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(54) **Title**: POLYPEPTIDES AND VECTORS FOR TARGETING HER2/NEU EXPRESSING CELLS AND USES THEREOF

(57) **Abstract**: Various aspects of the invention provide for capsids, parvovirus capsids, hybrid parvovirus capsids, parvovirus vectors, hybrid parvovirus vectors, hybrid parvovirus particles and parvovirus particles containing polypeptides in which the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into the VP2 loop of the B 19 capsid protein. Polypeptides in which the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into the VP2 loop of the B 19 capsid protein are also provided (e.g., SEQ ID NO:2). Other aspects of the invention provides capsids. parvovirus capsids, hybrid parvovirus capsids, parvovirus vectors, hybrid parvovirus vectors, hybrid parvovirus particles and parvovirus particles containing a polypeptide comprising SEQ I D NO: 2. Also provided in various aspects of the invention a pharmaceutical compositions and methods of delivering therapeutic agents and/or reporter peptides/proteins to target cells. Finally, methods of treating diseases characterized by cells expression HER2/*neu* receptors are also provided.

DESCRIPTION

POLYPEPTIDES AND VECTORS FOR TARGETING HER2/NEU EXPRESSING CELLS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application Serial No. 61/487,476, filed May 18, 2011, the disclosure of which is hereby incorporated by reference in its entirety, including all figures, tables and amino acid or nucleic acid sequences.

BACKGROUND OF THE INVENTION

Parvovirus B19 (B19) is a small ssDNA-containing virus that has evolved to be restricted in its replication to erythroid progenitor cells in the human bone marrow. B19 binds to erythroid cells through blood group P antigen or globoside (10). However, it has been demonstrated that P antigen alone is not sufficient for parvovirus B19 infection (54) and that functionally activated α5β1 integrin serves as a coreceptor for internalization into human cells (53). Subsequently, the Ku80 subunit of the DNA double-strand break repair protein Ku, which also functions as an adhesion receptor for fibronectin (31), was reported to also provide coreceptor activity (34).

The human epidermal growth factor receptor 2 (HER2/*neu*) has been reported to be overexpressed on ~25% of human breast cancers by up to 100-fold the levels present on non-cancerous cells and on ~30-40% of medulloblastomas by up to 5-fold (3, 11, 47). HER2-overexpression has been documented to result in constitutive activation of kinase signaling (57). HER2/*neu*-positive breast cancers are characterized by poor differentiation, high rates of proliferation, lymph node involvement, a relative resistance to certain types of chemotherapy and poor prognosis (11). About 30% of breast cancer patients present with bone marrow metastases at diagnosis (9). HER2/*neu* expression is seen in micrometastatic tumors in the bone marrow as well as in primary tumor cells and detection of HER2/*neu* in circulating tumor cells suggests a stable HER2/*neu* expression during metastatic spread of breast cancer (21, 35, 39, 50). Thus targeting HER2/*neu* on breast cancer cells is an attractive therapeutic approach and a humanized anti-HER2 monoclonal antibody (h4D5, trastazumab, Herceptin®) has been developed and tested in clinical trials (17, 25, 40, 42) (38). The mechanisms of Herceptin® action are thought to involve antibody-mediated cytotoxicity (5),

blocking of receptor dimerization, removal of HER2/*neu* from the cell surface by endocytosis, and inhibition of HER2/*neu* extracellular domain (ECD) shedding by blocking a proteolytic cleavage site, which prevents constitutive tyrosine kinase activation of ECD-less receptors (13, 30) (23, 26, 48). When used as single agent in patients with HER2/*neu*-positive tumors, however, Herceptin® treatment had relatively high relapse rates (36) and it is currently used with combination chemotherapy (38).

The complementarity-determining regions (CDRs) of antibodies mediate their high-affinity binding and specificity to antigens (4) and peptide analogs of CDRs have been developed for antibodies with known sequences and structures (43). The HER2/*neu*-binding peptidomimetic (AHNP) was derived from the structure of the CDR-H3 loop of the anti-HER2 antibody rhu 4D5, and was shown to bind to cell surface-expressed HER2/*neu*, albeit with lower affinity than whole antibodies, and to inhibit HER2/*neu* kinase activity, modestly down-modulate HER2/*neu* and sensitize tumor cells to apoptosis when used in conjunction with ionizing radiation or chemotherapeutic agents (7, 37) (28).

Integrins have been suggested to play a cooperative role during oncogenesis, partly attributed to their reciprocal signaling to growth factor receptors (1) (20, 32, 51). Targeted disruption of the β1 integrin chain in a transgenic mouse model of human breast cancer demonstrated a critical role of β1 integrin in initiation and maintenance of mammary tumor growth *in vivo* (56) and co-clustering of HER2/*neu* and integrin β1 was demonstrated to be critical for induction of cell migration and survival during breast cancer progression (52). Integrin β1 is know to play an important role in hematopoietic stem and progenitor cell interaction with the bone marrow microenvironment (44), and has recently been identified as one of the adhesion receptors involved in cancer cell survival during genotoxic stresses induced by ionizing radiation and cytotoxic drugs (14, 15, 46).

Equipping viral vectors with the capability to target both HER2/*neu* and β1 integrins, could allow the delivery of a cytotoxic insult to HER2/*neu*-positive cancer cells in microenvironments, such as the bone marrow, where prosurvival signals mediated through β1 integrins prevent tumor cell eradication with conventional radiation and chemotherapies. We report here the introduction of the AHNP peptide into the capsid of B19 vectors to replace the endogenous P antigen binding site and demonstrate retargeting of B19 vectors to HER2/*neu*-positive tumor cells.

BRIEF SUMMARY OF THE INVENTION

Various aspects of the invention provide for capsids, parvovirus capsids, hybrid parvovirus capsids, parvovirus vectors, hybrid parvovirus vectors, hybrid parvovirus particles and parvovirus particles containing polypeptides in which the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into the loop 4 of the B19 VP2 capsid protein or into loop 8 of a parvovirus VP2 capsid protein. Polypeptides in which the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into the loop 4 of the B19 VP2 capsid protein are also provided (e.g., SEQ ID NO: 2). Other aspects of the invention provides capsids, parvovirus capsids, hybrid parvovirus capsids, parvovirus vectors, hybrid parvovirus vectors, hybrid parvovirus particles and parvovirus particles containing a polypeptide comprising SEQ ID NO: 2. Also provided in various aspects of the invention are pharmaceutical compositions and methods of delivering therapeutic agents, DNA/RNA/peptides/proteins and/or reporter DNA/RNA/peptides/proteins to target cells. Finally, methods of treating diseases characterized by cells expression HER2/*neu* receptors are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D. Structural grafting of the AHNP peptide onto the B19 VP2 monomer. (A) A sequence alignment of the AHNP peptide sequence with the sequence of B19 VP2 region containing the proposed P antigen binding site amino acids. The residues shown in green were replaced with those shown in orange in the chimeric AHNP-B19 VP2. (B) A superimposition of the Cα trace of the loop region replaced for wt B19 (blue), the SWISS-generated model AHNP peptide loop model (green), the rhu 4D5 CDR-H3 loop structure on which the AHNP peptide design was based (purple), the interactive AHNP peptide loop model generated in the Coot program (orange) (left hand side) and the same loops with the amino acid positions of the DGFYA peptide important for receptor binding superimposed (right hand side). (C) Superimposition of the AHNP peptide loop model generated in the Coot program (orange) and the SWISS MODEL generated AHNP peptide loop model (green). (D) A ribbon diagram showing the secondary structure of the B19 VP2 monomer with the substituted region in loop 4 shown for wt (blue). A close-up view indicated by the arrow of the wt B19 region in loop 4 (blue) shows the AHNP peptide loop model generated in the Coot program (orange) and the SWISS MODEL generated AHNP

peptide loop model (green). The N- and C-terminal regions observed in the crystal structure, with the secondary structure elements and loop regions, and the approximate icosahedral 2-, 3-, and 5-fold axes are labeled. The stretch of amino acids that were disordered in the structure (amino acids 301–313) is indicated with the dashed lines.

Figures 2A-2C. Analysis of B19-*scGFP* and AHNP-B19-sc*GFP* fractions from iodixanol gradients. (A) Pooled and concentrated samples from fractions 7-9 of three separate iodixanol gradients (corresponding to the 40% phase) were analyzed by slot blot for presence of encapsidated genomes. Samples and control plasmids were loaded at 2-fold dilutions. (B) Western blot analysis of fractions 6-18 of B19-sc*GFP* (top panel) and AHNP-sc*GFP* vector preparations (bottom panel) using anti-B19 VP2 antibodies. Purified B19-sc*GFP* virions were included as control (lane C); B19 VP2, parvovirus B19 capsid protein 2 (58kDa); *, VP2 break-down product. (C) Infectious assay. Phase contrast (Phase) and fluorescent microscopy (GFP) images of murine MMTV-HER2/*neu*-transgenic mammary tumor cells exposed to fractions 7-9, 10-12, and 13-15 of iodixanol gradients. Images were acquired 24 h post infection.

Figure 3. B19-sc*GFP* and AHNP-B19-*scGFP*- mediated transgene expression in murine MMTV-HER2/*neu*-transgenic mammary tumor cells. Phase contrast and fluorescent microscopy images of murine MMTV-HER2/*neu*-transgenic mammary tumor cells exposed to B19-sc*GFP* (top left panels: GFP left, phase right) and AHNP-B19-*scGFP* vectors (top right panels: GFP left, phase right) 24 h post infection demonstrated significantly higher *GFP* transgene expression in AHNP-sc*GFP* transduced cells (bottom).

