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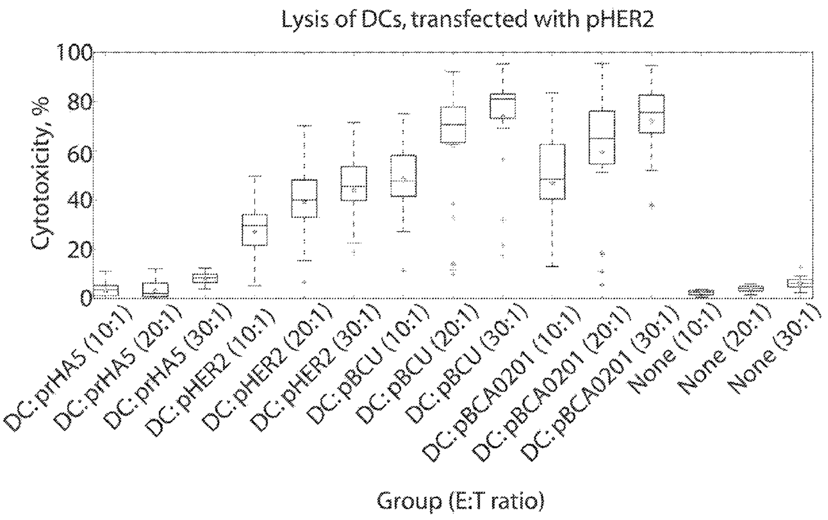
(54) **Title**: POLYEPITOPE CONSTRUCTS AND METHODS FOR THEIR PREPARATION AND USE

Figure 1A



(57) **Abstract**: The invention relates to immunogenic polyepitope constructs containing CTL and/or Th epitopes and optimized spacer sequences which improve processing and presentation of the epitopes leading to induction of high level of both CD4+ and CD8+ specific T-cell responses and specific types of cytokines, and high level of protection and therapeutic activity.

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POLYEPITOPE CONSTRUCTS AND METHODS FOR THEIR PREPARATION AND USE

FIELD OF THE INVENTION

The invention relates to novel immunogenic polyepitope constructs containing CTL and/or Th epitopes and optimized spacer sequences which improve processing and presentation of the epitopes leading to induction of high level of both CD4+ and CD8+ specific T-cell responses and specific types of cytokines, and high level of protection and therapeutic activity.

BACKGROUND OF THE INVENTION

Breast cancer is the most common cancer found in women. About 13% women in the US will develop breast cancer during their life. About 30% of such cases are advanced forms of cancer which are characterized by the enhanced expression of HER2 protein by tumor cells (Sequeira SJ et al., BMC Cell Biol. 2009, 10:64; Hawthorne VS et al., Mol Cancer Res. 2009, 7(4):592-600). HER2 is a member of the EGF family of receptors which control cell proliferation and survival and which is present in normal cells, but in much lower amounts than in cancer cells. Changes in regulation of activity of HER2 protein lead to suppression of apoptosis and active cell proliferation and can lead to cancer (Alroy I and Yarden Y. 2000 Breast Dis. 11:31-48; Harari D and Yarden Y. 2000 Oncogene 19(53):6102-14; Hudziak RM et al., 1987 PNAS, 84(20):7159-63). HER2 overexpression was also found in some other cancers, e.g. in 80% of metastatic prostate cancers (Mossoba ME et al., 2008, Mol. Ther. 16(3):607-617).

Many research groups are now trying to develop anti-cancer vaccines based on various cancer-specific antigens, including HER2. Development of anti-cancer vaccines is very promising, because cancer antigen-specific CTL response can efficiently destroy cancer cells and the mechanisms of immunological memory prevent re-emergence of cancer. Candidate anti-cancer vaccines that are currently being developed on the basis of HER2 utilize both extracellular and intracellular portions of the protein. Several of these candidate vaccines use a single peptide E75 (HER2 amino acids 369-377) (Gates JD et al., 2009 J Am Coll Surg. 208(2):193-201; Mittendorf EA et al., 2008 Cancer Immunol Immunother. 57(10):1511-21; Mittendorf EA et al., 2006 Ann Surg Oncol. 13(8):1085-98), others use several different peptides derived from HER2 (Matsueda S et al., 2009 Anticancer Res 29(7):2427-35; Li Y et al., 2009 Anticancer Res 29(1):41-58; Vertuani S et al., 2009 Cancer Immunol Immunother 58(5):653-64; Scardino A et al., 2007 Cancer Res 67(14):7028-36.) and yet others also contain epitopes from other antigens (Kavanagh B et al. 2007 J

Immunother 30(7):762-72). Several candidate vaccines demonstrated induction of humoral immune responses and good safety in primates (Renard V and Leach DR. 2007, Vaccine, 25(2):B17-23). Several CTL-inducing HER2-specific constructs showed low toxicity and lack of autoimmune reactions in clinical studies and also, in some cases, development of both cellular and humoral immune responses (Disis ML et al., 1998, Proc Am Soc Clin Oncol, 17:97a.; Zaks TZ et al., 1998, Cancer Res, 369–377; Knutson KL et al., 2001, J Clin Investig, 107: 477–484; Murray JL et al., 2000, Sem Oncol, 27:71–75; Salazar LG et al., 2003, Clin Cancer Res, 9:5559–5565; Disis ML et al., 2004, J Clin Oncol, 22:1916–1925; Limentani S et al., 2005, ASCO Proc, Abstr 2520).

HER2 peptide E75 (HER2 amino acids 369-377) was shown to be safe and effective in raising a dose-dependent HER2/neu immunity in HLA-A2 and HLA-A3 breast cancer patients (Peoples GE et al., 2005, J Clin Oncol, 23(30):7536-45) and was shown to prevent or delay cancer recurrences (Gates JD et al., 2009, J Am Coll Surg, 208(2):193-201; Peoples GE et al., 2008, Clin Cancer Res, 14(3):797-803; Peoples GE et al., 2005, J Clin Oncol, 23(30):7536-45) and reduce the number of circulating tumor cells (Stojadinovic A et al., 2007, Ann Surg Oncol, 14(12):3359-68). Evaluation of the in vitro immune response of peripheral blood lymphocytes isolated from six consecutive cancer patients immunized with E75 revealed a statistically significant increase in E75-stimulated lymphocytic proliferation. E75-stimulated lymphocytes demonstrated an E75-specific cytolytic response and moreover, these E75-specific lymphocytes also demonstrated tumor-specific lysis against HER2/neu-expressing cancer cell lines (Woll MM et al., 2004, Int J Oncol., 25:1769-1780). E75 vaccination was shown to result in CD4+ recruitment and was associated with a significant decrease in circulating regulatory T cells (Treg) and TGF-β levels (which are primary mediators of immunosuppression leading to tumor survival; see, e.g., Ueda R et al., 2009, Clin Cancer Res, 15(21):6551-6559; Takaku S et al., 2010, Int J Cancer, 126(7):1666-1674) in the majority of the vaccinated patients (Hueman MT et al., 2006, Breast Cancer Res Treat, 98(1):17-29).

Despite some advances described above, there is still no approved vaccine for breast cancer and most other cancers. Thus, there is still a great need for cancer-specific immunogens and vaccines that lead to efficient induction of both CD4+ and CD8+ T cell responses and thus are able to overcome immunosuppression and to provide protective immunity and therapeutic activity.

SUMMARY OF THE INVENTION

As specified above, there is a great need in the art to develop new immunogenic compositions for efficient induction of immune responses to various clinically relevant antigens. The present invention addresses this and other needs by providing novel polyepitope constructs.

Thus, in one aspect, the invention provides immunogenic polyepitope constructs comprising two or more T cell epitopes selected from the group consisting of:

AKFVAAWTLKAAA (SEQ ID NO: 1), AVVGILLVVVLGVVFGILIKRRQQKIR (SEQ ID NO: 7), PICTIDVYMIMVKCWMIDSE (SEQ ID NO: 8), AQMRILKETELRKVKVLGSGA (SEQ ID NO: 9), IKWMALESILRRRFTHQSDV (SEQ ID NO: 10), PICTIDVYMIMVKCWMIDS (SEQ ID NO: 11), CRWGLLLAL (SEQ ID NO: 21), LAALCRWGL (SEQ ID NO: 22), RELGSGLAL (SEQ ID NO: 23), WGLLLALLP (SEQ ID NO: 24), LVVVLGVVF (SEQ ID NO: 25), KITDFGLAR (SEQ ID NO: 26), QLFEDNYAL (SEQ ID NO: 27), YISAWPDSL (SEQ ID NO: 28), GDLTLGLEP (SEQ ID NO: 29), DVWSYGVTV (SEQ ID NO: 30), KIFGSLAFL (SEQ ID NO: 31), FDGDLGMGA (SEQ ID NO: 32), LVHRDLAAR (SEQ ID NO: 33), MELAALCRW (SEQ ID NO: 34), RASPLTSII (SEQ ID NO: 35), RGAPPSTFK (SEQ ID NO: 36), SIISAVVGI (SEQ ID NO: 37), LHCPALVTY (SEQ ID NO: 38), LRIVRGTQL (SEQ ID NO: 39), VKVLGSGAF (SEQ ID NO: 40), LQPEQLQVF (SEQ ID NO: 41), VKIPVAIKV (SEQ ID NO: 42), QLMPYGCLL (SEQ ID NO: 43), QETELVEPL (SEQ ID NO: 44), DIFHKNNQL (SEQ ID NO: 45), ASCVTACPY (SEQ ID NO: 46), TELVEPLTP (SEQ ID NO: 47), PLQRLRIVR (SEQ ID NO: 48), LQVIRGRIL (SEQ ID NO: 49), DEAYVMAGV (SEQ ID NO: 50), EECRVLQGL (SEQ ID NO: 51), TVCAGGCAR (SEQ ID NO: 52), YSEDPTVPL (SEQ ID NO: 53), RWGLLLALL (SEQ ID NO: 54), FEDNYALAV (SEQ ID NO: 55), QEVQGYVLI (SEQ ID NO: 56), LLALLPPGA (SEQ ID NO: 57), GSGAFGTVY (SEQ ID NO: 58), LGISWLGLR (SEQ ID NO: 59), ISAVVGILL (SEQ ID NO: 60), MQIAKGMSY (SEQ ID NO: 61), LSYMPIWKF (SEQ ID NO: 62), GVVKDVFAF (SEQ ID NO: 63), AIKVLRENT (SEQ ID NO: 64), SWLGLRSLR (SEQ ID NO: 65), ILLVVVLGV (SEQ ID NO: 66), FGPEADQCV (SEQ ID NO: 67), TLQGLGISW (SEQ ID NO: 68), TDFGLARLL (SEQ ID NO: 69), DSTFYRSLL (SEQ ID NO: 70), IISAVVGIL (SEQ ID NO: 71), TTPVTGASP (SEQ ID NO: 72), GMEHLREVR (SEQ ID NO: 73), ALCRWGLLL (SEQ ID NO: 74), RIVRGTQLF (SEQ ID NO: 75), GSCTLVCPL (SEQ ID NO: 76), DGENVKIPV (SEQ ID NO: 77), MELAALCRWGLLLALLPPGA (SEQ ID NO: 78), QEVQGYVLI (SEQ ID NO: 56), PLQRLRIVRGTQLFEDNYALAV (SEQ ID NO: 79),

TTPVTGASP (SEQ ID NO: 72), DIFHKNNQL (SEQ ID NO: 45), TVCAGGCAR (SEQ ID NO: 52), LHCPALVTY (SEQ ID NO: 38), ASCVTACPY (SEQ ID NO: 46), GSCTLVCPL (SEQ ID NO: 76), GMEHLREVR (SEQ ID NO: 73), KIFGSLAFL (SEQ ID NO: 31), LQPEQLQVF (SEQ ID NO: 41), YISAWPDSL (SEQ ID NO: 28), LQVIRGRIL (SEQ ID NO: 49), TLQGLGISWLGLRSLRELGSGLAL (SEQ ID NO: 80), EECRVLQGL (SEQ ID NO: 51), FGPEADQCV (SEQ ID NO: 67), LSYMPIWKF (SEQ ID NO: 62), RASPLTSIISAVVGILLVVVLGVVF (SEQ ID NO: 81), QETELVEPLTP (SEQ ID NO: 82), VKVLGSGAFGTVY (SEQ ID NO: 83), DGENVKIPVAIKVLRENT (SEQ ID NO: 84), DEAYVMAGV (SEQ ID NO: 50), QLMPYGCLL (SEQ ID NO: 43), MQIAKGMSY (SEQ ID NO: 61), LVHRDLAAR (SEQ ID NO: 33), KITDFGLARLL (SEQ ID NO: 85), DVWSYGVTV (SEQ ID NO: 30), DSTFYRSLL (SEQ ID NO: 70), GDLTLGLEP (SEQ ID NO: 29), FDGDLGMGA (SEQ ID NO: 32), YSEDPTVPL (SEQ ID NO: 53), GVVKDVFAF (SEQ ID NO: 63), RGAPPSTFK (SEQ ID NO: 36), LRHLYQGCQ (SEQ ID NO: 437), LRIVRGTQL (SEQ ID NO: 39), CLHFNHSGICELHCPALV (SEQ ID NO: 438), LQVFETLEE (SEQ ID NO: 439), LRSLRELGS (SEQ ID NO: 440), LCFVHTVPWDQ (SEQ ID NO: 441), LRGQECVEE (SEQ ID NO: 442), CPINCTHSC (SEQ ID NO: 443), IRKYTMRRL (SEQ ID NO: 444), MRILKETELRKVKVLGS (SEQ ID NO: 445), VKIPVAIKVLRENTSPK (SEQ ID NO: 446), YVMAGVGSPYVSRLLGICLTSTVQLV (SEQ ID NO: 447), VRLVHRDLA (SEQ ID NO: 448), FGLARLLDIDETEYH (SEQ ID NO: 449), WMALESILRRRFTHQS (SEQ ID NO: 450), CTIDVYMIMVKCWMI (SEQ ID NO: 451), CRPRFRELVSEFS (SEQ ID NO: 452), and FVVIQNEDL (SEQ ID NO: 359).

In one embodiment, the epitopes within the polyepitope constructs of the invention are connected end-to-end and/or are connected using spacer sequences which provide optimal processing and presentation of epitopes. In a specific embodiment, such spacer sequences are selected from the group consisting of K/R-K/R, A, AR, ARY, [ANRK][RQYW][YWFVI] (SEQ ID NO: 464), ADLVKV (SEQ ID NO: 2), ADLVAG (SEQ ID NO: 3), ADLAVK (SEQ ID NO: 4), AD, ADL, ADLV (SEQ ID NO: 5), ADLVK (SEQ ID NO: 6), [APRS] [DILT] [AGL] [AKV] (SEQ ID NO: 460), [ARSPNK] [DLITGV] [LGAVEK] [VKAFSI] [ALKSEI] [GVKLSE] (SEQ ID NO: 461), and [AGNRKP] [DIATVG] [LGANVE] [ASNVLK] [VIKAGP] [KAGVSE] (SEQ ID NO: 462).

In one embodiment, the polyepitope constructs of the invention further comprise one or more homologous or heterologous targeting signals which direct intracellular transport of the construct to a specific cellular compartment. In a specific embodiment, at least one of said targeting signals is selected from the group consisting of (i) a signal peptide of HER2 protein or a modified version thereof, (ii) an N-terminal portion or the whole sequence of the invariant chain associated with MHC class II molecules, (iii) a C-terminal portion of the human LAMP-1 protein, and (iv) the tyrosine-motif Y-X-X-hydrophobic amino acid, wherein X is any amino acid. In another specific embodiment, at least one of said targeting signals is selected from the group consisting of MELAALCRWGLLLALLPPGAP (SEQ ID NO: 13), MELAALCRWGLLLALLPPGAAS (SEQ ID NO: 14), RKRSHAGYQTI (SEQ ID NO: 15), IPIAVGGALAGLVLIVLIAYLVGRKRSHAGYQTI (SEQ ID NO: 16), LRMKLPKPPKPVSQMR (SEQ ID NO: 17), LRMKLPK (SEQ ID NO: 18), LRMK (SEQ ID NO: 19), and MHRRRSRSCREDQKPVMDDQRDLISNNEQLPMLGRRPGAPESKCSRGALYTGFSILVTLLLAGQATTAYFLYQQQGRLDKLTVTSQNLQLENLRMKLPKPPKPVSKMRMATPLLMQALPMGALPQGPMQNATKYGNMTEDHVMHLLQNADPLKVYPPLKGSFPENLRHLKNTMETIDWKVFESWMHHWLLFEMSRHSLEQKPTDAPPKVLTKCQEEVSHIPAVHPGSFRPKCDENGNYLPLQCYGSIGYCWCVFPNGTEVPNTRSRGHHNCSESLELEDPSSGLGVTKQDLGPVPM (SEQ ID NO: 454)

In one embodiment, the polyepitope constructs of the invention further comprise N-terminally conjugated ubiquitin. In a specific embodiment, the ubiquitin is UbV76 having the sequence MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGV (SEQ ID NO: 455). In one embodiment, the ubiquitin is conjugated directly to the N terminus of the polyepitope construct. In another embodiment, Arg or Val is inserted between the ubiquitin and the N terminus of the polyepitope construct.

In one embodiment, the polyepitope constructs of the invention comprise the sequence selected from the group consisting of:

CRWGLLLALLVVVLGVVFSIISAVVGIRELGSGLALMELAALCRWADLARDEAYVMAGVADLVEECRVLQGLADYSEDPTVPLAVKIPVAIKVAQLFEDNYALADVWSYGVTVAWGLLLALLPATVCAGGCARADIFHKNNQLADASCVTACPYADLLHCPALVTYATELVEPLTPADLKITDFGLARARGAPPSTFKADLYISAWPDSLAQETELVEPLALQVIRGRILALAALCRWGLADLQLMPYGCLLADKIFGSLAFLARGDLTLGLEPAVKVLGSGAFADLVHRDLAARADLQPEQLQVFADAFDGDLGMGAAPLQRLRIVRADLRIVRGTQLARASPLTSII (SEQ ID NO: 86);

QETELVEPLASCVTACPYADLVKVCRWGLLLALSIISAVVGIAARDEAYVMAGVADLVKLHCPALVTYARASPLTSIIADLVEECRVLQGLAFDGDLGMGAARGAPPSTFKADLKIFGSLAFLMELAALCRWADLVQLMPYGCLLAQLFEDNYALKITDFGLARADYISAWPDSLTVCAGGCARADLWGLLLALLPADLVHRDLAARADLYSEDPTVPLRELGSGLALARGDLTLGLEPAVKVLGSGAFADLQPEQLQVFADLDVWSYGVTVADLRIVRGTQLAPLQRLRIVRADLAALCRWGLAVKIPVAIKVADLQVIRGRILALVVVLGVVFADIFHKNNQLATELVEPLTP (SEQ ID NO: 87);

CRWGLLLALASCVTACPYADLYISAWPDSLAVKIPVAIKVAQLFEDNYALADVWSYGVTVAWGLLLALLPADIFHKNNQLATELVEPLTPADLLHCPALVTYAPLQRLRIVRADLQLMPYGCLLADKIFGSLAFLMELAALCRWADLVHRDLAARADLQPEQLQVFADAFDGDLGMGAALQVIRGRILAVKVLGSGAFADLRIVRGTQLARGAPPSTFKADLQETELVEPLRELGSGLALLVVVLGVVFSIISAVVGIARGDLTLGLEPADKITDFGLARALAALCRWGLADYSEDPTVPLTVCAGGCARARASPLTSIIADLVEECRVLQGLAARDEAYVMAGV (SEQ ID NO: 88);

CRWGLLLALAFGPEADQCVADLQLMPYGCLLADYSEDPTVPLAVKIPVAIKVAQLFEDNYALADVWSYGVTVAWGLLLALLPATVCAGGCARAISAVVGILLATLQGLGISWADSWLGLRSLRADLVKRWGLLLALLLLALLPPGARELGSGLALLVVVLGVVFSIISAVVGIILLVVVLGVAIISAVVGILAIKVLRENTADLVQETELVEPLALQVIRGRILAGVVKDVFAFADLARDEAYVMAGVADLPLQRLRIVRADLKITDFGLARALGISWLGLRADLQEVQGYVLIADLHCPALVTYAVKVLGSGAFADGMEHLREVRADTTPVTGASPADASCVTACPYADLYISAWPDSLARGDLTLGLEPADRGAPPSTFKADLRIVRGTQLATELVEPLTPADAFDGDLGMGAALAALCRWGLADLQPEQLQVFADAFEDNYALAVAMQIAKGMSYATDFGLARLLMELAALCRWADLVHRDLAARADGSGAFGTVYARDGENVKIPVADLVDSTFYRSLLADLVEECRVLQGLADKIFGSLAFLALCRWGLLLADIFHKNNQLADLSYMPIWKFADLVGSCTLVCPLARASPLTSIIADLRIVRGTQLF (SEQ ID NO: 89);

TTPVTGASPADLSWLGLRSLRADLVGSCTLVCPLAIKVLRENTADYSEDPTVPLMELAALCRWADLRWGLLLALLILLVVVLGVADLWGLLLALLPADLVHRDLAARADLDVWSYGVTVADLGISWLGLRADLVKVQETELVEPLTDFGLARLLRELGSGLALAIISAVVGILAFGPEADQCVADLVKVCRWGLLLALISAVVGILLGSGAFGTVYADLSYMPIWKFADLVEECRVLQGLGVVKDVFAFADLAFEDNYALAVADLKIFGSLAFLASCVTACPYADLVKVQLMPYGCLLAARDEAYVMAGVADLVKLHCPALVTYAVKVLGSGAFADLQPEQLQVFADLRIVRGTQLFADLVDSTFYRSLLADGMEHLREVRADLRIVRGTQLATVCAGGCARADLAALCRWGLAPLQRLRIVRADLQVIRGRILALVVVLGVVFADIFHKNNQLATLQGLGISWAQLFEDNYALARGDLTLGLEPAARDGENVKIPVADLVALCRWGLLLALLALLPPGAARGAPPSTFKADLKITDFGLARADMQIAKGMSYADAFDGDLGMGAAVKIPVAIKVARASPLTSIIADLQEVQGYVLIADYISAWPDSLSIISAVVGIATELVEPLTP (SEQ ID NO: 90);

CRWGLLLALISAVVGILLAFGPEADQCVADLQETELVEPLTDFGLARLLRELGSGLALLVVVLGVVFSIISAVVGIILLVVVLGVAIISAVVGILGSGAFGTVYAIKVLRENTADLRIVRGTQLFADLVKLHCPALVTYAVKVLGSGAFADGMEHLREVRADYISAWPDSLALCRWGLLLAVKIPVAIKVALAALCRWGLADTTPVTGASPADRGAPPSTFKADLYSEDPTVPLAFDGDLGMGALLALLPPGAARDGENVKIPVADLVDSTFYRSLLADGSCTLVCPLMELAALCRWADSWLGLRSLRADLVPLQRLRIVRADLKITDFGLARALGISWLGLRADLQEVQGYVLIADKIFGSLAFLASCVTACPYADLRASPLTSIIADLVEECRVLQGLAARDEAYVMAGVADLRWGLLLALLGVVKDVFAFADLQLMPYGCLLADLQPEQLQVFADLRIVRGTQLAMQIAKGMSYADVWSYGVTVAWGLLLALLPATVCAGGCARAQLFEDNYALARGDLTLGLEPADIFHKNNQLATELVEPLTPADLVHRDLAARADAFEDNYALAVALQVIRGRILATLQGLGISWADLSYMPIWKF (SEQ ID NO: 91);

TVCAGGCARADGMEHLREVRADGKEECRVLQGLADGRELGSGLALPQLFEDNYALSDGQETELVEPLPLVVVLGVVFARDGENVKIPVALLALLPPGAAQEVQGYVLIPDLARGDLTLGLEPAIKVLRENTADAFDGDLGMGAPDAKARDEAYVMAGVADIFHKNNQLAVKVLGSGAFATLQGLGISWAIAFGPEADQCVPDLKLSYMPIWKFADLKPLQRLRIVRAIISAVVGILMELAALCRWATGVVKDVFAFADLVKIPVAIKVSIISAVVGIPISAVVGILLPILQPEQLQVFADGKYSEDPTVPLADMQIAKGMSYARGAPPSTFKADLQVIRGRILPDGRASPLTSIIADLVHRDLAARADSWLGLRSLRADGKLGISWLGLRADGVKITDFGLARATDFGLARLLPDGDSTFYRSLLAILLVVVLGVADTTPVTGASPRDLRIVRGTQLATELVEPLTPPDLKASCVTACPYPILAALCRWGLADAFEDNYALAVAIDVWSYGVTVAWGLLLALLPRDAKQLMPYG

CLLAIKIFGSLAFLALCRWGLLLRDGRIVRGTQLFADLVGSGAFGTVYADGGSCTLVCPLPDGYISAWPDSLRDLHCPALVTYALLVCRWGLLLALRWGLLLALL (SEQ ID NO: 92);

MELAALCRWGLLLALLPPGAPDGENVKIPVAIKVLRENTADGKEECRVLQGLPDGKYSEDPTVPLPDDEAYVMAGVADLKQETELVEPLTPPDGRASPLTSIISAVVGILLVVVLGVVFPDAGMEHLREVRADGKDIFHKNNQLPDLQPEQLQVFRDAQEVQGYVLIPDLAFDGDLGMGAPDLQVIRGRILPDVKVLGSGAFGTVYPIGDLTLGLEPPDLKASCVTACPYATLQGLGISWLGLRSLRELGSGLALPMQIAKGMSYALFGPEADQCVPDLKLSYMPIWKFADLKPLQRLRIVRGTQLFEDNYALAVARGAPPSTFKAGVVKDVFAFRDLVKITDFGLARLLPLVHRDLAARADVWSYGVTVRDTTPVTGASPRDLYISAWPDSLRTVCAGGCARSDKIFGSLAFLPDLHCPALVTYADDSTFYRSLLADGKQLMPYGCLLADGGSCTLVCPL (SEQ ID NO: 93);

WGLLLALLP-RDA-YSEDPTVPL--ADIDETEYHA-PDLK-AREEGAGSDVFD--AYGVTVWELM-ALGK-ARDDDDMGDLVD-PLGK-AEITGYLYIS-ADGK-HLDMLRHLY-ADLK-AHSDCLACLH-AD-LTCSPQPEY-ADLK-QSDVWSYGV-AD-AYKDPPFCVA-PDL-ARDGDLGMGAA-PIAK-LLDIDETEY-AD-ARDGDPASNTA-AI-ARDGENVKIPV-ALL-GSGAFGTVY-PD-NASLSFLQD-PLLK-LHCPALVTY-AD-DSTFYRSLL-ADL-FSPAFDNLY-AILK-TIDVYMIMV (SEQ ID NO: 110);

TIDVYMIMV-PDLK-CRWGLLLAL-A-LLALLPPGA-ADG-AILDEAYVMA--ALIHHNTHL-PDL-RLVHRDLAA--LLLALLPPG-ADGK-QLFEDNYAL-P-ILHNGAYSL-P-SLTLQGLGI-R-LVDAEEYLV-R-ILLVVVLGV-ADA-SIISAVVGI-A-RLLQETELV-AD-AFEDNYALAV--AVVGILLVV-A-VVLGVVFGI-AD-ALLNWCMQIA-ADLV-ALCRWGLLL-AD-YISAWPDSL-RD-KIFGSLAFL-RDL-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 123);

MELAALCRWGLLLALLPPGAPPDLLALLPPGAPDATLEEITGYLAILDEAYVMAPILHNGAYSLPQLFEDNYALSIISAVVGIAQLMPYGCLLRLLVVVLGVVRDLQLRSLTEIAILLVVVLGVPDAVVGILLVVADALCRWGLLLADYISAWPDSLRDKIFGSLAFL (SEQ ID NO: 124);

LVPQQGFFC-ADLV-PCARVCYGL-PDLK-KHSDCLACL--ATLEEITGYL-A-TLSPGKNGV-PDL-DLVDAEEYL-P-ILHNGAYSL-A-SLPDLSVFQ-RD-QIAKGMSYL--AILDEAYVMA--ALIHHNTHL-AI-AFGPEADQCV-RDLK-LVDAEEYLV-A-QLFEDNYAL--SIISAVVGI-ADG-THLDMLRHL--ACLTSTVQLV-ADG-FRNPHQALL-ADG-RLLQETELV-ADL-KIFGSLAFL-A-YISAWPDSL-RD-AYSLTLQGL-RDL-TYLPTNASL-SDA-RWGLLLALL-A-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 138);

HYKDPPFCV-AIGK-AIQNEDLGPA-RDL-QIAKGMSYL-A-TLSPGKNGV-SD-LLALLPPGA-ADG-PYVSRLLGI--AYLSTDVGSC-AD-ILLVVVLGV-ADA-SIISAVVGI-AD-SLRELGSGL-PTG-RASPLTSII-A-LLVVVLGVV-RDL-AYLTPQGGAA--ALIHHNTHL-AD-ARPLTSIISAV-ADL-FRNPHQALL-ADGK-KIFGSLAFL--ALLNWCMQIA-ADLK-ACLTSTVQLV-ADG-YISAWPDSL-A-HLYQGCQVV-ADL-SLTLQGLGI-AD-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 148);

CRWGLLLAL-PD-AIQNEDLGPA--AVLDNGDPL--RLLQETELV-ADG-FRNPHQALL-PDLK-QVFETLEEI-PD-QIAKGMSYL-PD-VVLGVVFGI-ADA-TQLFEDNYA-AD-AVVGILLVV-AD-RASPLTSII-A-LLVVVLGVV-RD-LQLRSLTEI-A-ILLVVVLGV-ADA-SIISAVVGI-PD-YVLIAHNQV-AD-VKIPVAIKV--ALIHHNTHL-A-LAALCRWGL-A-SAVVGILLV-ADGK-KIFGSLAFL-A-IWIPDGENV-AD-TIDVYMIMV-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 156);

