	100	100	100	100
1/64	100	50	100	100	100	100
1/128	100	100	100	100	100	100

100 = 4/4 wells transformed

Figure 2

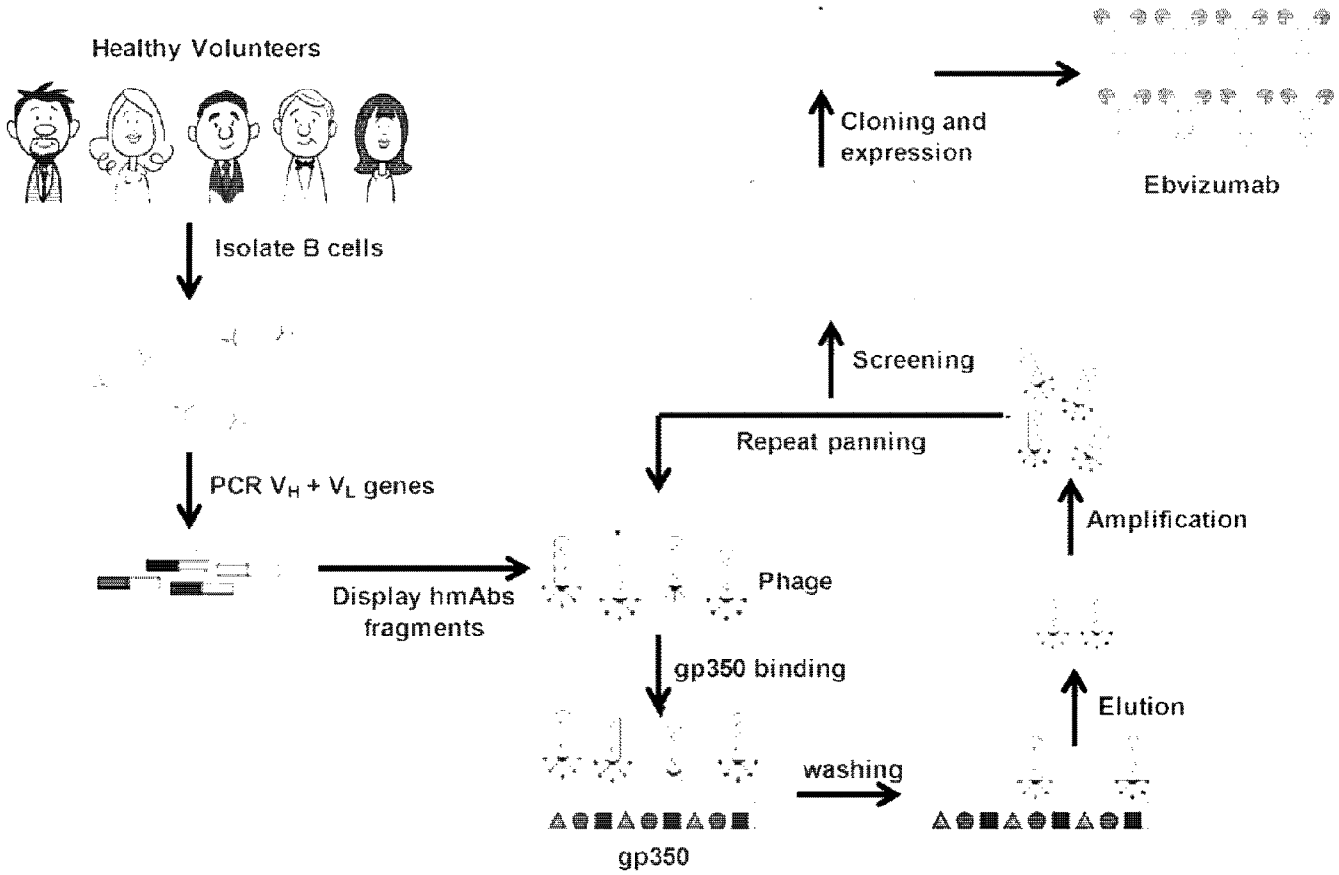

Healthy Volunteers
Isolate B cells
PCR V_H + V_L genes
Display hmAbs fragments
Phage
gp350 binding
gp350
washing
Elution
Amplification
Screening
Repeat panning
Cloning and expression
Ebvizumab