Figures 4A-4C. Dependence of AHNP-B19 infection on HER2 kinase activity. (A) Western blot detection of phosphorylated tyrosine 1248 (^{P1248}-HER2) and total HER2 in cell extracts from MMTV-HER2/*neu* mammary tumor cells in the absence and presence of peptides derived from the activation loop of the kinase domain (p1) or the transmembrane region (p2) of the HER2/*neu* receptor. (B) Phase contrast and fluorescent microscopy images of murine MMTV-HER2/*neu*-transgenic mammary tumor cells exposed to B19-sc*GFP* (left panels) and AHNP-B19-*scGFP* vectors (right panels) in the absence and presence of inhibitory β1 integrin antibodies (second panels); peptides derived from the activation loop of the HER2/*neu* kinase domain (third panels). For inhibition of P antigen binding, virions were pre-incubated with recombinant P antigen for 1.5 h on ice prior to addition to cells (fourth panels). (C) Quantitative analyses of B19-sc*GFP* and AHNP-B19-sc*GFP* transduction efficiencies. Images from five visual fields were analyzed quantitatively by ImageJ analysis

software. Transgene expression was assessed as total area of green fluorescence (pixel^2 per visual field) and mean ± SD are depicted. Students *t* test was used to compare the groups. * $p \leq 0.05$; ** $p \leq 0.001$.

Figure 5. B19-sc*GFP* and AHNP-B19-*scGFP*- mediated transgene expression in Daoy medulloblastoma cells. Quantitative analyses of B19-sc*GFP* and AHNP-B19-sc*GFP* transduction efficiencies. Images from three to five visual fields were analyzed quantitatively by ImageJ analysis software. Transgene expression was assessed as total area of green fluorescence (pixel^2 per visual field) and mean ± SD are depicted. Students *t* test was used to compare the groups. * $p \leq 0.05$.

DETAILED DISCLOSURE OF THE INVENTION

In a first aspect of the invention, a polypeptide in which the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into loop 4 of the B19 VP2 capsid protein is provided. In certain embodiments, amino acid positions 393-406 of SEQ ID NO: 1 (VP2 loop 4 residues 393-FPNKGTQQYTDQIE-406 of SEQ ID NO: 1) are substituted with YCDGFYACYMDV (SEQ ID NO: 3) (see, for example, SEQ ID NO: 2). Other aspects of the invention provide for the substitution of SEQ ID NO: 3 into loop 8 of a parvovirus VP2 capsid protein (for variable region/loop annotation please see Ng R. JVI 84: 12945-57, 2010). Another aspect of the invention provides capsids, parvovirus capsids, hybrid parvovirus capsids, parvovirus vectors, hybrid parvovirus vectors, hybrid parvovirus particles and parvovirus particles containing polypeptides in which the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into the VP2 loop of the B19 capsid protein or into loop 8 of a parvovirus VP2 capsid protein (for variable region/loop annotation please see Ng R. JVI 84: 12945-57, 2010). Yet another aspect of the invention provides capsids, parvovirus capsids, hybrid parvovirus capsids, parvovirus vectors, hybrid parvovirus vectors, hybrid parvovirus particles and parvovirus particles containing a polypeptide comprising SEQ ID NO: 2.

One aspect of the present invention provides parvovirus vectors for the *in vitro* and *in vivo* delivery of nucleic acids to cells. Another aspect of the invention provides novel capsid structures for use in the delivery of compounds and other therapeutic agents to cells expressing the HER2/*neu* receptor by conjugating cytotoxic drugs, including but not limited to, intercalators (including enediynes (59) and metallic intercalators (60)), taxanes (61), maytansine (62), methotrexate (63), anthracyclines (e.g. daunomycin) (64), and

isothiocyanates (65) to the outside (66) and/or inside of the HER2-AHNP B19 capsid, and/or infusing respective drugs into HER2-AHNP B19 capsids (67). The parvovirus vectors of the present invention can utilize properties associated with gene delivery by adeno-associated virus (AAV) vectors while specifically targeting cell populations expressing the HER2/*neu* receptor.

The term "parvovirus" as used herein encompasses all parvoviruses, including autonomously-replicating parvoviruses and dependoviruses. The autonomous parvoviruses include members of the genera *Parvovirus, Erythrovirus, Densovirus, Iteravirus,* and *Contravirus*. Exemplary autonomous parvoviruses include, but are not limited to, mouse minute virus, bovine parvovirus, canine parvovirus, chicken parvovirus, feline panleukopenia virus, feline parvovirus, goose parvovirus, and B19 virus. Other autonomous parvoviruses are known to those skilled in the art.

The genus *Dependovirus* contains the adeno-associated viruses (AAV), including but not limited to, AAV type 1, AAV type 2, AAV type 3, AAV type 4, AAV type 5, AAV type 6, AAV type 7, AAV type 8, AAV type 9, AAV type rh32.33, avian AAV, bovine AAV, canine AAV, equine AAV, and ovine AAV. In certain embodiments, parvovirus particles, capsids and genomes of the present invention can be from the B19 parvovirus.

The term "tropism" refers to entry of the virus into the cell. In certain aspects of the invention, expression of sequences carried by the viral genome in the cell, e.g., for a recombinant virus, expression of the heterologous nucleotide sequences(s), follows entry of the virus into the cell. Those skilled in the art will appreciate that transcription of a heterologous nucleic acid sequence from the viral genome may not be initiated in the absence of trans-acting factors, e.g., for an inducible promoter or otherwise regulated nucleic acid sequence. In the case of parvovirus and AAV, gene expression from the viral genome may be from a stably integrated provirus, from a non-integrated episome, as well as any other form in which the virus may take within the cell.

The parvovirus vectors of the present invention are useful for the delivery of nucleic acids to cells both *in vitro* and *in vivo*. In particular, the vectors described herein can be used to deliver or transfer nucleic acids to mammalian cells expressing a HER2/*neu* receptor. Nucleic acids of interest include nucleic acids encoding peptides and proteins that are, preferably, therapeutic (e.g., for medical or veterinary use). Alternatively, reporter peptides/proteins, such as green fluorescent protein or the like, can also be delivered to a target cell expressing the HER2/*neu* receptor. In certain embodiments, RNA molecules

including but not limited to siRNA, shRNA or antisense RNA molecules can be delivered using parvovirus vectors disclosed herein.

A "therapeutic agent" is a peptide or protein or a nucleic acid or a cytotoxic drug that can be used to induce cell death or inhibit metastasis in a HER2/*neu* receptor expressing cell (e.g., a breast cancer cell). Non-limiting examples of such proteins or peptides are encoded by suicide genes, tumor suppressor genes or genes encoding cytokines. Suicide genes can be defined as genes that are able to convert a nontoxic prodrug into a toxic drug and non-limiting examples of such genes are those encoding Herpes simplex virus thymidine kinase (HSV-tk)/acyclovir (ACV) or ganciclovir (GCV), and the bacterial or fungal cytosine deaminase (CD)/5-florocytosine (5-FC). Non-limiting examples of tumor suppressor genes include p53, p21WAF1/CIP1 (p21), p16INK4a (p16), p18INK4c (p18), p27KIP2 (p27), Rb, Wt-1, NF1, VHL, APC, and the like. Exemplary cytokines that can be delivered using the vectors disclosed herein include are IL-1, 2, 4, 5, and 12, IFN-α, β, and γ, GM-CSF and TNF.

As discussed above, the vectors disclosed herein can also be used to deliver chemical compounds to a cell expressing a HER2/*neu* receptor. Chemical compounds with cytotoxic properties are known in the art and include, but are not limited to, intercalators (including enediynes (59) and metallic intercalators (60), taxanes (61), maytansine (62), methotrexate (63), anthracyclines (e.g. daunomycin) (64), and isothiocyanates (65) and can be attached to the outside (66) and/or "packaged"/conjugated/infused inside of the HER2-AHNP B19 capsid (67).

The term "packaged" refers to the process by which recombinant viral DNA is inserted into the parvoviral capsid by a self-assembly process within the host cell (e.g., HEK293 cell) used for viral vector generation.

As discussed above, the vectors disclosed herein can also be used to deliver a heterologous nucleic acid sequence encoding a reporter peptide/protein to a cell expressing a HER2/*neu* receptor. Non-limiting examples of such reporter proteins include may encode a reporter peptide or protein (e.g., an enzyme). Reporter proteins are known in the art and include, but are not limited to, Green Fluorescent Protein, β-galactosidase, alkaline phosphatase, chloramphenicol acetyltransferase, and the like. Cytotoxic drugs delivered by the AHNP-B19 vector to HER2-expressing cells include, but are not limited to, intercalators, taxanes, maytansine, methotrexate, anthracyclines, and isothiocyanates conjugated to either the outside of the AHNP-B19 vector capsid or conjugated/packaged inside the AHNP-B19 vector capsid.

Except as otherwise indicated, standard methods known to those skilled in the art may be used for the construction of parvovirus genomes and particles suitable for the delivery of therapeutic agents to a HER2/*neu* receptor expressing cell.

Yet another aspect of the invention provides for a "hybrid parvovirus" vector for delivery of nucleic acids or other molecules to cells. The term "hybrid parvovirus" is used to denote an AAV genome encapsidated within a different parvovirus capsid (for example a B19 capsid). In particular preferred embodiments the parvovirus capsid is a B19 capsid as described herein. For example, a recombinant AAV type 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, rh32.33 or B19 genome may be encapsidated within an B19 capsid containing a sequence that targets the virus to HER2/*neu* receptor expressing cells. In embodiments, the hybrid parvovirus particle contains a rAAV genome that carries at least one heterologous nucleic acid sequence to be delivered to a cell. Those skilled in the art will appreciate that the rAAV genome can encode more than one heterologous nucleic acid sequence (*e.g.*, two, three or more heterologous nucleic acid sequences) and is, in general, only limited by the packaging capacity of the virus capsid. Heterologous nucleic acid sequence(s) for expressing within a target cell (e.g., a HER2/*neu* receptor expressing cell) are as described above and can be regulated with promoters/enhancers that are native or which may be inducible.

Any method of introducing a vector, as disclosed herein, into a target cell can be used, including but not limited to, electroporation, calcium phosphate precipitation, microinjection, cationic or anionic liposomes, and liposomes in combination with a nuclear localization signal. However, the parvovirus and hybrid parvovirus vectors disclosed herein can also be introduced into a target cell using the HER2/*neu* receptor.

Another aspect of the invention provides hybrid parvovirus vectors that contain chimeric capsids and/or capsids that have been modified by insertion of an amino acid sequence(s) into the capsid that confers the ability of the vectors to bind to the HER2/*neu* receptor (*e.g.*, substitution of VP2 loop residues 393-FPNKGTQQYTDQIE-406, containing with the sequence of the AHNP peptide, YCDGFYACYMDV (SEQ ID NO: 3) into the capsid polypeptide of the B19 virus).