CVNCSQFLR-AD-LVKSPNHVK-A-ILKETELRK-RDLK-ARILHNGAYS-AD-GVVFGILIK-ADG-AELMTFGAKP-PDGK-LELTYLPTN-ALGK-KIRKYTMRR-ADLV-LERPKTLSP-A-VLRENTSPK-A-LLLALLPPG-ADGK-RSLTEILKG--ALLHTANRP-A-ILIKRRQQK-ADGK-AGILLVVVLG-PDGK-TVWELMTFG-A-ILWKDIFHK-ADGK-RGAPPSTFK-ADL-QLVTQLMPY-A-VVVLGVVFG-PD-VMAGVGSPY-AILK-LAARNVLVK-ADL-YTMRRLLQE-ADGK-TFYRSLLED-RD-VVFGILIKR-A-LAFLPESFD-A-YLYISAWPD-AD-MTFGAKPYD (SEQ ID NO: 183);

RWGLLLALL-A-EYVNARHCL-R-DLLEKGERL--AEYHADGGKV-S-DIFHKNNQL-A-QLFEDNYAL-P-LAALCRWGL-AI-AYGVTVWELM-AI-LRIVRGTQL--ILLVVVLGV-ADA-TYLPTNASL-A-IWIPDGENV-RLL-VWSYGVTVW-AL-EYLVPQQGF-ADLK-DVWSYGVTV-PDLK-RFRELVSEF-PDLK-LSYMPIWKF-ADL-SYGVTVWEL-ADA-QCVNCSQFL-ADAK-VYMIMVKCW-AILK-KWMALESIL-AI-MIMVKCWMI (SEQ ID NO: 194);

AWPDSLPDL--DLLEKGERL-RDG-PYVSRLLGI-PDL-TLQGLGISW-A-SLAFLPESF-PDGK-AVVGILLVV-RT-LVVVLGVVF-A-IWIPDGENV-RLL-VWSYGVTVW-AL-EYLVPQQGF-ADLK-QLMPYGCLL-AD-SYGVTVWEL-ADL-TYLPTNASL-A-RIVRGTQLF-RWGLLLALL-A-KWMALESIL-AIGV-VYMIMVKCW (SEQ ID NO: 197);

RMARDPQRF-AD-AVRGTQLFED-RD-LQPEQLQVF-ADG-EYVNARHCL-ADA-RWGLLLALL--ASEGAGSDVF--AGEGLACHQL-PDLK-LQGLGISWL-AI-SYGVTVWEL-AD-AWPDSLPDL-PL-EYLVPQQGF-ADGK-HNGAYSLTL--AFNHSGICEL-A-YLVPQQGFF-ADGV-AYSLTLQGL-PDLK-RFRELVSEF-ADGK-ACYGLGMEHL-AL-VWSYGVTVW-AI-AFQNLQVIRG-ADG-VTVWELMTF-ADGK-AFYRSLLEDD-RDL-TYLPTNASL-AI-VYMIMVKCW-AILK-KWMALESIL-AD-RFTHQSDVW (SEQ ID NO: 211);

CTIDVYMIM-PI-ICELHCPAL-A-QLVTQLMPY-ADG-VSRLLGICL--ALCRWGLLL-PDLK-ARDEAYVMAGV-AD-ETLEEITGY-A-TEILKGGVL-P-QLFEDNYAL-PD-LQPEQLQVF-AD-KVPIKWMAL--SIISAVVGI-RD-DTILWKDIF-ALGV-AETHLDMLRH-A-DVFDGDLGM-PDLK-SLRELGSGL--STVQLVTQL-PLGK-ISWLGLRSL--AFDGDLGMGA-AD-CRWGLLLAL-PD-VTVWELMTF-ADGK-AFEDNYALAV-RDLK-HTVPWDQLF (SEQ ID NO: 224);

LHCPALVTY-SD-LTCSPQPEY-ADL-RLVHRDLAA-ALG-HLDMLRHLY-AD-LVVVLGVVF-PDGK-DIFHKNNQL-AD-LEEITGYLY-AD-GVVKDVFAF-AD-ARPGGLRELQL-AD-ETLEEITGY-ALL-THQSDVWSY-AD-AYLEDVRLVH-PDLK-QVVQGNLEL-AI-GSGAFGTVY-RL-VMAGVGSPY-AILK-LMTFGAKPY-AD-GTQLFEDNY-ADGK-CVTACPYNY-ADG-GTVYKGIWI-ADL-SMPNPEGRY-ADLK-HTVPWDQLF-ADLK-SLTLQGLGI-AD-MQIAKGMSY-A-ICLTSTVQL-SD-DVWSYGVTV-PDLK-MSYLEDVRL-RD-VCTGTDMKL-AD-FSPAFDNLY-AIL-SPAFDNLYY (SEQ ID NO: 239);

KIRKYTMRR-A-YLYISAWPD--LVKSPNHVK-PLLK-KVKVLGSGA-PDG-KETELRKVK-PD-AIKVLRENT-AD-GGKVPIKWM-ADG-NVKIPVAIK-AD-ARGGCLLDHVRE--AGLRSLRELG-ADG-RPKTLSPGK-AI-LQRLRIVRG-PDGV-KLRLPASPE-A-WGLLLALLP-AD-RSRACHPCS-AILK-KRRQQKIRK-ADLK-HVRENRGRL-AD-ARPGKNGVVKD-A-PLQRLRIVR-RDAK-AARNVLVKS-AD-MARDPQRFV-A-VLRENTSPK-ADL-VARCPSGVK-ADL-HYKDPPFCV-AD-KIFGSLAFL-A-STFKGTPTA-ADL-TQRCEKCSK (SEQ ID NO: 258);

SMPNPEGRY-ADL-KHSDCLACL--ADMGDLVDAE-RDGK-CVTACPYNY-AL-GGAVENPEY-AL-AVVKDVFAFG-PLAK-AEIPDLLEKG-PDGK-HLDMLRHLY-ADLK-TVWELMTFG-AD-LTCSPQPEY-ADL-RSSSTRSGG-ADGK-ETLEEITGY-AD-VLQGLPREY-AD-ARPLTSIISAV-AL-ASCVTACPY-PLL-SAVVGILLV-ADLV-AESFDGDPAS-R-DVFDGDLGM-PIL-AAPRSPLAPS-AI-GTQLFEDNY-AIG-ASLTEILKGG-AD-KGMSYLEDV-AD-VMAGVGSPY-ATLK-SLPDLSVFQ-RDLK-THQSDVWSY-ADA-SPAFDNLYY-ADL-FSPAFDNLY-ADLK-YYWDQDPPE-ADLV-LMTFGAKPY (SEQ ID NO: 270);

QALLHTANR-AIG-RQVPLQRLR-ADGK-QKIRKYTMR-ADGK-GVGSPYVSR--RILKETELR-ADL-LEDVRLVHR-ADG-TLIDTNRSR-ADL-GMEHLREVR-ADGK-REGPLPAAR-RIG-MALESILRR-PDGK-LGISWLGLR-ADGV-KITDFGLAR-A-PLQRLRIVR-ADG-VVFGILIKR-RDGK-LVHRDLAAR-A-TVCAGGCAR-RDG-KIRKYTMRR-ADG-AALCRWGLL-ADGK-KIFGSLAFL-PDG-KVPIKWMAL-SD-ASPLDSTFYR-ADL-VSEFSRMAR-ADLV-CVNCSQFLR-ADLK-LACHQLCAR-AD-VFQNLQVIR-AIL-SWLGLRSLR (SEQ ID NO: 285);

AAPRSPLAPS--ALPAARPAGA-PDG-ALPTHDPSPL-A-ALPASPETHL-SD-ASPETHLDML--AVLDNGDPL--ASPKANKEIL-P-GAVENPEYL--ASPGKNGVVK-AD-LPTNASLSF--ADPASNTAPL--AARPAGATL--AAPQPHPPPA-ADGV-LQVIRGRIL-

PDG-RASPLTSII-ADL-APPSPREGPL-RDLK-HVRENRGRL-SDL-AHPPPAFSPA-PDLK-AMPNQAQMRI-ADLV-RKYTMRRLL-A-GVVKDVFAF-AD-AVPLQRLRIV-ADGK-GSCTLVCPL-AI-ASPREGPLPA-ADL-RCEKCSKPC (SEQ ID NO: 304);

MELAALCRWGLLLALLPPGAPASPKANKEILAARPAGATLALPTHDPSPLAALPASPETHLSDASPETHLDMLADAPPSPREGPLRDLKHVRENRGRLADLACPSGVKPDLADGSTRSGGGDLPIASPLTSIISA (SEQ ID NO: 305);

YISAWPDSL-PDL-ECRPRFREL-AD-VGILLVVVL-PD-QQKIRKYTM-AD-LFRNPHQAL-AL-LIKRRQQKI-ADLK-AYGVTVWELM-PDLK-LGMEHLREV--ASPKANKEIL--ALIHHNTHL-A-DIFHKNNQL-AD-MVHHRHRSS-AD-AVPLQRLRIV-A-ILLVVVLGV-AD-VSRLLGICL--AFGLARLLDI-AI-LQRLRIVRG-AD-VVGILLVVV-PDG-KVPIKWMAL--SLAFLPESF-AI-LQVIRGRIL--LVVVLGVVF-A-MRILKETEL-RTG-VLIQRNPQL-PDLK-ILRRRFTHQ-AD-LAALCRWGL-AD-LDSTFYRSL-RD-LRIVRGTQL-PIAK-ISAVVGILL-AI-MIMVKCWMI (SEQ ID NO: 319);

MELAALCRWGLLLALLPPGAPAIGFHKNNQLALASPKANKEILRDGKDIFHKNNQLPDGKLGMEHLREVADLFRNPHQALALLGCKKIFGSLPDLRIVRGTQLADGVMRILKETELSDGQLRSLTEILADGKECRPRFRELADGQLMPYGCLLPDLK (SEQ ID NO: 320);

LVVVLGVVF-A-IQRNPQLCY-AILV-TQCVNCSQF-ADG-TLIDTNRSR--ASEGAGSDVF--ALIHHNTHL-AI-AYGVTVWELM-AIGK-ISWLGLRSL-S-VKVLGSGAF-A-QLFEDNYAL-PLG-RELGSGLAL--ASCVTACPY-AIL-VTSANIQEF-AIG-VQGNLELTY-AD-LTCSPQPEY-ADLK-QVVQGNLEL-AI-GSGAFGTVY-RL-VMAGVGSPY-ADGV-LQVIRGRIL--SLAFLPESF-ADG-VWSYGVTVW-ADA-RIVRGTQLF-WCMQIAKGM-AD-MQIAKGMSY-A-LMTFGAKPY-RDL-RACHPCSPM (SEQ ID NO: 327);

LRIVRGTQL--ASEGAGSDVF--ALDIDETEYH-ADLK-QETELVEPL-AD-ARPEYLTPQGG-ADGV-EEITGYLYI-PDGK-EECRVLQGL-ADG-RELGSGLAL--AEDLGPASPL-A-TEILKGGVL-P-LEEITGYLY-PLGK-AGDLGMGAAK-AD-LELTYLPTN-RDG-VKVLGSGAF-AD-TELVEPLTP-RDLK-SAWPDSLPD-AD-DVWSYGVTV-AD-MQIAKGMSY-AD-QRFVVIQNE (SEQ ID NO: 335);

GRILHNGAY-ADG-CRWGLLLAL--LQPEQLQVF--AILDEAYVMA-RD-AKGLQSLPT-AD-GRLGSQDLL-ADG-RELGSGLAL--AYLEDVRLVH-RD-AFAGCKKIFG-ADG-FRNPHQALL-PIGK-AGEGLACHQL-AD-ARPAGATLE-SL-RRLLQETEL--AAGCTGPKH-AD-AVRGTQLFED-RDLV-RKYTMRRLL-RD-LRIVRGTQL-PDLK-RNPQLCYQD-ADLK-RQVPLQRLR-ADAK-ARVCYGLGM-ADGV-HRDLAARNV-PD-QRASPLTSI-PLLK-HRHRSSSTR-ADLV-YLYISAWPD-ADAK-QRFVVIQNE-ADLV-RRQQKIRKY-ADLK-CRVLQGLPR-ADL-YTMRRLLQE-ADLK-RRFTHQSDV (SEQ ID NO: 351);

HTVPWDQLF-ADLV-CRWGLLLAL-RI-ALDIDETEYH-ADL-ARDGDLGMGAA-RD-LPTNASLSF--ADPASNTAPL--ALPTHDPSPL-AD-NKEILDEAY--ADPAPGAGGM-AI-AEPLTPSGAM-A-GVVKDVFAF-AD-LTCSPQPEY-ADLK-LVTYNTDTF-AD-LALLPPGAA-PD-EILDEAYVM-P-LVVVLGVVF--AECVGEGLAC-A-TPTAENPEY-AD-RSLLEDDDM-ALLV-FVVIQNEDL-AL-AMPNQAQMRI-ADLV-MSYLEDVRL-AI-LMTFGAKPY-AD-ICELHCPAL-ALGK-YYWDQDPPE-ADL-SPAFDNLYY-ADL-FSPAFDNLY-AILK-AMPYGCLLDH (SEQ ID NO: 363);

MELAALCRWGLLLALLPPGAPADGKTPTAENPEYAALPASPETHLPILKYSEDPTVPLPDGALPTHDPSPLADNKEILDEAYADEILDEAYVMPLVVVLGVVFADMQIAKGMSYALMTFGAKPYPLGKAPPPAFSPAFADLHCPALVTY (SEQ ID NO: 364);

MELAALCRW--RDLAARNVL-PDA-QETELVEPL--AEEEAPRSPL-PDGK-EECRVLQGL-ADA-GERLPQPPI-ADG-SETDGYVAP-PDA-AGEGLACHQL-ADG-RELGSGLAL-P-QLFEDNYAL-PD-ALEDDDMGDL-PDLK-REVRAVTSA--ASEGAGSDVF-A-TEILKGGVL-PL-EEITGYLYI-PDGK-AENPEYLGL-PDLK-QEVQGYVLI-AD-EQLQVFETL-A-QVVQGNLEL-A-QEFAGCKKI--ALCRWGLLL-RD-AFEDNYALAV (SEQ ID NO: 374);

ISWLGLRSL--AEEEAPRSPL--RDLAARNVL-RLG-GENVKIPVA-RLG-KHSDCLACL-AIG-GERLPQPPI-ADL-TGTDMKLRL-PDGK-AENPEYLGL-ADG-RELGSGLAL--REVRAVTSA-ADG-REYVNARHC-A-QEFAGCKKI-A-QETELVEPL-A-TELRKVKVL--TDMKLRLPA-ADLK-QEVQGYVLI-PDL-ARGGSRCWGESS-ALGV-KITDFGLAR-A-TDFGLARLL-PDA-RKYTMRRLL-ADG-RELQLRSLT-ADLK-LDSTFYRSL--MELAALCRW-A-TLQGLGISW-ADL-CQSLTRTVC-ALL-HYKDPPFCV-AIG-YISAWPDSL-AD-CRWGLLLAL-RDL-TRTVCAGGC-ADLK-TFYRSLLED (SEQ ID NO: 384);

TRTVCAGGC-ADG-GGGDLTLGL--ARPEADQCVAC-A-TLQGLGISW-AI-AFDGDLGMGA-PDAK-ARGDLTLGLEP-PDGK-IDSECRPRF-ADG-VKVLGSGAF-ADG-QETELVEPL-ADG-RELGSGLAL-A-QEVQGYVLI-ALG-ERGAPPSTF-A-QEFAGCKKI--MELAALCRW-ALG-VKIPVAIKV-AL-LHCPALVTY (SEQ ID NO: 389);

LRIVRGTQL-PIAA-GGGDLTLGL--ARPEADQCVAC-AI-AFDGDLGMGA-PDAK-ARGDLTLGLEP-PDLK-QETELVEPL-PI-VKVLGSGAF--ASEGAGSDVF-PDG-RELGSGLAL-A-QEVQGYVLI-ADGK-EECRVLQGL-PDLK-LEEITGYLY-A-TEILKGGVL-PL-EEITGYLYI-AD-MELAALCRW-AD-ARPDLSVFQNL-ADL-TDFGLARLL-PD-TRTVCAGGC (SEQ ID NO: 391);

CELHCPALV-ADG-GENVKIPVA--ALPASPETHL-RD-ARPEGRYTFGA-ADGK-IDSECRPRF-ADLK-GERLPQPPI-AIL-AEEAPRSPLA-ADGA-EEITGYLYI--ALPAARPAGA-PDGK-MEHLREVRA-PDG-RELQLRSLT-ADLK-KEILDEAYV-AT-AFDGDLGMGA-PDLK-REVRAVTSA--ALPSETDGYV-ADG-AEQRASPLT-ADG-AGEGLACHQL-ADG-RELGSGLAL-AD-CEKCSKPCA-ADGV-QEVQGYVLI-ADL-TSANIQEFA-AD-LDSTFYRSL--MELAALCRW-ATGK-AINCTHSCVD-RD-AFEDNYALAV-RD-LGMGAAKGL--VSRLLGICL-PD-VKIPVAIKV-AI-ASCVTACPY (SEQ ID NO: 403);

CRWGLLLAL-PD-ENVKIPVAI--AYGVTVWELM-A-ALPASPETHL--ARPDLSVFQNL-PD-LPTNASLSF-ADG-ALPTHDPSPL-PDL-ALPSETDGYV-PDLK-LGMEHLREV-AD-LPQPPICTI-ADGV-QEVQGYVLI-AD-EQLQVFETL-A-LGMGAAKGL-PD-KGMSYLEDV-A-QEFAGCKKI-S-VGILLVVVL--AMPNQAQMRI-ADLK-LQLRSLTEI-AD-VKIPVAIKV-A-TDFGLARLL (SEQ ID NO: 406);

ASPLDSTFYR-ADG-VENPEYLTP-A-ALPASPETHL--ARAGVGSPYVS-RD-LPTNASLSF-ADG-ALPTHDPSPL-ADL-LERPKTLSP-AL-AFDGDLGMGA-PDAK-ARGDLTLGLEP-PDL-ARDDMGDLVDA-PDL-ARPEDECVGE-A-TPTAENPEY-AL-AMPNQAQMRI-ADLK-LPQPPICTI-AD-ASPLTSIISA-AD-CRWGLLLAL--AGPLPAARPA-PD-AAPRSPLAPS-ALA-ASPQPEYVNQ-ALG-VKIPVAIKV-AD-ACPSGVKPDL-AD-LHCPALVTY-SDA-SPAFDNLYY (SEQ ID NO: 415);

AWKDIFHKNN-AD-AFDGDLGMGA-PDLK-REVRAVTSA-ALL-AEEAPRSPLA-ADG-ARDGDPASNTA--ALPAARPAGA-A-IWIPDGENV-SD-LRENTSPKA-RD-LVEPLTPSG-ADG-LTSIISAVV-A-RKVKVLGSG-ADGV-RELQLRSLT-ADLK-LPQPPICTI-AD-LQRLRIVRG-PDLK-RGRILHNGA-AD-ASPLTSIISA--ASPLAPSEGA--ACPALVTYNT-AD-AVPLQRLRIV-ADAA-AMPNQAQMRI-ADLK-AYKDPPFCVA-RDL-AMPIWKFPDE-ADG-AMPYGCLLDH-ADGK-WGLLLALLP (SEQ ID NO: 425);

MELAALCRW-A-VTSANIQEF-ALGK-ENVKIPVAI-ADGK-DIFHKNNQL-RD-ATLERPKTL--LVVVLGVVF-P-TLQGLGISW-A-DVFDGDLGM-RDLV-ALCRWGLLL-PDGK-ISWLGLRSL--RSLLEDDDM-ADG-GSGAFGTVY-ADA-GTQLFEDNY-RDLK-LSYMPIWKF-ADLK-PAFDNLYYW-ADL-QLMPYGCLL-PDLK-MSYLEDVRL-R-DVWSYGVTV-PDLK-RFTHQSDVW-ADLV-HTVPWDQLF (SEQ ID NO: 428);

PAFDNLYYW-AIL-CTIDVYMIM-ADLV-RMARDPQRF-AD-KGCPAEQRA-PDLK-LGSQDLLNW--AIISAVVGIL-AL-RCEKCSKPC-AIL-VTSANIQEF-ADL-GAMPNQAQM-AD-AVTGASPGGL-P-ISAVVGILL-PD-RSGGGDLTL--AYLSTDVGSC-A-LAALCRWGL-AL-ASCVTACPY-ADL-HTVPWDQLF-ADLK-LSYMPIWKF-ADG-RASPLTSII-ADG-VTVWELMTF-ADGV-

ARGQECVEEC-ADL-RIVRGTQLF-TRTVCAGGC-AD-KIFGSLAFL-PD-VCTGTDMKL-AD-LCYQDTILW (SEQ ID NO: 436), and

AKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDSRKRSHAGYQTI (SEQ ID NO: 453).

In a preferred embodiment, the polyepitope construct consists of the sequence

MELAALCRWGLLLALLPPGAPDGENVKIPVAIKVLRENTADGKEECRVLQGLPDGKYSEDPTVPLPDDEAYVMAGVADLKQETELVEPLTPPDGRASPLTSIISAVVGILLVVVLGVVFPDAGMEHLREVRADGKDIFHKNNQLPDLQPEQLQVFRDAQEVQGYVLIPDLAFDGDLGMGAPDLQVIRGRILPDVKVLGSGAFGTVYPIGDLTLGLEPPDLKASCVTACPYATLQGLGISWLGLRSLRELGSGLALPMQIAKGMSYALFGPEADQCVPDLKLSYMPIWKFADLKPLQRLRIVRGTQLFEDNYALAVARGAPPSTFKAGVVKDVFAFRDLVKITDFGLARLLPLVHRDLAARADVWSYGVTVRDTTPVTGASPRDLYISAWPDSLRTVCAGGCARSDKIFGSLAFLPDLHCPALVTYADDSTFYRSLLADGKQLMPYGCLLADGGSCTLVCPLAKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDSRKRSHAGYQTI (SEQ ID NO: 456 - universal).

In another preferred embodiment, the polyepitope construct consists of the sequence

MELAALCRWGLLLALLPPGAPPDLLALLPPGAPDATLEEITGYLAILDEAYVMAPILHNGAYSLPQLFEDNYALSIISAVVGIAQLMPYGCLLRLLVVVLGVVRDLQLRSLTEIAILLVVVLGVPDAVVGILLVVADALCRWGLLLADYISAWPDSLRDKIFGSLAFLAKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDSRKRSHAGYQTI (SEQ ID NO: 457 - HLA-A*0201-specific).

In yet another preferred embodiment, the polyepitope construct consists of the sequence

MELAALCRWGLLLALLPPGAPADGKTPTAENPEYAALPASPETHLPILKYSEDPTVPLPDGALPTHDPSPLADNKEILDEAYADEILDEAYVMPLVVVLGVVFADMQIAKGMSYALMTFGAKPYPLGKAPPPAFSPAFADLHCPALVTYAKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDSRKRSHAGYQTI (SEQ ID NO: 458 - HLA-B*3501-specific).

In conjunction with the polyepitope constructs of the invention, provided herein are pharmaceutical compositions comprising such polyepitope constructs and a pharmaceutically acceptable carrier or excipient.

Further provided herein are nucleic acids encoding such polyepitope constructs, pharmaceutical compositions comprising such nucleic acids and a pharmaceutically acceptable carrier or excepient, and host cells comprising such nucleic acids.

In another aspect, the invention provides a method for inducing T cell responses in mammals comprising administering to said mammals polyepitope constructs of the invention or nucleic acids encoding such polyepitope constructs.

In yet another aspect, the invention provides a method for treating a HER2-positive breast cancer in mammals comprising administering to said mammals polyepitope constructs of the invention or nucleic acids encoding such polyepitope constructs.

The present invention is further explained below using detailed disclosure and specific examples. Such description, materials, methods, and examples are illustrative only and not intended to be limiting. All cited literature references, patents and patent applications are incorporated herein in their entireties.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-B show the results of cytotoxicity assays. T-cell immunity was stimulated ex vivo by autologous dendritic cells (DCs) transfected either with pHER2 (positive control), or with plasmids coding for polyepitope constructs of the invention (pBCU – "universal" one – containing HER2 epitopes, predicted to be the most promiscuous MHC-binders, or pBCA0201 – containing HER2 epitopes restricted by HLA-A*0201), or with plasmid prHA5 coding for an unrelated protein rHA5 corresponding to a portion (aa 17-346) of Influenza A virus H5N1 hemagglutinin (HA). Unstimulated non-adherent mononuclear cells (None) were used as negative controls. Either autologous DCs transfected with pHER2 **(A)** or MCF-7 breast cancer cells (HER2+/HLA-A*0201+) **(B)** were used as target cells. Cytotoxicity was assessed at different ratio of effector to target cells (10:1, 20:1, 30:1). Statistical significance of observed differences between the groups was assessed using Wilcoxon rank-sum test. $P < 0.05$ was considered to be significant.

Figures 2A-B show the levels of γIFN production by T-cells determined by intracellular cytokine staining followed by flow cytometry. Results are represented as percent (%) of double-positive T-cells as compared to the total number of either CD8+ **(A)** or CD4+ **(B)** T-cells (1×10^5 cells). None –unstimulated non-adherent mononuclear cells (MNCs) (negative control); DC:prHA5 – MNCs stimulated by DCs transfected with prHA5 (negative control); DC:pHER2 – MNCs stimulated by DCs transfected with pHER2; DC:pBCU – MNCs stimulated by DCs transfected with pBCU; DC:pBCA0201 – MNCs stimulated by DCs transfected with pBCA0201. MCF-7 cancer cells were used as target cells in these expreiments. Statistical significance of observed differences between the groups was assessed using Wilcoxon rank-sum test. $P < 0.05$ was considered to be significant.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on development of new methods for arranging immunogenic epitopes into polyepitope constructs aimed at optimizing proteasome and/or immunoproteasome processing of the polyepitope and optimizing TAP-binding of released epitopes. The new methods of the invention are based on the novel algorithm of epitope arrangement which allows to choose appropriate epitope matchings and spacer sequences taking into account predicted efficiency of proteasomal processing, spacer length and the number of predicted "non-target" CTL-epitopes resulting from artificial junction of epitopes through the spacer. These new methods of the invention lead to generation of novel HER2-specific polyepitope constructs (also disclosed herein) which are characterized by greatly enhanced antigen presentation as compared to the native HER2 antigen.

The present invention provides immunogenic polyepitope constructs comprising two or more different T cell epitopes, which epitopes are CTL epitopes or T-helper (Th) epitopes and are derived from one or more disease-associated antigens or pathogens, and wherein the epitopes are optionally joined by spacer sequences which improve the immunogenicity of the polyepitope construct by providing efficient proteasome and/or immunoproteasome processing of the epitopes and enhancing their interaction with Transporters Associated with Antigen Processing (TAP). As compared to the use of whole protein antigens, the use of the spacer-containing polyepitope constructs of the invention results in an enhanced efficiency of epitope presentation by antigen presenting cells (APCs).

The polyepitope constructs of the invention can comprise CTL epitopes or Th epitopes or both. CTL and Th epitopes can be either mixed within a construct or can be arranged into separate CTL and Th epitope clusters. In a separate embodiment, the invention provides a combination of two or more polyepitope constructs, wherein at least one of the constructs is CTL epitope-only or Th epitope-only. Th epitopes are primarily useful to stimulate CD4+ T-cell responses, and CTL epitopes are primarily useful to stimulate CD8+ T-cell responses. The present invention also encompasses combinations of two or more different polyepitope constructs. To induce an effective

T-cell immune response, it is important to induce both CTL (CD8+) and Th (CD4+). Thus, the preferred polyepitope constructs of the present invention include both CTL and Th epitopes.

The sequences of the different epitopes within polyepitope constructs of the invention can be derived from any part of a polypeptide antigen and can overlap to some degree (i.e., share from at least one amino acid residue to all but one amino acid residue) or they can be non-overlapping. The epitopes used within the construct can be arranged in any order as compared to the antigen from which they are derived. Epitopes used within polyepitope constructs of the invention can be of any specified length but are preferably at least 8 amino acids in length. CTL epitopes are preferably 8-12 amino acids in length. Th epitopes are preferably 9-25 amino acids in length. The MHC class I alleles to which the epitopes in the polyepitope constructs of the present invention bind can be any human class I or II allomorphs, e.g., HLA-A*0101, HLA-A*0201, HLA-A*0301 etc. A given epitope may be promiscuous, i.e., bind more than one MHC allotype. Preferably, the epitopes used in the polyepitope constructs of the invention are promiscuous MHC-binders. A representative list of class I-binding epitopes of the HER2 protein, any of which can be included in the polyepitope constructs of the invention, is provided in Example 2.1.1, below. A representative list of class II-binding epitopes of the HER2 protein any of which could be included in the polyepitope constructs of the invention, is provided in Example 2.3.1.1, below. Examples of epitopes selected for 30 human MHC class I alleles are provided in Example 2.2.1, below. These epitopes can be used either to construct "universal" polyepitope constructs aimed to evoke cellular immune responses in the majority of humans, or to produce "allele-specific" polyepitope constructs specific for certain HLA alleles.

The polyepitope constructs of the invention can be specific for a particular disease-associated antigen or pathogen (including two or more strains of the same pathogen), or can contain epitopes derived from two or more different antigens or pathogens. In one preferred embodiment, the polyepitope constructs of the invention comprise epitopes of HER2 protein.

The use of individual epitopes within the constructs of the invention allows to achieve efficient MHC class I and MHC class II-dependent antigen presentation even when only a partial sequence of a disease-associated antigen or pathogen is available (e.g., in cases of newly discovered pathogens or tumor antigens). The use of individual epitopes as opposed to whole antigens also allows to avoid problems associated with interference with antigen presentation by certain protein antigens (e.g., viral or bacterial proteins down-regulating host immune responses, down-regulating expression of MHC molecules on the cellular surface, interfering with cytokine signaling etc.), or deleterious effects (e.g., toxicity) associated with over-expression of particular viral proteins or tumor antigens.

An important additional advantage of the present invention is that the assortment of epitopes within the polyepitope constructs increases the likelihood that at least one epitope will be presented by each of a variety of HLA allotypes. This allows for immunization of a population of individuals polymorphic at the HLA locus, using a single polyepitope construct or a nucleic acid encoding such polyepitope construct. Alternatively, the polyepitope construct can be specific for a particular HLA allotype (e.g., it can contain epitopes with certain HLA-specificity).

In a specific embodiment, the polyepitope constructs of the invention further comprise Th epitopes which are not derived from a disease-associated antigen or pathogen but enhance the CD4+ T-cell responses to the antigen or pathogen (e.g., Pan DR T Helper Epitope [PADRE epitope] AKFVAAWTLKAAA [SEQ ID NO: 1]).

