vaccination using mutant peptides could specifically ablate cancer cells expressing respective mutated targets, but sparing normal tissues unharmed. However, making antibodies one by one *in vitro* specifically against countless point mutations discovered so far is very difficult and not practical. Therefore, vaccination using mutant peptides could be a choice.

SUMMARY

As described herein, intracellular oncoproteins can be targeted with vaccination to prevent cancer and cancer metastasis.

As described herein, wild-type C57BL6 mice intravenously (i.v.) injected either with B16F0 or B16F10 melanoma cells, followed by i.v. injection with PRL-3 monoclonal antibody (mAb) showed that PRL-3 mAb can reduce tumors formed by B16F0 melanoma cells that express endogenous PRL-3 intracellular phosphatase,[1] but not those by B16F10 melanoma cells that do not express PRL-3.

C57BL6 mice i.v. injected with either EGFP-B 16F0 or EGFP-B16F10 cells in which both cell lines were engineered to overexpress EGFP, followed by i.v. injection with EGFP monoclonal antibody showed that regardless of PRL-3 expression status, EGFP mAb could eradicate tumors formed by both EGFP-B 16F0 and EGFP-B 16F 10 melanoma cell lines. Further, polyomavirus middle T (Py-mT)-transgenic young females i.v. injected with mT antibody showed that the mT antibody could effectively reduce the formation of mT-expressing breast tumors.

Moreover, C57BL6 mice vaccinated with PRL-3 or EGFP antigens showed significant reduction in the formation of metastatic tumors expressing either PRL-3 or EGFP protein respectively, as compared with un-immunized mice. Furthermore, Py-mT mice vaccinated with mT antigen significantly prevented tumor formation in mammary glands. The results obtained from 194 wild type mice demonstrate that cancer metastasis caused by intracellular oncoproteins can be successfully blocked with antibody therapy and vaccination.

As shown herein, young PymT transgenic mice injected with fragments of the middle T protein exhibited reduced incidence of breast tumor than mice immunized with GST (negative control). Moreover, immunization of mice with Her2/neu fragments containing only a fragment of the intracellular domain inhibited the formation of Her2/neu expressing tumors formed by B16F0 melanoma cancer cells.

Further, MMTV-PymT mice vaccinated with Estrogen Receptor peptides or with Estrogen Receptor antibody have reduced burden of mammary tumors compared to mice immunized with GST (negative control).

We therefore provide for methods of treating cancers and preventing cancer metastasis with fragments of intracellular oncoprotein. The term “intracellular oncoprotein” as used herein may include oncoproteins that have an intracellular region. For example, membrane anchored proteins may have extracellular, transmembrane, and intracellular domains or regions.

The fragment may be a fragment of a region of the oncoprotein that comprises a mutation as compared to the wild-type, or non-oncogenic protein. The mutation may be associated with, or causative of, cancer (i.e. an oncogenic mutation).

There is provided, according to an example of the present disclosure, use of a vaccine therapy comprising an intracellular oncoprotein fragment for vaccination to stimulate a host to produce its own antibodies to prevent tumor formation.

Also provided is a polypeptide for use in a method of stimulating the production of anti-intracellular oncoprotein antibodies in a subject in need thereof, the polypeptide being a fragment of the intracellular oncoprotein having between 10 and 150 amino acids, preferably between 10 and 100 amino acids. In one aspect, provided is a use of a polypeptide in the manufacture of a medicament for the treatment or prevention of cancer by stimulating the production of anti-intracellular oncoprotein antibodies in a subject in need thereof, the polypeptide being a fragment of an intracellular oncoprotein having between 10 and 150 amino acids, preferably between 10 and 100 amino acids, wherein the intracellular oncoprotein fragment does not comprise an extracellular domain or a transmembrane domain, wherein the intracellular oncoprotein is VHZ. In another aspect, provided is a use of a fragment of between 10 and 150 amino acids of an intracellular oncoprotein or peptide based on a fragment of said intracellular oncoprotein in the manufacture of a medicament for preventing cancer metastasis in a patient with cancer, wherein the use comprises: identification of the intracellular oncoprotein specific to the patients cancer for administration of the fragment to the patient, wherein the intracellular oncoprotein is VHZ. In another aspect, provided is a use of a fragment of an intracellular oncoprotein or peptide based on a fragment of said intracellular oncoprotein, the fragment or peptide being between 10 and 150 amino acids in length in the manufacture of a medicament for treating or preventing cancer