The methods of the present invention provide a means for delivering heterologous nucleic acid sequences and/or other compounds into cells expressing a HER2/*neu* receptor. The vectors and other reagents, methods and pharmaceutical formulations of the present invention are additionally useful in a method of delivering a therapeutic agent to a subject in a method of treatment of diseases, including, but not limited to breast cancer,

medulloblastoma, lung cancer (e.g., non-small cell lung cancer), uterine cancer (e.g., uterine serous endometrial carcinoma), stomach cancer and ovarian cancer. Therapeutic agents suitable for delivery to a subject in need of treatment are discussed above.

For the purposes of this invention, the term "subject" refers to mammals, such as humans, mice, rats, apes, chimpanzees, orangutans, monkey, dog, cat, guinea pig, hamster, rabbits, ferrets, cows, horses, goats and sheep and in which disease causing cells (e.g., cancer cells) expressing HER2/*neu* receptors are found. The disease causing cells can arise naturally in the subject (i.e., by the development of, for example, breast cancer in a human) or be introduced into the mammal via injection of cancer cells or cancer cell lines expressing HER2/*neu* receptors (*i.e.*, in animal models of disease).

In other aspects of the invention, a pharmaceutical composition comprising a virus particle, a polypeptide, a parvovirus vector or a hybrid parvovirus, as disclosed herein, in a pharmaceutically-acceptable carrier, adjuvant or diluent is provided. For injection, the carrier will typically be a liquid. Pharmaceutically acceptable carriers, adjuvants and diluents are well-known to those skilled in the art and can contain the additives usual for injection solutions, such as stabilizing agents, salts or saline, and/or buffers. Exemplary modes of administration include oral, rectal, transmucosal, topical, transdermal, inhalation, parenteral (e.g., intravenous, subcutaneous, intradermal, intramuscular, and intraarticular) administration. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Alternatively, one may administer the virus in a local rather than systemic manner, for example, in a depot or sustained-release formulation.

In certain embodiments of the invention, more than one administration (e.g., two, three, four, or more administrations) may be employed to achieve delivery of therapeutic agents to target cells expressing a HER2/*neu* receptor. As would be apparent to those skilled in the art, the parvovirus and hybrid parvovirus vectors provided herein can be administered alone or in combination with standard treatment protocols/regimens for a particular cancer characterized by expression of HER2/*neu* receptors (e.g., breast cancer). Furthermore, certain embodiments of the invention contemplate the administration of the parvovirus and hybrid parvovirus vectors provided herein to a subject after the completion of a treatment regimen for a cancer characterized by expression of HER2/*neu* receptors (e.g., breast cancer). In these embodiments, the parvovirus and hybrid parvovirus vectors provided herein can be

administered to a subject up to 5 years (or more) after the completion of a treatment regimen for a cancer characterized by expression of HER2/*neu* receptors (e.g., breast cancer).

Thus, the following non-limiting embodiments are provided:

1. An isolated polypeptide in which:

a) the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into loop 8 of a parvovirus VP2 capsid protein (for variable region/loop annotation please see Ng R. JVI 84: 12945-57, 2010); or

b) the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into loop 4 of a parvovirus B19 VP2 capsid protein for the P antigen binding site.

2. The isolated polypeptide according to embodiment 1, wherein said polypeptide comprises SEQ ID NO: 2.

3. A capsid comprising a polypeptide according to any one of embodiments 1 or 2.

4. A parvovirus or hybrid parvovirus particle comprising a polypeptide according to any one of embodiments 1 or 2.

5. A parvovirus or hybrid parvovirus vector comprising nucleic acid encoding one or more therapeutic agent or one or more reporter peptide or protein, said parvovirus vector comprising a capsid comprising a polypeptide according to any one of embodiments 1 or 2.

6. A parvovirus or hybrid parvovirus vector comprising a cytotoxic compound/drug (*e.g.*, including, but not limited to, chemotherapeutics such as intercalators, taxanes, maytansine, methotrexate, anthracyclines and isothiocyanates) packaged/conjugated/infused in and/or associated with said parvovirus vector comprising a caspid comprising a polypeptide according to any one of embodiments 1 or 2.

7. The parvovirus or hybrid parvovirus vector according to embodiment 5, wherein said one or more therapeutic agent is a suicide gene, a tumor suppressor gene or a gene encoding a cytokine.

8. The parvovirus or hybrid parvovirus vector according to embodiment 7, wherein said nucleic acid encodes one or more RNA molecule (*e.g.*, including, but not limited to antisense RNA, siRNA or shRNA) and/or one or more of the following polypeptides: Herpes simplex virus thymidine kinase (HSV-tk)/acyclovir (ACV), ganciclovir (GCV), bacterial or fungal cytosine deaminase (CD)/5-florocytosine (5-FC), p53, p21WAF1/CIP1 (p21), p16INK4a (p16), p18INK4c (p18), p27KIP2 (p27), Rb, Wt-1, NF1, VHL, APC, IL-1, IL-2, IL-4, IL-5, IL-12, IFN-α, IFN-β, IFN-γ, GM-CSF or TNF.

9. The parvovirus or hybrid parvovirus vector according to embodiment 6, wherein said one or more therapeutic agent is a cytotoxic drug, including but not limited to DNA intercalators, taxanes, maytansine, methotrexate, anthracyclines and isothiocyanates.

10. The parvovirus or hybrid parvovirus vector according to embodiment 5, wherein said one or more reporter peptide/protein is Green Fluorescent Protein, β-galactosidase, alkaline phosphatase or chloramphenicol acetyltransferase.

11. A pharmaceutical composition comprising a pharmaceutically-acceptable carrier, adjuvant or diluent and a polypeptide, parvovirus capsid, parvovirus particle or parvovirus vector according to any one of embodiments 1-10.

12. A method of delivering a nucleic acid, therapeutic agent, reporter peptide or cytotoxic drug to a cell expressing one or more HER2/*neu* receptor comprising contacting said cell expressing one or more HER2/*neu* receptor with a parvovirus vector or a hybrid parvovirus vector according to any one of embodiments 5-9, said parvovirus or hybrid parvovirus vector optionally being contained in a pharmaceutical composition.

13. A method of treating a cancer characterized by expression of HER2/*neu* receptors on a cell surface comprising the administration of a parvovirus vector or a hybrid parvovirus vector according to any one of embodiments 5-9 to a subject having a cancer characterized by expression of HER2/*neu* receptors on a cell surface, said parvovirus or hybrid parvovirus vector optionally being contained in a pharmaceutical composition.

14. The method according to embodiment 13, wherein said cancer is selected from breast cancer, medullablastoma, lung cancer, non-small cell lung cancer, uterine cancer, uterine serous endometrial carcinoma, stomach cancer or ovarian cancer.

15. The method according to embodiment 13 or 14, wherein said subject is a human.

16. The method according to embodiment 15, wherein said parvovirus vector or a hybrid parvovirus vector is administered alone or in combination with a treatment regimen for said cancer.

17. The method according to embodiment 15, wherein said parvovirus vector or a hybrid parvovirus vector is administered to a subject after a treatment regimen for a cancer has been completed.

18. The method according to embodiment 16 or 17, wherein said cancer is breast cancer.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLES

Material and Methods

Structural Modeling of the chimeric AHNP-B19 VP2.

The crystal structure of the B19 capsid VP2 (PDB accession No. 1S58) was used as a template onto which the HER2/*neu* peptide, AHNP, was inserted using two strategies: (I) interactive substitution mutation of VP2 loop residues 393-FPNKGTQQYTDQIE-406, containing the proposed P-antigen binding site footprint, 399-QQYTDQIE-406 (12), with the

sequence of the AHNP peptide, YCDGFYACYMDV (SEQ ID NO: 3), in the program Coot (18) and (II) automatic 3D model generation using the SWISS-MODEL online molecular modeling program, SMR-Pipeline (45). In the first approach, the coordinates of the anti-HER2/*neu* antibody rhu 4D5 CDR-H3 loop amino acids RWGGDGFYAMDV (SEQ ID NO: 4), on which the AHNP-peptide design is based, was extracted from the crystal structure of the antibody (PDB accession No. 1FVD) and compared to the structure of the B19 VP2 loop containing the P antigen binding site to identify the optimal amino acids to be substituted with the AHNP peptide to maintain the structural conformation of the DGFYA sequence essential for the HER2/*neu* receptor recognition and eliminate the P antigen binding site. Sequence comparison of the B19 VP2 loop amino acids with the AHNP peptide and rhu 4D5 CDR-H3 loop sequences as well as the structural comparison to the rhu 4D5 CDR-H3 loop structure (by superimposition) identified the stretch of amino acids from 393-406 for the substitution. B19 VP2 amino acids 393-404 were interactively substituted with the AHNP peptide amino acids, residues 405 and 406 were deleted, the Cα position of the tyrosine 398 and alanine 399 were rotated to be the same as the equivalent residues in the rhu 4D5 crystal structure, and the model geometry was regularized (18). In the second approach, B19 VP2 amino acids 393-406 were deleted from the sequence (NCBI Accession No. M13178), replaced with those of the AHNP peptide amino acids (YCDGFYACYMDV, SEQ ID NO: 3), and submitted to the SMR-Pipeline along with the coordinates for the B19 VP2 crystal structure supplied as the user identified template for automated model building (45). The final total energy for the model generated was -7919.273 KJ/mol. The main chain conformation of the energy minimized model generated from this second approach was essentially identical to that obtained by the interactive mutation in Coot with the exception of the Cα positions for amino acid positions 398 and 399 which were adjusted as described above.

Cells, plasmids, peptides.

Murine mammary gland tumor cells derived from MMTV-HER2/*neu* transgenic mice were a kind gift from Dr. Hao-Yuan Yiang (Indiana University) and were maintained in Iscove's Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum and 1% penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO). Cells were subcultured every 3 days to avoid tumor nodule formation. Replacement of amino acids 393 to 406 of B19 VP2 by an anti-HER2/*neu* peptide was performed by PCR-based mutagenesis and the resulting AHNP-

B19 plasmid was confirmed by sequencing. Peptides derived from HER2 were purchased from CHI Scientific (Maynard, MA).