Figure 3

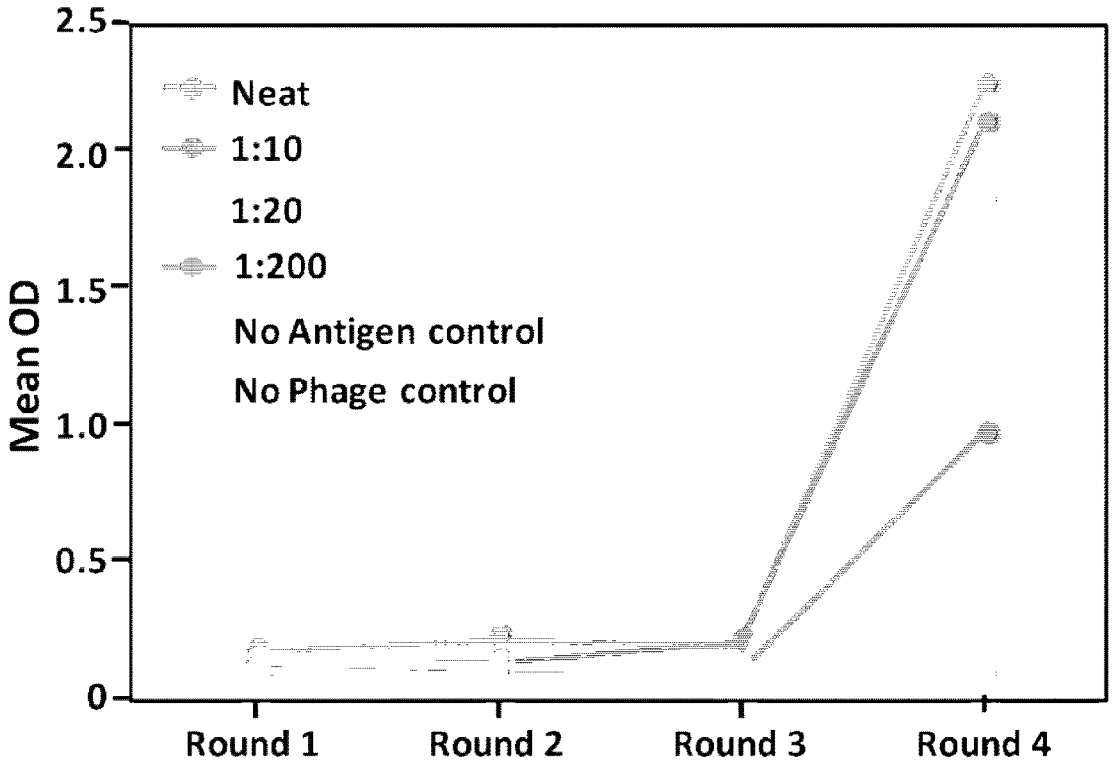

2.5
2.0
1.5
1.0
0.5
0
Mean OD
Neat
1:10
1:20
1:200
No Antigen control
No Phage control
Round 1
Round 2
Round 3
Round 4

Figure 4

```
                Signal Sequence
B8_HC           MGWSCIILFLVATATGVHSEVQLVESAGEVQKPGESLRISCKASGYTFSHYWIGWVRQLP
A11_HC          MGWSCIILFLVATATGVHSEMQLVQSGAEVKKPGASVKVSCKTSGYNFNHQGISWLRQAP
E10_HC          MGWSCIILFLVATATGVHSEVQLVQSGAEVKKPGASVEVSCKASGYTFNAYYIHWVRQAP
B7_HC           MGWSCIILFLVATATGVHSEVQLVQSGAEVKKPGASVEVSCKASGYTFNAYYIHWVRQAP
D7_HC           MGWSCIILFLVATATGVHSEVQLVQSGAEVKKPGASVEVSCKASGYTFNAYYIHWVRQAP
C10_HC          MGWSCIILFLVATATGVHSEVQLVQSGAEVKKPGASVKVSCKASGYTFNAYYIHWVRQAP
                ********************:***:*..**:*** *:.:***:***.*.   * *:** *

B8_HC           GKGLEWMGIIYPDDSDSRYSPSFQGQVTMSVDKSINTAYLQWNSLKVSDTATYYCVRHWL
A11_HC          GQGLEWMGWISGFNGKTNYAQKFQGRVTMTADRSTTTAYMELRSLRSDDTAVYYCASGG-
E10_HC          GQGLDWMGWINPNSGGTNYAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCATERG
B7_HC           GQGLDWMGWINPNSGGTNYAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCATERG
D7_HC           GQGLDWMGWINPNSGGTNYAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCATERG
C10_HC          GQGLDWMGWINPNSGGTNYAQNFKGRVTMTRDTSISTAYLELRSLTSDDTAVYFCATERG
                *:**:*** *    .. :.*: .*:*:***: * * .***:: .**   .***.*:*.

B8_HC           KRGSNFGFGFDPWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
A11_HC          --EQWLVQNFVHWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
E10_HC          YTSSFRRDAFDKWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
B7_HC           YTSSFRRDAFDKWGQGTMLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
D7_HC           YTSSFRRDAFDKWGQGTMLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
C10_HC          YTSSFRRDAFDKWGQGTMLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
                *  ***** ****************************************

B8_HC           TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
A11_HC          TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
E10_HC          TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
B7_HC           TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
D7_HC           TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
C10_HC          TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
                ************************************************************

B8_HC           VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
A11_HC          VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
E10_HC          VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
B7_HC           VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
D7_HC           VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
C10_HC          VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
                ************************************************************

B8_HC           FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
A11_HC          FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
E10_HC          FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
B7_HC           FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
D7_HC           FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
C10_HC          FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