The use of the spacer sequences in the polyepitope constructs of the invention is optional, and two or more of the epitopes can be contiguous (i.e., joined end-to-end) with no spacer between them.

The spacer sequences used in the polyepitope constructs of the invention are degenerate spacer motifs which are optimized for every pair of epitopes to provide the best processing efficiency using novel algorithms of epitope arrangement and sequence optimization. The spacer sequences useful in the polyepitope constructs of the invention can consist of a single amino acid residue or a sequence of two or more amino acids inserted between two neighboring epitopes (or between an epitope and other sequences) of the construct. Preferably, such spacer sequences consist of up to 6 amino acids. However, spacer sequences of up to 7, 8, 10, 15, 20, 30, or 50 amino acids and even longer sequences are also possible. Spacer sequences are useful for promoting proteolytic processing of polyepitope constructs to release individual epitopes for antigen presentation. The spacers sequences are typically removed from the epitope sequences by proteolytic processing within antigen-presenting cell (APC). This leaves the epitopes intact for binding to MHC molecules. Occasionally, a spacer amino acid or part of a spacer sequence will remain attached to an epitope through incomplete processing. This generally will have little or no effect on binding to the MHC molecule. In one preferred embodiment, the spacer used to connect two or more Th epitopes within the polyepitope construct has the core sequence K/R-K/R, which corresponds to cleavage sites recognized by cathepsins B and L.

In another preferred embodiment, the spacer connecting two CTL epitopes can be derived from the following amino acids in the corresponding positions: [AGKNPRS] [ADGILTV] [AEGKLNV] [AFIKLNSV] [AEGIKLPSV] [AEGKLSV] (SEQ ID NO: 463). This degenerate motif can be used as a basis for selection of spacer sequences for optimizing processing. While preferred length of spacer sequences is about 3-4 amino acids, the invention encompasses both shorter and longer sequences. E.g. two epitopes would be joined without any spacer (using blank spacer) if they could be joined end-to-end according to the scoring function.

In a specific embodiment, polyepitope constructs of the invention further comprise N-terminally conjugated modified ubiquitin (e.g., ubiquitin with G76V substitution [UbV76]), which further enhances proteasomal processing of the epitopes contained in the construct and also enhances CTL-responses. UbV76 can be fused directly to the amino terminus of the polyepitope construct or Arg or Val residue can be inserted between UbV76 and polyepitope construct to stabilize the resulting chimeric constructs (Andersson H.A., Barry M.A., 2004, Mol Ther, 10(3):432-446).

In a specific embodiment, the polyepitope constructs of the invention further comprise one or more targeting signals which direct intracellular transport of the construct to the specific compartment of the cell. Non-limiting examples of useful targeting signals include, for example, (i) homologous or heterologous signal peptides targeting constructs to the secretory pathway via the endoplasmic reticulum (ER) and trans-Golgi network (e.g., the signal peptide of HER2 protein) and (ii) endosome-targeting signals (e.g., a portion or the whole sequence of the invariant chain associated with MHC class II molecules; C-terminal portion of the human LAMP-1 protein, the tyrosine-motif Y-X-X-hydrophobic amino acid, wherein X is any amino acid). A preferred targeting signal useful in the polyepitope constructs of the invention includes both C-terminal portion of LAMP-1 and the signal peptide of HER2 protein. This targeting signal is useful for upregulating MHC class II-dependent antigen presentation and CTL response (because the signal peptide of HER2 protein contains CTL epitopes). The targeting signals used in the constructs of the present invention can be optionally modified to introduce an amino acid substitution or spacer sequences at the junction(s) between the targeting signal and the adjacent segment(s) to promote cleavage of the targeting sequence(s) from the epitopes by, e.g., a signal peptidase. The targeting sequences useful in the polyepitope constructs of the invention can contain substitutions of any amino acid except those relevant for targeting.

In conjunction with the polyepitope polypeptide constructs of the invention, provided herein are nucleic acids encoding such polyepitope polypeptide constructs, vectors comprising such nucleic acids (e.g., plasmid, bacterial, and viral vectors), and host cells which comprise such nucleic acids or vectors (e.g., dendritic cells (DC), Langerhans cells, or other antigen presenting cells). When the polyepitope constructs of the invention are administered as nucleic acids and/or using various delivery vehicles (e.g., microparticles, virus-like particles, etc.), such nucleic acids and/or delivery vehicles can further enhance the antigen-specific immune responses (e.g., by promoting IL-12 and γ-interferon (γIFN) release from macrophages, NK cells, and T cells).

The present invention further provides pharmaceutical compositions comprising (i) the polyepitope polypeptide constructs of the invention or nucleic acids encoding such polyepitope polypeptide constructs or vectors comprising such nucleic acids and (ii) a pharmaceutically acceptable carrier or excipient. Such compositions can further comprise a delivery vehicle (such as, e.g., a microparticle).

The polypeptide and nucleic acid constructs and compositions of the invention can be administered via different routes. For example, they can be administered to mucosal tissue (e.g., vaginal, nasal, lower respiratory, or gastrointestinal tissue [e.g., rectal]). Alternatively, they can be administered systemically, for example, intravenously, intramuscularly, intradermally, orally, or subcutaneously.

1.1 Definitions

As used herein, the term "tumor antigen" refers to a protein which is expressed exclusively in tumor cells, or is highly upregulated in tumor cells as compared to non-tumor homologs of the tumor cells. Such tumor antigens frequently serve as markers for differentiating tumor cells from their normal counterparts.

The term "epitope" as used herein refers to a T-cell epitope, e.g. an oligopeptide able to bind to either MHC class I or class II molecules and to stimulate T-cell immune responses of appropriate T-lymphocytes. The terms "universal epitope" and "universal polyepitope construct" are used herein to refer to epitopes and polyepitope constructs which evoke cellular immune responses in the majority of immunized population (e.g., humans). The terms "allele-specific epitope" and "allele-specific polyepitope construct" refer to epitopes and polyepitope constructs which evoke cellular immune responses in immunized subjects (e.g., humans) having certain MHC haplotype(s) (e.g., certain HLA alleles).

As used herein, the term "polyepitope" or "polyepitope construct" refers to an immunogenic construct including two or more different epitopes. Such different epitopes may have completely unrelated or related sequences and may overlap in their sequences to some degree (e.g., share at least one amino acid residue or share up to all but one residue), or they may be non-overlapping. A given epitope within the polyepitope need not be of any specified length but is preferably between 8 and 12 amino acids in length for MHC class I-restricted epitopes and preferably between 8 and 25 amino acids in length for MHC class II-restricted epitopes. In the polyepitope constructs of the present invention, two or more adjacent epitopes can be joined end-to-end, with no spacer between them. Alternatively, any two adjacent epitopes can be linked by a spacer sequence, as defined below. The epitopes within the polyepitope constructs of the present invention can be arranged in any order (e.g., such order does not have to reflect the order of these epitopes within the protein they are derived from). The polyepitope constructs of the invention can contain any number of epitopes, but preferably contain at least 5 epitopes (in case of allele-specific constructs) or at least 20 epitopes (in case of universal constructs).

The term "polyCTL" refers to a polyepitope construct including either known or predicted epitopes for CD8+ T-lymphocytes.

The terms "polyThelper" or "polyTh" refer to a polyepitope construct including either known or predicted epitopes for CD4+ T-lymphocytes.

The term "junk epitope" refers to an epitope, not found in original antigen(s) of interest, generated due to artificial conjunction of chosen epitopes and/or spacer sequences within the polyepitope construct.

The term "targeting signal" refers to a sequence which directs intracellular transport of the polyepitope construct to a specific compartment of an antigen-presenting cell (APC).

The terms "spacer sequence", "spacer" and "flanking sequence" are used interchangeably to refer to a single amino acid residue or a sequence of two or more amino acids inserted between two neighboring epitopes or an epitope and another sequence within a polyepitope construct which improve the immunogenicity of the polyepitope construct by providing efficient proteasome and/or immunoproteasome processing of the epitopes and enhancing their interaction with Transporters Associated with Antigen Processing (TAP).

The term "therapeutically effective" applied to dose or amount refers to that quantity of a polyepitope construct or pharmaceutical composition or vaccine that is sufficient to result in a desired activity upon administration to a mammal in need thereof. As used herein with respect to polyepitope construct-containing compositions or vaccines, the term "therapeutically effective amount/dose" is used interchangeably with the term "immunogenically effective amount/dose" and refers to the amount/dose of a polyepitope construct or pharmaceutical composition or vaccine that is sufficient to produce an effective immune response upon administration to a mammal. According to the present invention, a preferred immunogenically effective amount of the polyepitope construct is in the range of 1-950 μg per kg of the body weight.

The phrase "pharmaceutically acceptable", as used in connection with compositions of the invention, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce unwanted reactions when administered to a human. Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

The term "carrier" applied to pharmaceutical or vaccine compositions of the invention refers to a diluent, excipient, or vehicle with which a compound (e.g., an antigen and/or an MHC molecule) is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution, saline solutions, and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin, 18th Edition.

The term "about" or "approximately" usually means within 20%, more preferably within 10%, and most preferably still within 5% of a given value or range. Alternatively, especially in biological systems (e.g., when measuring an immune response), the term "about" means within about a log (i.e., an order of magnitude) preferably within a factor of two of a given value.

In accordance with the present invention, conventional molecular biology, microbiology, and recombinant DNA techniques may be employed within the skill of the art. Such techniques are well-known and are explained fully in the literature. See, e.g., Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (herein "Sambrook et al., 1989"); DNA Cloning: A Practical Approach, Volumes I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); Nucleic Acid Hybridization [B. D. Hames & S. J. Higgins eds. (1985)]; Transcription And Translation [B. D. Hames & S. J. Higgins, eds. (1984)]; Animal Cell Culture [R. I. Freshney, ed. (1986)]; Immobilized Cells And Enzymes [IRL Press, (1986)]; B. Perbal, A Practical Guide To Molecular Cloning (1984); F. M. Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, Inc. (1994).

All other terms found here are used in their common meaning in the specified fields of interest: molecular biology, immunology, cytology, bioinformatics.

1.2 Antigens Used as a Source of Epitopes of the Invention

While the specific polyepitope constructs disclosed herein are based on HER2-specific epitopes and are useful for inducing immune response to HER2-expressing breast cancer cells, the same principals as described herein are applicable to all other disease-specific polyepitope constructs. The antigens useful as a source of epitopes in the polyepitope constructs of the present invention include without limitation various viral, bacterial, fungal, parasite-specific, and tumor-specific antigens. Non-limiting examples of viral antigens of the invention include antigens derived from influenza virus (e.g., surface glycoproteins hemagglutinin (HA) and neuramimidase (NA)); immunodeficiency virus (e.g., a human immunodeficiency virus antigens (HIV) such as gp120, gp160, p18 antigen Gag p17/p24, Tat, Pol, Nef, and Env); herpesvirus (e.g., a glycoprotein from herpes simplex virus (HSV), Marek's Disease Virus, cytomegalovirus (CMV), or Epstein-Barr virus); hepatitis virus (e.g., Hepatitis B surface antigen (HBsAg)); papilloma virus; rous associated virus (e.g., RAV-1 env); infectious bronchitis virus (e.g., matrix and/or preplomer); flavivirus (e.g., a Japanese encephalitis virus (JEV) antigen, a Yellow Fever antigen, or a Dengue virus antigen); Morbillivirus (e.g., a canine distemper virus antigen, a measles antigen, or rinderpest antigen such as HA or F); rabies (e.g., rabies glycoprotein G); parvovirus (e.g., a canine parvovirus antigen); poxvirus (e.g., an ectromelia antigen, a canary poxvirus antigen, or a fowl poxvirus antigen); chicken pox virus (varicella zoster antigen); infectious bursal disease virus (e.g., VP2, VP3, or VP4); Hantaan virus, and mumps virus. Non-limiting examples of bacterial antigens of the invention include lipopolysaccharides isolated from gram-negative bacterial cell walls and staphylococcus-specific, streptococcus-specific, pneumococcus-specific (e.g., PspA; see PCT Publication No. WO 92/14488), Neisseria gonorrhea-specific, Borrelia-specific (e.g., OspA, OspB, OspC antigens of Borrelia associated with Lyme disease such as Borrelia burgdorferi, Borrelia afzelli, and Borrelia garinii [see, e.g., U.S. Pat. No. 5,523,089; PCT Publication Nos. WO 90/04411, WO 91/09870, WO 93/04175, WO 96/06165, WO93/08306; PCT/US92/08697; Bergstrom et al., Mol. Microbiol., 1999; 3: 479486; Johnson et al., Infect. and Immun. 1992; 60: 1845-1853; Johnson et al., Vaccine 1995; 13: 1086-1094; The Sixth International Conference on Lyme Borreliosis: Progress on the Development of Lyme Disease Vaccine, Vaccine 1995; 13: 133-135]), and pseudomonas-specific proteins or peptides. Non-limiting example of malaria-specific antigen is malarial circumsporozoite (CS) protein. Non-limiting examples of fungal antigens include those isolated from candida (e.g., MP65 from Candida albicans), trichophyton, and ptyrosporum. Non-limiting examples of tumor-specific antigens include WT-1 antigen (in lymphoma and other solid tumors), ErbB receptors, Melan A [MART1], gp 100, tyrosinase, TRP-1/gp 75, and TRP-2 (in melanoma); MAGE-1 and MAGE-3 (in bladder, head and neck, and non-small cell carcinoma); HPV EG and E7 proteins (in cervical cancer); Mucin [MUC-1] (in breast, pancreas, colon, and prostate cancers); prostate-specific antigen [PSA] (in prostate cancer); carcinoembryonic antigen [CEA] (in colon, breast, and gastrointestinal cancers) and such shared tumor-specific antigens as MAGE-2, MAGE-4, MAGE-6, MAGE-10, MAGE-12, BAGE-1, CAGE-1,2,8, CAGE-3 TO 7, LAGE-1, NY-ESO-1/LAGE-2, NA-88, GnTV, and TRP2-INT2. Non-limiting examples of autoimmune disease-specific antigens include GAD 65, IA-2 and insulin B chain (for type 1-diabetes), and myelin basic protein and glatiramer acetate (GA) (for multiple sclerosis).

1.3 Algorithm of Epitope Selection

The epitopes useful in the polyepitope constructs of the present invention can be determined using computational methods.

Useful computational methods include, for example, the original TEpredict software (Antonets D.V., Maksyutov A.Z., 2010, MolBiol 44(1):130-139; http://tepredict.sourceforge.net).

Predictive models for TEpredict were built using partial least squares (PLS) regression on the basis of known peptide-HLA binding data, taken from IEDB (Immune Epitope Database, http://www.epimmune.org). Models, included in TEpredict, use scales of physicochemical properties of aminoacids to parametrize peptides.

Predictive models useful for the present invention can be represented with the following general formula:

$$pIC_{50} = \sum_{i=1}^{9} \omega_i P_i + const$$

, where pIC_{50} is the measure of MHC-peptide binding affinity, P_i is a vector of properties, encoding aminoacid a at position i in the peptide; ω_i is a vector with weights of these properties.

There are numerous other algorithms which can be used for defining T-cell epitopes useful in the polyepitope constructs of the present invention. One non-limiting example is artificial neural network-based methods developed by Lundegaard et al. (Lundegaard C. et al. 2008. *NAR*, 36:W509-512.).

In one embodiment of the present invention, predictions of MHC class I-binding epitopes were made for 30 different HLA alleles (HLA-A*0101, A*0201, A*0202, A*0203, A*0206, A*0301, A*2301, A*2402, A*2403, A*2601, A*2902, A*3001, A*3002, A*3101, B*0702, B*0801, B*1501, B*1801, B*2705, B*3501, B*4001, B*4002, B*4402, B*4403, B*4501, B*5101, B*5301, B*5401, B*5701, B*5801). The predicted value of pIC50 greater then 6.8 was chosen to differentiate binders from non-binders.

Making prediction of peptide-TAP binding affinity before the prediction of MHC class I-binding epitopes was shown to lower the rate of false positive prediction results (Peters B et al., 2003, J. Immunol, 171:1741–1749), thus the prediction of peptide-to-TAP binding can be used for selection of potential T-cell epitopes in the methods of the present invention. TAP-binding prediction can be used as a filter to avoid selecting epitopes which inefficiently interact with TAP or as a ranking function to weight peptides according to their predicted TAP-binding affinity. Prediction of peptide-TAP binding can be done using algorithms implemented in TEpredict or using other relevant computational tools. E.g., in one of the specific embodiments of the present invention, from 1247 peptides of the HER2 protein 860 peptides were selected using TAP-binding affinity prediction. TAP binding prediction implemented in TEpredict is based on predictive model and algorithms developed by Peters et al. (J. Immunol, 2003, 171:1741–1749).

Prediction of proteasome and/or immunoproteasome cleavage of protein antigen of interest can be applied to choose peptides possessing a cleavage site at their C-terminus (proteasome was shown to generate C-terminus of naturally occurring MHC I-binding epitopes). Prediction of proteasome and/or immunoproteasome processing can also be used either as a filter or as a ranking function. In one embodiment of the present invention, 338 peptides from HER2 protein were selected using a combination of proteasome and immunoproteasome filters. Algorithms for predicting proteasomal and/or immunoproteasomal processing of protein antigens which were implemented in TEpredict software were based on predictive models developed by Toes et al. (Toes RE et al., 2001, J. Exp. Med, 194:1-12). Determination of threshold levels for predicting proteasome processing is described in, e.g., Singh and Raghava (Singh H and Raghava GP, 2003, Bioinformatics, 19:1009-1014).

While such additional steps of selection can lead to false negative results, they can be advantageous in terms of immunodominance. E.g., peptides, selected using these filters and predicted to bind to TAP and to have proteasomal cleavage site on their C-terminus, are likely to be more efficiently released in vivo. Indeed, Peters et al. (J. Immunol, 2003, 171:1741–1749) and Doytchinova et al. (J. Immunol, 2004, 173:6813-6819) had shown that preselection of peptides predicted to efficiently bind to TAP lowered the number of false-positive results when predicting T-cell epitopes.

Specific non-limiting examples of predicted epitopes chosen for inclusion into polyepitope constructs of the present invention are provided in Examples, below.

In one embodiment of the present invention, promiscuous MHC class I- or class II-binders were selected using greedy algorithm. This algorithm allows to choose the minimal number of peptides to cover the diversity of selected MHC allotypes. The epitopes were selected with five-fold redundancy, i.e., at most five potential epitopes for every MHC allotype, used for predictions, were contained in the created set. This was thought to be important due to extremely high polymorphism of HLA genes. This algorithm was created to cover the majority of individuals in the populations of interest by the smallest number of peptides; to create a redundant set of promiscuous epitopes to construct a “universal” set of peptides able to evoke immune responses in the majority of humans. Non-limiting examples of selected MHC class I and class II-binders are provided in Examples, below.

In an alternative set of embodiments, HLA allele-specific polyepitope constructs were created for vaccination of individuals with specified HLA alleles. In one such embodiment, HLA allele-specific sets were created for 30 different HLA class I alleles. Two different sets were created for each allele using two different prediction algorithms. These sets are listed in Table 3, below.

1.4 Algorithms for Combining Epitopes into Polyepitope Constructs of the Invention

1.4.1 Methods for optimizing Epitope interaction with TAP

To make processing of epitopes within polyepitope constructs of the invention more efficient the present inventors have developed novel spacer (flanking) sequences aimed to optimize peptide binding to TAP. In the specific computational methods disclosed herein, TAP-binding affinity was predicted for every epitope within the polyepitope construct and spacer sequences were added only to peptides predicted to be inefficient TAP-binders.

In one specific embodiment of the present invention, an algorithm for choosing spacer sequences to optimize TAP binding is based on matrices and methods developed by Peters et al. (J. Immunol, 2003, 171:1741–1749) included in TEpredict. In this algorithm, affinity of peptide-TAP binding is calculated according the formula: N1+N2+N3+C, where N1 corresponds to contribution of the first N-terminal amino acid, N2 – of the second amino acid from the N-terminus of the peptide, N3 – of the third amino acid from the N-terminus of the peptide, and C is the contribution of the last (C-terminal) amino acid. In this algorithm, C-terminus needs to be unchanged (because it was shown that there are no active carboxypeptidases within endoplasmic reticulum (ER), and thus proteasomal processing is believed to provide C-terminus of the epitope while the N-terminus of the peptide could be trimmed by ERAPs (ER aminopeptidases)) and only N-terminal amino acids can be added to improve TAP binding. In one specific embodiment, ARY motif and its shorter derivatives were chosen as the N-terminal spacer sequence. First, Ala (A) residue was added to the epitope and if that peptide was predicted to be inefficient TAP binder, Ala-Arg (AR) motif was added to the epitope. If that peptide was predicted to bind to TAP with low affinity then Ala-Arg-Tyr (ARY) motif was added to the epitope. For many of the epitopes used in the polyepitope constructs of the present invention, only a single Ala residue was needed for efficient interaction with TAP. In another embodiment, a degenerate motif for optimization of peptide binding to TAP was used, e.g. [ANRK][RQYW][YWFVI] (SEQ ID NO: 464).

1.4.2 Methods for Optimizing Proteasome and/or Immunoproteasome Processing of Epitopes

In the methods of the present invention, to optimize proteasome and/or immunoproteasome release of epitopes from the polyepitope constructs of the invention, spacer sequences need to be determined for every pair of epitopes. This can be done using, for example, the two different algorithms described below.

The first algorithm is based on the use of 6 amino acid – long consensus spacer sequence ADLVKV (SEQ ID NO: 2), which is optimal for both proteasome and immunoproteasome processing. For optimization of the release of C-termini of epitopes, ProPred1 **matrices** can be used (Toes RE et al., 2001, J. Exp. Med, 194:1-12; Singh H., Raghava G.P., 2003, Bioinformatics, 19(8):1009-14). For combination analysis and data presentation, directed graphs can be used, where peptides are nodes of the graph and edges connecting nodes A and B define the combinations, where the necessary cleavage site is present at the C-terminus of peptide A.

Other spacer sequences can be used with the same algorithm. For example, sequence ADLVAG (SEQ ID NO: 3) can be used to optimize proteasome processing, and sequence ADLAVK (SEQ ID NO: 4) can be used to optimize immunoproteasome processing. Degenerate variants of these spacer sequences can be also used, wherein any amino acid from the sequence can be replaced by any of the 20 naturally occurring amino acids. All amino acids within the spacer can be replaced simultaneously. Furthermore, the spacer can be shorter or longer than 6 amino acids in length. However, the spacer selection is not random, since the selection of spacer sequence for every pair of epitopes is made according to the scoring function. When a spacer sequence between epitopes A and B is predicted, the preference is given to amino acids providing the most efficient release of the C-terminus of epitope A. Determination can be performed using models incorporated within TEpredict or any other model for predicting proteasome and/or immunoproteasome processing.

This version of algorithm for constructing a polyepitope construct of the invention can be presented by the following sequence of steps:

1. addition of spacer sequences (for optimization of epitope interaction with TAP) for all chosen epitopes (if needed);

2. testing of spacer sequences from the group consisting of ' ', 'A', 'AD', 'ADL', 'ADLV' (SEQ ID NO: 5), 'ADLVK' (SEQ ID NO: 6), 'ADLVKV' (SEQ ID NO: 2), until the resulting construct contains all requisite chosen epitopes or until all spacer sequences are tested.

If the resulting construct does not include all requisite chosen epitopes:

2.1. a graph is constructed;

2.2. if the graph contains adjacent vertices, choose the path with the maximal length;

2.3. exclude vertices corresponding to peptides included in the chosen path;

2.4. add to the selection peptide(s) corresponding to the chosen path;

2.5. see point 2.2;

2.6. if the graph does not contain adjacent vertices, create a new selection of peptides consisting of chosen paths and remaining nodes of the graph; go back to the new cycle (point 2).

3. as a result, a sequence of the polyepitope construct should be obtained; if the path was not chosen, which included all epitopes, repeat algorithm from point 2 at a lower stringency of proteasome/immunoproteasome filter.

The present invention also encompasses various modifications of the above algorithm. For example, an additional cycle can be included which uses different values of stringency of proteasome/immunoproteasome filter.

The second approach is based on the use of a degenerate optimal spacer sequence [APRS] [DILT] [AGL] [AKV] (SEQ ID NO: 460) for optimizing proteasome and/or immunoproteasome processing. This sequence is used to create a selection of spacer sequences of 1-4 amino acids in length, which selection includes more than 150 different sequences. Other degenerate optimal spacer sequences can be also used. For example, [ARSPNK] [DLITGV] [LGAVEK] [VKAFSI] [ALKSEI] [GVKLSE] (SEQ ID NO: 461) can be used as a basis for selection of spacer sequences for optimizing proteasome processing, and [AGNRKP] [DIATVG] [LGANVE] [ASNVLK] [VIKAGP] [KAGVSE] (SEQ ID NO: 462) can be used as a basis for selection of spacer sequences for optimizing immunoproteasome processing. While preferred length of spacer sequences is about 3-4 amino acids, the invention encompasses both shorter and longer sequences. Degenerate variants of the spacer sequences can be also used with amino acid changes in positions which do not affect proteasome and/or immunoproteasome processing.

When the above second approach is used, for each combination of epitopes A and B, the selected spacer sequence is the sequence which allows for efficient proteasome cleavage at the C-terminus of epitope A, predicted at a given level of stringency of the proteasome filter. Briefly, the filter works as follows: for any overlapping nanomeric peptides extracted from the antigen sequence the probability of proteasomal cleavage site on its C-terminus is predicted; if predicted score is less than selected threshold value then the peptide is excluded from further analysis. See also Toes RE et al., 2001, J. Exp. Med, 194:1-12; Singh H., Raghava G.P., 2003, Bioinformatics, 19(8):1009-14. For all selected variants, epitope prediction is conducted, and one prediction is chosen for each pair of peptides (using criteria described below). Then a polyepitope construct is assembled, wherein the first peptide is used as a function argument, or is selected automatically (as the best based on chosen criteria). If any given peptide is not included in the final polyepitope construct, the algorithm searches for peptides, which can be used for insertion of this omitted peptide. If no place for insertion is found, the omitted peptide is used as a starting peptide.

The following criteria can be used for choosing the spacer sequence for peptides A and B: the number of junk epitopes predicted for a given spacer; the number of MHC allomorphs, which interact with these junk epitopes; the length of the spacer (normally, the shorter spacers are preferred). All variants of spacer sequences are arranged by predicted efficiency of the release of the C-terminus of peptide A. These criteria can be used as filters; they can be used together or separately, and in different sequence. Also, the stringency of prediction of potential T-cell epitopes and proteasome and/or immunoproteasome processing of peptide fragments can be varied.

The above criteria are used for selecting the first pair of peptides (if the first peptide was not previously defined) and for selecting each following peptide.

1.4.3 Methods for Minimizing the Number of "Junk" Epitopes

While literature describes induction of T-cell immune responses to all antigenic peptides which can be presented by allelic variants of MHC molecules of a given organism, the present inventors believe that it is important to minimize the number of "junk" epitopes which are formed at the junctions of epitopes within the polyepitope constructs of the present invention. Minimizing the number of junk epitopes is important, because such epitopes can gain immunologic advantage by being heterologous for a given organism, and T lymphocytes which can interact with them have not been subjected to negative selection. The second algorithm for constructing the polyepitope constructs of the present invention provided above was created in part for solving this problem. See also, Example 2.1.2, below.

1.5 The Algorithm for Selection and Joining of Th Epitopes

The above methods address selection and arrangement of CTL epitopes which are used for induction of CD8+ T-lymphocytes. Preferably, the polyepitope constructs of the present invention also contain Th epitopes which are used for induction of CD4+ T-lymphocutes.

Th epitopes can be predicted using, for example, TEpredict. Also, a universal immunogenic peptide PADRE (Pan DR T Helper Epitope) can be used, since it interacts with a large number of common HLA-DR allomorphs as well as murine I-A^b.

The following fragments containing Th epitopes for most ErbB2 MHC II allomorphs were chosen for predictions:

AVVGILLVVVLGVVFGILIKRRQQKIR, PICTIDVYMIMVKCWMIDSE, AQMRILKETELRKVKVLGSGA, IKWMALESILRRRFTHQSDV, PICTIDVYMIMVKCWMIDS (SEQ ID NOS: 7, 8, 9, 10, 11 respectively)

When these fragments were chosen, 3-5 amino acids flanking the epitope were included as potentially important for interaction with certain T-cell receptors.

The peptides were joined by KK motifs which correspond to sites for cleavage by lysosomal catepsins B and L.

AKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDS (PADRE is in bold; spacer sequence are underlined) (SEQ ID NO: 12)

1.6 Targeting Signals Useful in the Polyepitope Constructs of the Invention

Numerous studies have demonstrated that inclusion of N-terminal signal sequences of various proteins and C-terminal lysosomal sorting sequence from human LAMP-1 protein in immunogenic constructs results in high level of Th response as compared to constructs which do not contain such targeting signals (Bonini C. et al. Greenberg P.D. *Jour Immunol,* 2001, 166(8):5250-5257; Su Z. et al. 2002, 62(17):5041-5048; Bonehill A. et al. *Jour Immunol,* 2004, 172(11):6649-57; Fassnacht M. et al. *Clinical Cancer Res,* 2005, 11(15):5566-71). The use of N-terminal signal sequences ensures targeting to ER and secretory pathway, while the use of the C-terminal lysosomal sorting sequence from human LAMP-1 protein ensures targeting of the associated immunogen from the secretory pathway into lysosomes for degradation, where peptide fragments bind to MHC-II molecules leading to their presentation on the cell surface.

A preferred N-terminal targeting signal used in the polyepitope constructs of the present invention is a slightly modified version of the HER2 signal peptide: MELAALCRWGLLLALLPPGAP (SEQ ID NO: 13) or the original HER2 signal peptide MELAALCRWGLLLALLPPGAAS (SEQ ID NO: 14).

Carboxy terminal sorting signal can be the last 11 amino acids of the LAMP-1 protein: RKRSHAGYQTI (SEQ ID NO: 15). A longer fragment of LAMP-1 can be also used as a sorting signal, e.g. the last 34 amino acids: IPIAVGGALAGLVLIVLIAYLVGRKRSHAGYQTI (SEQ ID NO: 16) – transmembrane and cytoplasmic domains.