in a patient, wherein the use comprises: determination of whether a patient is suffering from, or likely to develop, a cancer associated with an intracellular oncoprotein before administration to that person the fragment of that intracellular oncoprotein or peptide based on the fragment of that intracellular oncoprotein, wherein the intracellular oncoprotein is VHZ.A method of stimulating the production of anti-intracellular oncoprotein antibodies in a subject in need thereof, comprising administering a polypeptide which is a fragment of the intracellular oncoprotein, having between 10 and 150 amino acids is also provided.

The polypeptide may be a fragment of an oncoprotein that is intracellular, or a fragment of an intracellular region of an oncoprotein. The fragment may comprise an oncogenic mutation.

The polypetide may comprise a plurality of fragments. Thus, more than one fragment of an oncoprotein may be provided in one polypeptide. More than one polypeptide may be administered to a patient. Some or all of those polypeptides may comprise more than one oncoprotein fragment. The one or more oncoprotein fragments may be fragments of the same oncoprotein, or may be fragments from different oncoproteins. In some cases, the polypeptide comprises more than one copy of the same fragment.

We provide, according to another example of the present disclosure, a method of treatment of cancer, preferably metastatic cancer, in an individual suffering or suspected to be suffering from cancer. In one aspect, provided is a use according to the disclosure, wherein the method is a method for preventing or reducing cancer metastasis. The method may comprise administering a therapeutically effective amount of a fragment of an intracellular oncoprotein to the individual. The intracellular oncoprotein may have been determined as being expressed in the cancer that the individual is suffering from.

The fragment may correspond to a region of the oncoprotein that comprises a mutation. That is, the fragment may comprise an oncogenic mutation. An advantage of targeting an oncogenic mutation, for example using a peptide that comprises a fragment of an oncoprotein that includes an oncogenic mutation, is that an immune response specific to a cancer may be generated, leaving normal cells unharmed. The patient may be administered a plurality of oncoprotein fragments. The plurality of oncoprotein fragments may be combined into one or more peptides that each comprises a plurality of different peptides, each optionally linked by a peptide linker. The fragments may be from the same oncoprotein, or different oncoproteins. The method may involve inducing the patient to produce anti-cancer antibodies, for example antibodies against the oncoprotein or oncogenic

mutation of the oncoprotein fragment. The method may involve administering the fragment or peptide with an adjuvant.
The intracellular oncoprotein fragment may be such that it does not comprise an extracellular domain or a transmembrane domain. The intracellular oncoprotein fragment may comprise a cytoplasmic or nuclear oncoprotein fragment.

The intracellular oncoprotein fragment may comprise a portion of an Estrogen Receptor protein, for example ER (estrogen receptor), or a Hepatitis B virus protein such as HBV X-protein, PRL-3 protein, VHZ, Her2/neu or polyomavirus middle T (Py-mT) protein,, Kras, EGFR, B-raf, PI3KCA, beta-catenin, GNAS, Ret, EZH2 or GNAQ.

The cancer may be associated with or caused by over-expression of the intracellular oncoprotein of which an oncoprotein fragment is used for immunization. The cancer may be selected from the group consisting of: breast cancer, hepatocellular cancer such as hepatocellular carcinoma, ovarian cancer, liver cancer, pancreatic cancer, prostate cancer, gastric cancer, lung cancer, penis cancer, cervical cancer, brain cancer, esophageal cancer, bladder carcinoma, kidney renal cell carcinoma, ovary lymphoma and skin melanoma.
The cancer may comprise a metastatic cancer.

The method may be such that the number of metastatic tumours in a treated individual is reduced by at least 50%, at least 60%, at least 70%, at least 80% or at least 90%, compared to an untreated individual. In some cases, metastasis of the cancer is entirely prevented.