                ************************************************************

B8_HC           TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
A11_HC          TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
E10_HC          TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
B7_HC           TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
D7_HC           TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
C10_HC          TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
                ************************************************************

B8_HC           PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
A11_HC          PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
E10_HC          PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
B7_HC           PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
D7_HC           PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
C10_HC          PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
                ***************************************************
```

Figure 5A

```
                    Signal Sequence
A11_LC(Lambda)      MGWSCIILFLVATATGVHSETVLTQEPSLTVSPGGTVTLTCASSTGAVTSGYYPNWFQQK
B8_LC(Lambda)       MGWSCIILFLVATATGVHSESALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQH
C10_LC (Lambda)     MGWSCIILFLVATATGVHSEAVVTQPPSASGTPGQGVTISCSGSSSNIG-SNFVYWYQQL
E10_LC(Lambda)      MGWSCIILFLVATATGVHSESVLTQPPSISAAPGQRVTIPCSGSSSDIG-NHYVSWYQQL
B7_LC(Lambda)       MGWSCIILFLVATATGVHSESVLTQPPSVSAAPGQKVTISCSGSSSNIG-NNYVSWYQQL
D7_LC(Lambda)       MGWSCIILFLVATATGVHSESVLTQPPSVSAAPGQKVTMSCSGSTSNIG-SNSVSWYQHL
                    ********************:.:**  * : :**  :*: *:.::. :       *:*:

A11_LC(Lambda)      PGQAPRALIYSTSNKHSWTPARFSGSLLGGKAALTLSGVQPEDEAEYYCLLYYGG--AWV
B8_LC(Lambda)       PGKAPKLMIYDVSNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYCSSYTSS-STLV
C10_LC(Lambda)      PGTAPKLLIYRNNQRPSGVPDRFSGSKSATSASLAISGLRSEDEADYYCATWDDSLSGYV
E10_LC(Lambda)      PGAAPKLLIYEDNKRPSGIPDRFSGSKSGTSASLGITGLQTGDEADYYCGTWDNSLRSGF
B7_LC(Lambda)       PGTAPKLLIYENNKRPSGIPDRFSGSKSGTSATLGITGLQTGDEAGYYCGTWDSSLSAVV
D7_LC(Lambda)       PGTAPKLLLFDNAKRPSGIPDRFSGSKSGTSATLGITGLQTGDEADYYCGTWDSGLSVMV
                    ** **: :::   :: *    *****  . .*:* ::*::  *** ***  :  .    .

A11_LC(Lambda)      FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
B8_LC(Lambda)       FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
C10_LC (Lambda)     FGTGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
E10_LC (Lambda)     FGGGTKVTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
B7_LC(Lambda)       FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
D7_LC (Lambda)      FGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
                    ** ***:**************************************************

A11_LC(Lambda)      AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS-
B8_LC(Lambda)       AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS*
C10_LC(Lambda)      AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS-
E10_LC(Lambda)      AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS-
B7_LC(Lambda)       AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS-
D7_LC(Lambda)       AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS-
                    ************************************************************
```