Recombinant B19 vector generation and purification.

Recombinant wt-VP2 and AHNP-VP2 B19 vectors containing self-complementary AAV2 genomes encoding a chicken β-actin promoter-driven green fluorescent protein (B19-sc*GFP* and AHNP-B19-sc*GFP*) were generated by triple plasmid transfection of HEK293 cells as previously described (54). Viral vectors were purified using discontinuous iodixanol gradient centrifugation. One milliliter fractions were collected from the bottom of the tube and dialyzed against phosphate buffered saline (PBS) overnight at 4°C.

Western blot analysis.

The localization of assembled B19 capsids within the iodixanol gradient was evaluated by SDS-PAGE and western blot using a anti-B19 VP2 antibody (Millipore, Billerica, MA). Analysis of the phoshorylation status of HER2/*neu* receptors on MMTV-HER2/*neu* cells following exposure to either HER2 kinase domain (p1)- or HER2/*neu* transmembrane domain (p2)-derived peptides was evaluated using antibodies specific for phosphorylated tyrosine 1248 (^{P1248}HER2) and antibodies detecting total levels of HER2/*neu* (CHI Scientific, Maynard, MA).

Infectious assays.

Fractions containing the 60/40%, 25% and 15% phases of the iodixanol gradient were pooled and concentrated using Amicon Ultra-4 centrifugal filter devices (100,000 MWCO, Millipore, Billerica, MA). Physical genome titers were determined using slot blot analysis as previously described (54). The presence of infectious viral particles was evaluated in HER2/*neu*-overexpressing MMTV-HER2/*neu* murine tumor cells plated in 12-well plates and exposed to 100 µl of dialyzed and pooled iodixanol fractions for 2 h at 37°C in serum-free medium.

Inhibitor studies.

MMTV-HER2/*neu* cells were plated in 12-well plates and exposed to 10 µg/ml of P4C10 monoclonal antibodies that inhibit β1 integrin function by stabilizing its inactive conformation (Millipore, Billerica, MA), or 1µg/ml of HER2 peptides derived from either the

kinase domain (p1) or the transmembrane domain (p2) of the receptor (CHI Scientific, Maynard, MA) for 30 min prior to infection with B19-*scGFP*- or AHNP-B19-sc*GFP* vectors at MOI 10 for 2 h at 37C in serum-free medium. For P antigen binding inhibition, viral vectors were pre-incubated with 10 μg of globoside (globotetraosylceramide, Sigma-Aldrich, St. Louis, MO) for 90 min on ice before addition to the cells. Fluorescent microscopy and phase contrast images were collected on a Zeiss Fluorescent Microscope 24 h after infection. Images from three to five visual fields were analyzed quantitatively by ImageJ analysis software (National Institutes of Health, Bethesda, MD). Transgene expression was assessed as total area of green fluorescence (pixels squared per visual field) (mean ± SD). *P* values were calculated by Student *t* test.

Results

Structural modeling shows that the putative P antigen binding site on B19 VP2 can be replaced with the AHNP peptidomimetic.

In order to generate parvovirus B19 vectors that target cancer cells and not erythroid progenitor cells, the P antigen binding epitope on B19 capsids (12) was replaced with an alternative peptide, AHNP, that promotes binding to the cell surface receptor HER2/*neu*, which is highly expressed on a subgroup of breast cancers and medulloblastomas (3, 11), based on structural modeling. A model of the B19 VP2 was built in which residues 393-406 in loop 4, carrying the putative P antigen binding site footprint (aa 393-406), were replaced with the 12 amino acid sequence of AHNP (Figs. 1A and 1C). This required the deletion of residues 405 and 406 to ensure that all the residues proposed as the P antigen binding site were eliminated. Comparison of the VP2 model resulting from the two different approaches (as described in Materials and Methods) to the crystal structure of the CDR-H3 loop of the rhu 4D5 (Fig. 1B, purple) on which the AHNP peptide was based showed structural superimposition of residues DGF, thus the conformation of residues Y and A were adjusted to be the same as those in the rhu 4D5 structure (Fig. 1B and C, green = SWISS MODEL generated AHNP loop; orange = AHNP generated by interactive mutation in the Coot program based on a superimposition of the B19 P antigen loop (blue) and the rhu 4D5 crystal structure (purple)). These adjustments were made without affecting conformation of the main chain for the remainder of the residues in this loop in the wild-type B19 VP2 structure. The modeled loop is easily accommodated within the VP2 monomer structure (Fig. 1C). Based on

the structural model, 14 amino acids of B19 VP2 were replaced with the 12 amino acid AHNP peptide using PCR-based mutagenesis and sequence confirmed by DNA sequencing.

Packaging and purification of recombinant wt and AHNP-B19-sc*GFP* virions.

Self-complementary AAV2 genomes containing a chicken β-actin promoter-driven green fluorescent protein gene were packaged into capsids comprised of either wt B19 VP2 (B19-sc*GFP*) or AHNP-VP2 proteins (AHNP-B19-sc*GFP*) as reported previously (53, 54). Virus preparations were purified on discontinuous iodixanol gradients and physical titers were determined by slot blot analysis following DNAse treatment. Physical titers of AHNP-B19-sc*GFP* vectors were comparable to titers of B19-sc*GFP* vectors (~1-2 x 10^9/ml, (54) (Fig. 2A). SDS-PAGE electrophoresis and western blot analysis of fractions 7-18 of the gradients revealed a single band at 58 kDa representative of B19 VP2 (Fig. 2B, lane C purified virus control) and additional bands of lower molecular weight (Fig. 2B, lanes 14-18), representing break-down products of B19 VP2. The presence of DNA-containing particles in gradient fractions was determined by infectious assays. Murine mammary gland tumor cells from mice transgenic for a mouse mammary tumor virus-LTR driven HER2/*neu* gene (MMTV-LTR HER2/*neu*-transgenic mice) (33) were incubated with pooled, dialyzed and concentrated fractions from the lower (fractions 7-9), middle (fractions 10-12) and upper (fractions 13-15) sections of the iodixanol gradient. Fluorescent microscopy images of cells 24 h after infection revealed *GFP*-positive cells only in cells exposed to fractions 7-9 of the AHNP-B19-sc*GFP* gradient (Fig. 2C, upper panel on the right) and not cells exposed to B19-sc*GFP* fractions (Fig. 2C, panels on the left) or AHNP-B19-sc*GFP* fractions 10-15 (Fig. 2C, middle and lower panel on the right). These results indicated that DNA-containing infectious AHNP-B19-sc*GFP* particles were present in the 40% iodixanol phase of the gradient (fractions 7-9) and B19 VP2 signals detected in fractions 10-18 represented either empty particles (likely in fractions 10-12) or free VP2 capsid proteins (fractions 12 and above) (Fig. 2B). Based on these results, the partial replacement of loop 4 of B19 VP2 by an AHNP peptide did not affect assembly or packaging of mutant vectors.

Efficient transduction of HER2/*neu* expressing cells with AHNP-B19-sc*GFP* vectors.

Murine MMTV-HER2/*neu* mammary tumor epithelial cells have a tendency to form small tumor nodules upon continued culture *in vitro* (Fig. 3, phase contrast microscopy images) and the efficiency of B19-sc*GFP* and AHNP-B19-sc*GFP* vectors to transduce

MMTV-HER2/*neu* cells and nodules was evaluated. MMTV-HER2/*neu* cells were incubated with B19-sc*GFP* and AHNP-B19-sc*GFP* vectors at MOI 10 and cells observed under a fluorescent microscope 48 h after infection. Low levels of *GFP*-positive cells were detected in cells exposed to B19-sc*GFP* virions (Fig. 3, top, columns on the left), suggesting the presence of P antigen receptors and β1 integrin co-receptors on these cells. We had previously observed low-level transduction of murine fibroblasts (55) and murine hematopoietic progenitor cells (Weigel-Van Aken KA unpublished observation) with B19-sc*GFP* vectors, suggesting that murine P antigen (which could not be quantified due to unavailability of antibodies to murine P antigen) and murine β1 integrins (which are expressed abundantly on murine fibroblasts (55) are functional receptors and co-receptors for B19-sc*GFP* vector entry. When MMTV-HER2/*neu* cells were exposed to AHNP-B19-sc*GFP* vectors, a ~10-fold increase in *GFP* transgene expression was observed (Fig, 3, bottom) and *GFP*-positive cells were observed in cells growing as monolayer and cells within tumor nodules (Fig. 3, top, columns on the right). These results documented enhanced vector targeting to HER2/*neu*-positive cells by incorporation of 60 copies of an anti-HER2 antibody mimic peptide (AHNP) into the B19 capsid.

Dependence of AHNP-B19-sc*GFP* vector transduction on HER2 receptor activation.

It is well documented that HER2/*neu* receptors undergo endocytosis only after activation which involves (i) receptor homo- or hetero-dimerization with other members of the EGFR family; (ii) enhanced tyrosine kinase activity leading to HER2/*neu* autophosphorylation at cytoplasmic tyrosine residues (including Tyr-1248) and phosphorylation of other protein substrates (58). Phosphorylation of the cytoplasmic Tyr-1248, which is located within a NP*X*Y internalization signal, has been demonstrated to be sufficient to trigger the internalization of dimerized HER2/*neu* via coated pits (22). In order to determine whether AHNP-B19-sc*GFP* transduction required HER2/*neu* activation, peptides derived from the activation loop of the HER2/*neu* kinase domain (p1; 49) and from the transmembrane domain of the receptor (p2; 29) were used in inhibitor studies.