Two non-limiting examples of preferred polyepitope constructs of the present invention are as follows:

1. N-signal|PolyTh|PolyCTL|LAMP-1
2. N-signal|PolyCTL|PolyTh|LAMP-1

As specified above, combinations of all-CTL and all-Th constructs as well as intermixed arrangements of CTL and Th epitopes are also encompassed.

Another example of useful endosomal targeting signal is a portion (first 110 amino acids) or the whole sequence of the invariant chain (Ii) associated with MHC class II molecules. This signal enhances the efficiency of induction of CD4+ T-cell response. Also, Th epitopes may be associated with the immunoregulatory fragment of Ii, LRMKLPKPPKPVSQMR (SEQ ID NO: 17, Ii 77-92), or its shorter fragments such as, e.g., LRMKLPK (SEQ ID NO: 18) or LRMK (SEQ ID NO: 19).

N-terminally conjugated ubiquitin (e.g., ubiquitin with G76V substitution [UbV76]) can be used in the polyepitope constructs of the present invention to further enhance proteasomal processing of the epitopes contained in the constructs and also to enhance CTL (CD8+) responses.

UbV76 can be conjugated directly to the amino terminus of the polyepitope construct or Val or Arg residue can be inserted between UbV76 and polyepitope construct to further stabilize the resulting chimeric constructs. See Example 2.4.5, below.

1.7 Production of the Polyepitope Constructs of the Invention

The polyepitope constructs of the present invention can be produced synthetically using various methods well known in the art (e.g., exclusive solid phase synthesis, automated solid phase synthesis, partial solid phase synthesis methods, fragment condensation, classical solution synthesis, etc.; see, *e.g.*, Merrifield J. Am. Chem. Soc. 1963 85:2149 and Merrifield et al., 1982, Biochemistry, 21:502; Stewart, *Solid Phase Peptide Syntheses*, Freeman and Co.: San Francisco, 1969; 2002/2003 General Catalog from Novabiochem Corp, San Diego, USA; Goodman, *Synthesis of Peptides and Peptidomimetics*, Houben-Weyl, Stuttgart 2002) or can be expressed in a prokaryotic or eukaryotic host cell using various expression vectors encoding such constructs. Thus, provided herein are isolated polynucleotides that encode the polyepitope constructs of the present invention as well as recombinant vectors and host cells (both eukaryotic and prokaryotic) that have been genetically modified to express or overexpress the polyepitope constructs of the present invention. The host cells may be cultured or otherwise maintained under conditions permitting expression of the polyepitope polypeptide from the nucleic acid, e.g., the plasmid, encoding it.

The polyepitope constructs of the invention can be modified in various ways to improve their pharmacokinetic and other properties (e.g., to generate constructs with more favorable solubility, stability, and/or susceptibility to hydrolysis and/or proteolysis). Polyepitope constructs can be modified at the amino (N-) terminus, and/or carboxy (C-) terminus and/or by replacement of one or more of the naturally occurring genetically encoded amino acids with an unconventional amino acid, modification of the side chain of one or more amino acid residues, peptide phosphorylation, and the like.

Amino terminus modifications include methylation (*e.g.*, $--NHCH_3$ or $--N(CH_3)_2$), acetylation (*e.g.*, with acetic acid or a halogenated derivative thereof such as α-chloroacetic acid, α-bromoacetic acid, or α-iodoacetic acid), adding a benzyloxycarbonyl (Cbz) group, or blocking the amino terminus with any blocking group containing a carboxylate functionality defined by RCOO-- or sulfonyl functionality defined by $R--SO_2--$, where R is selected from alkyl, aryl, heteroaryl, alkyl aryl, and the like, and similar groups. One can also incorporate a desamino acid at the N-terminus (so that there is no N-terminal amino group) to decrease susceptibility to proteases or to restrict the conformation of the peptide compound.

Carboxy terminus modifications include replacing the free acid with a carboxamide group or forming a cyclic lactam at the carboxy terminus to introduce structural constraints. One can also incorporate a desamino or descarboxy residue at the termini of the construct, so that there is no terminal amino or carboxyl group, to decrease susceptibility to proteases.

One can also replace any of the 20 naturally occurring amino acids. Common examples of conventional amino acid replacements include stereoisomers (*e.g.*, D-amino acids) and unnatural amino acids such as, for example, L-ornithine, L-homocysteine, L-homoserine, L-citrulline, 3-sulfino-L-alanine, N-(L-arginino)succinate, 3,4-dihydroxy-L-phenylalanine, 3-iodo-L-tyrosine, 3,5-diiodo-L-tyrosine, triiodothyronine, L-thyroxine, L-selenocysteine, N-(L-arginino)taurine, 4-aminobutylate, (R,S)-3-amino-2-methylpropanoate, a,a-disubstituted amino acids, N-alkyl amino acids, lactic acid, β-alanine, 3-pyridylalanine, 4-hydroxyproline, O-phosphoserine, N-methylglycine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, nor-leucine, and other similar amino acids and imino acids. A general method for site-specific incorporation of unnatural amino acids into proteins and peptides is described in Noren et al., Science, 244:182-188 (April 1989).

1.8 Pharmaceutical and Immunogenic Compositions and Methods for Delivery of the Polyepitope Constructs of the Invention

The polyepitope constructs of the invention can be administered directly, but are preferably administered as part of immunogenic compositions comprising pharmaceutically acceptable carrier(s) and/or excipient(s). In a specific embodiment, the polyepitope constructs of the invention are administered conjointly (together in one composition or separately in two different compositions, which can be administered simultaneously or sequentially to the same or different site) with an adjuvant. Any adjuvant known in the art can be used. Non-limiting examples of adjuvants useful in the immunogenic compositions of the present invention include oil-emulsion and emulsifier-based adjuvants such as complete Freund's adjuvant, incomplete Freund's adjuvant, AS03, MF59, or SAF; mineral gels such as aluminum hydroxide (alum), aluminum phosphate or calcium phosphate; microbially-derived adjuvants such as cholera toxin (CT), pertussis toxin, Escherichia coli heat-labile toxin (LT), mutant toxins (e.g., LTK63 or LTR72), Bacille Calmette-Guerin (BCG), Corynebacterium parvum, DNA CpG motifs, muramyl dipeptide, or monophosphoryl lipid A; particulate adjuvants such as immunostimulatory complexes (ISCOMs), liposomes, biodegradable microspheres, or saponins (e.g., QS-21); cytokines such as IFN-γ, IL-2, IL-12 or GM-CSF; synthetic adjuvants such as nonionic block copolymers, muramyl peptide analogues (e.g., N-acetyl-muramyl-L-threonyl-D-isoglutanine [thr-MDP], N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-[1'-2'-dipalmitoyl-s- n-glycero-3-hydroxyphosphoryloxy]-ethylamine), polyphosphazenes, or synthetic polynucleotides, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, hydrocarbon emulsions, or keyhole limpet hemocyanins (KLH). Preferably, these adjuvants are pharmaceutically acceptable for use in humans.

The polyepitope constructs of the invention can be also administered in the form of nucleic acids encoding such polyepitope constructs (e.g., a plasmid, viral or any other appropriate vector). To achieve expression of the polyepitope construct in a target cell (e.g., dendritic cell (DC), Langerhans cell, or other antigen presenting cell (APC), or any other host cell), such vectors should contain one or more regulatory sequences which permit expression in such cells. Such regulatory sequence(s) can be operatively linked to the sequence encoding the polyepitope construct, such that they drive expression of the latter.

The polyepitope constructs of the invention or nucleic acids encoding them can be delivered in a microparticle that also includes a polymeric matrix or in a synthetic viral vector. Any suitable viral vector can be used (e.g., Adenovirus, Poxvirus, Lentivirus, etc.). See also http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed&cmd=DetailsSearch&term=microparticle+polymeric+antigen.

When the polyepitope constructs of the invention are administered as nucleic acids and/or using various delivery vehicles (e.g., microparticles, virus-like particles), such nucleic acids and/or delivery vehicles can further enhance the antigen-specific immune responses (e.g., by promoting IL-12 and γ-interferon (IFN) release from macrophages, NK cells, and T cells).

The polyepitope constructs of the invention can be used to produce antigen presenting cells (APCs, e.g., dendritic cells (DC), Langerhans cells, or other type), capable to present desired epitopes to the lymphocytes. Desired APCs can be obtained using any method known in the art, e.g., in vitro by transfecting e.g. DCs (derived from e.g. monocytes of the patient) with polynucleotides (either DNA or mRNA), coding for the polyepitope, or by pulsing with corresponding polyepitope polypeptide, or by infecting with recombinant vector microorganism bearing corresponding gene coding for the polyepitope, or some other similar technique known in the art. Produced APCs can be used either as a therapeutic cellular vaccine, or to produce ex vivo autologous effector T-cells for using them as a therapeutic cellular vaccine.

The polypeptide and nucleic acid constructs and compositions of the invention can be administered via different routes. For example, they can be administered to mucosal tissue (e.g., vaginal, nasal, lower respiratory, or gastrointestinal tissue [e.g., rectal]). Alternatively, they can be administered systemically, for example, intravenously, intramuscularly, intradermally, orally, or subcutaneously.

1.9 Effective Dose and Safety Evaluations

According to the methods of the present invention, the pharmaceutical and immunogenic compositions described herein are administered to a patient at immunogenically effective doses, preferably, with minimal toxicity.

Following methodologies which are well-established in the art (see, e.g., Goldenthal et al., National Cooperative Vaccine Development Working Group. AIDS Res. Hum. Retroviruses 1993, 9:545-9), effective doses and toxicity of the compounds and compositions of the instant invention can be first determined in preclinical studies using small animal models (e.g., mice) in which these compounds and compositions can be reproducibly immunized by the same route proposed for the human clinical trials. Specifically, for any pharmaceutical composition or vaccine used in the methods of the invention, the therapeutically effective dose can be estimated initially from animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms). Dose-response curves derived from animal systems are then used to determine testing doses for the initial clinical studies in humans. In safety determinations for each composition, the dose and frequency of immunization should meet or exceed those anticipated for use in the clinical trial.

As disclosed herein, the dose of polyepitope constructs and other components in the compositions of the present invention is determined to ensure that the dose administered continuously or intermittently will not exceed a certain amount in consideration of the results in test animals and the individual conditions of a patient. A specific dose naturally varies depending on the dosage procedure, the conditions of a patient or a subject animal such as age, body weight, sex, sensitivity, feed, dosage period, drugs used in combination, seriousness of the disease. The appropriate dose and dosage times under certain conditions can be determined by the test based on the above-described indices and should be decided according to the judgment of the practitioner and each patient's circumstances according to standard clinical techniques. In this connection, the preferred dose of a polyepitope construct is generally in the range of 1-950 μg per kg of the body weight depending on the mode of delivery and immunization.

Toxicity and therapeutic efficacy of polyepitope constructs in immunogenic compositions of the invention can be determined by standard pharmaceutical procedures in experimental animals, e.g., by determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compositions that exhibit large therapeutic indices are preferred. While therapeutics that exhibit toxic side effects can be used (e.g., when treating severe forms of cancer, life-threatening infections or autoimmune diseases), care should be taken to design a delivery system that targets such immunogenic compositions to the specific site in order to minimize potential damage to other tissues and organs and, thereby, reduce side effects. As disclosed herein, the polyepitope constructs of the invention are highly immunostimulating and possess low toxicity.

As specified above, the data obtained from the animal studies can be used in formulating a range of dosage for use in humans. The therapeutically effective dosage of polyepitope constructs of the present invention for use in humans lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. Ideally, a single dose should be used.

2 EXAMPLES

The present invention is further described by way of the following particular examples. However, the use of such examples is illustrative only and is not intended to limit the scope or meaning of this invention or of any exemplified term. Nor is the invention limited to any particular preferred embodiment(s) described herein. Indeed, many modifications and variations of the invention will be apparent to those skilled in the art upon reading this specification, and such "equivalents" can be made without departing from the invention in spirit or scope. The invention is therefore limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

2.0 Sequence of human full-length ErbB2 (HER2) protein:

```
>gi|119533|sp|P04626.1|ERBB2_HUMAN   RecName:   Full=Receptor   tyrosine-protein
kinase  erbB-2;  AltName:  Full=p185erbB2;  AltName:  Full=C-erbB-2;  AltName:
Full=NEU  proto-oncogene;  AltName:  Full=Tyrosine  kinase-type  cell  surface
receptor HER2; AltName: Full=MLN 19; AltName: CD_antigen=CD340; Flags: Precursor
MELAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNAS
LSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNTTPVTGASPGGLREL
QLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGESSE
DCQSLTRTVCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTFE
SMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKCSKPCARVCYGLGMEHL
REVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSLP
DLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLALIHHNTHLCFVHTVPWDQLFRNPH
QALLHTANRPEDECVGEGLACHQLCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHC
LPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINC
THSCVDLDDKGCPAEQRASPLTSIISAVVGILLVVVLGVVFGILIKRRQQKIRKYTMRRLLQETELVEPL
TPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENTSPKANKEILDE
AYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHVRENRGRLGSQDLLNWCMQIAKGMSYLEDVR
LVHRDLAARNVLVKSPNHVKITDFGLARLLDIDETEYHADGGKVPIKWMALESILRRRFTHQSDVWSYGV
TVWELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTIDVYMIMVKCWMIDSECRPRFRELVSEFSRMA
RDPQRFVVIQNEDLGPASPLDSTFYRSLLEDDDMGDLVDAEEYLVPQQGFFCPDPAPGAGGMVHHRHRSS
STRSGGGDLTLGLEPSEEEAPRSPLAPSEGAGSDVFDGDLGMGAAKGLQSLPTHDPSPLQRYSEDPTVPL
PSETDGYVAPLTCSPQPEYVNQPDVRPQPPSPREGPLPAARPAGATLERPKTLSPGKNGVVKDVFAFGGA
VENPEYLTPQGGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLGLDVPV
```

(SEQ ID NO: 20)

The following Examples illustrate the invention without limiting its scope.

2.1 Universal CTL Epitopes

2.1.1 A List of Universal CTL Epitopes

CRWGLLLAL, LAALCRWGL, RELGSGLAL, WGLLLALLP, LVVVLGVVF, KITDFGLAR, QLFEDNYAL, YISAWPDSL, GDLTLGLEP, DVWSYGVTV, KIFGSLAFL, FDGDLGMGA, LVHRDLAAR, MELAALCRW, RASPLTSII, RGAPPSTFK, SIISAVVGI, LHCPALVTY, LRIVRGTQL, VKVLGSGAF, LQPEQLQVF, VKIPVAIKV, QLMPYGCLL, QETELVEPL, DIFHKNNQL, ASCVTACPY, TELVEPLTP, PLQRLRIVR, LQVIRGRIL, DEAYVMAGV, EECRVLQGL, TVCAGGCAR, YSEDPTVPL, RWGLLLALL, FEDNYALAV, QEVQGYVLI, LLALLPPGA, GSGAFGTVY, LGISWLGLR, ISAVVGILL, MQIAKGMSY, LSYMPIWKF,

GVVKDVFAF, AIKVLRENT, SWLGLRSLR, ILLVVVLGV, FGPEADQCV, TLQGLGISW, TDFGLARLL, DSTFYRSLL, IISAVVGIL, TTPVTGASP, GMEHLREVR, ALCRWGLLL, RIVRGTQLF, GSCTLVCPL, DGENVKIPV (SEQ ID NOS: 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, and 77, respectively)

2.1.2 A List of Peptide Fragments Containing Overlapping ErbB2 Epitopes

MELAALCRWGLLLALLPPGA, QEVQGYVLI, PLQRLRIVRGTQLFEDNYALAV, TTPVTGASP, DIFHKNNQL, TVCAGGCAR, LHCPALVTY, ASCVTACPY, GSCTLVCPL, GMEHLREVR, KIFGSLAFL, LQPEQLQVF, YISAWPDSL, LQVIRGRIL, TLQGLGISWLGLRSLRELGSGLAL, EECRVLQGL, FGPEADQCV, LSYMPIWKF, RASPLTSIISAVVGILLVVVLGVVF, QETELVEPLTP, VKVLGSGAFGTVY, DGENVKIPVAIKVLRENT, DEAYVMAGV, QLMPYGCLL, MQIAKGMSY, LVHRDLAAR, KITDFGLARLL, DVWSYGVTV, DSTFYRSLL, GDLTLGLEP, FDGDLGMGA, YSEDPTVPL, GVVKDVFAF, RGAPPSTFK (SEQ ID NOS: 78, 56, 79, 72, 45, 52, 38, 46, 76, 73, 31, 41, 28, 49, 80, 51, 67, 62, 81, 82, 83, 84, 50, 43, 61, 33, 85, 30, 70, 29, 32, 53, 63, and 36, respectively)

2.1.3 Several Versions of Universal PolyCTL Constructs

Constructs with fixed optimal spacer sequence:

For 33 peptides selected with 3-fold excess (overall sequence length 297 aa):

2.1.3.1 polyCTL construct with spacer sequences which optimize TAP interaction and proteasome processing:

CRWGLLLALLVVVLGVVFSIISAVVGIRELGSGLALMELAALCRWADLARDEAYVMAGVADLVEECRVLQGLADYSEDPTVPLAVKIPVAIKVAQLFEDNYALADVWSYGVTVAWGLLLALLPATVCAGGCARADIFHKNNQLADASCVTACPYADLLHCPALVTYATELVEPLTPADLKITDFGLARARGAPPSTFKADLYISAWPDSLAQETELVEPLALQVIRGRILALAALCRWGLADLQLMPYGCLLADKIFGSLAFLARGDLTLGLEPAVKVLGSGAFADLVHRDLAARADLQPEQLQVFADAFDGDLGMGAAPLQRLRIVRADLRIVRGTQLARASPLTSII (SEQ ID NO: 86)

(overall length is 349 aa; spacers constitute 17.5% of the sequence)

2.1.3.2. polyCTL construct with spacer sequences which optimize TAP interaction and immunoproteasome processing:

QETELVEPLASCVTACPYADLVKVCRWGLLLALSIISAVVGIAARDEAYVMAGVADLVKLHCPALVTYARASPLTSIIADLV
EECRVLQGLAFDGDLGMGAARGAPPSTFKADLKIFGSLAFLMELAALCRWADLVQLMPYGCLLAQLFEDNYALKITDFGLAR
ADYISAWPDSLTVCAGGCARADLWGLLLALLPADLVHRDLAARADLYSEDPTVPLRELGSGLALARGDLTLGLEPAVKVLGS
GAFADLQPEQLQVFADLDVWSYGVTVADLRIVRGTQLAPLQRLRIVRADLAALCRWGLAVKIPVAIKVADLQVIRGRILALV
VVLGVVFADIFHKNNQLATELVEPLTP (SEQ ID NO: 87)

(overall length is 355 aa; spacers constitute 19.5% of the length)

2.1.3.3. polyCTL construct with spacer sequences which optimize TAP interaction and proteasome and immunoproteasome processing:

CRWGLLLALASCVTACPYADLYISAWPDSLAVKIPVAIKVAQLFEDNYALADVWSYGVTVAWGLLLALLPADIFHKNNQLAT
ELVEPLTPADLLHCPALVTYAPLQRLRIVRADLQLMPYGCLLADKIFGSLAFLMELAALCRWADLVHRDLAARADLQPEQLQ
VFADAFDGDLGMGAALQVIRGRILAVKVLGSGAFADLRIVRGTQLARGAPPSTFKADLQETELVEPLRELGSGLALLVVVLG
VVFSIISAVVGIARGDLTLGLEPADKITDFGLARALAALCRWGLADYSEDPTVPLTVCAGGCARARASPLTSIIADLVEECR
VLQGLAARDEAYVMAGV (SEQ ID NO: 88)

(overall length is 345 aa; spacers constitute 16.1% of the length)

For 57 peptides selected with 5-fold excess:

2.1.3.4. polyCTL construct with spacer sequences which optimize TAP interaction and proteasome processing:

CRWGLLLALAFGPEADQCVADLQLMPYGCLLADYSEDPTVPLAVKIPVAIKVAQLFEDNYALADVWSYGVTVAWGLLLALLP
ATVCAGGCARAISAVVGILLATLQGLGISWADSWLGLRSLRADLVKRWGLLLALLLLALLPPGARELGSGLALLVVVLGVVF
SIISAVVGIILLVVVLGVAIISAVVGILAIKVLRENTADLVQETELVEPLALQVIRGRILAGVVKDVFAFADLARDEAYVMA
GVADLPLQRLRIVRADLKITDFGLARALGISWLGLRADLQEVQGYVLIADLHCPALVTYAVKVLGSGAFADGMEHLREVRAD
TTPVTGASPADASCVTACPYADLYISAWPDSLARGDLTLGLEPADRGAPPSTFKADLRIVRGTQLATELVEPLTPADAFDGD
LGMGAALAALCRWGLADLQPEQLQVFADAFEDNYALAVAMQIAKGMSYATDFGLARLLMELAALCRWADLVHRDLAARADGS
GAFGTVYARDGENVKIPVADLVDSTFYRSLLADLVEECRVLQGLADKIFGSLAFLALCRWGLLLADIFHKNNQLADLSYMPI
WKFADLVGSCTLVCPLARASPLTSIIADLRIVRGTQLF (SEQ ID NO: 89)

(overall length is 612 aa; spacers constitute 19.3% of the length)

2.1.3.5. polyCTL construct with spacer sequences which optimize TAP interaction and immunoproteasome processing:

TTPVTGASPADLSWLGLRSLRADLVGSCTLVCPLAIKVLRENTADYSEDPTVPLMELAALCRWADLRWGLLLALLILLVVVL
GVADLWGLLLALLPADLVHRDLAARADLDVWSYGVTVADLGISWLGLRADLVKVQETELVEPLTDFGLARLLRELGSGLALA
IISAVVGILAFGPEADQCVADLVKVCRWGLLLALISAVVGILLGSGAFGTVYADLSYMPIWKFADLVEECRVLQGLGVVKDV
FAFADLAFEDNYALAVADLKIFGSLAFLASCVTACPYADLVKVQLMPYGCLLAARDEAYVMAGVADLVKLHCPALVTYAVKV

LGSGAFADLQPEQLQVFADLRIVRGTQLFADLVDSTFYRSLLADGMEHLREVRADLRIVRGTQLATVCAGGCARADLAALCR
WGLAPLQRLRIVRADLQVIRGRILALVVVLGVVFADIFHKNNQLATLQGLGISWAQLFEDNYALARGDLTLGLEPAARDGEN
VKIPVADLVALCRWGLLLALLALLPPGAARGAPPSTFKADLKITDFGLARADMQIAKGMSYADAFDGDLGMGAAVKIPVAIK
VARASPLTSIIADLQEVQGYVLIADYISAWPDSLSIISAVVGIATELVEPLTP (SEQ ID NO: 90)

(overall length is 627 aa; spacers constitute 22.2% of the length)

2.1.3.6. polyCTL construct with spacer sequences which optimize TAP interaction and proteasome and immunoproteasome processing:

CRWGLLLALISAVVGILLAFGPEADQCVADLQETELVEPLTDFGLARLLRELGSGLALLVVVLGVVFSIISAVVGIILLVVV
LGVAIISAVVGILGSGAFGTVYAIKVLRENTADLRIVRGTQLFADLVKLHCPALVTYAVKVLGSGAFADGMEHLREVRADYI
SAWPDSLALCRWGLLLAVKIPVAIKVALAALCRWGLADTTPVTGASPADRGAPPSTFKADLYSEDPTVPLAFDGDLGMGALL
ALLPPGAARDGENVKIPVADLVDSTFYRSLLADGSCTLVCPLMELAALCRWADSWLGLRSLRADLVPLQRLRIVRADLKITD
FGLARALGISWLGLRADLQEVQGYVLIADKIFGSLAFLASCVTACPYADLRASPLTSIIADLVEECRVLQGLAARDEAYVMA
GVADLRWGLLLALLGVVKDVFAFADLQLMPYGCLLADLQPEQLQVFADLRIVRGTQLAMQIAKGMSYADVWSYGVTVAWGLL
LALLPATVCAGGCARAQLFEDNYALARGDLTLGLEPADIFHKNNQLATELVEPLTPADLVHRDLAARADAFEDNYALAVALQ
VIRGRILATLQGLGISWADLSYMPIWKF (SEQ ID NO: 91)

(overall length is 602 aa; spacers constitute 17.3% of the length)

Using degenerate spacer sequence:

2.1.3.7. Before selection of spacer sequences for optimal proteasome processing of 57 selected epitopes, selection of spacers optimal for TAP interaction was conducted. Selection was optimized to minimize the number of junk epitopes and to maximaize the number of interacting MHC I alleles, keeping the spacer sequences of the minimal size as preferred.

TVCAGGCARADGMEHLREVRADGKEECRVLQGLADGRELGSGLALPQLFEDNYALSDGQETELVEPLPLVVVLGVVFARDGE
NVKIPVALLALLPPGAAQEVQGYVLIPDLARGDLTLGLEPAIKVLRENTADAFDGDLGMGAPDAKARDEAYVMAGVADIFHK
NNQLAVKVLGSGAFATLQGLGISWAIAFGPEADQCVPDLKLSYMPIWKFADLKPLQRLRIVRAIISAVVGILMELAALCRWA
TGVVKDVFAFADLVKIPVAIKVSIISAVVGIPISAVVGILLPILQPEQLQVFADGKYSEDPTVPLADMQIAKGMSYARGAPP
STFKADLQVIRGRILPDGRASPLTSIIADLVHRDLAARADSWLGLRSLRADGKLGISWLGLRADGVKITDFGLARATDFGLA
RLLPDGDSTFYRSLLAILLVVVLGVADTTPVTGASPRDLRIVRGTQLATELVEPLTPPDLKASCVTACPYPILAALCRWGLA
DAFEDNYALAVAIDVWSYGVTVAWGLLLALLPRDAKQLMPYGCLLAIKIFGSLAFLALCRWGLLLRDGRIVRGTQLFADLVG
SGAFGTVYADGGSCTLVCPLPDGYISAWPDSLRDLHCPALVTYALLVCRWGLLLALRWGLLLALL (SEQ ID NO: 92)

(the overall length is 639 aa with spacer sequences constituting 22% of the overall length; in this construct, with chosen stringency of proteasome filter, 29 junk epitopes were predicted keeping all predicted epitopes; spacer sequences are underlined)

2.1.3.8. Before selection of spacer sequences for optimal proteasome processing of 34 selected overlapping epitopes, selection of spacers optimal for TAP interaction was conducted. Selection was optimized to minimize the number of junk epitopes and to maximaize the number of interacting MHC I alleles:

MELAALCRWGLLLALLPPGAPDGENVKIPVAIKVLRENTADGKEECRVLQGLPDGKYSEDPTVPLPDDEAYVMAGVADLKQETELVEPLTPPDGRASPLTSIISAVVGILLVVVLGVVFPDAGMEHLREVRADGKDIFHKNNQLPDLQPEQLQVFRDAQEVQGYVLIPDLAFDGDLGMGAPDLQVIRGRILPDVKVLGSGAFGTVYPIGDLTLGLEPPDLKASCVTACPYATLQGLGISWLGLRSLRELGSGLALPMQIAKGMSYALFGPEADQCVPDLKLSYMPIWKFADLKPLQRLRIVRGTQLFEDNYALAVARGAPPSTFKAGVVKDVFAFRDLVKITDFGLARLLPLVHRDLAARADVWSYGVTVRDTTPVTGASPRDLYISAWPDSLRTVCAGGCARSDKIFGSLAFLPDLHCPALVTYADDSTFYRSLLADGKQLMPYGCLLADGGSCTLVCPL (SEQ ID NO: 93)

(the overall length is 461 aa with spacer sequences constituting 22% of the overall length; in this construct, with chosen stringency of proteasome filter, 18 initially chosen epitopes are not predicted, but there are only 9 junk epitopes not present in ErbB2; with minimal stringency of proteasome filter, only 7 initially chosen epitopes are not predicted, but the number of junk epitopes increases to 106; spacer sequences are underlined)

2.2 Allele-Specific CTL Epitopes

2.2.1 Table of Chosen Allele-Specific Epitopes and Polyepitope Constructs

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
A*0101	LTCSPQPEY, GSGAFGTVY, EGAGSDVFD, YKDPPFCVA, TIDVYMIMV, YGVTVWELM, DGENVKIPV, LLDIDETEY, QSDVWSYGV, HLDMLRHLY, DGDPASNTA, NASLSFLQD, DGDLGMGAA, FSPAFDNLY, DSTFYRSLL, WGLLLALLP, YSEDPTVPL, LHCPALVTY, EITGYLYIS, DDDMGDLVD,	WGLLLALLP-RDA-YSEDPTVPL-ADIDETEYHA-PDLK-AREEGAGSDVFD-AYGVTVWELM-ALGK-ARDDDDMGDLVD-PLGK-AEITGYLYIS-ADGK-HLDMLRHLY-ADLK-AHSDCLACLH-AD-LTCSPQPEY-ADLK-QSDVWSYGV-AD-AYKDPPFCVA-PDL-ARDGDLGMGAA-PIAK-LLDIDETEY-AD-ARDGDPASNTA-AI-ARDGENVKIPV-ALL-GSGAFGTVY-PD-NASLSFLQD-PLLK-LHCPALVTY-AD-DSTFYRSLL-ADL-FSPAFDNLY-AILK-TIDVYMIMV (SEQ ID NO: 110)