Figure 5B

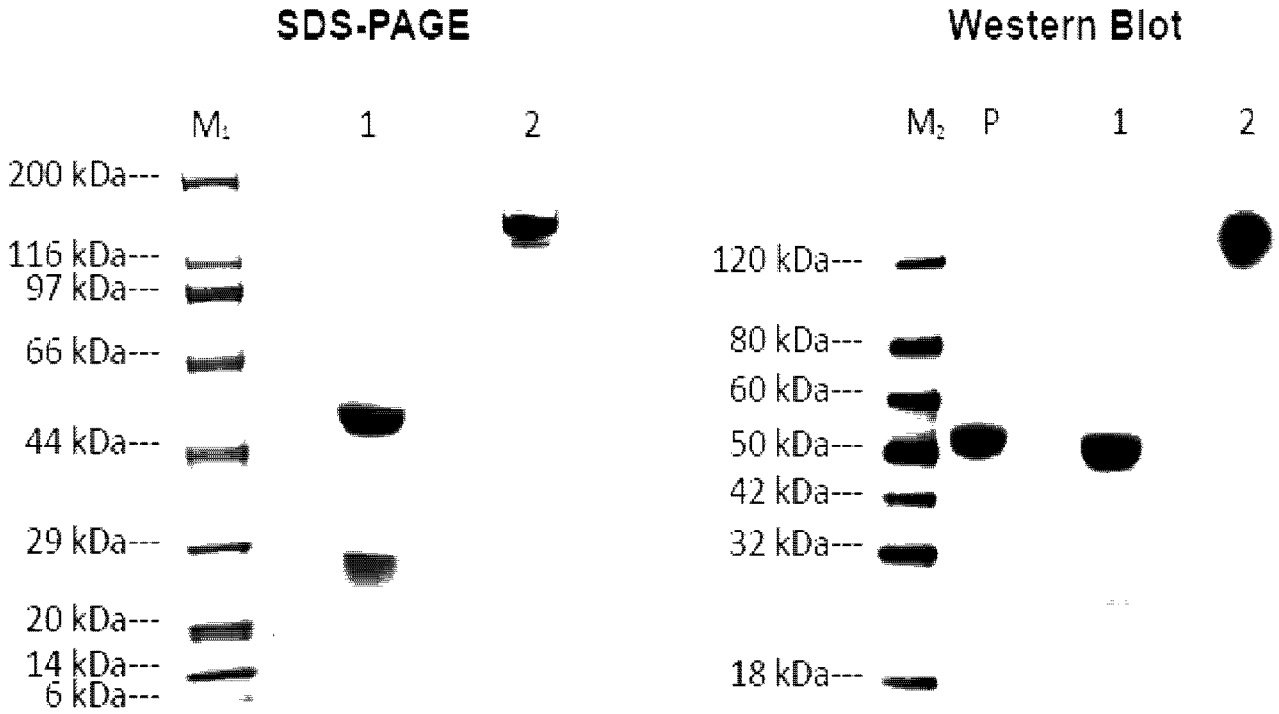
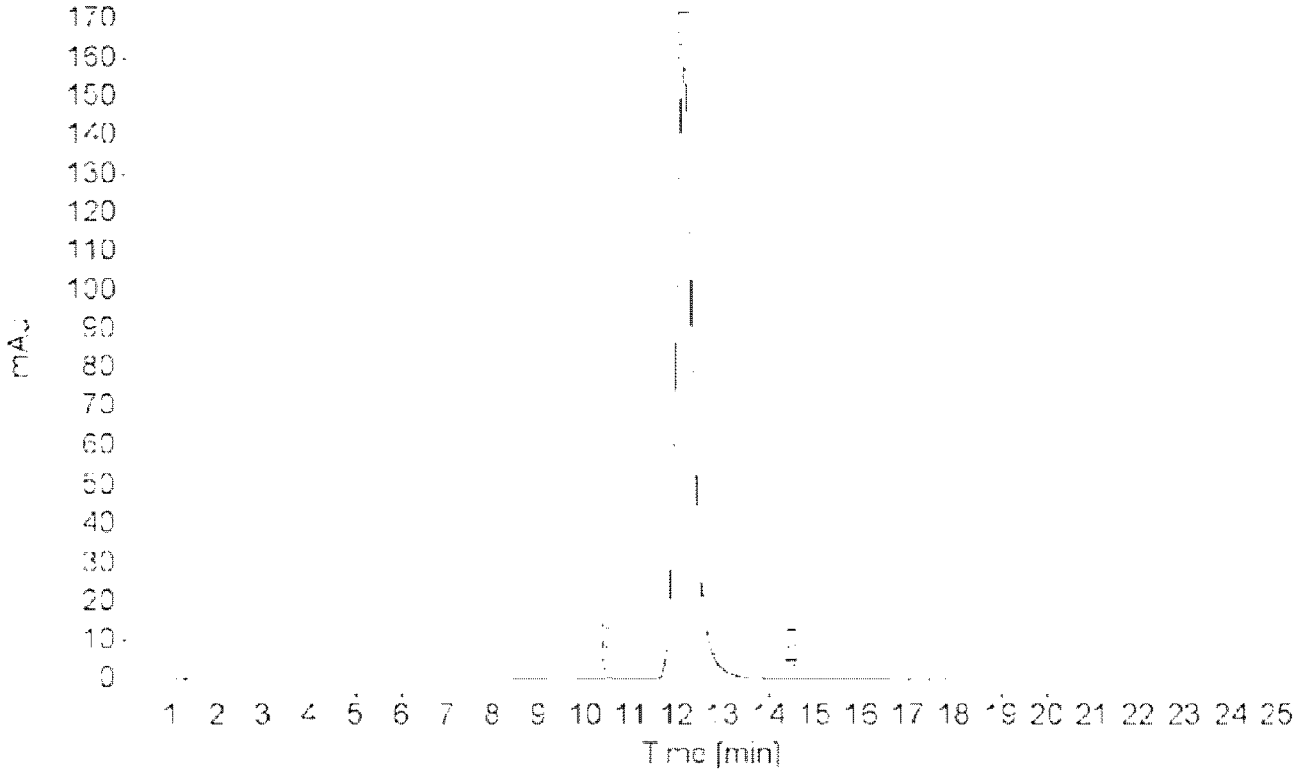