Reduced phosphorylation of Tyr-1248 was observed in MMTV-HER2/*neu* cells exposed to the HER2 kinase domain-derived peptide (p1), but not to the transmembrane domain-derived peptide (p2) (Fig. 4A). Transduction of MMTV-HER2/*neu* cells with AHNP-*B19scGFP* vectors was ~10-fold higher than transduction with B19-sc*GFP* (Fig. 4C) and it was 90% reduced following exposure of the cells to the HER2 kinase domain-derived peptide

(p1) (Fig. 4B, third panel on the right; Fig. 4C, AHNP+p1), suggesting that kinase activity of HER2/*neu* was required for AHNP-B19-*scGFP* entry. A 40% reduction in transduction efficiency was observed with the transmembrane domain-derived peptide (p2) (Fig. 4C, AHNP+p2). Transduction with AHNP-B19-sc*GFP* vectors was integrin β1 coreceptor-dependent, as demonstrated by a ~96% inhibition following incubation with a function-blocking integrin β1 antibody (Fig. 4B, second panel on the right; Fig. 4C, AHNP+β1 Ab) that we demonstrated previously abrogates β1 integrin coreceptor function for B19 entry (53). As expected, preincubation of the virions with P antigen did not affect AHNP-B19-sc*GFP* transduction (Fig. 4B, fourth panel on the right; Fig. 4C, AHNP+P Ag). This observation demonstrated that the chimeric B19 capsid was no longer able to bind P antigen and validates the previously mapped binding site.

Somewhat surprising, transduction efficiency of B19-sc*GFP* vectors was ~7-fold enhanced by exposure of MMTV-HER2/*neu* cells to the HER2 kinase domain-derived peptide (p1) (Fig. 4B, third panel on the left; Fig. 4C, wt+p1), and was ~73% and ~93% inhibited, as expected, by preincubation of the cells with a function-blocking integrin β1 antibody and pre-incubation of the virions with P antigen, respectively (Fig. 4B, second and fourth panels on the left, Fig. 4C, wt+β1 Ab, wt+P Ag). Taken together these results demonstrated that replacement of the P antigen binding epitope on B19 capsids by an AHNP peptide had successfully removed vector dependence on P antigen as primary receptor for binding to the cell surface and introduced binding to HER2/*neu* as receptor for AHNP-B19 vector entry. Importantly, HER2/*neu*-binding AHNP-B19 vectors still used β1 integrins as co-receptors for infection.

AHNP vector transduction of medulloblastoma cells.

Expression levels of HER2/*neu* receptors on breast cancer cells and MMTV-HER2/*neu* transgenic murine tumor cells can reach 100-fold the levels on non-cancerous cells, frequently leading to receptor homo-dimerization and self-activation (2). It was therefore of interest whether AHNP-B19 vectors could transduce cells expressing lower levels of HER2/*neu*, precluding receptor homo-dimerization and self-activation. Human medulloblastoma cells (Daoy) overexpress HER2/*neu* in about 30-40% of tumors by ~5-fold, which is substantially lower than the up to 100-fold overexpression found on breast cancer cells (3) and are not sensitive to trastazumab treatment (3, 24). When Daoy cells were exposed to B19-sc*GFP* and AHNP-B19-sc*GFP* vectors, very low levels of *GFP* transgene

expression were observed in B19-*scGFP* exposed and ~29-fold higher levels with AHNP-B19-*scGFP* (Fig. 5). Interestingly, incubation of Daoy cells with the HER2 kinase domain-derived peptide (p1) did not result in increased transduction with B19-sc*GFP* vectors, as was observed in MMTV- HER2/*neu* murine breast tumor cells (Fig. 5, compare Figs. 4B and 4C). Although the overall levels of transgene expression were lower in AHNP-B19-sc*GFP*-transduced Daoy compared to MMTV- HER2/*neu* cells, transduction was also inhibited by pre-incubation of cells with either the HER2 kinase domain-derived peptide (p1) or the β1 integrin function-blocking antibody (Fig. 5). Preincubation of AHNP-B19-sc*GFP* virions with P antigen, in contrast, had no effect on transduction efficiency (Fig. 5). These results corroborated that AHNP-B19-sc*GFP* virions infected target cells through HER2/*neu* receptors and β1 integrins co-receptors. The results also documented that cells with lower expression levels of HER2/*neu* receptors that preclude receptor homodimerization and self-activation can be successfully targeted by AHNP-B19 vectors.

Discussion

Interaction of parvovirus B19 with P antigen has been mapped to a 6 amino acid peptide on the surface-exposed loop 4 of VP2 by cryo-electron microscopy (12). We provide support for this finding by replacing this peptide and demonstrating loss of competitive inhibition of B19 infection with P antigen. Although we could not directly measure the levels of P antigen on murine MMTV- HER2/*neu* transgenic breast tumor cells, the fact that B19-sc*GFP* vectors could transduce these cells, especially when HER2/*neu* receptors were functionally silenced, provides evidence for the functionality of murine P antigen and murine β1 integrins for B19 transduction, and corroborates our previous observations of murine fibroblast and murine hematopoietic progenitor cell transduction by B19-sc*GFP* vectors ((55) and unpublished observation).

The anti-HER2/*neu* peptide (AHNP) was chosen for insertion into B19 VP2, since antibodies to HER2/*neu* (Herceptin®/ trastazumab) have been used successfully in clinical trials to target HER2/*neu*-positive breast cancer cells and short peptides derived from the complementarity-determining region (CDR-H3) of the HER2 antibody have demonstrated favorable HER2/*neu* binding affinity (8, 37). In addition, the CDR-H3-derived AHNP peptide could be structurally aligned with the B19 VP2 loop 4 in such a way that the HER2/*neu*-binding residues (DGFYA) were maximally exposed on the loop when replacing the P antigen-binding residues. Introduction of AHNP into B19 VP2 loop 4 significantly increased

the transduction of murine HER2/*neu*-overexpressing (MMTV-HER2/*neu*) and human HER2/*neu*-expressing (Daoy) cells, retained dependence on β1 integrin coreceptors and restricted viral entry to cells expressing functionally active HER2/*neu* kinase receptors. These results corroborate the feasibility of changing the tropism of parvovirus B19 for P antigen-positive cells and retargeting B19 vectors to alternative cell surface receptors. The dependence of AHNP-B19 entry on both, activated HER2/*neu* kinases and activated β1 integrins, might have importance for the applicability of AHNP-B19 vectors, since cells with HER2/*neu* amplification overexpress HER2/*neu* receptors up to 100-fold above normal levels (11) and HER2/*neu* overexpression has been shown to lead to receptor homo-dimerization and self-activation (2), making these cells good targets for AHNP-B19 vectors. Normal cells, in contrast, express low levels of HER2/*neu*, lack homo-dimerization and receptor self-activation and are less likely targets. The dependence of AHNP-B19 transduction on functionally active β1 integrins will likely contribute to its feasibility to target HER2/*neu*-positive breast cancer metastases in the bone marrow, the occurrence of which has been documented in ~30% of breast cancer patients at diagnosis (9), since bone marrow-resident cancer cells use β1 integrins for survival under genotoxic stress conditions including irradiation and chemotherapy (14, 46) (15).

Intriguing was the observation that HER2/*neu* receptor kinase activity negatively affected B19-sc*GFP* transduction, specifically of cells with high levels of HER2/*neu* overexpression and that inhibition of the HER2/*neu* kinase using a kinase domain-derived peptide significantly enhanced B19-sc*GFP* transduction. Overexpression of HER2/*neu* in cultured cells has been demonstrated to lead to HER2/*neu* activation (16) and transfection of an inducible homo-dimerizing HER2-construct into non-malignant human mammary epithelial cells induced morphological changes characteristic of an epithelial-mesenchymal conversion and functional impairment of β1 integrins; an effect that could be reversed with β1 integrin activating antibodies (6) (27). Although a HER2/*neu*-dependent suppression of β1 integrin function could have caused the low transduction of MMTV-HER2/*neu* cells with B19-sc*GFP*, it did not prevent the efficient transduction with AHNP-B19 vectors, which was clearly β1 integrin coreceptor-dependent. Alternatively, a potential effect of constitutively activated HER2/*neu* on glycolipid receptors such as P antigen could be involved, especially since P antigen has been shown to be internalized through caveolae (41) and a negative reciprocal regulation between HER2/*neu* and caveolin-1 affecting caveolae-mediated internalization processes has been documented (19).

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

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CLAIMS

We claim:

1. An isolated polypeptide in which:

a) the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into loop 8 of a parvovirus VP2 capsid protein; or

b) the sequence YCDGFYACYMDV (SEQ ID NO: 3) has been substituted into loop 4 of a parvovirus B19 VP2 capsid protein for the P antigen binding site.

2. The isolated polypeptide according to claim 1, wherein said polypeptide comprises SEQ ID NO: 2.

3. A capsid comprising a polypeptide according to any one of claims 1 or 2.

4. A parvovirus or hybrid parvovirus particle comprising a polypeptide according to any one of claims 1 or 2.

5. A parvovirus or hybrid parvovirus vector comprising nucleic acid encoding one or more therapeutic agent or one or more reporter peptide or protein, said parvovirus vector comprising a capsid comprising a polypeptide according to any one of claims 1 or 2.

6. A parvovirus or hybrid parvovirus vector comprising a cytotoxic compound or drug packaged, conjugated or infused in and/or associated with said parvovirus vector comprising a caspid comprising a polypeptide according to any one of claims 1 or 2.

7. The parvovirus or hybrid parvovirus vector according to claim 5, wherein said one or more therapeutic agent is a suicide gene, a tumor suppressor gene or a gene encoding a cytokine.

8. The parvovirus or hybrid parvovirus vector according to claim 7, wherein said nucleic acid encodes one or more RNA molecule, such as antisense RNA, siRNA, or shRNA and/or one or more of the following polypeptides: Herpes simplex virus thymidine kinase (HSV-tk)/acyclovir (ACV), ganciclovir (GCV), bacterial or fungal cytosine deaminase

(CD)/5-florocytosine (5-FC), p53, p21WAF1/CIP1 (p21), p16INK4a (p16), p18INK4c (p18), p27KIP2 (p27), Rb, Wt-1, NF1, VHL, APC, IL-1, IL-2, IL-4, IL-5, IL-12, IFN-α, IFN-β, IFN-γ, GM-CSF or TNF.

9. The parvovirus or hybrid parvovirus vector according to claim 6, wherein said one or more therapeutic agent is a cytotoxic drug selected from the group consisting ofDNA intercalators, taxanes, maytansine, methotrexate, anthracyclines and isothiocyanates.

10. The parvovirus or hybrid parvovirus vector according to claim 5, wherein said one or more reporter peptide/protein is Green Fluorescent Protein, β-galactosidase, alkaline phosphatase or chloramphenicol acetyltransferase.