HLA allele	Peptides	Example of poly CTL construct(s)
	HSDCLACLH, DIDETEYHA (SEQ ID NOS: _94, 58, 95, 96, 97, 98, 77, 99, 100, 101, 102, 103, 104, 105, 70, 24, 53, 38, 106, 107, 108, and 109, respectively)	
A*0201	LLLALLPPG, ILDEAYVMA, ILHNGAYSL, RLLQETELV, CRWGLLLAL, TIDVYMIMV, MIMVKCWMI, LVDAEEYLV, RLVHRDLAA, ALCRWGLLL, LLNWCMQIA, ALIHHNTHL, LLALLPPGA, QLFEDNYAL, AVVGILLVV, KIFGSLAFL, QLMPYGCLL, FEDNYALAV, VVLGVVFGI, ILLVVVLGV, SIISAVVGI, SLTLQGLGI, YISAWPDSL (SEQ ID NOS: 111, 112, 113, 114, 21, 97, 115, 116, 117, 74, 118, 119, 57, 27, 120, 31, 43, 55, 121, 66, 37, 122, and 28_, respectively)	***Var1:*** TIDVYMIMV-PDLK-CRWGLLLAL-A-LLALLPPGA-ADG-AILDEAYVMA-ALIHHNTHL-PDL-RLVHRDLAA-LLLALLPPG-ADGK-QLFEDNYAL-P-ILHNGAYSL-P-SLTLQGLGI-R-LVDAEEYLV-R-ILLVVVLGV-ADA-SIISAVVGI-A-RLLQETELV-AD-AFEDNYALAV-AVVGILLVV-A-VVLGVVFGI-AD-ALLNWCMQIA-ADLV-ALCRWGLLL-AD-YISAWPDSL-RD-KIFGSLAFL-RDL-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 123) ***Var2:*** MELAALCRWGLLLALLPPGAPPDLLALLPPGAPDATLEEITGYLAILDEAYVMAPILHNGAYSLPQLFEDNYALSIISAVVGIAQLMPYGCLLRLLVVVLGVVRDLQLRSLTEIAILLVVVLGVPDAVVGILLVVADALCRWGLLLADYISAWPDSLRDKIFGSLAFL (SEQ ID NO: 124)
A*0202	CLTSTVQLV, ILDEAYVMA, ILHNGAYSL, QIAKGMSYL, PCARVCYGL, RLLQETELV, KHSDCLACL, MIMVKCWMI, RWGLLLALL, TYLPTNASL, LVDAEEYLV, SLPDLSVFQ, FRNPHQALL, TLEEITGYL, DLVDAEEYL, ALIHHNTHL,	LVPQQGFFC-ADLV-PCARVCYGL-PDLK-KHSDCLACL--ATLEEITGYL-A-TLSPGKNGV-PDL-DLVDAEEYL-P-ILHNGAYSL-A-SLPDLSVFQ-RD-QIAKGMSYL-AILDEAYVMA-ALIHHNTHL-AI-AFGPEADQCV-RDLK-LVDAEEYLV-A-QLFEDNYAL-SIISAVVGI-ADG-THLDMLRHL-ACLTSTVQLV-ADG-FRNPHQALL-ADG-RLLQETELV-ADL-KIFGSLAFL-A-YISAWPDSL-RD-AYSLTLQGL-RDL-TYLPTNASL-SDA-RWGLLLALL-A-

HLA allele	Peptides	Example of poly CTL construct(s)
	QLFEDNYAL, AYSLTLQGL, KIFGSLAFL, QLMPYGCLL, YISAWPDSL, FGPEADQCV, LVPQQGFFC, SIISAVVGI, THLDMLRHL, TLSPGKNGV (SEQ ID NOS: 125, 112, 113, 126, 127, 114, 128, 115, 54, 129, 116, 130, 131, 132, 133, 119, 27, 134, 31, 43, 28, 67, 135, 37, 136, and 137, respectively)	QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 138)
A*0203	HYKDPPFCV, CLTSTVQLV, YLTPQGGAA, QIAKGMSYL, SLRELGSGL, HLYQGCQVV, MIMVKCWMI, PLTSIISAV, PYVSRLLGI, FRNPHQALL, RASPLTSII, ILLVVVLGV, LLNWCMQIA, ALIHHNTHL, LLALLPPGA, IQNEDLGPA, KIFGSLAFL, YLSTDVGSC, QLMPYGCLL, LLVVVLGVV, TLSPGKNGV, SIISAVVGI, SLTLQGLGI, YISAWPDSL (SEQ ID NOS: 139, 125, 140, 126, 141, 142, 115, 143, 144, 131, 35, 66, 118, 119, 57, 145, 31, 146, 43, 147, 137, 37, 122, and 28_, respectively)	HYKDPPFCV-AIGK-AIQNEDLGPA-RDL-QIAKGMSYL-A-TLSPGKNGV-SD-LLALLPPGA-ADG-PYVSRLLGI-AYLSTDVGSC-AD-ILLVVVLGV-ADA-SIISAVVGI-AD-SLRELGSGL-PTG-RASPLTSII-A-LLVVVLGVV-RDL-AYLTPQGGAA-ALIHHNTHL-AD-ARPLTSIISAV-ADL-FRNPHQALL-ADGK-KIFGSLAFL-ALLNWCMQIA-ADLK-ACLTSTVQLV-ADG-YISAWPDSL-A-HLYQGCQVV-ADL-SLTLQGLGI-AD-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 148)
A*0206	QVFETLEEI, LQLRSLTEI, YVLIAHNQV, QIAKGMSYL,	CRWGLLLAL-PD-AIQNEDLGPA-AVLDNGDPL-RLLQETELV-ADG-FRNPHQALL-PDLK-QVFETLEEI-PD-

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
	LLVVVLGVV, RLLQETELV, CRWGLLLAL, TIDVYMIMV, MIMVKCWMI, LAALCRWGL, AVLDNGDPL, FRNPHQALL, RASPLTSII, ALIHHNTHL, IWIPDGENV, TQLFEDNYA, SAVVGILLV, IQNEDLGPA, AVVGILLVV, KIFGSLAFL, QLMPYGCLL, VKIPVAIKV, VVLGVVFGI, ILLVVVLGV, SIISAVVGI (SEQ ID NOS: 149, 150, 151, 126, 147, 114, 21, 97, 115, 22, 152, 131, 35, 119, 153, 154, 155, 145, 120, 31, 43, 42, 121, 66, and 37, respectively)	QIAKGMSYL-PD-VVLGVVFGI-ADA-TQLFEDNYA-AD-AVVGILLVV-AD-RASPLTSII-A-LLVVVLGVV-RD-LQLRSLTEI-A-ILLVVVLGV-ADA-SIISAVVGI-PD-YVLIAHNQV-AD-VKIPVAIKV--ALIHHNTHL-A-LAALCRWGL-A-SAVVGILLV-ADGK-KIFGSLAFL-A-IWIPDGENV-AD-TIDVYMIMV-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 156)
A*0301	LAARNVLVK, VVFGILIKR, VMAGVGSPY, RILHNGAYS, LLLALLPPG, TFYRSLLED, VVVLGVVFG, QLVTQLMPY, GILLVVVLG, LELTYLPTN, LVKSPNHVK, ELMTFGAKP, TVWELMTFG, YLYISAWPD, ILKETELRK, YTMRRLLQE, RSLTEILKG, GVVFGILIK, VLRENTSPK, CVNCSQFLR, ILIKRRQQK, LERPKTLSP, MTFGAKPYD, ALLHTANRP, KIRKYTMRR, RGAPPSTFK, ILWKDIFHK, LAFLPESFD (SEQ ID NOS: 157, 158, 159, 160, 111, 161, 162, 163, 164,	CVNCSQFLR-AD-LVKSPNHVK-A-ILKETELRK-RDLK-ARILHNGAYS-AD-GVVFGILIK-ADG-AELMTFGAKP-PDGK-LELTYLPTN-ALGK-KIRKYTMRR-ADLV-LERPKTLSP-A-VLRENTSPK-A-LLLALLPPG-ADGK-RSLTEILKG-ALLHTANRP-A-ILIKRRQQK-ADGK-AGILLVVVLG-PDGK-TVWELMTFG-A-ILWKDIFHK-ADGK-RGAPPSTFK-ADL-QLVTQLMPY-A-VVVLGVVFG-PD-VMAGVGSPY-AILK-LAARNVLVK-ADL-YTMRRLLQE-ADGK-TFYRSLLED-RD-VVFGILIKR-A-LAFLPESFD-A-YLYISAWPD-AD-MTFGAKPYD (SEQ ID NO: 183)

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
	165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 36, 181, and 182, respectively)	
A*2301	VYMIMVKCW, DVWSYGVTV, RWGLLLALL, DIFHKNNQL, SYGVTVWEL, MIMVKCWMI, EYLVPQQGF, TYLPTNASL, EYHADGGKV, IWIPDGENV, YGVTVWELM, QLFEDNYAL, RFRELVSEF, EYVNARHCL, KWMALESIL, DLLEKGERL, LSYMPIWKF, LRIVRGTQL, VWSYGVTVW, ILLVVVLGV, QCVNCSQFL, LAALCRWGL (SEQ ID NOS: _184, 30, 54, 45, 185, 115, 186, 129, 187, 153, 98, 27, 188, 189, 190, 191, 62, 39, 192, 66, 193, and 22, respectively)	RWGLLLALL-A-EYVNARHCL-R-DLLEKGERL-AEYHADGGKV-S-DIFHKNNQL-A-QLFEDNYAL-P-LAALCRWGL-AI-AYGVTVWELM-AI-LRIVRGTQL-ILLVVVLGV-ADA-TYLPTNASL-A-IWIPDGENV-RLL-VWSYGVTVW-AL-EYLVPQQGF-ADLK-DVWSYGVTV-PDLK-RFRELVSEF-PDLK-LSYMPIWKF-ADL-SYGVTVWEL-ADA-QCVNCSQFL-ADAK-VYMIMVKCW-AILK-KWMALESIL-AI-MIMVKCWMI (SEQ ID NO: 194)
A*2402	VYMIMVKCW, LVVVLGVVF, TLQGLGISW, SYGVTVWEL, EYLVPQQGF, SLAFLPESF, TYLPTNASL, PYVSRLLGI, RIVRGTQLF, IWIPDGENV, RWGLLLALL, KWMALESIL, DLLEKGERL, QLMPYGCLL, VWSYGVTVW, AVVGILLVV, AWPDSLPDL (SEQ ID NOS: 184, 25, 68, 185, 186, 195,	AWPDSLPDL-DLLEKGERL-RDG-PYVSRLLGI-PDL-TLQGLGISW-A-SLAFLPESF-PDGK-AVVGILLVV-RT-LVVVLGVVF-A-IWIPDGENV-RLL-VWSYGVTVW-AL-EYLVPQQGF-ADLK-QLMPYGCLL-AD-SYGVTVWEL-ADL-TYLPTNASL-A-RIVRGTQLF-RWGLLLALL-A-KWMALESIL-AIGV-VYMIMVKCW (SEQ ID NO: 197)

HLA allele	Peptides	Example of poly CTL construct(s)
	129, 144, 75, 153, 54, 190, 191, 43, 192, 120, and 196_, respectively)	
A*2403	VYMIMVKCW, FYRSLLEDD, CYGLGMEHL, LQGLGISWL, SEGAGSDVF, SYGVTVWEL, EYLVPQQGF, LQPEQLQVF, TYLPTNASL, RMARDPQRF, VTVWELMTF, YLVPQQGFF, RWGLLLALL, HNGAYSLTL, GEGLACHQL, RFRELVSEF, EYVNARHCL, KWMALESIL, FQNLQVIRG, AYSLTLQGL, VWSYGVTVW, RFTHQSDVW, FNHSGICEL, VRGTQLFED, AWPDSLPDL (SEQ ID NOS: 184, 198, 199, 200, 201, 185, 186, 41, 129, 202, 203, 204, 54, 205, 206, 188, 189, 190, 207, 134, 192, 208, 209, 210, and 196_, respectively)	RMARDPQRF-AD-AVRGTQLFED-RD-LQPEQLQVF-ADG-EYVNARHCL-ADA-RWGLLLALL-ASEGAGSDVF-AGEGLACHQL-PDLK-LQGLGISWL-AI-SYGVTVWEL-AD-AWPDSLPDL-PL-EYLVPQQGF-ADGK-HNGAYSLTL-AFNHSGICEL-A-YLVPQQGFF-ADGV-AYSLTLQGL-PDLK-RFRELVSEF-ADGK-ACYGLGMEHL-AL-VWSYGVTVW-AI-AFQNLQVIRG-ADG-VTVWELMTF-ADGK-AFYRSLLEDD-RDL-TYLPTNASL-AI-VYMIMVKCW-AILK-KWMALESIL-AD-RFTHQSDVW (SEQ ID NO: 211)
A*2601	SLRELGSGL, ISWLGLRSL, CRWGLLLAL, ETLEEITGY, LQPEQLQVF, VTVWELMTF, DTILWKDIF, HTVPWDQLF, ICELHCPAL, VSRLLGICL, ALCRWGLLL, QLFEDNYAL, DVFDGDLGM, ETHLDMLRH, FDGDLGMGA, KVPIKWMAL, DEAYVMAGV, CTIDVYMIM,	CTIDVYMIM-PI-ICELHCPAL-A-QLVTQLMPY-ADG-VSRLLGICL-ALCRWGLLL-PDLK-ARDEAYVMAGV-AD-ETLEEITGY-A-TEILKGGVL-P-QLFEDNYAL-PD-LQPEQLQVF-AD-KVPIKWMAL-SIISAVVGI-RD-DTILWKDIF-ALGV-AETHLDMLRH-A-DVFDGDLGM-PDLK-SLRELGSGL-STVQLVTQL-PLGK-ISWLGLRSL--AFDGDLGMGA-AD-CRWGLLLAL-PD-VTVWELMTF-ADGK-AFEDNYALAV-RDLK-HTVPWDQLF

HLA allele	Peptides	Example of poly CTL construct(s)
	TEILKGGVL, QLVTQLMPY, STVQLVTQL, SIISAVVGI, FEDNYALAV (SEQ ID NOS: 141, 212, 21, 213, 41, 203, 214, 215, 216, 217, 74, 27, 218, 219, 32, 220, 50, 221, 222, 163, 223, 37, and 55_, respectively)	(SEQ ID NO: 224)
A*2902	DVWSYGVTV, LTCSPQPEY, GSGAFGTVY, ICLTSTVQL, VMAGVGSPY, DIFHKNNQL, YLEDVRLVH, THQSDVWSY, GTVYKGIWI, ETLEEITGY, GVVKDVFAF, SMPNPEGRY, GTQLFEDNY, VCTGTDMKL, MQIAKGMSY, HTVPWDQLF, LEEITGYLY, RLVHRDLAA, LVVVLGVVF, LMTFGAKPY, HLDMLRHLY, QVVQGNLEL, SLTLQGLGI, SPAFDNLYY, FSPAFDNLY, PGGLRELQL, LHCPALVTY, CVTACPYNY, MSYLEDVRL (SEQ ID NOS: 30, 94, 58, 225, 159, 45, 226, 227, 228, 213, 63, 229, 230, 231, 61, 215, 232, 117, 25, 233, 101, 234, 122, 235, 105, 236, 38, 237, and 238__, respectively)	LHCPALVTY-SD-LTCSPQPEY-ADL-RLVHRDLAA-ALG-HLDMLRHLY-AD-LVVVLGVVF-PDGK-DIFHKNNQL-AD-LEEITGYLY-AD-GVVKDVFAF-AD-ARPGGLRELQL-AD-ETLEEITGY-ALL-THQSDVWSY-AD-AYLEDVRLVH-PDLK-QVVQGNLEL-AI-GSGAFGTVY-RL-VMAGVGSPY-AILK-LMTFGAKPY-AD-GTQLFEDNY-ADGK-CVTACPYNY-ADG-GTVYKGIWI-ADL-SMPNPEGRY-ADLK-HTVPWDQLF-ADLK-SLTLQGLGI-AD-MQIAKGMSY-A-ICLTSTVQL-SD-DVWSYGVTV-PDLK-MSYLEDVRL-RD-VCTGTDMKL-AD-FSPAFDNLY-AIL-SPAFDNLYY (SEQ ID NO: 239)

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
A*3001	HYKDPPFCV, GGKVPIKWM, RSRACHPCS, HVRENRGRL, MARDPQRFV, AARNVLVKS, LVKSPNHVK, TQRCEKCSK, LQRLRIVRG, STFKGTPTA, KLRLPASPE, YLYISAWPD, KRRQQKIRK, KETELRKVK, NVKIPVAIK, RPKTLSPGK, KIFGSLAFL, VARCPSGVK, AIKVLRENT, GCLLDHVRE, WGLLLALLP, KIRKYTMRR, PLQRLRIVR, PGKNGVVKD, VLRENTSPK, GLRSLRELG, KVKVLGSGA (SEQ ID NOS: 139, 240, 241, 242, 243, 244, 166, 245, 246, 247, 248, 169, 249, 250, 251, 252, 31, 253, 64, 254, 24, 180, 48, 255, 174, 256, and 257_, respectively)	KIRKYTMRR-A-YLYISAWPD--LVKSPNHVK-PLLK-KVKVLGSGA-PDG-KETELRKVK-PD-AIKVLRENT-AD-GGKVPIKWM-ADG-NVKIPVAIK-AD-ARGGCLLDHVRE-AGLRSLRELG-ADG-RPKTLSPGK-AI-LQRLRIVRG-PDGV-KLRLPASPE-A-WGLLLALLP-AD-RSRACHPCS-AILK-KRRQQKIRK-ADLK-HVRENRGRL-AD-ARPGKNGVVKD-A-PLQRLRIVR-RDAK-AARNVLVKS-AD-MARDPQRFV-A-VLRENTSPK-ADL-VARCPSGVK-ADL-HYKDPPFCV-AD-KIFGSLAFL-A-STFKGTPTA-ADL-TQRCEKCSK (SEQ ID NO: 258)
A*3002	ESFDGDPAS, KGMSYLEDV, LTCSPQPEY, VMAGVGSPY, VVKDVFAFG, DMGDLVDAE, THQSDVWSY, GGAVENPEY, SLTEILKGG, ETLEEITGY, SMPNPEGRY, GTQLFEDNY, SLPDLSVFQ, RSSSTRSGG, HLDMLRHLY, LMTFGAKPY, VLQGLPREY, PLTSIISAV, ASCVTACPY, DVFDGDLGM, SAVVGILLV, TVWELMTFG, SPAFDNLYY, FSPAFDNLY, YYWDQDPPE, EIPDLLEKG,	SMPNPEGRY-ADL-KHSDCLACL--ADMGDLVDAE-RDGK-CVTACPYNY-AL-GGAVENPEY-AL-AVVKDVFAFG-PLAK-AEIPDLLEKG-PDGK-HLDMLRHLY-ADLK-TVWELMTFG-AD-LTCSPQPEY-ADL-RSSSTRSGG-ADGK-ETLEEITGY-AD-VLQGLPREY-AD-ARPLTSIISAV-AL-ASCVTACPY-PLL-SAVVGILLV-ADLV-AESFDGDPAS-R-DVFDGDLGM-PIL-AAPRSPLAPS-AI-GTQLFEDNY-AIG-ASLTEILKGG-AD-KGMSYLEDV-AD-VMAGVGSPY-ATLK-SLPDLSVFQ-RDLK-THQSDVWSY-ADA-SPAFDNLYY-ADL-FSPAFDNLY-ADLK-YYWDQDPPE-ADLV-LMTFGAKPY (SEQ ID NO: 270)

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
	APRSPLAPS, KHSDCLACL, CVTACPYNY (SEQ ID NOS: 259, 260, 94, 159, 261, 262, 227, 263, 264, 213, 229, 230, 130, 265, 101, 233, 266, 143, 46, 218, 155, 168, 235, 105, 267, 268, 269, 128, and 237_, respectively)	
A*3101	VVFGILIKR, KVPIKWMAL, GMEHLREVR, QKIRKYTMR, TVCAGGCAR, MALESILRR, SPLDSTFYR, GVGSPYVSR, KITDFGLAR, RILKETELR, LVHRDLAAR, LACHQLCAR, PLQRLRIVR, VSEFSRMAR, LEDVRLVHR, VFQNLQVIR, LGISWLGLR, AALCRWGLL, QALLHTANR, KIFGSLAFL, CVNCSQFLR, REGPLPAAR, KIRKYTMRR, TLIDTNRSR, RQVPLQRLR, SWLGLRSLR (SEQ ID NOS: _ 158, 220, 73, 271, 52, 272, 273, 274, 26, 275, 33, 276, 48, 277, 278, 279, 59, 280, 281, 31, 175, 282, 180, 283, 284, and 65, respectively)	QALLHTANR-AIG-RQVPLQRLR-ADGK-QKIRKYTMR-ADGK-GVGSPYVSR-RILKETELR-ADL-LEDVRLVHR-ADG-TLIDTNRSR-ADL-GMEHLREVR-ADGK-REGPLPAAR-RIG-MALESILRR-PDGK-LGISWLGLR-ADGV-KITDFGLAR-A-PLQRLRIVR-ADG-VVFGILIKR-RDGK-LVHRDLAAR-A-TVCAGGCAR-RDG-KIRKYTMRR-ADG-AALCRWGLL-ADGK-KIFGSLAFL-PDG-KVPIKWMAL-SD-ASPLDSTFYR-ADL-VSEFSRMAR-ADLV-CVNCSQFLR-ADLK-LACHQLCAR-AD-VFQNLQVIR-AIL-SWLGLRSLR (SEQ ID NO: 285)
B*0702	RCEKCSKPC, SPKANKEIL, SPETHLDML, PPSPREGPL,	***Var1:*** AAPRSPLAPS-ALPAARPAGA-PDG-ALPTHDPSPL-A-ALPASPETHL-SD-ASPETHLDML-

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
	GAVENPEYL, GVVKDVFAF, SPGKNGVVK, AVLDNGDPL, HVRENRGRL, AARPAGATL, MPNQAQMRI, LPTHDPSPL, RASPLTSII, RKYTMRRLL, SPREGPLPA, GSCTLVCPL, DPASNTAPL, LPAARPAGA, APQPHPPPA, HPPPAFSPA, LPTNASLSF, VPLQRLRIV, LPASPETHL, APRSPLAPS, LQVIRGRIL (SEQ ID NOS: 286, 287, 288, 289, 290, 63, 291, 152, 242, 292, 293, 294, 35, 295, 296, 76, 297, 298, 299, 300, 301, 302, 303, 269, and 49, respectively)	AVLDNGDPL--ASPKANKEIL-P-GAVENPEYL--ASPGKNGVVK-AD-LPTNASLSF-ADPASNTAPL--AARPAGATL-AAPQPHPPPA-ADGV-LQVIRGRIL-PDG-RASPLTSII-ADL-APPSPREGPL-RDLK-HVRENRGRL-SDL-AHPPPAFSPA-PDLK-AMPNQAQMRI-ADLV-RKYTMRRLL-A-GVVKDVFAF-AD-AVPLQRLRIV-ADGK-GSCTLVCPL-AI-ASPREGPLPA-ADL-RCEKCSKPC (SEQ ID NO: 304) ***Var2:*** MELAALCRWGLLLALLPPGAPASPKANKEILAARPAGATLALPTHDPSPLAALPASPETHLSDASPETHLDMLADAPPSPREGPLRDLKHVRENRGRLADLACPSGVKPDLADGSTRSGGGDLPIASPLTSIISA (SEQ ID NO: 305)
B*0801	LVVVLGVVF, VVGILLVVV, ILRRRFTHQ, VLIQRNPQL, QQKIRKYTM, ISAVVGILL, SPKANKEIL, DIFHKNNQL, FGLARLLDI, MIMVKCWMI, YGVTVWELM, SLAFLPESF, LAALCRWGL, LQRLRIVRG, LGMEHLREV, MVHHRHRSS, LDSTFYRSL, ALIHHNTHL, MRILKETEL, VSRLLGICL, ECRPRFREL, LFRNPHQAL, KVPIKWMAL, LRIVRGTQL, VPLQRLRIV, YISAWPDSL, LIKRRQQKI, LQVIRGRIL, VGILLVVVL, ILLVVVLGV (SEQ ID NOS: _25, 306,	***Var1:*** YISAWPDSL-PDL-ECRPRFREL-AD-VGILLVVVL-PD-QQKIRKYTM-AD-LFRNPHQAL-AL-LIKRRQQKI-ADLK-AYGVTVWELM-PDLK-LGMEHLREV-ASPKANKEIL-ALIHHNTHL-A-DIFHKNNQL-AD-MVHHRHRSS-AD-AVPLQRLRIV-A-ILLVVVLGV-AD-VSRLLGICL-AFGLARLLDI-AI-LQRLRIVRG-AD-VVGILLVVV-PDG-KVPIKWMAL-SLAFLPESF-AI-LQVIRGRIL-LVVVLGVVF-A-MRILKETEL-RTG-VLIQRNPQL-PDLK-ILRRRFTHQ-AD-LAALCRWGL-AD-LDSTFYRSL-RD-LRIVRGTQL-PIAK-ISAVVGILL-AI-MIMVKCWMI (SEQ ID NO: 319) ***Var2:*** MELAALCRWGLLLALLPPGAPAIGFHKNNQLALASPKANKEILRDGKDIFHKNNQLPDGKLGMEHLREVADLFRNPHQALALLG

HLA allele	Peptides	Example of poly CTL construct(s)
	307, 308, 309, 60, 287, 45, 310, 115, 98, 195, 22, 246, 311, 312, 313, 119, 314, 217, 315, 316, 220, 39, 302, 28, 317, 49, 318, and 66, respectively)	CKKIFGSLPDLRIVRGTQLADGVMRILKETELSDGQLRSLTEILADGKECRPRFRELADGQLMPYGCLLPDLK (SEQ ID NO: 320)
B*1501	WCMQIAKGM, LTCSPQPEY, GSGAFGTVY, VMAGVGSPY, IQRNPQLCY, ISWLGLRSL, SEGAGSDVF, YGVTVWELM, TQCVNCSQF, SLAFLPESF, LQVIRGRIL, VTSANIQEF, VQGNLELTY, RIVRGTQLF, MQIAKGMSY, ALIHHNTHL, LVVVLGVVF, LMTFGAKPY, RELGSGLAL, ASCVTACPY, VKVLGSGAF, RACHPCSPM, QVVQGNLEL, VWSYGVTVW, QLFEDNYAL, TLIDTNRSR (SEQ ID NOS: _321, 94, 58, 159, 322, 212, 201, 98, 323, 195, 49, 324, 325, 75, 61, 119, 25, 233, 23, 46, 40, 326, 234, 192, 27, and 283, respectively)	LVVVLGVVF-A-IQRNPQLCY-AILV-TQCVNCSQF-ADG-TLIDTNRSR-ASEGAGSDVF-ALIHHNTHL-AI-AYGVTVWELM-AIGK-ISWLGLRSL-S-VKVLGSGAF-A-QLFEDNYAL-PLG-RELGSGLAL-ASCVTACPY-AIL-VTSANIQEF-AIG-VQGNLELTY-AD-LTCSPQPEY-ADLK-QVVQGNLEL-AI-GSGAFGTVY-RL-VMAGVGSPY-ADGV-LQVIRGRIL-SLAFLPESF-ADG-VWSYGVTVW-ADA-RIVRGTQLF-WCMQIAKGM-AD-MQIAKGMSY-A-LMTFGAKPY-RDL-RACHPCSPM (SEQ ID NO: 327)
B*1801	DVWSYGVTV, SEGAGSDVF, LELTYLPTN, SAWPDSLPD, TELVEPLTP, QETELVEPL, EECRVLQGL, MQIAKGMSY, LEEITGYLY, LDIDETEYH, PEYLTPQGG, QRFVVIQNE,	LRIVRGTQL-ASEGAGSDVF-ALDIDETEYH-ADLK-QETELVEPL-AD-ARPEYLTPQGG-ADGV-EEITGYLYI-PDGK-EECRVLQGL-ADG-RELGSGLAL-AEDLGPASPL-A-TEILKGGVL-P-LEEITGYLY-PLGK-AGDLGMGAAK-AD-LELTYLPTN-RDG-VKVLGSGAF-AD-TELVEPLTP-RDLK-SAWPDSLPD-AD-DVWSYGVTV-AD-MQIAKGMSY-AD-

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
	GDLGMGAAK, RELGSGLAL, LRIVRGTQL, VKVLGSGAF, EEITGYLYI, TEILKGGVL, EDLGPASPL (SEQ ID NOS: 30, 201, 165, 328, 47, 44, 51, 61, 232, 329, 330, 331, 332, 23, 39, 40, 333, 222, and 334, respectively)	QRFVVIQNE (SEQ ID NO: 335)
B*2705	GRILHNGAY, RRLLQETEL, ILDEAYVMA, ARPAGATLE, GRLGSQDLL, YLEDVRLVH, CRWGLLLAL, RRFTHQSDV, HRDLAARNV, FAGCKKIFG, RRQQKIRKY, AAGCTGPKH, FRNPHQALL, ARVCYGLGM, RKYTMRRLL, QRFVVIQNE, QRASPLTSI, YTMRRLLQE, CRVLQGLPR, AKGLQSLPT, LQPEQLQVF, VRGTQLFED, RNPQLCYQD, YLYISAWPD, LRIVRGTQL, GEGLACHQL, HRHRSSSTR, RELGSGLAL, RQVPLQRLR (SEQ ID NOS: 336, 337, 112, 338, 339, 226, 21, 340, 341, 342, 343, 344, 131, 345, 295, 331, 346, 171, 347, 348, 41, 210, 349, 169, 39, 206, 350, 23, and 284, respectively)	GRILHNGAY-ADG-CRWGLLLAL-LQPEQLQVF-AILDEAYVMA-RD-AKGLQSLPT-AD-GRLGSQDLL-ADG-RELGSGLAL-AYLEDVRLVH-RD-AFAGCKKIFG-ADG-FRNPHQALL-PIGK-AGEGLACHQL-AD-ARPAGATLE-SL-RRLLQETEL-AAGCTGPKH-AD-AVRGTQLFED-RDLV-RKYTMRRLL-RD-LRIVRGTQL-PDLK-RNPQLCYQD-ADLK-RQVPLQRLR-ADAK-ARVCYGLGM-ADGV-HRDLAARNV-PD-QRASPLTSI-PLLK-HRHRSSSTR-ADLV-YLYISAWPD-ADAK-QRFVVIQNE-ADLV-RRQQKIRKY-ADLK-CRVLQGLPR-ADL-YTMRRLLQE-ADLK-RRFTHQSDV (SEQ ID NO: 351)