SDS-PAGE
M1
1
2
200 kDa---
116 kDa---
97 kDa---
66 kDa---
44 kDa---
29 kDa---
20 kDa---
14 kDa---
6 kDa---
Western Blot
M2
P
1
2
120 kDa---
80 kDa---
60 kDa---
50 kDa---
42 kDa---
32 kDa---
18 kDa---

Figure 6A



VWD1 A, Wavelength=280 nm

RT [min]	Height	Width [min]	Area	Area%
10.516	0.3292	1.1675	22.9863	0.5422
12.131	156.9809	0.4434	4205.4116	99.1935
14.486	0.1659	1.1256	11.2070	0.2643
		Sum	4239.6049	

Figure 6B

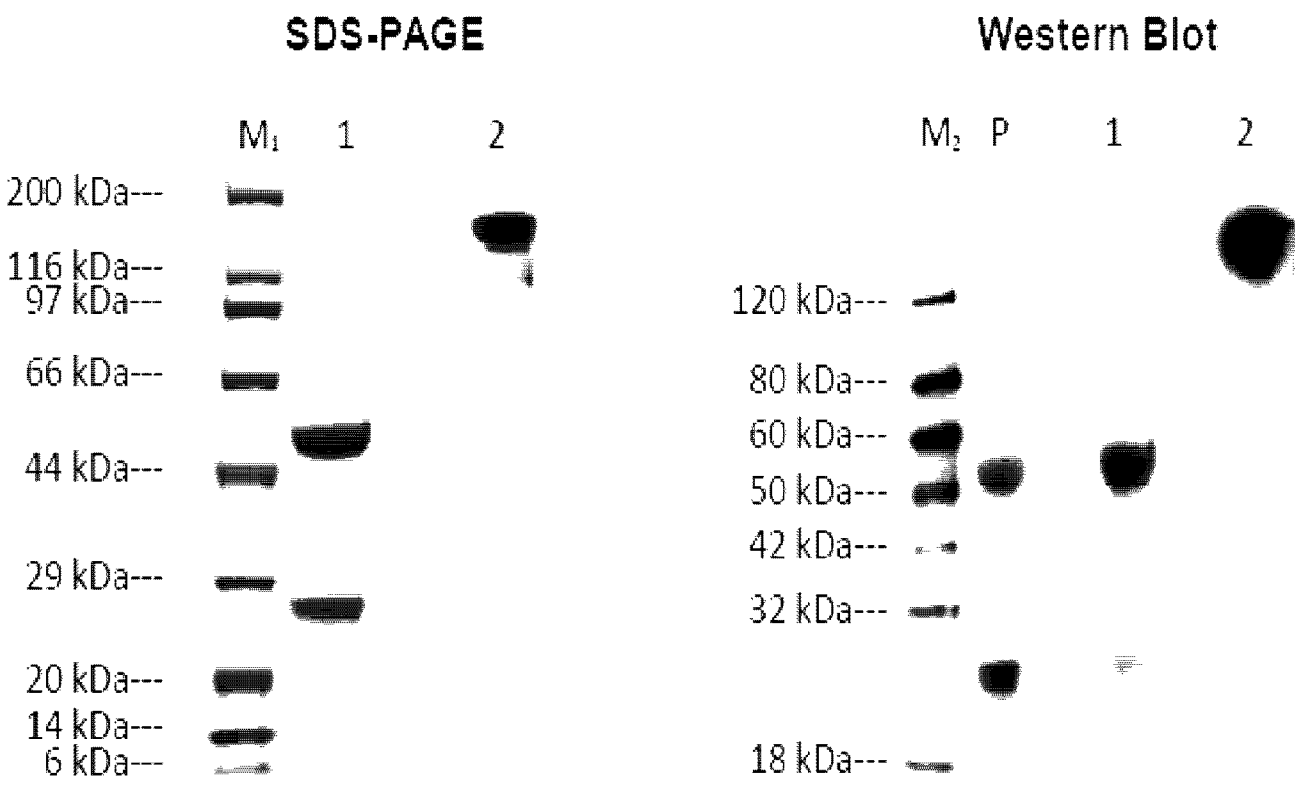
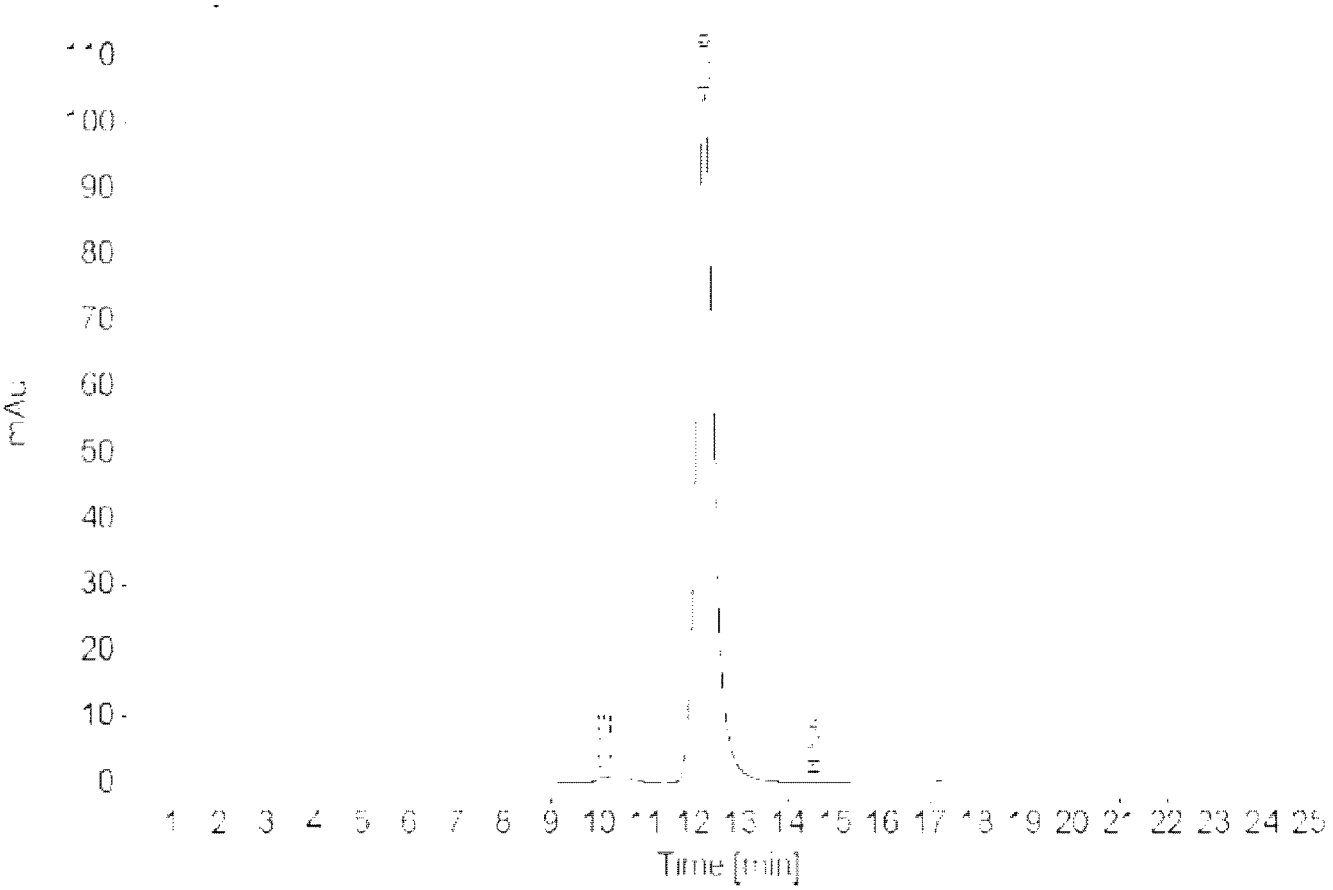