11. A pharmaceutical composition comprising a pharmaceutically-acceptable carrier, adjuvant or diluent and a polypeptide, parvovirus capsid, parvovirus particle or parvovirus vector according to any one of claims 1-10.

12. A method of delivering a nucleic acid, therapeutic agent, reporter protein/peptide or cytotoxic drug to a cell expressing one or more HER2/*neu* receptor comprising contacting said cell expressing one or more HER2/*neu* receptor with a parvovirus vector or a hybrid parvovirus vector according to any one of claims 5-9, said parvovirus or hybrid parvovirus vector optionally being contained in a pharmaceutical composition.

13. A method of treating a cancer characterized by expression of HER2/*neu* receptors on a cell surface comprising the administration of a parvovirus vector or a hybrid parvovirus vector according to any one of claims 5-9 to a subject having a cancer characterized by expression of HER2/*neu* receptors on a cell surface, said parvovirus or hybrid parvovirus vector optionally being contained in a pharmaceutical composition.

14. The method according to claim 13, wherein said cancer is selected from breast cancer, medullablastoma, lung cancer, non-small cell lung cancer, uterine cancer, uterine serous endometrial carcinoma, stomach cancer or ovarian cancer.

15. The method according to claim 13 or 14, wherein said subject is a human.

16. The method according to claim 15, wherein said parvovirus vector or a hybrid parvovirus vector is administered alone or in combination with a treatment regimen for said cancer.

17. The method according to claim 15, wherein said parvovirus vector or a hybrid parvovirus vector is administered to a subject after a treatment regimen for a cancer has been completed.

18. The method according to claim 16 or 17, wherein said cancer is breast cancer.

19. The use of a parvovirus vector or a hybrid parvovirus vector according to any one of claims 5-9, said parvovirus or hybrid parvovirus vector optionally being contained in a pharmaceutical composition, for delivering a nucleic acid, therapeutic agent, reporter protein/peptide or cytotoxic drug to a cell expressing one or more HER2/*neu* receptor.

20. The use of a parvovirus vector or a hybrid parvovirus vector according to any one of claims 5-9, said parvovirus or hybrid parvovirus vector optionally being contained in a pharmaceutical composition, for treating a cancer characterized by expression of HER2/*neu* receptors on a cell surface.

21. The use of claim 20, wherein said cancer is selected from breast cancer, medullablastoma, lung cancer, non-small cell lung cancer, uterine cancer, uterine serous endometrial carcinoma, stomach cancer or ovarian cancer.

22. The use of claim 20 or 21, wherein said subject is a human.

23. The use according to claim 20, wherein said parvovirus vector or a hybrid parvovirus vector is administered alone or in combination with a treatment regimen for said cancer.

24. The use according to claim 20, wherein said parvovirus vector or a hybrid parvovirus vector is administered to a subject after a treatment regimen for a cancer has been completed.

25. The use according to claim 21, 23 or 24, wherein said cancer is breast cancer.

26. A parvovirus vector or a hybrid parvovirus vector according to any one of claims 5-9, said parvovirus or hybrid parvovirus vector optionally being contained in a pharmaceutical composition, for delivering a nucleic acid, therapeutic agent, reporter protein/peptide or cytotoxic drug to a cell expressing one or more HER2/*neu* receptor.

27. A parvovirus vector or a hybrid parvovirus vector according to any one of claims 5-9, said parvovirus or hybrid parvovirus vector optionally being contained in a pharmaceutical composition, for treating a cancer characterized by expression of HER2/*neu* receptors on a cell surface.

28. The use of claim 27, wherein said cancer is selected from breast cancer, medullablastoma, lung cancer, non-small cell lung cancer, uterine cancer, uterine serous endometrial carcinoma, stomach cancer or ovarian cancer.

29. The method according to claim 27 or 28, wherein said parvovirus vector or a hybrid parvovirus vector is administered alone or in combination with a treatment regimen for said cancer.

30. The method according to claim 27 or 28, wherein said parvovirus vector or a hybrid parvovirus vector is administered to a subject after a treatment regimen for a cancer has been completed.

31. The use of claim 27, 28, 29 or 30, wherein said subject is a human.

32. The use according to any of claims 27-31, wherein said cancer is breast cancer.

33. The use of a parvovirus particle, parvovirus or hybrid parvovirus according to any one of claims 4-10 for the manufacture of a medicament.

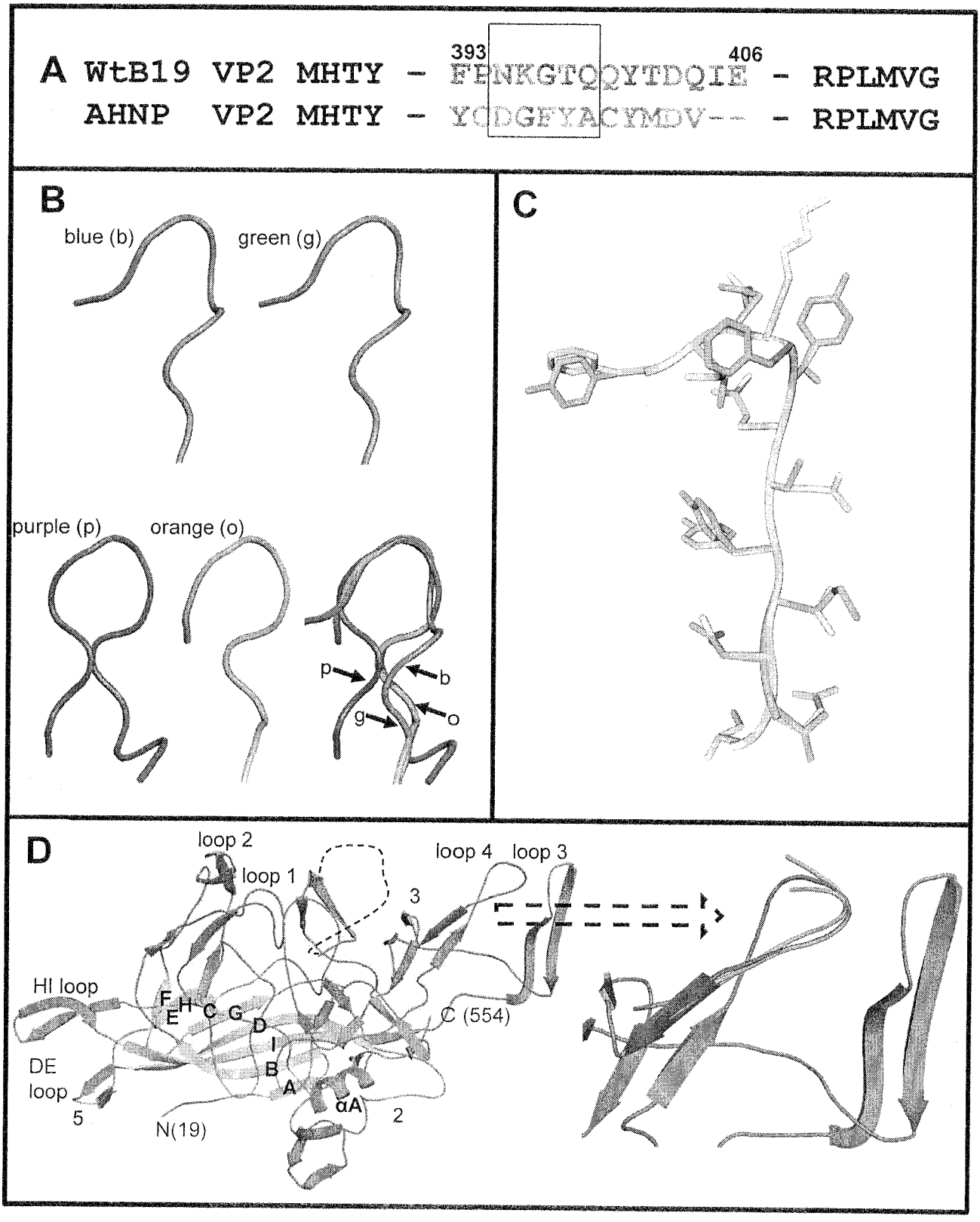

A
WtB19 VP2 MHTY - FPNKGTQQYTDQIE - RPLMVG
393
406
AHNP VP2 MHTY - YQDGFYACYMDV-- - RPLMVG
B
blue (b)
green (g)
purple (p)
orange (o)
p
b
g
o
C
D
loop 2
loop 1
loop 4
loop 3
3
HI loop
F
E
H
C
G
D
I
B
A
αA
2
C (554)
DE
loop
5
N(19)

FIG. 1

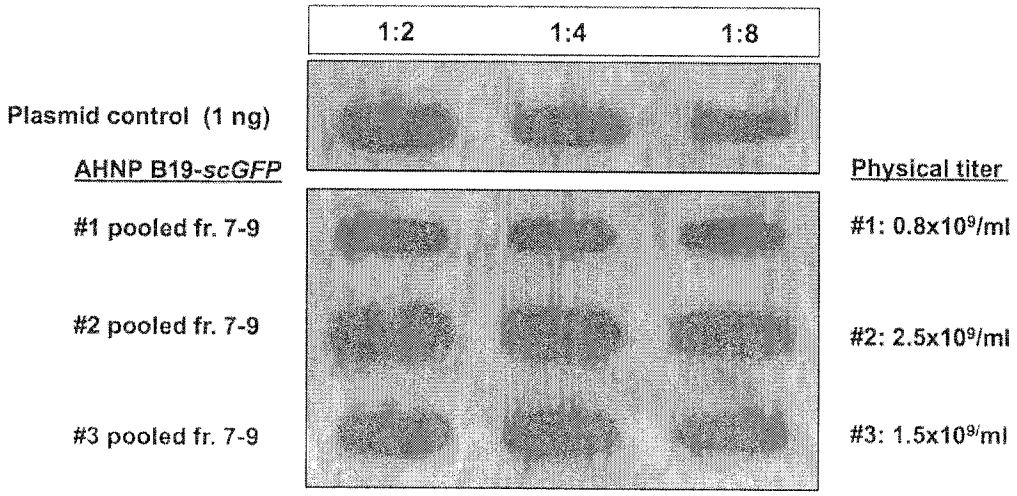

1:2
1:4
1:8
Plasmid control (1 ng)
AHNP B19-scGFP
Physical titer
#1 pooled fr. 7-9
#1: 0.8x10⁹/ml
#2 pooled fr. 7-9
#2: 2.5x10⁹/ml
#3 pooled fr. 7-9
#3: 1.5x10⁹/ml