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
B*3501	LTCSPQPEY, LALLPPGAA, EPLTPSGAM, DPASNTAPL, CRWGLLLAL, MPYGCLLDH, DGDLGMGAA, GVVKDVFAF, MSYLEDVRL, EILDEAYVM, LVTYNTDTF, MPNQAQMRI, LPTHDPSPL, TPTAENPEY, ICELHCPAL, DPAPGAGGM, LVVVLGVVF, FSPAFDNLY, LMTFGAKPY, HTVPWDQLF, FVVIQNEDL, RSLLEDDDM, SPAFDNLYY, NKEILDEAY, LPTNASLSF, ECVGEGLAC, YYWDQDPPE, LDIDETEYH (SEQ ID NOS: 94, 352, 353, 297, 21, 354, 104, 63, 238, 355, 356, 293, 294, 357, 216, 358, 25, 105, 233, 215, 359, 360, 235, 361, 301, 362, 267, and 329, respectively)	***Var1:*** HTVPWDQLF-ADLV-CRWGLLLAL-RI-ALDIDETEYH-ADL-ARDGDLGMGAA-RD-LPTNASLSF-ADPASNTAPL-ALPTHDPSPL-AD-NKEILDEAY--ADPAPGAGGM-AI-AEPLTPSGAM-A-GVVKDVFAF-AD-LTCSPQPEY-ADLK-LVTYNTDTF-AD-LALLPPGAA-PD-EILDEAYVM-P-LVVVLGVVF-AECVGEGLAC-A-TPTAENPEY-AD-RSLLEDDDM-ALLV-FVVIQNEDL-AL-AMPNQAQMRI-ADLV-MSYLEDVRL-AI-LMTFGAKPY-AD-ICELHCPAL-ALGK-YYWDQDPPE-ADL-SPAFDNLYY-ADL-FSPAFDNLY-AILK-AMPYGCLLDH (SEQ ID NO: 363) ***Var2:*** MELAALCRWGLLLALLPPGAPADGKTPTAENPEYAALPASPETHLPILKYSEDPTVPLPDGALPTHDPSPLADNKEILDEAYADEILDEAYVMPLVVVLGVVFADMQIAKGMSYALMTFGAKPYPLGKAPPPAFSPAFADLHCPALVTY (SEQ ID NO: 364)
B*4001	REVRAVTSA, SETDGYVAP, SEGAGSDVF, QVVQGNLEL, AENPEYLGL, RDLAARNVL, LEDDDMGDL, QETELVEPL, EEEAPRSPL, RELGSGLAL, ALCRWGLLL, MELAALCRW, EECRVLQGL, QLFEDNYAL, QEVQGYVLI, QEFAGCKKI, EQLQVFETL, EEITGYLYI, GEGLACHQL, TEILKGGVL, GERLPQPPI, FEDNYALAV (SEQ ID NOS: 365, 366,	MELAALCRW-RDLAARNVL-PDA-QETELVEPL-AEEEAPRSPL-PDGK-EECRVLQGL-ADA-GERLPQPPI-ADG-SETDGYVAP-PDA-AGEGLACHQL-ADG-RELGSGLAL-P-QLFEDNYAL-PD-ALEDDDMGDL-PDLK-REVRAVTSA-ASEGAGSDVF-A-TEILKGGVL-PL-EEITGYLYI-PDGK-AENPEYLGL-PDLK-QEVQGYVLI-AD-EQLQVFETL-A-QVVQGNLEL-A-QEFAGCKKI--ALCRWGLLL-RD-AFEDNYALAV (SEQ ID NO: 374)

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
	201, 234, 367, 368, 369, 44, 370, 23, 74, 34, 51, 27, 56, 371, 372, 333, 206, 222, 373, and 55, respectively)	
B*4002	HYKDPPFCV, CQSLTRTVC, RELQLRSLT, REVRAVTSA, TFYRSLLED, ISWLGLRSL, TLQGLGISW, KHSDCLACL, AENPEYLGL, CRWGLLLAL, TRTVCAGGC, TDMKLRLPA, RDLAARNVL, QETELVEPL, EEEAPRSPL, RKYTMRRLL, MELAALCRW, RELGSGLAL, QEFAGCKKI, TDFGLARLL, GENVKIPVA, LDSTFYRSL, QEVQGYVLI, KITDFGLAR, TELRKVKVL, GSRCWGESS, TGTDMKLRL, REYVNARHC, GERLPQPPI, YISAWPDSL (SEQ ID NOS: 139, 375, 376, 365, 161, 212, 68, 128, 367, 21, 377, 378, 368, 44, 370, 295, 34, 23, 371, 69, 379, 313, 56, 26, 380, 381, 382, 383, 373, and 28, respectively)	ISWLGLRSL-AEEEAPRSPL-RDLAARNVL-RLG-GENVKIPVA-RLG-KHSDCLACL-AIG-GERLPQPPI-ADL-TGTDMKLRL-PDGK-AENPEYLGL-ADG-RELGSGLAL--REVRAVTSA-ADG-REYVNARHC-A-QEFAGCKKI-A-QETELVEPL-A-TELRKVKVL-TDMKLRLPA-ADLK-QEVQGYVLI-PDL-ARGGSRCWGESS-ALGV-KITDFGLAR-A-TDFGLARLL-PDA-RKYTMRRLL-ADG-RELQLRSLT-ADLK-LDSTFYRSL-MELAALCRW-A-TLQGLGISW-ADL-CQSLTRTVC-ALL-HYKDPPFCV-AIG-YISAWPDSL-AD-CRWGLLLAL-RDL-TRTVCAGGC-ADLK-TFYRSLLED (SEQ ID NO: 384)
B*4402	TRTVCAGGC, TLQGLGISW, VKVLGSGAF, QETELVEPL, ERGAPPSTF, IDSECRPRF, RELGSGLAL, MELAALCRW, FDGDLGMGA, GGGDLTLGL,	TRTVCAGGC-ADG-GGGDLTLGL-ARPEADQCVAC-A-TLQGLGISW-AI-AFDGDLGMGA-PDAK-ARGDLTLGLEP-PDGK-IDSECRPRF-ADG-VKVLGSGAF-ADG-QETELVEPL-ADG-RELGSGLAL-A-QEVQGYVLI-ALG-ERGAPPSTF-A-QEFAGCKKI-MELAALCRW-ALG-

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
	QEFAGCKKI, QEVQGYVLI, LHCPALVTY, PEADQCVAC, VKIPVAIKV, GDLTLGLEP (SEQ ID NOS: _ 377, 68, 40, 44, 385, 386, 23, 34, 32, 387, 371, 56, 38, 388, 42, and 29, respectively)	VKIPVAIKV-AL-LHCPALVTY (SEQ ID NO: 389)
B*4403	FDGDLGMGA, TRTVCAGGC, SEGAGSDVF, VKVLGSGAF, PDLSVFQNL, QETELVEPL, EECRVLQGL, LEEITGYLY, LRIVRGTQL, RELGSGLAL, MELAALCRW, GDLTLGLEP, GGGDLTLGL, TDFGLARLL, EEITGYLYI, QEVQGYVLI, TEILKGGVL, PEADQCVAC (SEQ ID NOS: 32, 377, 201, 40, 390, 44, 51, 232, 39, 23, 34, 29, 387, 69, 333, 56, 222, and 388, respectively)	LRIVRGTQL-PIAA-GGGDLTLGL-ARPEADQCVAC-AI-AFDGDLGMGA-PDAK-ARGDLTLGLEP-PDLK-QETELVEPL-PI-VKVLGSGAF-ASEGAGSDVF-PDG-RELGSGLAL-A-QEVQGYVLI-ADGK-EECRVLQGL-PDLK-LEEITGYLY-A-TEILKGGVL-PL-EEITGYLYI-AD-MELAALCRW-AD-ARPDLSVFQNL-ADL-TDFGLARLL-PD-TRTVCAGGC (SEQ ID NO: 391)
B*4501	PEGRYTFGA, RELQLRSLT, MEHLREVRA, FDGDLGMGA, REVRAVTSA, VSRLLGICL, GERLPQPPI, LGMGAAKGL, LPAARPAGA, TSANIQEFA, LDSTFYRSL, IDSECRPRF, LPSETDGYV, RELGSGLAL, ASCVTACPY, QEVQGYVLI, AEQRASPLT, MELAALCRW, GENVKIPVA, INCTHSCVD, EEITGYLYI, GEGLACHQL, LPASPETHL, FEDNYALAV,	CELHCPALV-ADG-GENVKIPVA-ALPASPETHL-RD-ARPEGRYTFGA-ADGK-IDSECRPRF-ADLK-GERLPQPPI-AIL-AEEAPRSPLA-ADGA-EEITGYLYI-ALPAARPAGA-PDGK-MEHLREVRA-PDG-RELQLRSLT-ADLK-KEILDEAYV-AT-AFDGDLGMGA-PDLK-REVRAVTSA--ALPSETDGYV-ADG-AEQRASPLT-ADG-AGEGLACHQL-ADG-RELGSGLAL-AD-CEKCSKPCA-ADGV-QEVQGYVLI-ADL-TSANIQEFA-AD-LDSTFYRSL-MELAALCRW-ATGK-AINCTHSCVD-RD-AFEDNYALAV-RD-LGMGAAKGL--VSRLLGICL-PD-VKIPVAIKV-AI-ASCVTACPY

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
	EEAPRSPLA, CELHCPALV, KEILDEAYV, VKIPVAIKV, CEKCSKPCA (SEQ ID NOS: 392, 376, 393, 32, 365, 217, 373, 394, 298, 395, 313, 386, 396, 23, 46, 56, 397, 34, 379, 398, 333, 206, 303, 55, 399, 400, 401, 42, and 402, respectively)	(SEQ ID NO: 403)
B*5101	LQLRSLTEI, LPQPPICTI, KGMSYLEDV, CRWGLLLAL, PDLSVFQNL, YGVTVWELM, LGMEHLREV, LGMGAAKGL, MPNQAQMRI, LPTHDPSPL, ENVKIPVAI, QEFAGCKKI, TDFGLARLL, EQLQVFETL, LPTNASLSF, QEVQGYVLI, LPASPETHL, LPSETDGYV, VKIPVAIKV, VGILLVVVL (SEQ ID NOS: 150, 404, 260, 21, 390, 98, 311, 394, 293, 294, 405, 371, 69, 372, 301, 56, 303, 396, 42, and 318, respectively)	CRWGLLLAL-PD-ENVKIPVAI-AYGVTVWELM-A-ALPASPETHL-ARPDLSVFQNL-PD-LPTNASLSF-ADG-ALPTHDPSPL-PDL-ALPSETDGYV-PDLK-LGMEHLREV-AD-LPQPPICTI-ADGV-QEVQGYVLI-AD-EQLQVFETL-A-LGMGAAKGL-PD-KGMSYLEDV-A-QEFAGCKKI-S-VGILLVVVL--AMPNQAQMRI-ADLK-LQLRSLTEI-AD-VKIPVAIKV-A-TDFGLARLL (SEQ ID NO: 406)
B*5301	DDMGDLVDA, LPQPPICTI, CRWGLLLAL, SPLDSTFYR, RPEDECVGE, MPNQAQMRI, LPTHDPSPL, TPTAENPEY, SPQPEYVNQ, VENPEYLTP, AGVGSPYVS, SPLTSIISA, FDGDLGMGA, SPAFDNLYY,	ASPLDSTFYR-ADG-VENPEYLTP-A-ALPASPETHL-ARAGVGSPYVS-RD-LPTNASLSF-ADG-ALPTHDPSPL-ADL-LERPKTLSP-AL-AFDGDLGMGA-PDAK-ARGDLTLGLEP-PDL-ARDDMGDLVDA-PDL-ARPEDECVGE-A-TPTAENPEY-AL-AMPNQAQMRI-ADLK-LPQPPICTI-AD-ASPLTSIISA-AD-CRWGLLLAL-AGPLPAARPA-PD-AAPRSPLAPS-ALA-ASPQPEYVNQ-

HLA allele	**Peptides**	**Example of poly CTL construct(s)**
	LERPKTLSP, LPTNASLSF, GDLTLGLEP, LPASPETHL, LHCPALVTY, APRSPLAPS, GPLPAARPA, VKIPVAIKV, CPSGVKPDL (SEQ ID NOS: 407, 404, 21, 273, 408, 293, 294, 357, 409, 410, 411, 412, 32, 235, 177, 301, 29, 303, 38, 269, 413, 42, and 414, respectively)	ALG-VKIPVAIKV-AD-ACPSGVKPDL-AD-LHCPALVTY-SDA-SPAFDNLYY (SEQ ID NO: 415)
B*5401	LVEPLTPSG, IWIPDGENV, RELQLRSLT, LPQPPICTI, REVRAVTSA, LTSIISAVV, RKVKVLGSG, YKDPPFCVA, SPLAPSEGA, LQRLRIVRG, MPYGCLLDH, RGRILHNGA, MPNQAQMRI, CPALVTYNT, MPIWKFPDE, WKDIFHKNN, LPAARPAGA, SPLTSIISA, FDGDLGMGA, DGDPASNTA, VPLQRLRIV, WGLLLALLP, EEAPRSPLA, LRENTSPKA (SEQ ID NOS: 416, 153, 376, 404, 365, 417, 418, 96, 419, 246, 354, 420, 293, 421, 422, 423, 298, 412, 32, 102, 302, 24, 399, and 424, respectively)	AWKDIFHKNN-AD-AFDGDLGMGA-PDLK-REVRAVTSA-ALL-AEEAPRSPLA-ADG-ARDGDPASNTA-ALPAARPAGA-A-IWIPDGENV-SD-LRENTSPKA-RD-LVEPLTPSG-ADG-LTSIISAVV-A-RKVKVLGSG-ADGV-RELQLRSLT-ADLK-LPQPPICTI-AD-LQRLRIVRG-PDLK-RGRILHNGA-AD-ASPLTSIISA-ASPLAPSEGA-ACPALVTYNT-AD-AVPLQRLRIV-ADAA-AMPNQAQMRI-ADLK-AYKDPPFCVA-RDL-AMPIWKFPDE-ADG-AMPYGCLLDH-ADGK-WGLLLALLP (SEQ ID NO: 425)
B*5701	DVWSYGVTV, ATLERPKTL, GSGAFGTVY, ISWLGLRSL, PAFDNLYYW, DIFHKNNQL,	MELAALCRW-A-VTSANIQEF-ALGK-ENVKIPVAI-ADGK-DIFHKNNQL-RD-ATLERPKTL-LVVVLGVVF-P-TLQGLGISW-A-DVFDGDLGM-RDLV-ALCRWGLLL-PDGK-

HLA allele	Peptides	Example of poly CTL construct(s)
	MSYLEDVRL, VTSANIQEF, HTVPWDQLF, LVVVLGVVF, TLQGLGISW, ALCRWGLLL, DVFDGDLGM, MELAALCRW, RSLLEDDDM, LSYMPIWKF, QLMPYGCLL, ENVKIPVAI, RFTHQSDVW, GTQLFEDNY (SEQ ID NOS: 30, 426, 58, 212, 427, 45, 238, 324, 215, 25, 68, 74, 218, 34, 360, 62, 43, 405, 208, and 230, respectively)	ISWLGLRSL-RSLLEDDDM-ADG-GSGAFGTVY-ADA-GTQLFEDNY-RDLK-LSYMPIWKF-ADLK-PAFDNLYYW-ADL-QLMPYGCLL-PDLK-MSYLEDVRL-R-DVWSYGVTV-PDLK-RFTHQSDVW-ADLV-HTVPWDQLF (SEQ ID NO: 428)
B*5801	RSGGGDLTL, RCEKCSKPC, VTSANIQEF, TRTVCAGGC, PAFDNLYYW, ISAVVGILL, RMARDPQRF, VCTGTDMKL, VTVWELMTF, RIVRGTQLF, RASPLTSII, HTVPWDQLF, KGCPAEQRA, IISAVVGIL, LCYQDTILW, ASCVTACPY, GAMPNQAQM, CTIDVYMIM, VTGASPGGL, YLSTDVGSC, LSYMPIWKF, LGSQDLLNW, LAALCRWGL, RGQECVEEC, KIFGSLAFL (SEQ ID NOS: 429, 286, 324, 377, 427, 60, 202, 231, 203, 75, 35, 215, 430, 71, 431, 46, 432, 221, 433, 146, 62, 434, 22, 435, and 31, respectively)	PAFDNLYYW-AIL-CTIDVYMIM-ADLV-RMARDPQRF-AD-KGCPAEQRA-PDLK-LGSQDLLNW-AIISAVVGIL-AL-RCEKCSKPC-AIL-VTSANIQEF-ADL-GAMPNQAQM-AD-AVTGASPGGL-P-ISAVVGILL-PD-RSGGGDLTL--AYLSTDVGSC-A-LAALCRWGL-AL-ASCVTACPY-ADL-HTVPWDQLF-ADLK-LSYMPIWKF-ADG-RASPLTSII-ADG-VTVWELMTF-ADGV-ARGQECVEEC-ADL-RIVRGTQLF-TRTVCAGGC-AD-KIFGSLAFL-PD-VCTGTDMKL-AD-LCYQDTILW (SEQ ID NO: 436)

2.3 Th Epitopes

2.3.1 List of Th Epitopes:

2.3.1.1:

LRHLYQGCQ, LRIVRGTQL, CLHFNHSGICELHCPALV, LQVFETLEE, LRSLRELGS, LCFVHTVPWDQ, LRGQECVEE, CPINCTHSC, IRKYTMRRL, MRILKETELRKVKVLGS, VKIPVAIKVLRENTSPK, YVMAGVGSPYVSRLLGICLTSTVQLV, VRLVHRDLA, FGLARLLDIDETEYH, WMALESILRRRFTHQS, CTIDVYMIMVKCWMI, CRPRFRELVSEFS, FVVIQNEDL (SEQ ID NOS: 437, 39, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, and 359, respectively)

2.3.1.2:

AVVGILLVVVLGVVFGILIKRRQQKIR, PICTIDVYMIMVKCWMIDSE, AQMRILKETELRKVKVLGSGA, IKWMALESILRRRFTHQSDV, PICTIDVYMIMVKCWMIDS (SEQ ID NOS: _7, 8, 9, 10, and 11, respectively)

2.3.1.3:

AKFVAAWTLKAAA (SEQ ID NO: 1)

2.3.2 Polyepitope Th Constructs.

AKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDS**RKRSHAGYQTI** (SEQ ID NO: 453) (PADRE sequence is in bold and Italics; C-terminal fragment of LAMP-1 is in bold)

2.4 Targeting Sequences

2.4.1 Leader peptide of human ErbB2 protein

MELAALCRWGLLLALLPPGAAS (SEQ ID NO: 14)

2.4.2 Fragment of leader peptide of human ErbB2 protein used in targeted polyepitope constructs

MELAALCRWGLLLALLPPGAP (SEQ ID NO: 13)

2.4.3 C-terminal fragment of human LAMP-1 protein (11 last aa) used in targeted polyepitope constructs

RKRSHAGYQTI (SEQ ID NO: 15)

2.4.4 *Complete sequence of HLA-DR invariant chain (γ-chain, Ii)*

MHRRRSRSCREDQKPVMDDQRDLISNNEQLPMLGRRPGAPESKCSRGALYTGFSILVTLLLAGQATTAYFLYQQQGRLDKLTVTSQNLQLEN**LRMKLPKPPKPVSKMRM**ATPLLMQALPMGALPQGPMQNATKYGNMTEDHVMHLLQNADPLKVYPPLKGSFPENLRHLKNTMETIDWKVFESWMHHWLLFEMSRHSLEQKPTDAPPKVLTKCQEEVSHIPAVHPGSFRPKCDENGNYLPLQCYGSIGYCWCVFPNGTEVPNTRSRGHHNCSESLELEDPSSGLGVTKQDLGPVPM (SEQ ID NO: 454) (immunoregulatory fragment Ii-key is shown in bold)

2.4.5 Ubiquitin V76

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGV (SEQ ID NO: 455)

2.5 Complete Constructs

2.5.1 Universal Polyepitope Construct:

*MELAALCRWGLLLALLPPGAP*DGENVKIPVAIKVLRENTADGKEECRVLQGLPDGKYSEDPTVPLPDDEAYVMAGVADLKQETELVEPLTPPDGRASPLTSIISAVVGILLVVVLGVVFPDAGMEHLREVRADGKDIFHKNNQLPDLQPEQLQVFRDAQEVQGYVLIPDLAFDGDLGMGAPDLQVIRGRILPDVKVLGSGAFGTVYPIGDLTLGLEPPDLKASCVTACPYATLQGLGISWLGLRSLRELGSGLALPMQIAKGMSYALFGPEADQCVPDLKLSYMPIWKFADLKPLQRLRIVRGTQLFEDNYALAVARGAPPSTFKAGVVKDVFAFRDLVKITDFGLARLLPLVHRDLAARADVWSYGVTVRDTTPVTGASPRDLYISAWPDSLRTVCAGGCARSDKIFGSLAFLPDLHCPALVTYADDSTFYRSLLADGKQLMPYGCLLADGGSCTLVCPL***AKFVAAWTLKAAA***KKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDS**RKRSHAGYQTI** (SEQ ID NO: 456)

*2.5.2 HLA-A*0201-specific polyepitope construct:*

*MELAALCRWGLLLALLPPGAP*PDLLALLPPGAPDATLEEITGYLAILDEAYVMAPILHNGAYSLPQLFEDNYALSIISAVVGIAQLMPYGCLLRLLVVVLGVVRDLQLRSLTEIAILLVVVLGVPDAVVGILLVVADALCRWGLLLADYISAWPDSLRDKIFGSLAFL***AKFVAAWTLKAAA***KKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDS**RKRSHAGYQTI** (SEQ ID NO: 457)

*2.5.3 HLA-B*3501- specific polyepitope construct:*

*MELAALCRWGLLLALLPPGAP*ADGKTPTAENPEYAALPASPETHLPILKYSEDPTVPLPDGALPTHDPSPLADNKEILDEAYADEILDEAYVMPLVVVLGVVFADMQIAKGMSYALMTFGAKPYPLGKAPPPAFSPAFADLHCPALVTY***AKFVAAWTLKAAA***KKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDS**RKRSHAGYQTI** (SEQ ID NO: 458)

2.5.4 Unrelated protein rHA5 (corresponding to a portion (aa 17-346) of Influenza A virus H5N1 hemagglutinin (HA) GenBank Accession no. ABL31766)

DQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPTNDLCYPGSFNDYEELKHLLSRINHFEKIQIIPKSSWSDHEASSGVSSACPYLGSPSFFRNVVWLIKKNSTYPTIKKSYNNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISIGTSTLNQRLVPKIATRSKVNGQSGRMEFFWAILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQRESRRKKR (SEQ ID NO: 459)

Results and Discussion

To obtain designed polyepitope constructs provided in Examples 2.5.1, 2.5.2 and 2.5.3, corresponding nucleic acids encoding such constructs were produced. The nucleic acid sequences were optimized for expression in human cells by exclusion of rare codons and by minimizing mRNA secondary structure.

The encoding nucleic acids were inserted into pDNAVACC-Ultra plasmid (pDNAVACC5, NBC, USA, http://www.natx.com/). Also, two control plasmids were produced: pHER2–pDNAVACC encoding the full-length HER2 protein (GenBank Accession No. P04626) (positive control) and pDNAVACC-rHA5 encoding an unrelated protein, rHA5, corresponding to a portion (aa 17-346) of hemagglutinin (HA) of Influenza A virus of H5N1 subtype (GenBank Accession No. ABL31766) (negative control). Another negative control was empty plasmid pDNAVACC5.

Four constructs were created and tested:

1. pBCU – pDNAVACC containing the sequence encoding universal polyepitope construct of Example 2.1.3.8;

2. pBCA0201 – pDNAVACC containing the sequence encoding polyepitope construct for HLA-A*0201 (3.2-A*0201-Var2);

3. pHER2 – pDNAVACC containing the sequence encoding HER2 protein (3.2-B*3501-Var2);

4. prHA5 – pDNAVACC containing the sequence encoding a portion of influenza virus H5N1 hemagglutinin (see Example 2.5.4) that is unrelated to HER2.

A recombinant pQE30 plasmid (Qiagen, Germany) was also created for expression of the common C-terminal fragment of polyepitope constructs (polyECt). This C-terminal fragment was expressed in E.coli cells, purified and used for immunizing animals (BALB/c mice) to generate polyclonal antibodies recognizing polyepitope antigens of the invention. The efficiency of antibody binding was confirmed using ELISA. These antibodies were used to monitor the efficiency of transfection of dendritic cells (DCs) and the efficiency of polyepitope antigen expression after transfection. For detection of HER2 and unrelated protein (rHA5) expression, corresponding polyclonal murine antibodies were used Antibodies were generated by immunizing BALB/c mice i.p. with 20 µg of corresponding antigen (either rHA5 or polyECt) in complete Freund's adjuvant (Sigma, USA) and boosted twice with the same amount of the antigen in incomplete Freund's adjuvant (Sigma, USA) at 14 days interval. Blood was collected 10 days after the last immunization and antiserum was prepared. Each group consisted of six animals, the serum was pooled. Both antigens used for immunization were produced in prokaryotic expression system (E. coli) and purified by affinity chromatography using Ni-NTI agarose (Qiagen, Germany). rHA5 was expressed also using pQE30 expression vector.

The efficiency of induction of T cell response by each of the constructs was determined using the following *in vitro* assay.

28 healthy donors expressing HLA-A*0201 were selected using PCR assay ALLSET™ GOLD HLA A LOW RES SSP (Invitrogen, USA). This MHC I allomorph is one of the most

frequently found in human population. Mononuclear cells (MCs) were fractionated from the peripheral blood of HLA-A2+ normal donors by centrifugation in the ficoll-urografin (Sigma-Aldrich, USA; Schering, Germany) gradient density. Obtained MCs were plated on plastic culture dishes (Nunc, Denmark), and monocyte-enriched adherent cells were observed after a 1-h incubation at 37°C. The nonadherent cells were removed and cryopreserved, and the adherent cells were cultured in the presence of 50 ng/ml rhGM-CSF (BioVision, USA) and 200 ng/ml rhIL-4 (BioVision, USA) in AIM-V medium (Invitrogen, USA) (Obermaier B. et.al, Biol Proced Online, 2003, (5):197-203). After 24 hours LPS (E. coli 055:B5, Sigma, USA) was added (5 μg/ml) to stimulate maturation of DCs. After 24-hour incubation the LPS-treated cells were harvested and used as mature DCs. DCs were labeled using FITC- or PE-conjugated mAb specific to CD3, CD11c, CD14, CD83, CD86, and HLA-DR (all from BD Biosciences, USA). The fluorescence intensity was measured with a FACSCalibur (BD Biosciences, USA). The phagocytosing ability of DCs was assessed using FITC-labeled dextran (Sigma, USA) (Della Bella S. et. al, J. Leukocyte Biol., 2004, 75(1):106-16; Kato M. et.al. Int. Immunol., 2000, 11:1511-1519).

The resulting mature DCs were transfected with the constructs using MATra (Magnet assisted transfection, Promokine, Germany) following producer recommendations (http://www.promokine.info/fileadmin/PDFs/Cell_Transfection/MATra_handbook_PromoKine.pdf). Transfection efficiency was determined using dot-blot analysis (using polyclonal antibodies specific to the common C-terminal portion of polyepitopes of the invention, see above) or using fluorescent microscopy. Fluorescent plasmids were prepared with nick-translation labeling kit (PromoKine, Germany). DCs, transfected with labeled plasmids, were analyzed using fluorescent microscopy. Based on these determinations, efficient transfection and antigen expression was achieved.

The generated mature DCs were co-cultured for 48 hours with previously obtained fractions of autologous non-adherent mononuclear cells (MCs) (in 1:10 ratio) in the presence of recombinant human 40 ng/ml IL-18 and 10 ng/ml IL-12 (BioVision, USA) to stimulate cellular immune response in vitro. Five groups were created:

1. DC: prHA5 + non-adherent MCs
2. DC:pHER2 + non-adherent MCs
3. DC:pBCU + non-adherent MCs

4. DC:pBCA0201 + non-adherent MCs

5. unstimulated non-adherent MCs

To study the T cell response, MCF-7 breast cancer cells (Russian Cell Culture Collection; Institute of Cytology of the Russian Academy of Sciences; Ref. Nos. ECACC 86012803; ICLC HTL95021) were used as target cells (as well as autologous DCs transiently transfected with pHER2). MCF-7 cells express both ErbB2 and HLA-A*0201 (i.e., are HLA-A*0201$^+$/ErbB2$^+$). This is important, because T-lymphocytes of the majority of selected donors express the same HLA-A allele.

Levels of antigen-specific γIFN and IL-4 production were assayed using intracellular cytokine staining followed by flow cytometry. PBMCs were harvested and resuspended at 2×10^6 cells/ml in RPMI 1640 and 10% HS. The cultures were restimulated with either MCF-7 cancer cells or autologous DCs, transfected with pHER2 at 2×10^6 cells/ml. After 2 hours of incubation GolgiPlug™ Protein Transport Inhibitor (containing brefeldin A) solution (BD Biosciences, USA) was added, and the incubation period was extended to 12 hours at 37°C, 5% CO_2. For intracellular labeling, cells were fixed and permeabilized for 30 min at room temperature using BD FACS Permeabilizing Solution (BD Biosciences, USA) followed by washing. Cells were then labeled with PE- or FITC-conjugated monoclonal antibodies specific to γIFN or IL-4 and CD4 or CD8 (all from BD Biosciences, USA) for 30 min at room temperature in the dark. After washing, stained cells were analyzed by flow cytometry (FACSCalibur, BD Biosciences, USA). (Description of protocol could be found at http://www.bdbiosciences.com/support/resources/protocols/cytokines_fca.jsp).

Induced ex vivo cytotoxic responses were tested by measuring activity of lactate dehydrogenase (LDH) released from lysed target cells (either MCF-7 breast cancer cells or autologous APCs, transfected with pHER2) in different experimental and control groups. The CytoTox 96® Non-Radioactive Cytotoxicity Assay is a colorimetric alternative to radioactive cytotoxicity assays. The CytoTox 96® Assay quantitatively measures lactate dehydrogenase (LDH), a stable cytosolic enzyme that is released upon cell lysis, in much the same way as [^{51}Cr] is released in radioactive assays. Released LDH in culture supernatants was measured with a 30-minute coupled enzymatic assay that results in the conversion of a tetrazolium salt into a red formazan product. (Description of the protocol could be found in http://www.promega.com/tbs/tb163/tb163.pdf) The amount of color formed is proportional to the number of lysed cells. Visible wavelength absorbance data were collected using multimode microplate reader LB 941 TriStar (Berthold Technologies, Germany). Statistical significance of observed differences between the groups was assessed using Wilcoxon rank-sum test. $P < 0.05$ was considered to be significant.