SDS-PAGE
M1
1
2
200 kDa---
116 kDa---
97 kDa---
66 kDa---
44 kDa---
29 kDa---
20 kDa---
14 kDa---
6 kDa---
Western Blot
M2
P
1
2
120 kDa---
80 kDa---
60 kDa---
50 kDa---
42 kDa---
32 kDa---
18 kDa---

Figure 7A



VWD1 A, Wavelength=280 nm

RT [min]	Height	Width [min]	Area	Area%
10.123	0.9797	0.7958	46.7904	1.6633
12.209	103.9365	0.4432	2763.7512	98.2463
14.536	0.0712	0.6017	2.5710	0.0914
		Sum	2813.1126	

Figure 7B

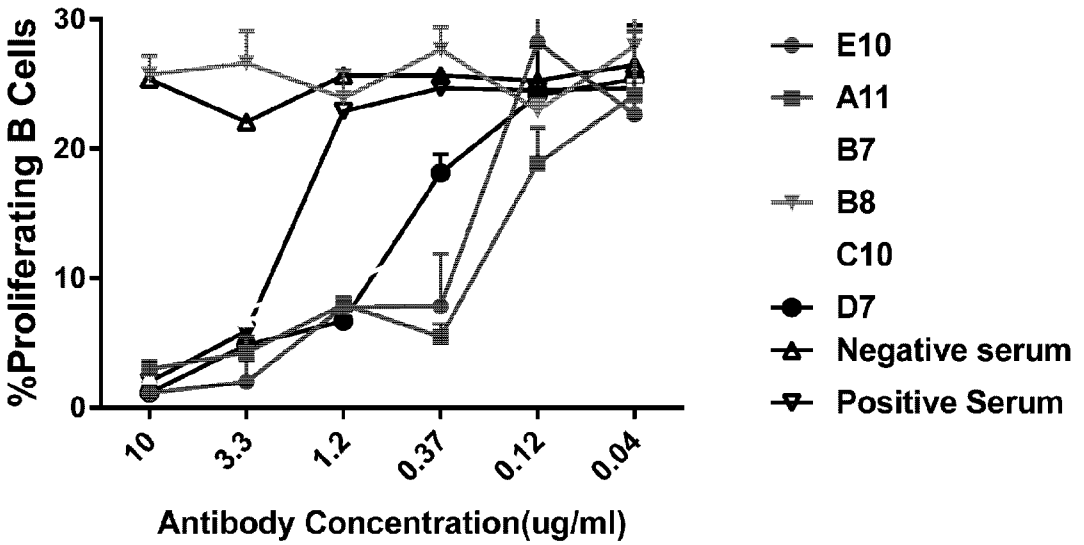


Figure 8

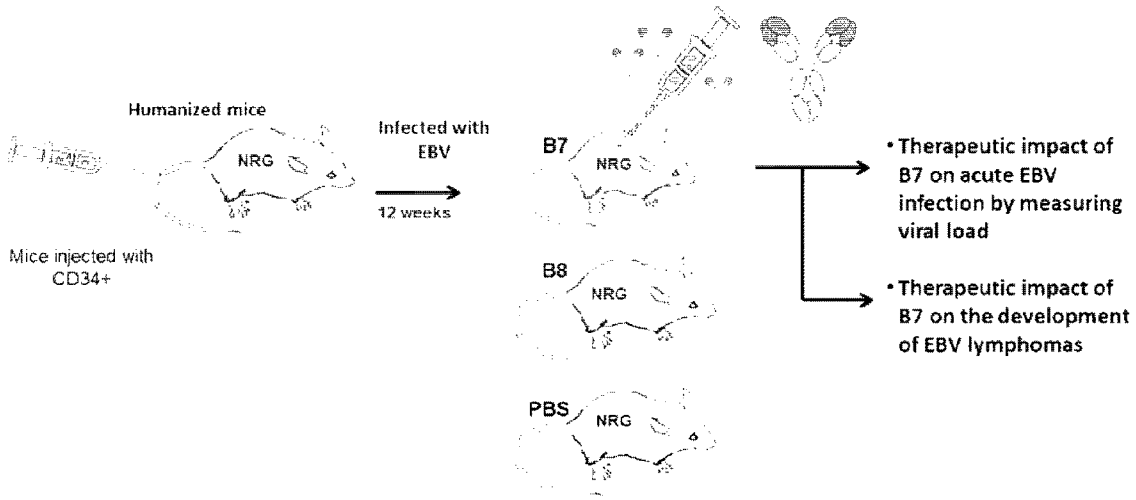


Figure 9

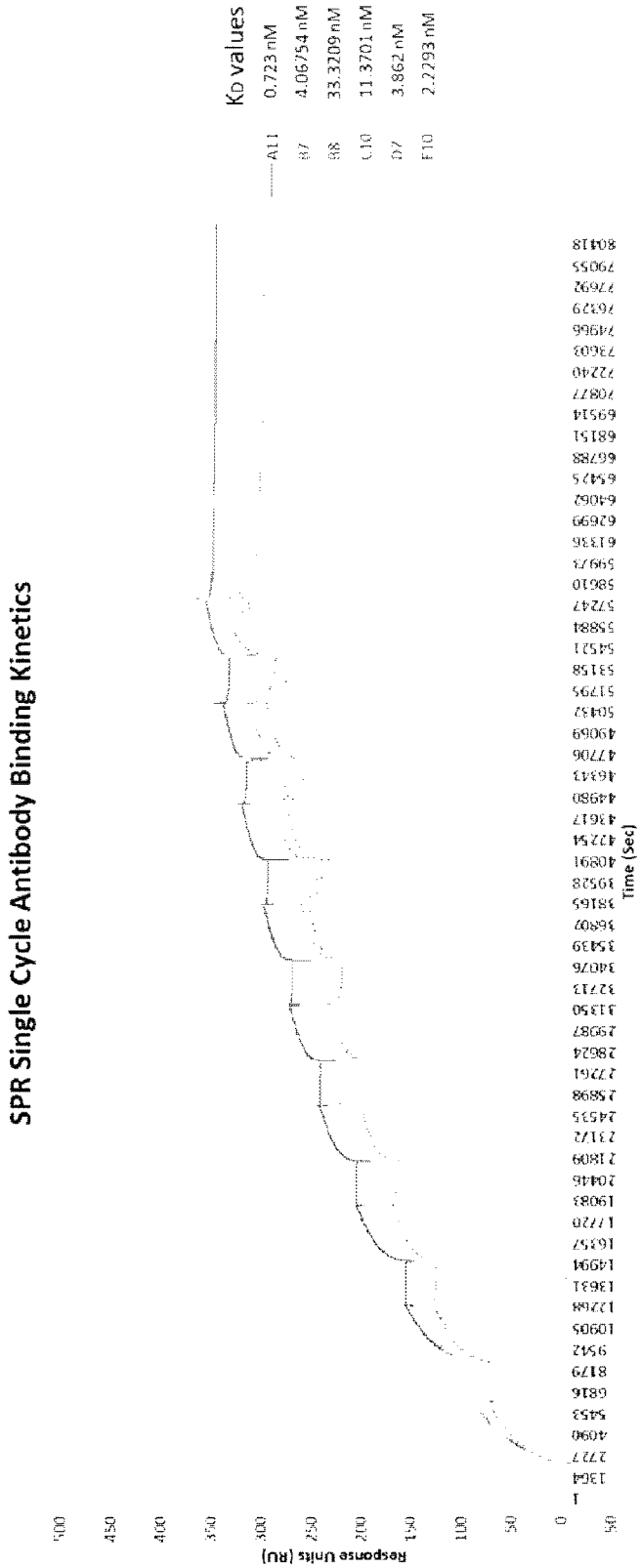

SPR Single Cycle Antibody Binding Kinetics
KD values
A11 0.723 nM
4.06754 nM
33.3209 nM
11.3701 nM
3.862 nM
2.2293 nM
Response Units (RU)
Time (Sec)