FIG. 2A

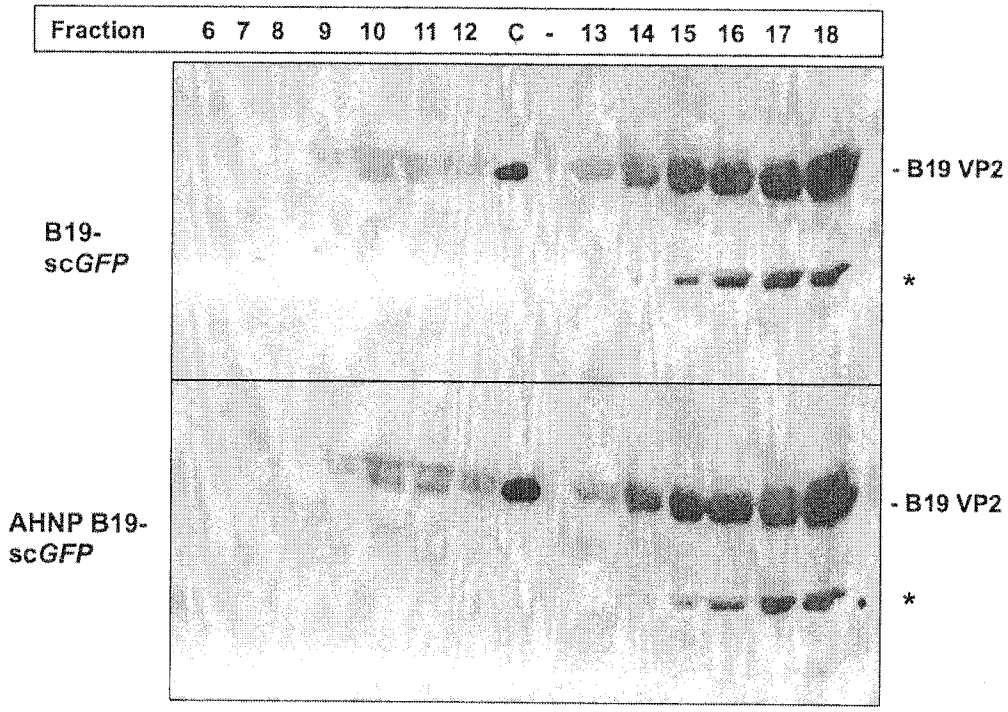

Fraction 6 7 8 9 10 11 12 C - 13 14 15 16 17 18
B19-scGFP
- B19 VP2
*
AHNP B19-scGFP
- B19 VP2
*

FIG. 2B

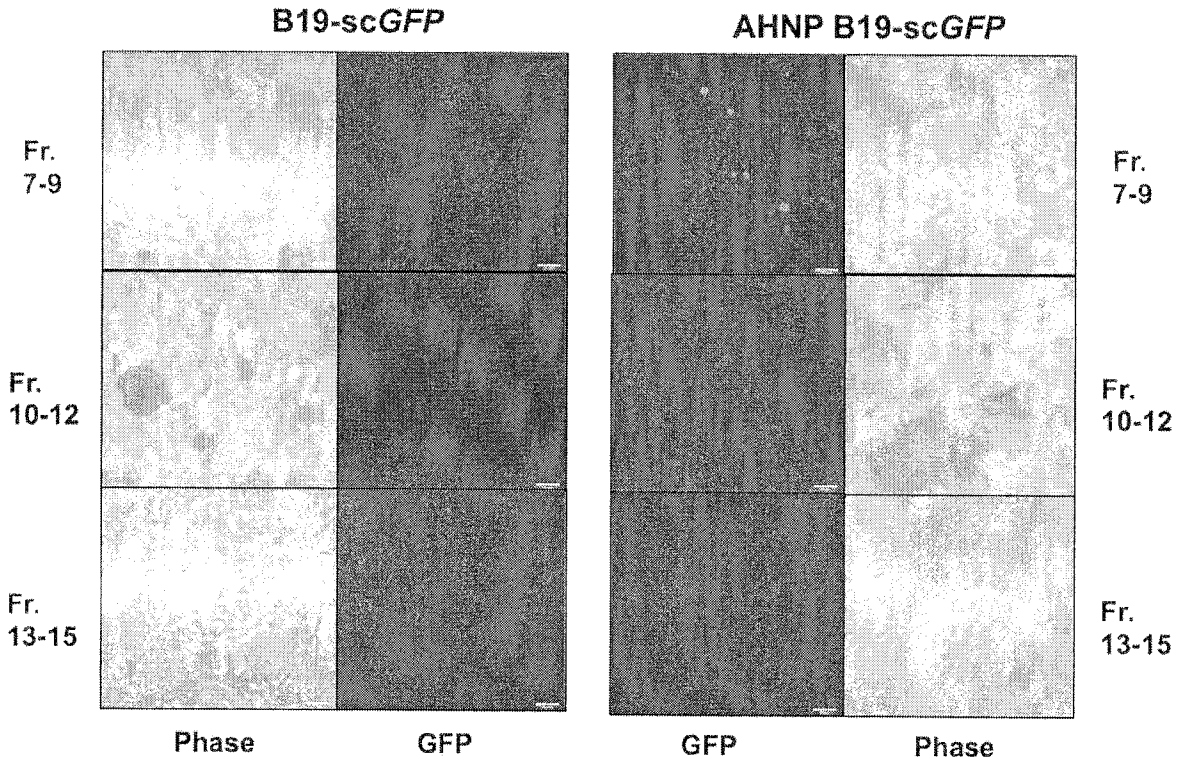

B19-scGFP
AHNP B19-scGFP
Fr. 7-9
Fr. 10-12
Fr. 13-15
Fr. 7-9
Fr. 10-12
Fr. 13-15
Phase
GFP
GFP
Phase

FIG. 2C

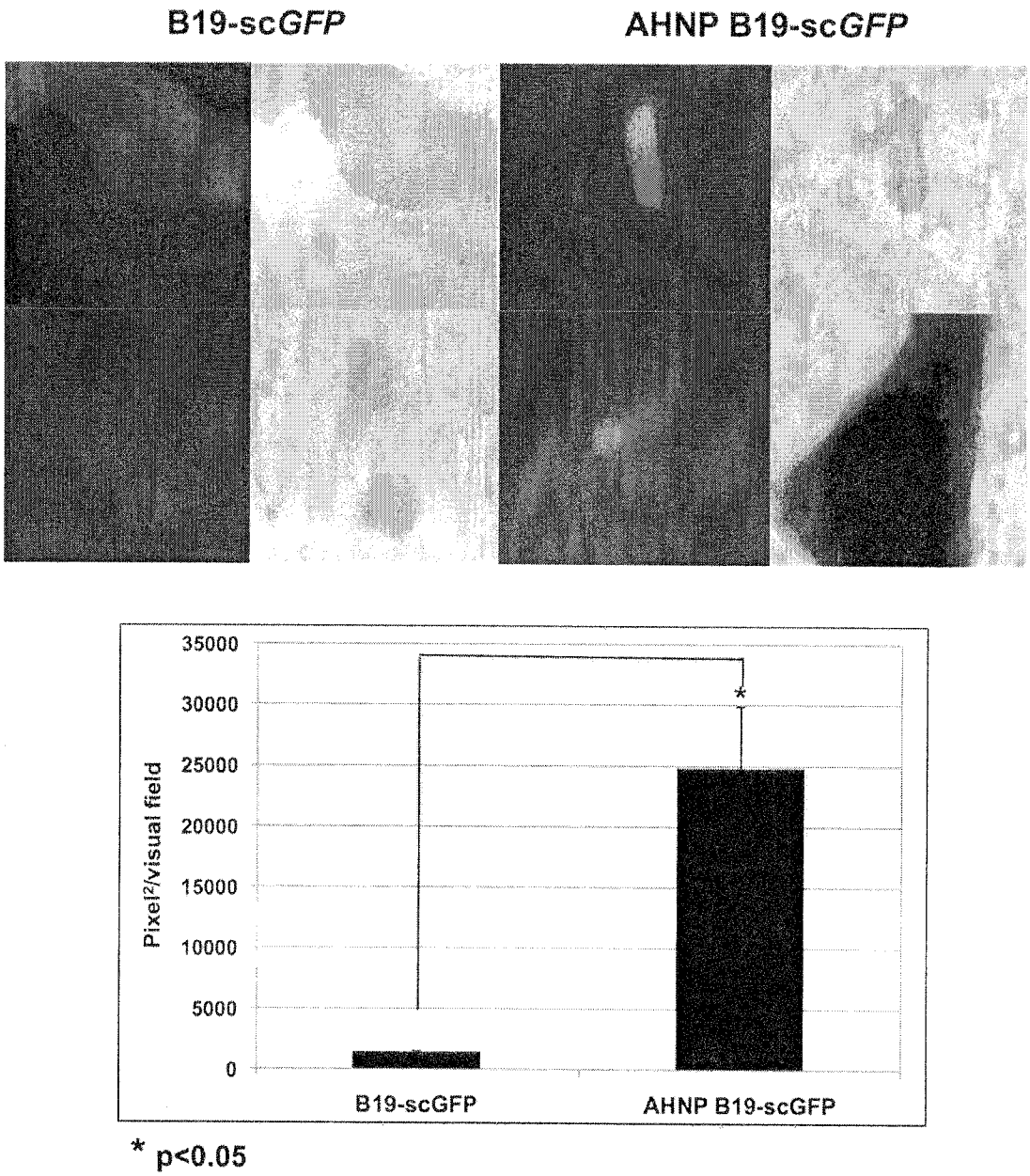

B19-scGFP
AHNP B19-scGFP
35000
30000
25000
20000
15000
10000
5000
0
Pixel²/visual field
*
B19-scGFP
AHNP B19-scGFP
* p<0.05