The poyepitope constructs demonstrated higher efficiency of induction of T cell immune responses as compared to the pHER2 construct and the negative control constructs; with the universal construct pBCU demonstrating slightly higher efficiency than the allele-specific construct pBCA0201. Specifically, in the cytotoxicity assays, all experimental groups showed significantly ($p < 0.001$) higher cytotoxicity as compared to both negative controls. In experiments using autologous DCs as target cells (Figure 1A), there were no statistically significant differences between each of pBCU and pBCA0201 while in both experimental groups cytotocic activity was found to be greater than in corresponding groups of pHER2 ($p < 0.001$); furthermore, when the ratio of effector-to-target cells was ≥ 20:1 both experimental groups demonstrated superior results as compared to pHER2(30:1) ($p < 0.01$). Using MCF-7 cells as targets (Figure 1B) revealed that pBCU construct induced slightly higher cytotoxicity than pBCA0201 (with 10:1 effector-to-target ratio the p value was < 0.013 and with 20:1 – $p < 0.042$; at 30:1 effector-to-target ratio the difference between these two experimental groups was found to be insignificant). When the ratio of effector-to-target cells was ≥ 20:1 both experimental groups demonstrated superior results as compared to pHER2(30:1) ($p < 0.01$). Numbers of γIFN producing CD8+ T-cells, stimulated by the presence of MCF-7 cancer cells, differed significantly between groups stimulated by DCs transfected with pHER2, pBCU and pBCA0201 ($p < 0.01$) (Figure 2A). Antigen-specific production of γIFN (stimulated by the presence of MCF-7 cells) by CD4+ T-lymphocytes in groups stimulated with DCs transfected with either pBCU or pBCA0201 was found to differ insignificantly, while both these groups demonstrated significantly greater numbers of γIFN-secreting CD4+ T cells than it was found in CD4+ T-cell stimulated with DCs transfected with pHER2 ($p < 0.001$) (Figure 2B). Thus, the determined in vitro efficiency of the tested constructs for T cell response induction was as follows: pBCU ≥ pBCA0201 >> pHER2.

* * *

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

It is further to be understood that all values are approximate, and are provided for description.

Patents, patent applications, publications, product descriptions, and protocols are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties for all purposes.

CLAIMS:

1. An isolated immunogenic polyepitope construct comprising two or more T cell epitopes selected from the group consisting of:

AKFVAAWTLKAAA (SEQ ID NO: 1), AVVGILLVVVLGVVFGILIKRRQQKIR (SEQ ID NO: 7), PICTIDVYMIMVKCWMIDSE (SEQ ID NO: 8), AQMRILKETELRKVKVLGSGA (SEQ ID NO: 9), IKWMALESILRRRFTHQSDV (SEQ ID NO: 10), PICTIDVYMIMVKCWMIDS (SEQ ID NO: 11), CRWGLLLAL (SEQ ID NO: 21), LAALCRWGL (SEQ ID NO: 22), RELGSGLAL (SEQ ID NO: 23), WGLLLALLP (SEQ ID NO: 24), LVVVLGVVF (SEQ ID NO: 25), KITDFGLAR (SEQ ID NO: 26), QLFEDNYAL (SEQ ID NO: 27), YISAWPDSL (SEQ ID NO: 28), GDLTLGLEP (SEQ ID NO: 29), DVWSYGVTV (SEQ ID NO: 30), KIFGSLAFL (SEQ ID NO: 31), FDGDLGMGA (SEQ ID NO: 32), LVHRDLAAR (SEQ ID NO: 33), MELAALCRW (SEQ ID NO: 34), RASPLTSII (SEQ ID NO: 35), RGAPPSTFK (SEQ ID NO: 36), SIISAVVGI (SEQ ID NO: 37), LHCPALVTY (SEQ ID NO: 38), LRIVRGTQL (SEQ ID NO: 39), VKVLGSGAF (SEQ ID NO: 40), LQPEQLQVF (SEQ ID NO: 41), VKIPVAIKV (SEQ ID NO: 42), QLMPYGCLL (SEQ ID NO: 43), QETELVEPL (SEQ ID NO: 44), DIFHKNNQL (SEQ ID NO: 45), ASCVTACPY (SEQ ID NO: 46), TELVEPLTP (SEQ ID NO: 47), PLQRLRIVR (SEQ ID NO: 48), LQVIRGRIL (SEQ ID NO: 49), DEAYVMAGV (SEQ ID NO: 50), EECRVLQGL (SEQ ID NO: 51), TVCAGGCAR (SEQ ID NO: 52), YSEDPTVPL (SEQ ID NO: 53), RWGLLLALL (SEQ ID NO: 54), FEDNYALAV (SEQ ID NO: 55), QEVQGYVLI (SEQ ID NO: 56), LLALLPPGA (SEQ ID NO: 57), GSGAFGTVY (SEQ ID NO: 58), LGISWLGLR (SEQ ID NO: 59), ISAVVGILL (SEQ ID NO: 60), MQIAKGMSY (SEQ ID NO: 61), LSYMPIWKF (SEQ ID NO: 62), GVVKDVFAF (SEQ ID NO: 63), AIKVLRENT (SEQ ID NO: 64), SWLGLRSLR (SEQ ID NO: 65), ILLVVVLGV (SEQ ID NO: 66), FGPEADQCV (SEQ ID NO: 67), TLQGLGISW (SEQ ID NO: 68), TDFGLARLL (SEQ ID NO: 69), DSTFYRSLL (SEQ ID NO: 70), IISAVVGIL (SEQ ID NO: 71), TTPVTGASP (SEQ ID NO: 72), GMEHLREVR (SEQ ID NO: 73), ALCRWGLLL (SEQ ID NO: 74), RIVRGTQLF (SEQ ID NO: 75), GSCTLVCPL (SEQ ID NO: 76), DGENVKIPV (SEQ ID NO: 77), MELAALCRWGLLLALLPPGA (SEQ ID NO: 78), QEVQGYVLI (SEQ ID NO: 56), PLQRLRIVRGTQLFEDNYALAV (SEQ ID NO: 79), TTPVTGASP (SEQ ID NO: 72), DIFHKNNQL (SEQ ID NO: 45), TVCAGGCAR (SEQ ID NO: 52), LHCPALVTY (SEQ ID NO: 38), ASCVTACPY (SEQ ID NO: 46), GSCTLVCPL (SEQ ID NO: 76), GMEHLREVR (SEQ ID NO: 73), KIFGSLAFL (SEQ ID NO: 31), LQPEQLQVF (SEQ

ID NO: 41), YISAWPDSL (SEQ ID NO: 28), LQVIRGRIL (SEQ ID NO: 49), TLQGLGISWLGLRSLRELGSGLAL (SEQ ID NO: 80), EECRVLQGL (SEQ ID NO: 51), FGPEADQCV (SEQ ID NO: 67), LSYMPIWKF (SEQ ID NO: 62), RASPLTSIISAVVGILLVVVLGVVF (SEQ ID NO: 81), QETELVEPLTP (SEQ ID NO: 82), VKVLGSGAFGTVY (SEQ ID NO: 83), DGENVKIPVAIKVLRENT (SEQ ID NO: 84), DEAYVMAGV (SEQ ID NO: 50), QLMPYGCLL (SEQ ID NO: 43), MQIAKGMSY (SEQ ID NO: 61), LVHRDLAAR (SEQ ID NO: 33), KITDFGLARLL (SEQ ID NO: 85), DVWSYGVTV (SEQ ID NO: 30), DSTFYRSLL (SEQ ID NO: 70), GDLTLGLEP (SEQ ID NO: 29), FDGDLGMGA (SEQ ID NO: 32), YSEDPTVPL (SEQ ID NO: 53), GVVKDVFAF (SEQ ID NO: 63), RGAPPSTFK (SEQ ID NO: 36), LRHLYQGCQ (SEQ ID NO: 437), LRIVRGTQL (SEQ ID NO: 39), CLHFNHSGICELHCPALV (SEQ ID NO: 438), LQVFETLEE (SEQ ID NO: 439), LRSLRELGS (SEQ ID NO: 440), LCFVHTVPWDQ (SEQ ID NO: 441), LRGQECVEE (SEQ ID NO: 442), CPINCTHSC (SEQ ID NO: 443), IRKYTMRRL (SEQ ID NO: 444), MRILKETELRKVKVLGS (SEQ ID NO: 445), VKIPVAIKVLRENTSPK (SEQ ID NO: 446), YVMAGVGSPYVSRLLGICLTSTVQLV (SEQ ID NO: 447), VRLVHRDLA (SEQ ID NO: 448), FGLARLLDIDETEYH (SEQ ID NO: 449), WMALESILRRRFTHQS (SEQ ID NO: 450), CTIDVYMIMVKCWMI (SEQ ID NO: 451), CRPRFRELVSEFS (SEQ ID NO: 452), and FVVIQNEDL (SEQ ID NO: 359).

2. The polyepitope construct of claim 1, wherein said epitopes are connected end-to-end or are connected using spacer sequences which provide optimal processing and presentation of epitopes.

3. The polyepitope construct of claim 2, wherein said spacer sequences are selected from the group consisting of K/R-K/R, A, AR, ARY, [ANRK][RQYW][YWFVI] (SEQ ID NO: 464), ADLVKV (SEQ ID NO: 2), ADLVAG (SEQ ID NO: 3), ADLAVK (SEQ ID NO: 4), AD, ADL, ADLV (SEQ ID NO: 5), ADLVK (SEQ ID NO: 6), [APRS] [DILT] [AGL] [AKV] (SEQ ID NO: 460), [ARSPNK] [DLITGV] [LGAVEK] [VKAFSI] [ALKSEI] [GVKLSE] (SEQ ID NO: 461), and [AGNRKP] [DIATVG] [LGANVE] [ASNVLK] [VIKAGP] [KAGVSE] (SEQ ID NO: 462).

4. The polyepitope construct of anyone of claims 1-3, further comprising one or more homologous or heterologous targeting signals which direct intracellular transport of the construct to a specific cellular compartment.

5. The polyepitope construct of claim 4, wherein at least one of said targeting signals is selected from the group consisting of (i) a signal peptide of HER2 protein or a modified version thereof, (ii) an N-terminal portion or the whole sequence of the invariant chain associated with MHC class II molecules, (iii) a C-terminal portion of the human LAMP-1 protein, and (iv) the tyrosine-motif Y-X-X-hydrophobic amino acid, wherein X is any amino acid.

6. The polyepitope construct of claim 4, wherein at least one of said targeting signals is selected from the group consisting of MELAALCRWGLLLALLPPGAP (SEQ ID NO: 13), MELAALCRWGLLLALLPPGAAS (SEQ ID NO: 14), RKRSHAGYQTI (SEQ ID NO: 15), IPIAVGGALAGLVLIVLIAYLVGRKRSHAGYQTI (SEQ ID NO: 16), LRMKLPKPPKPVSQMR (SEQ ID NO: 17), LRMKLPK (SEQ ID NO: 18), LRMK (SEQ ID NO: 19), and
MHRRRSRSCREDQKPVMDDQRDLISNNEQLPMLGRRPGAPESKCSRGALYTGFSILVTLLL
AGQATTAYFLYQQQGRLDKLTVTSQNLQLENLRMKLPKPPKPVSKMRMATPLLMQALPM
GALPQGPMQNATKYGNMTEDHVMHLLQNADPLKVYPPLKGSFPENLRHLKNTMETIDW
KVFESWMHHWLLFEMSRHSLEQKPTDAPPKVLTKCQEEVSHIPAVHPGSFRPKCDENGNY
LPLQCYGSIGYCWCVFPNGTEVPNTRSRGHHNCSESLELEDPSSGLGVTKQDLGPVPM
(SEQ ID NO: 454)

7. The polyepitope construct of anyone of claims 1-3, further comprising N-terminally conjugated ubiquitin.

8. The polyepitope construct of claim 7, wherein the ubiquitin is UbV76 having the sequence MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQK
ESTLHLVLRLRGV (SEQ ID NO: 455).

9. The polyepitope construct of claim 7, wherein the ubiquitin is conjugated directly to the N terminus of the polyepitope construct.

10. The polyepitope construct of claim 7, wherein Arg or Val is inserted between the ubiquitin and the N terminus of the polyepitope construct.

11. An isolated polyepitope construct comprising the sequence selected from the group consisting of:

CRWGLLLALLVVVLGVVFSIISAVVGIRELGSGLALMELAALCRWADLARDEAYVMAGVADLVEECRVLQGLADYSEDPTVPLAVKIPV
AIKVAQLFEDNYALADVWSYGVTVAWGLLLALLPATVCAGGCARADIFHKNNQLADASCVTACPYADLLHCPALVTYATELVEPLTPAD

LKITDFGLARARGAPPSTFKADLYISAWPDSLAQETELVEPLALQVIRGRILALAALCRWGLADLQLMPYGCLLADKIFGSLAFLARGDLTLGLEPAVKVLGSGAFADLVHRDLAARADLQPEQLQVFADAFDGDLGMGAAPLQRLRIVRADLRIVRGTQLARASPLTSII (SEQ ID NO: 86);

QETELVEPLASCVTACPYADLVKVCRWGLLLALSIISAVVGIAARDEAYVMAGVADLVKLHCPALVTYARASPLTSIIADLVEECRVLQGLAFDGDLGMGAARGAPPSTFKADLKIFGSLAFLMELAALCRWADLVQLMPYGCLLAQLFEDNYALKITDFGLARADYISAWPDSLTVCAGGCARADLWGLLLALLPADLVHRDLAARADLYSEDPTVPLRELGSGLALARGDLTLGLEPAVKVLGSGAFADLQPEQLQVFADLDVWSYGVTVADLRIVRGTQLAPLQRLRIVRADLAALCRWGLAVKIPVAIKVADLQVIRGRILALVVVLGVVFADIFHKNNQLATELVEPLTP (SEQ ID NO: 87);

CRWGLLLALASCVTACPYADLYISAWPDSLAVKIPVAIKVAQLFEDNYALADVWSYGVTVAWGLLLALLPADIFHKNNQLATELVEPLTPADLLHCPALVTYAPLQRLRIVRADLQLMPYGCLLADKIFGSLAFLMELAALCRWADLVHRDLAARADLQPEQLQVFADAFDGDLGMGAALQVIRGRILAVKVLGSGAFADLRIVRGTQLARGAPPSTFKADLQETELVEPLRELGSGLALLVVVLGVVFSIISAVVGIARGDLTLGLEPADKITDFGLARALAALCRWGLADYSEDPTVPLTVCAGGCARARASPLTSIIADLVEECRVLQGLAARDEAYVMAGV (SEQ ID NO: 88);

CRWGLLLALAFGPEADQCVADLQLMPYGCLLADYSEDPTVPLAVKIPVAIKVAQLFEDNYALADVWSYGVTVAWGLLLALLPATVCAGGCARAISAVVGILLATLQGLGISWADSWLGLRSLRADLVKRWGLLLALLLLALLPPGARELGSGLALLVVVLGVVFSIISAVVGIILLVVVLGVAIISAVVGILAIKVLRENTADLVQETELVEPLALQVIRGRILAGVVKDVFAFADLARDEAYVMAGVADLPLQRLRIVRADLKITDFGLARALGISWLGLRADLQEVQGYVLIADLHCPALVTYAVKVLGSGAFADGMEHLREVRADTTPVTGASPADASCVTACPYADLYISAWPDSLARGDLTLGLEPADRGAPPSTFKADLRIVRGTQLATELVEPLTPADAFDGDLGMGAALAALCRWGLADLQPEQLQVFADAFEDNYALAVAMQIAKGMSYATDFGLARLLMELAALCRWADLVHRDLAARADGSGAFGTVYARDGENVKIPVADLVDSTFYRSLLADLVEECRVLQGLADKIFGSLAFLALCRWGLLLADIFHKNNQLADLSYMPIWKFADLVGSCTLVCPLARASPLTSIIADLRIVRGTQLF (SEQ ID NO: 89);

TTPVTGASPADLSWLGLRSLRADLVGSCTLVCPLAIKVLRENTADYSEDPTVPLMELAALCRWADLRWGLLLALLILLVVVLGVADLWGLLLALLPADLVHRDLAARADLDVWSYGVTVADLGISWLGLRADLVKVQETELVEPLTDFGLARLLRELGSGLALAIISAVVGILAFGPEADQCVADLVKVCRWGLLLALISAVVGILLGSGAFGTVYADLSYMPIWKFADLVEECRVLQGLGVVKDVFAFADLAFEDNYALAVADLKIFGSLAFLASCVTACPYADLVKVQLMPYGCLLAARDEAYVMAGVADLVKLHCPALVTYAVKVLGSGAFADLQPEQLQVFADLRIVRGTQLFADLVDSTFYRSLLADGMEHLREVRADLRIVRGTQLATVCAGGCARADLAALCRWGLAPLQRLRIVRADLQVIRGRILALVVVLGVVFADIFHKNNQLATLQGLGISWAQLFEDNYALARGDLTLGLEPAARDGENVKIPVADLVALCRWGLLLALLALLPPGAARGAPPSTFKADLKITDFGLARADMQIAKGMSYADAFDGDLGMGAAVKIPVAIKVARASPLTSIIADLQEVQGYVLIADYISAWPDSLSIISAVVGIATELVEPLTP (SEQ ID NO: 90);

CRWGLLLALISAVVGILLAFGPEADQCVADLQETELVEPLTDFGLARLLRELGSGLALLVVVLGVVFSIISAVVGIILLVVVLGVAIISAVVGILGSGAFGTVYAIKVLRENTADLRIVRGTQLFADLVKLHCPALVTYAVKVLGSGAFADGMEHLREVRADYISAWPDSLALCRWGLLLAVKIPVAIKVALAALCRWGLADTTPVTGASPADRGAPPSTFKADLYSEDPTVPLAFDGDLGMGALLALLPPGAARDGENVKIPVADLVDSTFYRSLLADGSCTLVCPLMELAALCRWADSWLGLRSLRADLVPLQRLRIVRADLKITDFGLARALGISWLGLRADLQEVQGYVLIADKIFGSLAFLASCVTACPYADLRASPLTSIIADLVEECRVLQGLAARDEAYVMAGVADLRWGLLLALLGVVKDVFAFADLQLMPYGCLLADLQPEQLQVFADLRIVRGTQLAMQIAKGMSYADVWSYGVTVAWGLLLALLPATVCAGGCARAQLFEDNYALARGDLTLGLEPADIFHKNNQLATELVEPLTPADLVHRDLAARADAFEDNYALAVALQVIRGRILATLQGLGISWADLSYMPIWKF (SEQ ID NO: 91);

TVCAGGCARADGMEHLREVRADGKEECRVLQGLADGRELGSGLALPQLFEDNYALSDGQETELVEPLPLVVVLGVVFARDGENVKIPVALLALLPPGAAQEVQGYVLIPDLARGDLTLGLEPAIKVLRENTADAFDGDLGMGAPDAKARDEAYVMAGVADIFHKNNQLAVKVLGSGAFATLQGLGISWAIAFGPEADQCVPDLKLSYMPIWKFADLKPLQRLRIVRAIISAVVGILMELAALCRWATGVVKDVFAFADLVKIPVAIK

VSIISAVVGIPISAVVGILLPILQPEQLQVFADGKYSEDPTVPLADMQIAKGMSYARGAPPSTFKADLQVIRGRILPDGRASPLTSIIADLVHRDLAARADSWLGLRSLRADGKLGISWLGLRADGVKITDFGLARATDFGLARLLPDGDSTFYRSLLAILLVVVLGVADTTPVTGASPRDLRIVRGTQLATELVEPLTPPDLKASCVTACPYPILAALCRWGLADAFEDNYALAVAIDVWSYGVTVAWGLLLALLPRDAKQLMPYGCLLAIKIFGSLAFLALCRWGLLLRDGRIVRGTQLFADLVGSGAFGTVYADGGSCTLVCPLPDGYISAWPDSLRDLHCPALVTYALLVCRWGLLLALRWGLLLALL (SEQ ID NO: 92);

MELAALCRWGLLLALLPPGAPDGENVKIPVAIKVLRENTADGKEECRVLQGLPDGKYSEDPTVPLPDDEAYVMAGVADLKQETELVEPLTPPDGRASPLTSIISAVVGILLVVVLGVVFPDAGMEHLREVRADGKDIFHKNNQLPDLQPEQLQVFRDAQEVQGYVLIPDLAFDGDLGMGAPDLQVIRGRILPDVKVLGSGAFGTVYPIGDLTLGLEPPDLKASCVTACPYATLQGLGISWLGLRSLRELGSGLALPMQIAKGMSYALFGPEADQCVPDLKLSYMPIWKFADLKPLQRLRIVRGTQLFEDNYALAVARGAPPSTFKAGVVKDVFAFRDLVKITDFGLARLLPLVHRDLAARADVWSYGVTVRDTTPVTGASPRDLYISAWPDSLRTVCAGGCARSDKIFGSLAFLPDLHCPALVTYADDSTFYRSLLADGKQLMPYGCLLADGGSCTLVCPL (SEQ ID NO: 93);

WGLLLALLP-RDA-YSEDPTVPL--ADIDETEYHA-PDLK-AREEGAGSDVFD--AYGVTVWELM-ALGK-ARDDDDMGDLVD-PLGK-AEITGYLYIS-ADGK-HLDMLRHLY-ADLK-AHSDCLACLH-AD-LTCSPQPEY-ADLK-QSDVWSYGV-AD-AYKDPPFCVA-PDL-ARDGDLGMGAA-PIAK-LLDIDETEY-AD-ARDGDPASNTA-AI-ARDGENVKIPV-ALL-GSGAFGTVY-PD-NASLSFLQD-PLLK-LHCPALVTY-AD-DSTFYRSLL-ADL-FSPAFDNLY-AILK-TIDVYMIMV (SEQ ID NO: 110);

TIDVYMIMV-PDLK-CRWGLLLAL-A-LLALLPPGA-ADG-AILDEAYVMA--ALIHHNTHL-PDL-RLVHRDLAA--LLLALLPPG-ADGK-QLFEDNYAL-P-ILHNGAYSL-P-SLTLQGLGI-R-LVDAEEYLV-R-ILLVVVLGV-ADA-SIISAVVGI-A-RLLQETELV-AD-AFEDNYALAV--AVVGILLVV-A-VVLGVVFGI-AD-ALLNWCMQIA-ADLV-ALCRWGLLL-AD-YISAWPDSL-RD-KIFGSLAFL-RDL-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 123);

MELAALCRWGLLLALLPPGAPPDLLALLPPGAPDATLEEITGYLAILDEAYVMAPILHNGAYSLPQLFEDNYALSIISAVVGIAQLMPYGCLLRLLVVVLGVVRDLQLRSLTEIAILLVVVLGVPDAVVGILLVVADALCRWGLLLADYISAWPDSLRDKIFGSLAFL (SEQ ID NO: 124);

LVPQQGFFC-ADLV-PCARVCYGL-PDLK-KHSDCLACL--ATLEEITGYL-A-TLSPGKNGV-PDL-DLVDAEEYL-P-ILHNGAYSL-A-SLPDLSVFQ-RD-QIAKGMSYL--AILDEAYVMA--ALIHHNTHL-AI-AFGPEADQCV-RDLK-LVDAEEYLV-A-QLFEDNYAL--SIISAVVGI-ADG-THLDMLRHL--ACLTSTVQLV-ADG-FRNPHQALL-ADG-RLLQETELV-ADL-KIFGSLAFL-A-YISAWPDSL-RD-AYSLTLQGL-RDL-TYLPTNASL-SDA-RWGLLLALL-A-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 138);

HYKDPPFCV-AIGK-AIQNEDLGPA-RDL-QIAKGMSYL-A-TLSPGKNGV-SD-LLALLPPGA-ADG-PYVSRLLGI--AYLSTDVGSC-AD-ILLVVVLGV-ADA-SIISAVVGI-AD-SLRELGSGL-PTG-RASPLTSII-A-LLVVVLGVV-RDL-AYLTPQGGAA--ALIHHNTHL-AD-ARPLTSIISAV-ADL-FRNPHQALL-ADGK-KIFGSLAFL--ALLNWCMQIA-ADLK-ACLTSTVQLV-ADG-YISAWPDSL-A-HLYQGCQVV-ADL-SLTLQGLGI-AD-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 148);

CRWGLLLAL-PD-AIQNEDLGPA--AVLDNGDPL--RLLQETELV-ADG-FRNPHQALL-PDLK-QVFETLEEI-PD-QIAKGMSYL-PD-VVLGVVFGI-ADA-TQLFEDNYA-AD-AVVGILLVV-AD-RASPLTSII-A-LLVVVLGVV-RD-LQLRSLTEI-A-ILLVVVLGV-ADA-SIISAVVGI-PD-YVLIAHNQV-AD-VKIPVAIKV--ALIHHNTHL-A-LAALCRWGL-A-SAVVGILLV-ADGK-KIFGSLAFL-A-IWIPDGENV-AD-TIDVYMIMV-QLMPYGCLL-ADG-MIMVKCWMI (SEQ ID NO: 156);

CVNCSQFLR-AD-LVKSPNHVK-A-ILKETELRK-RDLK-ARILHNGAYS-AD-GVVFGILIK-ADG-AELMTFGAKP-PDGK-LELTYLPTN-ALGK-KIRKYTMRR-ADLV-LERPKTLSP-A-VLRENTSPK-A-LLLALLPPG-ADGK-RSLTEILKG--

ALLHTANRP-A-ILIKRRQQK-ADGK-AGILLVVVLG-PDGK-TVWELMTFG-A-ILWKDIFHK-ADGK-RGAPPSTFK-ADL-QLVTQLMPY-A-VVVLGVVFG-PD-VMAGVGSPY-AILK-LAARNVLVK-ADL-YTMRRLLQE-ADGK-TFYRSLLED-RD-VVFGILIKR-A-LAFLPESFD-A-YLYISAWPD-AD-MTFGAKPYD (SEQ ID NO: 183);

RWGLLLALL-A-EYVNARHCL-R-DLLEKGERL--AEYHADGGKV-S-DIFHKNNQL-A-QLFEDNYAL-P-LAALCRWGL-AI-AYGVTVWELM-AI-LRIVRGTQL--ILLVVVLGV-ADA-TYLPTNASL-A-IWIPDGENV-RLL-VWSYGVTVW-AL-EYLVPQQGF-ADLK-DVWSYGVTV-PDLK-RFRELVSEF-PDLK-LSYMPIWKF-ADL-SYGVTVWEL-ADA-QCVNCSQFL-ADAK-VYMIMVKCW-AILK-KWMALESIL-AI-MIMVKCWMI (SEQ ID NO: 194);

AWPDSLPDL--DLLEKGERL-RDG-PYVSRLLGI-PDL-TLQGLGISW-A-SLAFLPESF-PDGK-AVVGILLVV-RT-LVVVLGVVF-A-IWIPDGENV-RLL-VWSYGVTVW-AL-EYLVPQQGF-ADLK-QLMPYGCLL-AD-SYGVTVWEL-ADL-TYLPTNASL-A-RIVRGTQLF-RWGLLLALL-A-KWMALESIL-AIGV-VYMIMVKCW (SEQ ID NO: 197);

RMARDPQRF-AD-AVRGTQLFED-RD-LQPEQLQVF-ADG-EYVNARHCL-ADA-RWGLLLALL--ASEGAGSDVF--AGEGLACHQL-PDLK-LQGLGISWL-AI-SYGVTVWEL-AD-AWPDSLPDL-PL-EYLVPQQGF-ADGK-HNGAYSLTL--AFNHSGICEL-A-YLVPQQGFF-ADGV-AYSLTLQGL-PDLK-RFRELVSEF-ADGK-ACYGLGMEHL-AL-VWSYGVTVW-AI-AFQNLQVIRG-ADG-VTVWELMTF-ADGK-AFYRSLLEDD-RDL-TYLPTNASL-AI-VYMIMVKCW-AILK-KWMALESIL-AD-RFTHQSDVW (SEQ ID NO: 211);

CTIDVYMIM-PI-ICELHCPAL-A-QLVTQLMPY-ADG-VSRLLGICL--ALCRWGLLL-PDLK-ARDEAYVMAGV-AD-ETLEEITGY-A-TEILKGGVL-P-QLFEDNYAL-PD-LQPEQLQVF-AD-KVPIKWMAL--SIISAVVGI-RD-DTILWKDIF-ALGV-AETHLDMLRH-A-DVFDGDLGM-PDLK-SLRELGSGL--STVQLVTQL-PLGK-ISWLGLRSL--AFDGDLGMGA-AD-CRWGLLLAL-PD-VTVWELMTF-ADGK-AFEDNYALAV-RDLK-HTVPWDQLF (SEQ ID NO: 224);

LHCPALVTY-SD-LTCSPQPEY-ADL-RLVHRDLAA-ALG-HLDMLRHLY-AD-LVVVLGVVF-PDGK-DIFHKNNQL-AD-LEEITGYLY-AD-GVVKDVFAF-AD-ARPGGLRELQL-AD-ETLEEITGY-ALL-THQSDVWSY-AD-AYLEDVRLVH-PDLK-QVVQGNLEL-AI-GSGAFGTVY-RL-VMAGVGSPY-AILK-LMTFGAKPY-AD-GTQLFEDNY-ADGK-CVTACPYNY-ADG-GTVYKGIWI-ADL-SMPNPEGRY-ADLK-HTVPWDQLF-ADLK-SLTLQGLGI-AD-MQIAKGMSY-A-ICLTSTVQL-SD-DVWSYGVTV-PDLK-MSYLEDVRL-RD-VCTGTDMKL-AD-FSPAFDNLY-AIL-SPAFDNLYY (SEQ ID NO: 239);

KIRKYTMRR-A-YLYISAWPD--LVKSPNHVK-PLLK-KVKVLGSGA-PDG-KETELRKVK-PD-AIKVLRENT-AD-GGKVPIKWM-ADG-NVKIPVAIK-AD-ARGGCLLDHVRE--AGLRSLRELG-ADG-RPKTLSPGK-AI-LQRLRIVRG-PDGV-KLRLPASPE-A-WGLLLALLP-AD-RSRACHPCS-AILK-KRRQQKIRK-ADLK-HVRENRGRL-AD-ARPGKNGVVKD-A-PLQRLRIVR-RDAK-AARNVLVKS-AD-MARDPQRFV-A-VLRENTSPK-ADL-VARCPSGVK-ADL-HYKDPPFCV-AD-KIFGSLAFL-A-STFKGTPTA-ADL-TQRCEKCSK (SEQ ID NO: 258);