Figure 10

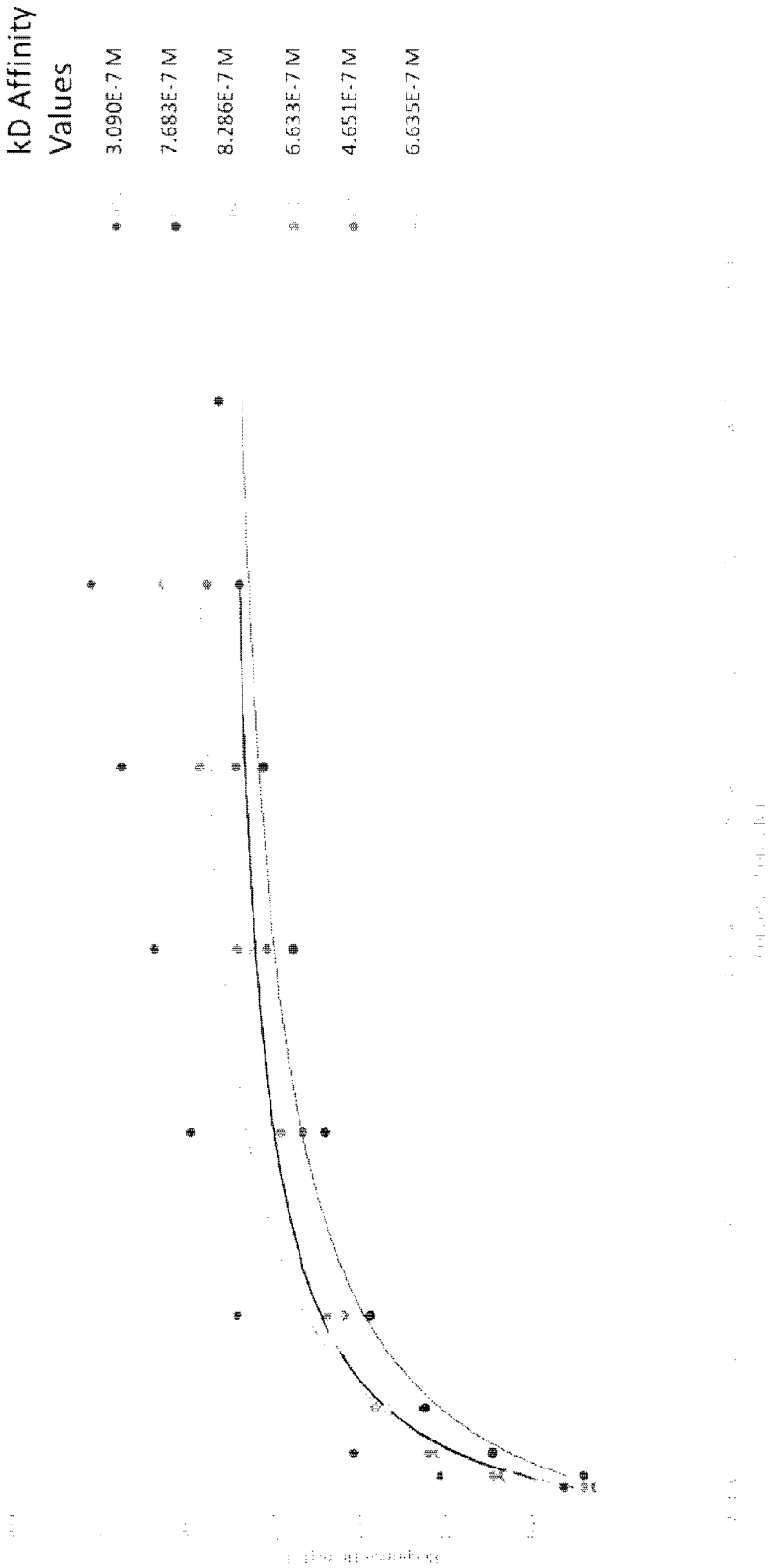

kD Affinity Values
3.090E-7 M
7.683E-7 M
8.286E-7 M
6.633E-7 M
4.651E-7 M
6.635E-7 M

Figure 11

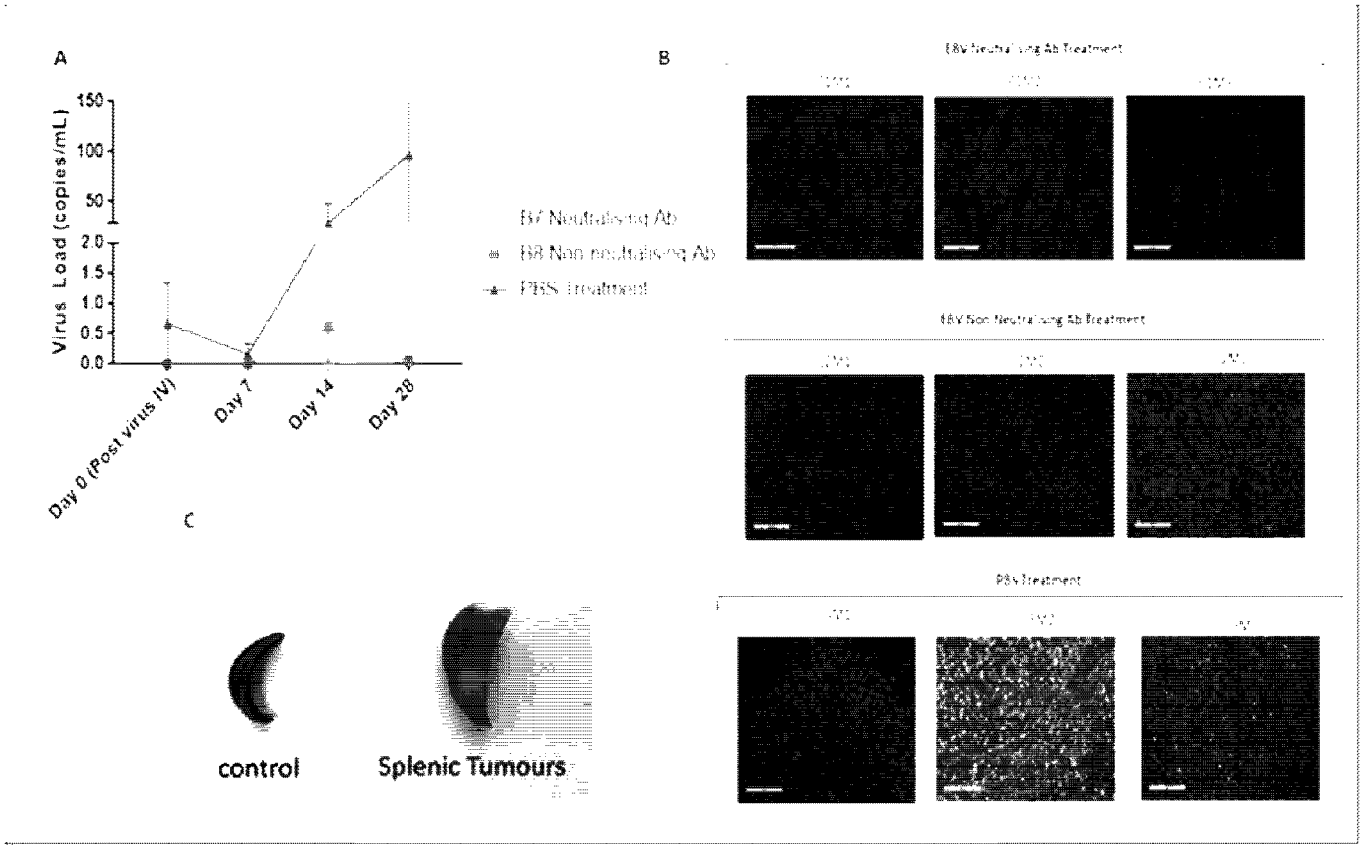

A
Virus Load (copies/mL)
150
100
50
2.0
1.5
1.0
0.5
0.0
Day 0 (Post virus IV)
Day 7
Day 14
Day 28
B
C
control
Splenic Tumours