As a further example of the present disclosure, there is provided an intracellular oncoprotein fragment, or a variant, homologue, or derivative thereof, for use in a method of prevention of cancer, such as metastatic cancer, the method comprising administering a prophylactically effective amount of an intracellular oncoprotein fragment, variant, homologue, or derivative thereof, to an individual suffering or suspected to be suffering from cancer.

We provide, according to another example of the present disclosure, a pharmaceutical composition comprising (a) an intracellular oncoprotein fragment, or a variant, homologue orderivative thereof, together with a pharmaceutically acceptable excipient, diluent or carrier.

The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and

T. Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Second Edition, Books 1 -3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; Current Protocols in Molecular Biology, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, DNA Isolation and Sequencing: Essential Techniques, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, In Situ Hybridization: Principles and Practice; Oxford University Press; M. J. Gait (Editor), 1984, Oligonucleotide Synthesis: A Practical Approach, Irl Press; D. M. J. Lilley and J. E. Dahlberg, 1992, Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis ofDNA Methods in Enzymology, Academic Press; Using Antibodies: A Laboratory Manual: Portable Protocol NO. I by Edward Harlow, David Lane, Ed Harlow (1999, Cold Spring Harbor Laboratory Press, ISBN 0-87969- 544-7); Antibodies: A Laboratory Manual by Ed Harlow (Editor), David Lane (Editor) (1988, Cold Spring Harbor Laboratory Press, ISBN 0-87969-314-2), 1855. Handbook of Drug Screening, edited by Ramakrishna Seethala, Prabhavathi B. Fernandes (2001 , New York, NY, Marcel Dekker, ISBN 0-8247-0562-9); and Lab Ref: A Handbook of Recipes, Reagents, and Other Reference Tools for Use at the Bench, Edited Jane Roskams and Linda Rodgers, 2002, Cold Spring Harbor Laboratory, ISBN 0-87969-630-3. Each of these general texts is herein incorporated by reference.

The disclosure includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Aspects and examples of the present disclosure will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and examples will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments and experiments illustrating the principles of the invention will now be discussed with reference to the accompanying figures in which:

Figure 1 is a diagram showing that PRL-3 mAb effectively inhibits the formation of metastatic tumors formed by PRL-3 expressing cancer cells. A, Total cell lysates were prepared from B16F0 and B16F10 melanoma cancer cells and endogenous PRL-3 protein expression was determined using immunoblot. GAPDH was used as a loading control. B, C57BL6 mice were injected with lxl O^6 B16F0 cells (on day 1) followed by PRL-3 mouse antibody (mAb, clone #318) treatment with therapeutic plan. C. On day 17-20, tumors were frequently found in the lung, liver, adrenal gland, kidney, and bone in untreated mice (n=5), while the PRL-3 mAb eliminates the formation of tumors in most tissues of treated mice (n=5). D, The body weight of PRL-3 mAb 'treated' B 16F0 recipient mice constantly increased. throughout the duration of the experiment. E, Parallel experiments were performed with non-PRL-3 expressing BI 6F10 melanoma cancer cell line and no therapeutic outcome was obtained with PRL-3 mAb therapy. F, The body weights of both treated and untreated B16F10 receipt mice constantly decreased over the duration of the experiment.

Figure 2 is a diagram showing that B-cells are important in mediating the antibody therapeutic event. A. Kaplan-Meier survival curves were used to compare the 'treated' and 'untreated' groups of B-cell deficient muMT mice injected with B16F0 cells. No difference in median survival rate for untreated (18.5 days) and treated (19 days) groups was evident. B. Kaplan-Meier analysis of wild-type C57BL6 mice injected with B16F0 had a statistically significant difference (.P=0.0002) in median, survival between untreated (17 days) and treated (21.5 days) groups. C. Kaplan-Meier analysis of wild-type C57BL6 mice C57BL6

CLAIMS

1. Use of a polypeptide in the manufacture of a medicament for the treatment or prevention of cancer by stimulating the production of anti-intracellular oncoprotein antibodies in a subject in need thereof, the polypeptide being a fragment of an intracellular oncoprotein having between 10 and 150 amino acids, preferably between 10 and 100 amino acids, wherein the intracellular oncoprotein fragment does not comprise an extracellular domain or a transmembrane domain, wherein the intracellular oncoprotein is VHZ.