SMPNPEGRY-ADL-KHSDCLACL--ADMGDLVDAE-RDGK-CVTACPYNY-AL-GGAVENPEY-AL-AVVKDVFAFG-PLAK-AEIPDLLEKG-PDGK-HLDMLRHLY-ADLK-TVWELMTFG-AD-LTCSPQPEY-ADL-RSSSTRSGG-ADGK-ETLEEITGY-AD-VLQGLPREY-AD-ARPLTSIISAV-AL-ASCVTACPY-PLL-SAVVGILLV-ADLV-AESFDGDPAS-R-DVFDGDLGM-PIL-AAPRSPLAPS-AI-GTQLFEDNY-AIG-ASLTEILKGG-AD-KGMSYLEDV-AD-VMAGVGSPY-ATLK-SLPDLSVFQ-RDLK-THQSDVWSY-ADA-SPAFDNLYY-ADL-FSPAFDNLY-ADLK-YYWDQDPPE-ADLV-LMTFGAKPY (SEQ ID NO: 270);

QALLHTANR-AIG-RQVPLQRLR-ADGK-QKIRKYTMR-ADGK-GVGSPYVSR--RILKETELR-ADL-LEDVRLVHR-ADG-TLIDTNRSR-ADL-GMEHLREVR-ADGK-REGPLPAAR-RIG-MALESILRR-PDGK-LGISWLGLR-ADGV-KITDFGLAR-A-PLQRLRIVR-ADG-VVFGILIKR-RDGK-LVHRDLAAR-A-TVCAGGCAR-RDG-KIRKYTMRR-ADG-AALCRWGLL-ADGK-

KIFGSLAFL-PDG-KVPIKWMAL-SD-ASPLDSTFYR-ADL-VSEFSRMAR-ADLV-CVNCSQFLR-ADLK-LACHQLCAR-AD-
VFQNLQVIR-AIL-SWLGLRSLR (SEQ ID NO: 285);

AAPRSPLAPS--ALPAARPAGA-PDG-ALPTHDPSPL-A-ALPASPETHL-SD-ASPETHLDML--AVLDNGDPL--ASPKANKEIL-
P-GAVENPEYL--ASPGKNGVVK-AD-LPTNASLSF--ADPASNTAPL--AARPAGATL--AAPQPHPPPA-ADGV-LQVIRGRIL-
PDG-RASPLTSII-ADL-APPSPREGPL-RDLK-HVRENRGRL-SDL-AHPPPAFSPA-PDLK-AMPNQAQMRI-ADLV-
RKYTMRRLL-A-GVVKDVFAF-AD-AVPLQRLRIV-ADGK-GSCTLVCPL-AI-ASPREGPLPA-ADL-RCEKCSKPC (SEQ ID
NO: 304);

MELAALCRWGLLLALLPPGAPASPKANKEILAARPAGATLALPTHDPSPLAALPASPETHLSDASPETHLDMLADAPPSPREGPLRDLK
HVRENRGRLADLACPSGVKPDLADGSTRSGGGDLPIASPLTSIISA (SEQ ID NO: 305);

YISAWPDSL-PDL-ECRPRFREL-AD-VGILLVVVL-PD-QQKIRKYTM-AD-LFRNPHQAL-AL-LIKRRQQKI-ADLK-
AYGVTVWELM-PDLK-LGMEHLREV--ASPKANKEIL--ALIHHNTHL-A-DIFHKNNQL-AD-MVHHRHRSS-AD-AVPLQRLRIV-
A-ILLVVVLGV-AD-VSRLLGICL--AFGLARLLDI-AI-LQRLRIVRG-AD-VVGILLVVV-PDG-KVPIKWMAL--SLAFLPESF-
AI-LQVIRGRIL--LVVVLGVVF-A-MRILKETEL-RTG-VLIQRNPQL-PDLK-ILRRRFTHQ-AD-LAALCRWGL-AD-
LDSTFYRSL-RD-LRIVRGTQL-PIAK-ISAVVGILL-AI-MIMVKCWMI (SEQ ID NO: 319);

MELAALCRWGLLLALLPPGAPAIGFHKNNQLALASPKANKEILRDGKDIFHKNNQLPDGKLGMEHLREVADLFRNPHQALALLGCKKIF
GSLPDLRIVRGTQLADGVMRILKETELSDGQLRSLTEILADGKECRPRFRELADGQLMPYGCLLPDLK (SEQ ID NO: 320);

LVVVLGVVF-A-IQRNPQLCY-AILV-TQCVNCSQF-ADG-TLIDTNRSR--ASEGAGSDVF--ALIHHNTHL-AI-AYGVTVWELM-
AIGK-ISWLGLRSL-S-VKVLGSGAF-A-QLFEDNYAL-PLG-RELGSGLAL--ASCVTACPY-AIL-VTSANIQEF-AIG-
VQGNLELTY-AD-LTCSPQPEY-ADLK-QVVQGNLEL-AI-GSGAFGTVY-RL-VMAGVGSPY-ADGV-LQVIRGRIL--
SLAFLPESF-ADG-VWSYGVTVW-ADA-RIVRGTQLF-WCMQIAKGM-AD-MQIAKGMSY-A-LMTFGAKPY-RDL-RACHPCSPM
(SEQ ID NO: 327);

LRIVRGTQL--ASEGAGSDVF--ALDIDETEYH-ADLK-QETELVEPL-AD-ARPEYLTPQGG-ADGV-EEITGYLYI-PDGK-
EECRVLQGL-ADG-RELGSGLAL--AEDLGPASPL-A-TEILKGGVL-P-LEEITGYLY-PLGK-AGDLGMGAAK-AD-LELTYLPTN-
RDG-VKVLGSGAF-AD-TELVEPLTP-RDLK-SAWPDSLPD-AD-DVWSYGVTV-AD-MQIAKGMSY-AD-QRFVVIQNE (SEQ ID
NO: 335);

GRILHNGAY-ADG-CRWGLLLAL--LQPEQLQVF--AILDEAYVMA-RD-AKGLQSLPT-AD-GRLGSQDLL-ADG-RELGSGLAL--
AYLEDVRLVH-RD-AFAGCKKIFG-ADG-FRNPHQALL-PIGK-AGEGLACHQL-AD-ARPAGATLE-SL-RRLLQETEL--
AAGCTGPKH-AD-AVRGTQLFED-RDLV-RKYTMRRLL-RD-LRIVRGTQL-PDLK-RNPQLCYQD-ADLK-RQVPLQRLR-ADAK-
ARVCYGLGM-ADGV-HRDLAARNV-PD-QRASPLTSI-PLLK-HRHRSSSTR-ADLV-YLYISAWPD-ADAK-QRFVVIQNE-ADLV-
RRQQKIRKY-ADLK-CRVLQGLPR-ADL-YTMRRLLQE-ADLK-RRFTHQSDV (SEQ ID NO: 351);

HTVPWDQLF-ADLV-CRWGLLLAL-RI-ALDIDETEYH-ADL-ARDGDLGMGAA-RD-LPTNASLSF--ADPASNTAPL--
ALPTHDPSPL-AD-NKEILDEAY--ADPAPGAGGM-AI-AEPLTPSGAM-A-GVVKDVFAF-AD-LTCSPQPEY-ADLK-
LVTYNTDTF-AD-LALLPPGAA-PD-EILDEAYVM-P-LVVVLGVVF--AECVGEGLAC-A-TPTAENPEY-AD-RSLLEDDDM-
ALLV-FVVIQNEDL-AL-AMPNQAQMRI-ADLV-MSYLEDVRL-AI-LMTFGAKPY-AD-ICELHCPAL-ALGK-YYWDQDPPE-ADL-
SPAFDNLYY-ADL-FSPAFDNLY-AILK-AMPYGCLLDH (SEQ ID NO: 363);

MELAALCRWGLLLALLPPGAPADGKTPTAENPEYAALPASPETHLPILKYSEDPTVPLPDGALPTHDPSPLADNKEILDEAYADEILDE
AYVMPLVVVLGVVFADMQIAKGMSYALMTFGAKPYPLGKAPPPAFSPAFADLHCPALVTY (SEQ ID NO: 364);

MELAALCRW--RDLAARNVL-PDA-QETELVEPL--AEEEAPRSPL-PDGK-EECRVLQGL-ADA-GERLPQPPI-ADG-SETDGYVAP-PDA-AGEGLACHQL-ADG-RELGSGLAL-P-QLFEDNYAL-PD-ALEDDDMGDL-PDLK-REVRAVTSA--ASEGAGSDVF-A-TEILKGGVL-PL-EEITGYLYI-PDGK-AENPEYLGL-PDLK-QEVQGYVLI-AD-EQLQVFETL-A-QVVQGNLEL-A-QEFAGCKKI--ALCRWGLLL-RD-AFEDNYALAV (SEQ ID NO: 374);

ISWLGLRSL--AEEEAPRSPL--RDLAARNVL-RLG-GENVKIPVA-RLG-KHSDCLACL-AIG-GERLPQPPI-ADL-TGTDMKLRL-PDGK-AENPEYLGL-ADG-RELGSGLAL--REVRAVTSA-ADG-REYVNARHC-A-QEFAGCKKI-A-QETELVEPL-A-TELRKVKVL--TDMKLRLPA-ADLK-QEVQGYVLI-PDL-ARGGSRCWGESS-ALGV-KITDFGLAR-A-TDFGLARLL-PDA-RKYTMRRLL-ADG-RELQLRSLT-ADLK-LDSTFYRSL--MELAALCRW-A-TLQGLGISW-ADL-CQSLTRTVC-ALL-HYKDPPFCV-AIG-YISAWPDSL-AD-CRWGLLLAL-RDL-TRTVCAGGC-ADLK-TFYRSLLED (SEQ ID NO: 384);

TRTVCAGGC-ADG-GGGDLTLGL--ARPEADQCVAC-A-TLQGLGISW-AI-AFDGDLGMGA-PDAK-ARGDLTLGLEP-PDGK-IDSECRPRF-ADG-VKVLGSGAF-ADG-QETELVEPL-ADG-RELGSGLAL-A-QEVQGYVLI-ALG-ERGAPPSTF-A-QEFAGCKKI--MELAALCRW-ALG-VKIPVAIKV-AL-LHCPALVTY (SEQ ID NO: 389);

LRIVRGTQL-PIAA-GGGDLTLGL--ARPEADQCVAC-AI-AFDGDLGMGA-PDAK-ARGDLTLGLEP-PDLK-QETELVEPL-PI-VKVLGSGAF--ASEGAGSDVF-PDG-RELGSGLAL-A-QEVQGYVLI-ADGK-EECRVLQGL-PDLK-LEEITGYLY-A-TEILKGGVL-PL-EEITGYLYI-AD-MELAALCRW-AD-ARPDLSVFQNL-ADL-TDFGLARLL-PD-TRTVCAGGC (SEQ ID NO: 391);

CELHCPALV-ADG-GENVKIPVA--ALPASPETHL-RD-ARPEGRYTFGA-ADGK-IDSECRPRF-ADLK-GERLPQPPI-AIL-AEEAPRSPLA-ADGA-EEITGYLYI--ALPAARPAGA-PDGK-MEHLREVRA-PDG-RELQLRSLT-ADLK-KEILDEAYV-AT-AFDGDLGMGA-PDLK-REVRAVTSA--ALPSETDGYV-ADG-AEQRASPLT-ADG-AGEGLACHQL-ADG-RELGSGLAL-AD-CEKCSKPCA-ADGV-QEVQGYVLI-ADL-TSANIQEFA-AD-LDSTFYRSL--MELAALCRW-ATGK-AINCTHSCVD-RD-AFEDNYALAV-RD-LGMGAAKGL--VSRLLGICL-PD-VKIPVAIKV-AI-ASCVTACPY (SEQ ID NO: 403);

CRWGLLLAL-PD-ENVKIPVAI--AYGVTVWELM-A-ALPASPETHL--ARPDLSVFQNL-PD-LPTNASLSF-ADG-ALPTHDPSPL-PDL-ALPSETDGYV-PDLK-LGMEHLREV-AD-LPQPPICTI-ADGV-QEVQGYVLI-AD-EQLQVFETL-A-LGMGAAKGL-PD-KGMSYLEDV-A-QEFAGCKKI-S-VGILLVVVL--AMPNQAQMRI-ADLK-LQLRSLTEI-AD-VKIPVAIKV-A-TDFGLARLL (SEQ ID NO: 406);

ASPLDSTFYR-ADG-VENPEYLTP-A-ALPASPETHL--ARAGVGSPYVS-RD-LPTNASLSF-ADG-ALPTHDPSPL-ADL-LERPKTLSP-AL-AFDGDLGMGA-PDAK-ARGDLTLGLEP-PDL-ARDDMGDLVDA-PDL-ARPEDECVGE-A-TPTAENPEY-AL-AMPNQAQMRI-ADLK-LPQPPICTI-AD-ASPLTSIISA-AD-CRWGLLLAL--AGPLPAARPA-PD-AAPRSPLAPS-ALA-ASPQPEYVNQ-ALG-VKIPVAIKV-AD-ACPSGVKPDL-AD-LHCPALVTY-SDA-SPAFDNLYY (SEQ ID NO: 415);

AWKDIFHKNN-AD-AFDGDLGMGA-PDLK-REVRAVTSA-ALL-AEEAPRSPLA-ADG-ARDGDPASNTA--ALPAARPAGA-A-IWIPDGENV-SD-LRENTSPKA-RD-LVEPLTPSG-ADG-LTSIISAVV-A-RKVKVLGSG-ADGV-RELQLRSLT-ADLK-LPQPPICTI-AD-LQRLRIVRG-PDLK-RGRILHNGA-AD-ASPLTSIISA--ASPLAPSEGA--ACPALVTYNT-AD-AVPLQRLRIV-ADAA-AMPNQAQMRI-ADLK-AYKDPPFCVA-RDL-AMPIWKFPDE-ADG-AMPYGCLLDH-ADGK-WGLLLALLP (SEQ ID NO: 425);

MELAALCRW-A-VTSANIQEF-ALGK-ENVKIPVAI-ADGK-DIFHKNNQL-RD-ATLERPKTL--LVVVLGVVF-P-TLQGLGISW-A-DVFDGDLGM-RDLV-ALCRWGLLL-PDGK-ISWLGLRSL--RSLLEDDDM-ADG-GSGAFGTVY-ADA-GTQLFEDNY-RDLK-LSYMPIWKF-ADLK-PAFDNLYYW-ADL-QLMPYGCLL-PDLK-MSYLEDVRL-R-DVWSYGVTV-PDLK-RFTHQSDVW-ADLV-HTVPWDQLF (SEQ ID NO: 428);

PAFDNLYYW-AIL-CTIDVYMIM-ADLV-RMARDPQRF-AD-KGCPAEQRA-PDLK-LGSQDLLNW--AIISAVVGIL-AL-RCEKCSKPC-AIL-VTSANIQEF-ADL-GAMPNQAQM-AD-AVTGASPGGL-P-ISAVVGILL-PD-RSGGGDLTL--AYLSTDVGSC-A-LAALCRWGL-AL-ASCVTACPY-ADL-HTVPWDQLF-ADLK-LSYMPIWKF-ADG-RASPLTSII-ADG-VTVWELMTF-ADGV-ARGQECVEEC-ADL-RIVRGTQLF-TRTVCAGGC-AD-KIFGSLAFL-PD-VCTGTDMKL-AD-LCYQDTILW (SEQ ID NO: 436), and

AKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDSRKRSHAGYQTI (SEQ ID NO: 453).

12. An isolated polyepitope construct consisting of the sequence

MELAALCRWGLLLALLPPGAPDGENVKIPVAIKVLRENTADGKEECRVLQGLPDGKYSEDPTVPLPDDEAYVMAGVADLKQETELVEPLTPPDGRASPLTSIISAVVGILLVVVLGVVFPDAGMEHLREVRADGKDIFHKNNQLPDLQPEQLQVFRDAQEVQGYVLIPDLAFDGDLGMGAPDLQVIRGRILPDVKVLGSGAFGTVYPIGDLTLGLEPPDLKASCVTACPYATLQGLGISWLGLRSLRELGSGLALPMQIAKGMSYALFGPEADQCVPDLKLSYMPIWKFADLKPLQRLRIVRGTQLFEDNYALAVARGAPPSTFKAGVVKDVFAFRDLVKITDFGLARLLPLVHRDLAARADVWSYGVTVRDTTPVTGASPRDLYISAWPDSLRTVCAGGCARSDKIFGSLAFLPDLHCPALVTYADDSTFYRSLLADGKQLMPYGCLLADGGSCTLVCPLAKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDSRKRSHAGYQTI (SEQ ID NO: 456 - universal).

13. An isolated polyepitope construct consisting of the sequence

MELAALCRWGLLLALLPPGAPPDLLALLPPGAPDATLEEITGYLAILDEAYVMAPILHNGAYSLPQLFEDNYALSIISAVVGIAQLMPYGCLLRLLVVVLGVVRDLQLRSLTEIAILLVVVLGVPDAVVGILLVVADALCRWGLLLADYISAWPDSLRDKIFGSLAFLAKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDSRKRSHAGYQTI (SEQ ID NO: 457 - HLA-A*0201-specific).

14. An isolated polyepitope construct consisting of the sequence

MELAALCRWGLLLALLPPGAPADGKTPTAENPEYAALPASPETHLPILKYSEDPTVPLPDGALPTHDPSPLADNKEILDEAYADEILDEAYVMPLVVVLGVVFADMQIAKGMSYALMTFGAKPYPLGKAPPPAFSPAFADLHCPALVTYAKFVAAWTLKAAAKKAVVGILLVVVLGVVFGILIKRRQQKIRKKPICTIDVYMIMVKCWMIDSEKKAQMRILKETELRKVKVLGSGAKKIKWMALESILRRRFTHQSDVKKPICTIDVYMIMVKCWMIDSRKRSHAGYQTI (SEQ ID NO: 458 - HLA-B*3501-specific).

15. A pharmaceutical composition comprising the polyepitope construct of any one of claims 1-3 and 11-14 and a pharmaceutically acceptable carrier or excipient.

16. An isolated nucleic acid comprising a sequence encoding the polyepitope construct of any one of claims 1-3 and 11-14.

17. A pharmaceutical composition comprising the nucleic acid of claim 16 and a pharmaceutically acceptable carrier or excepient.

18. A host cell comprising the nucleic acid of claim 16.

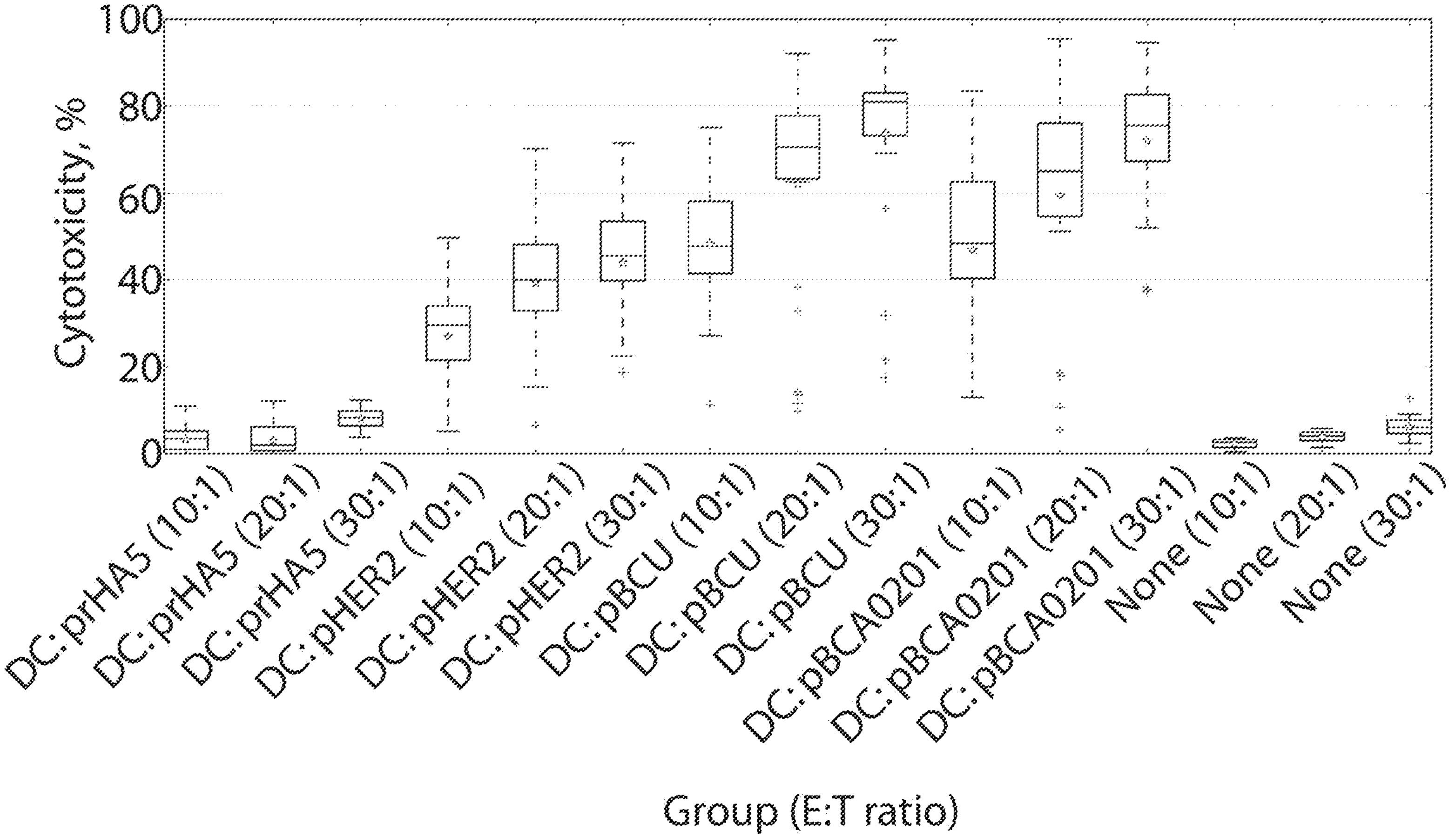
19. A method for inducing a T cell response in a mammal comprising administering to said mammal the pharmaceutical composition of claim 15.

20. A method for inducing a T cell response in a mammal comprising administering to said mammal the pharmaceutical composition of claim 17.

21. A method for treating a HER2-positive breast cancer in a mammal comprising administering to said mammal the pharmaceutical composition of claim 15.

22. A method for treating a HER2-positive breast cancer in a mammal comprising administering to said mammal the pharmaceutical composition of claim 17.

Figure 1A



Lysis of DCs, transfected with pHER2

Figure 1B

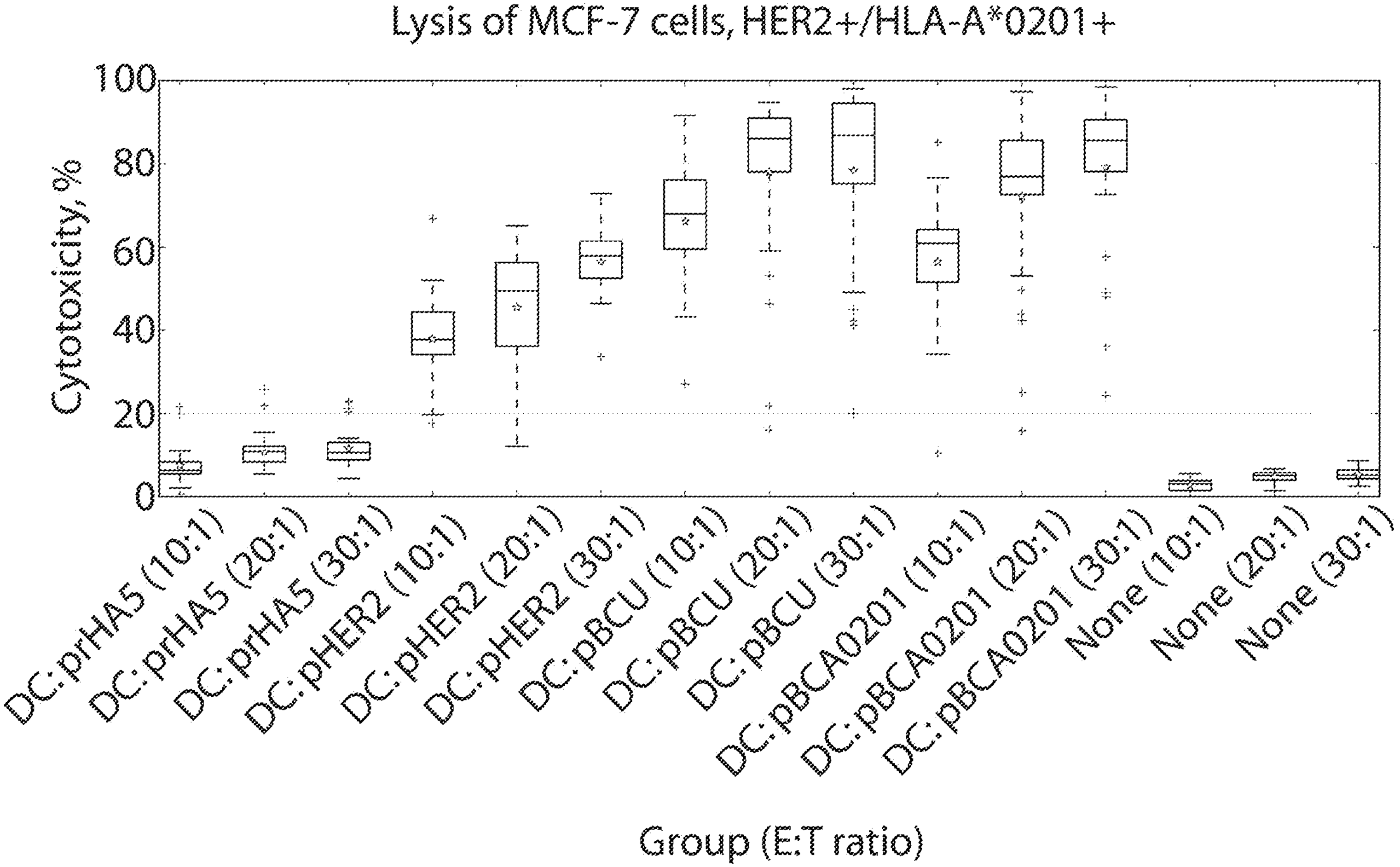

Lysis of MCF-7 cells, HER2+/HLA-A*0201+
Cytotoxicity, %
100
80
60
40
20
0
DC:prHA5 (10:1)
DC:prHA5 (20:1)
DC:prHA5 (30:1)
DC:pHER2 (10:1)
DC:pHER2 (20:1)
DC:pHER2 (30:1)
DC:pBCU (10:1)
DC:pBCU (20:1)
DC:pBCU (30:1)
DC:pBCA0201 (10:1)
DC:pBCA0201 (20:1)
DC:pBCA0201 (30:1)
None (10:1)
None (20:1)
None (30:1)
Group (E:T ratio)

Figure 2A

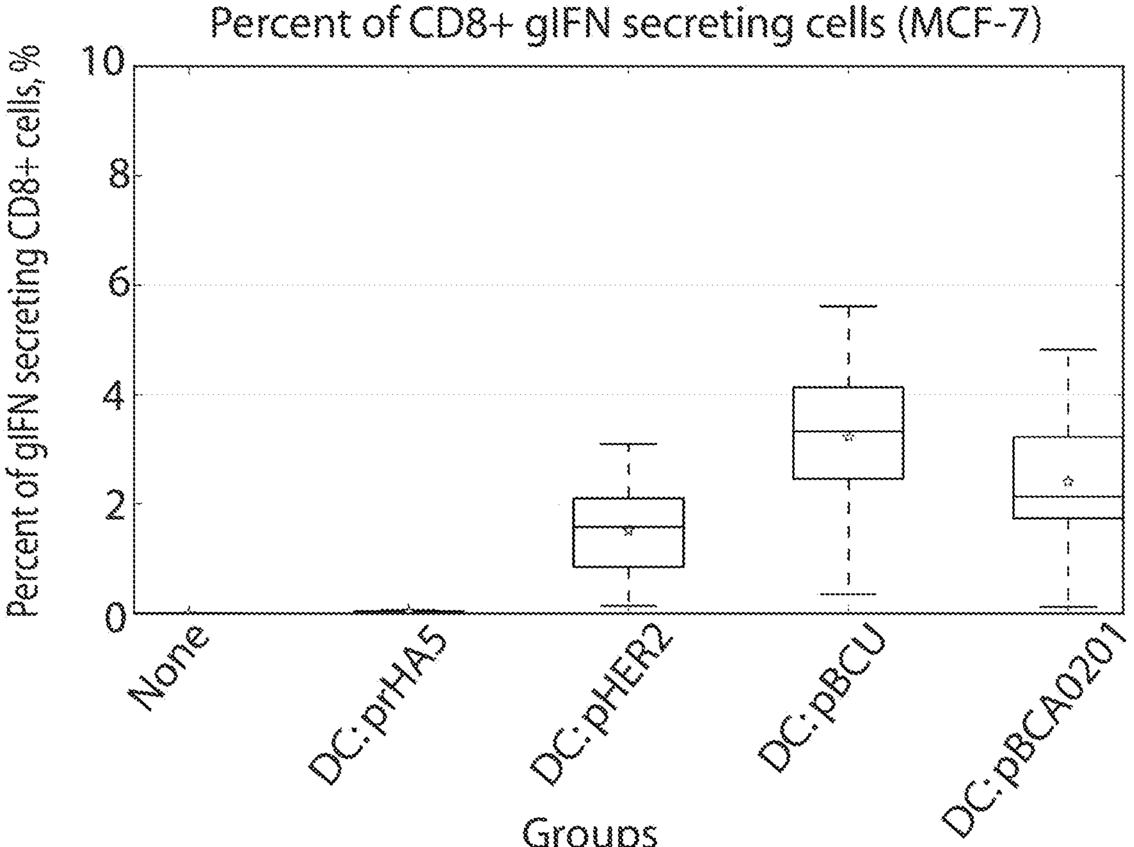


Figure 2B