FIG. 3

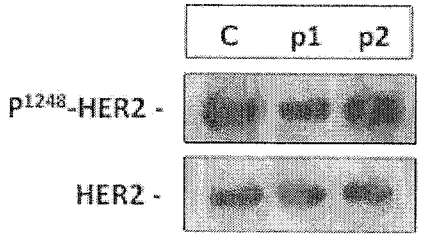

C
p1
p2
P1248-HER2 -
HER2 -

FIG. 4A

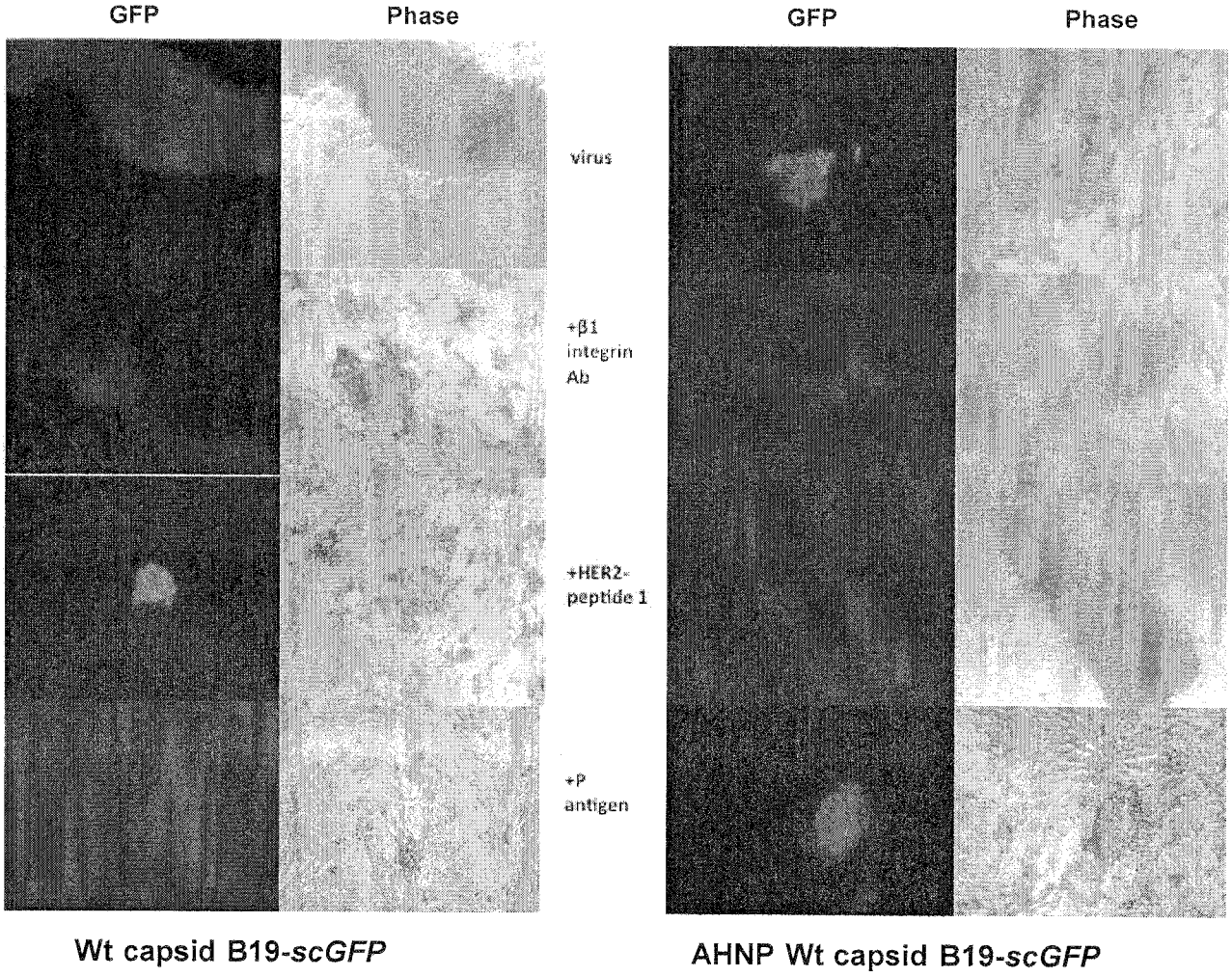
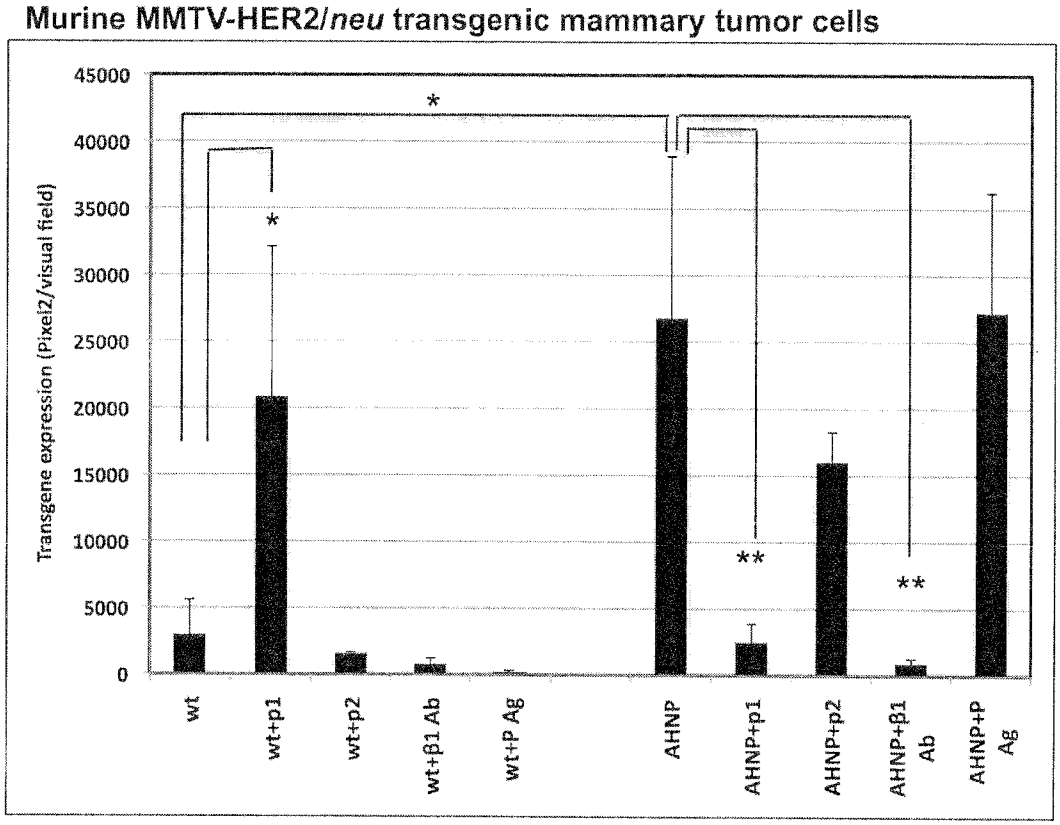

GFP
Phase
GFP
Phase
virus
+β1 integrin Ab
+HER2-peptide 1
+P antigen
Wt capsid B19-scGFP
AHNP Wt capsid B19-scGFP

FIG. 4B



* p<0.05

** p<0.01

FIG. 4C

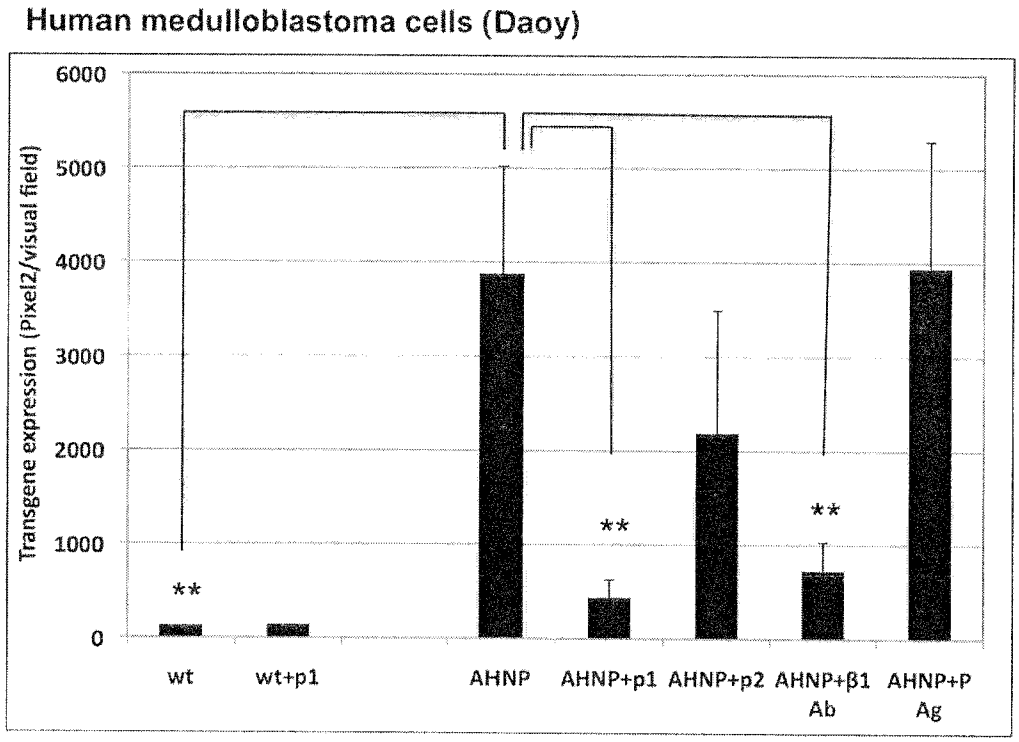

Human medulloblastoma cells (Daoy)
Transgene expression (Pixel2/visual field)
6000
5000
4000
3000
2000
1000
0
**
**
**
wt
wt+p1
AHNP
AHNP+p1
AHNP+p2
AHNP+β1 Ab
AHNP+P Ag
** p<0.01

FIG. 5