Figure 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2018/050851

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395 (2006.01) A61P 31/22 (2006.01) C07K 16/08 (2006.01) G01N 33/563 (2006.01) C12N 15/13 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: PATENW (all English language databases), $Abs (TI, AB, TXT, IW, AW), HCaplus, Embase, Biosis, Medline, Genomequest, Espacenet, Patentscope, Auspat, PubMed, IP Australia internal databases

Search terms: Epstein Barr Virus, EBV, glycoprotein 350, gp350, antibody, immunoglobulin, FAB fragment, scFv, antigen binding protein, humanize, human, inhbit, decrease, suppress, block (and related terms). SEQ ID NOs 1-156. KHANNA Rajiv, HUA Kristine (inventor search). Queensland Institute of Medical Research (applicant search).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 2018	31 October 2018
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Safiea Goolam AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832795

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2018/050851**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/130565 A1 (THE BRIGHAM AND WOMEN'S HOSPITAL, INC.) 06 September 2013 Abstract; claim 1; figure 6; [0007]; [000140]; [00045]; [00060]; Example 1 at [000126]; claims 8-9; [00099]; [000137]-[000140]	1, 2, 5, 9-18, 24
X	WO 2015/117244 A1 (VALORISATION-HSJ, LIMITED PARTNERSHIP; VALORISATION-RECHERCHE, LIMITED PARTNERSHIP) 13 August 2015 Abstract; page 7 lines 31-36; claim 15; page 19 lines 5-27; page 20 line 20-page 21 line 11; claims 17-20; Example 1 at pages 24-25	1, 2, 5, 9-18, 24
X	HAQUE T, et al., 'A Mouse Monoclonal Antibody against Epstein-Barr Virus Envelope Glycoprotein 350 Prevents Infection Both In Vitro and In Vivo,' *The Journal of Infectious Diseases,* (2006), vol 194, pp 584-587. Page 585 column 1; page 587 column 1; page 586 column 2; page 585 column 2-page 586 column 1; page 587 column 2	1, 2, 5, 9-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2018/050851

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☐ Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Supplemental Box

Continuation of: **Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-2 (partially), 5 (partially), 9-18 (partially), 24 (partially), 3-4 (fully), 6-8 (fully) and 25 (fully) are directed to recombinant human or humanised antibodies against EBV gp350 that comprises six CDRs or a heavy and light chain encoding antibody clone B8. The feature of a human/humanised antibody against EBV defined by antibody clone B8 (see SEQ ID NOs in tables at Box VIII of the written opinion) is specific to this group of claims.

- Claims 1-2 (partially), 5 (partially), 9-18 (partially), 24(partially), 3-4 (fully), 6-8 (fully) and 25 (fully) are directed to recombinant human or humanised antibodies against EBV gp350 that comprises six CDRs or a heavy and light chain encoding antibody clone A11. The feature of a human/humanised antibody against EBV defined by antibody clone A11 (see SEQ ID NOs in tables at Box VIII of the written opinion) is specific to this group of claims.

- Claims 1-2 (partially), 5 (partially), 9-18 (partially), 24(partially), 3-4 (fully), 6-8 (fully) and 25 (fully) are directed to recombinant human or humanised antibodies against EBV gp350 that comprises six CDRs or a heavy and light chain encoding antibody clone E10. The feature of a human/humanised antibody against EBV defined by antibody clone E10 (see SEQ ID NOs in tables at Box VIII) is specific to this group of claims.

- Claims 1-2 (partially), 5 (partially), 9-18 (partially), 24 (partially), 3-4 (fully), 6-8 (fully) and 25 (fully) are directed to recombinant human or humanised antibodies against EBV gp350 that comprises six CDRs or a heavy and light chain encoding antibody clone B7. The feature of a human/humanised antibody against EBV defined by antibody clone B7 (see SEQ ID NOs in tables at Box VIII of the written opinion) is specific to this group of claims.

- Claims 1-2 (partially), 5 (partially), 9-18 (partially), 24 (partially), 3-4 (fully), 6-8 (fully) and 25 (fully) are directed to recombinant human or humanised antibodies against EBV gp350 that comprises six CDRs or a heavy and light chain encoding antibody clone D7. The feature of a human/humanised antibody against EBV defined by antibody clone D7 (see SEQ ID NOs in tables at Box VIII of the written opinion) is specific to this group of claims.

- Claims 1-2 (partially), 5 (partially), 9-18 (partially), 24 (partially), 3-4 (fully), 6-8 (fully) and 25 (fully) are directed to recombinant human or humanised antibodies against EBV gp350 that comprises six CDRs or a heavy and light chain encoding antibody clone C10. The feature of a human/humanised antibody against EBV defined by antibody clone C10 (see SEQ ID NOs in tables at Box VIII of the written opinion) is specific to this group of claims.

- Claims 1-2 (partially), 5 (partially) and 9-18 (partially) and 24 (partially) are directed to recombinant human or humanised antibodies against EBV gp350. The feature of a human/humanised antibody against EBV glycoprotein 350 is specific to this group of claims.

- Claims 19-23 (fully) are directed to a method of determining the efficacy of an anti-EBV antibody. The feature of a method of determining the efficacy of an anti-EBV antibody is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to

Supplemental Box

all of the claimed inventions and which provides a technical relationship among them is an anti-EBV antibody. The only feature common to the first-seventh inventions is a human/humanised antibody that is directed against Epstein Barr Virus glycoprotein 350.

However this feature does not make a contribution over the prior art because it is disclosed in:

WO 2013/130565A1 D1 discloses a recombinant humanized EBV neutralizing antibody which binds to gp350 and is capable of preventing entry of EBV into a human cell (see abstract, claim 1, Figure 6 [0007], [000140]).

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

The International Searching Authority believes that a search and examination for the second-eighth invention will not involve more than negligible additional search and examination effort over that for the first invention and so no additional search fee is required. Therefore the search and opinion is not limited and provides comment on all eight inventions.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2018/050851**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2013/130565 A1	06 September 2013	WO 2013130565 A1	06 Sep 2013
		US 2015064174 A1	05 Mar 2015
		US 9376485 B2	28 Jun 2016
WO 2015/117244 A1	13 August 2015	WO 2015117244 A1	13 Aug 2015
		EP 3102590 A1	14 Dec 2016
		US 2017189517 A1	06 Jul 2017
		US 10016494 B2	10 Jul 2018

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.