2. Use according to claim 1 wherein the polypeptide is a fragment comprising an oncogenic mutation.

3. Use according to any one of the preceding claims wherein the patient is to be administered a plurality of fragments.

4. Use according to claim 3 wherein the patient is to be administered a plurality of fragments of the same oncoprotein.

5. Use according to claim 4 wherein the patient is to be administered a plurality of fragments from two or more oncoproteins.

6. Use according to any one of the preceding claims, wherein the method is a method for preventing or reducing cancer metastasis.

7. Use according to any one of the preceding claims wherein the patient has a primary cancer that expresses the intracellular oncoprotein.

8. Use of a fragment of between 10 and 150 amino acids of an intracellular oncoprotein or peptide based on a fragment of said intracellular oncoprotein in the manufacture of a medicament for preventing cancer metastasis in a patient with cancer, wherein the use comprises: identification of the intracellular oncoprotein specific to the patients cancer for administration of the fragment to the patient, wherein the intracellular oncoprotein is VHZ.

9. Use of a fragment of an intracellular oncoprotein or peptide based on a fragment of said intracellular oncoprotein, the fragment or peptide being between 10 and 150 amino acids in length in the manufacture of a medicament for treating or preventing cancer in a patient, wherein the use comprises: determination of whether a patient is suffering from, or likely to develop, a cancer associated with an intracellular oncoprotein before administration to that person the fragment of that intracellular oncoprotein or peptide based on the fragment of that intracellular oncoprotein, wherein the intracellular oncoprotein is VHZ.

10. The use of claim 8 or claim 9 wherein the fragment comprises an oncogenic mutation.

11. The use of any one of claims 8 to 10 wherein the patient is to be administered a plurality of oncoprotein fragments.

12. The use of claim 11 wherein the plurality of oncoprotein fragments are combined into one or more peptides that each comprises a plurality of different peptides, each optionally linked by a peptide linker.

13. The use of any one of claims 8 to 12 wherein the patient is to be induced to produce antibodies against the peptide.

14. The use of any one of claims 9 to 13 wherein the patient is to be administered with the intracellular oncoprotein fragment or peptide before they develop the cancer.

15. The use of any one of claims 8 to 14 wherein the patient is to be administered with a further dose of the intracellular oncoprotein fragment or peptide.

16. The use of any one of claims 9 to 15 wherein the patient is identified as being likely to suffer from a cancer associated with an intracellular oncoprotein by assessing the prevalence of the cancer in family members.

17. The use according to any preceding claim, in which the cancer is associated with or caused by over-expression of the intracellular oncoprotein.

18. The use according to any preceding claim, in which the cancer is selected from the group consisting of: breast cancer, hepatocellular cancer, acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), adrenocortical cancer, anal cancer, bladder cancer, blood cancer, bone cancer, brain tumor, cancer of the female genital system, cancer of the male genital system, central nervous system lymphoma, cervical cancer, childhood rhabdomyosarcoma, childhood sarcoma, chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), colon and rectal cancer, colon cancer, endometrial cancer, endometrial sarcoma, esophageal cancer, eye cancer, gallbladder cancer, gastric cancer, gastrointestinal tract cancer, hairy cell leukemia, head and neck cancer, Hodgkin's disease, hypopharyngeal cancer, Kaposi's sarcoma, kidney cancer, laryngeal cancer, leukemia, leukemia, liver cancer, lung cancer, malignant fibrous histiocytoma, malignant thymoma, melanoma, mesothelioma, multiple myeloma, myeloma, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, nervous system cancer, neuroblastoma, non-Hodgkin's lymphoma, oral cavity cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pituitary tumor,

plasma cell neoplasm, primary CNS lymphoma, prostate cancer, rectal cancer, respiratory system, retinoblastoma, salivary gland cancer, skin cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, stomach cancer, testicular cancer, thyroid cancer, urinary system cancer, uterine sarcoma, vaginal cancer, vascular system, Waldenstrom's macroglobulinemia and Wilms' tumor.