SUMMARY

[0004] The present inventors discovered that an animal undergoing cancer therapy with anti-neoplastic drug-loaded, bispecific antibody-targeted, minicells exhibits a greater anti-tumor response to the drug when the animal is suffering from a concomitant viral infection. Further investigation revealed that the observed enhancement in the therapeutic efficacy of an anti-cancer drug in this context arose from synergism between the tumor-killing capability of the administered, drug-loaded, bispecific antibody-targeted minicells and an activated host-immune response against tumor cells, itself due to increased expression of interferon-gamma (IFN-gamma or IFNγ) that the viral infection triggered.

[0005] IFN-gamma itself has been investigated for its potential anti-neoplastic use, both in monotherapy and in combination with other anti-neoplastic agents. Such investigations have not led to clinical success, however. For instance, the combination treatment of IFN-alpha and IFN-gamma failed to exhibit an improvement over treatment with IFN-alpha alone. See, *e.g.*, Kloke *et al.*, *Eur. J. Haematol.* 48: 93-8 (1992), and Wandl *et al.*, *Semin. Oncol.* 19: 88-94 (1992). The only IFN-gamma indications approved by the U.S. Food and Drug Administration (FDA) are for treating chronic granulomatous disease (CGD) and severe malignant osteopetrosis (bone disease).

[0005a] In one aspect of the invention, there is provided the use of a first composition in the manufacture of a medicament for treating a tumor in a subject, wherein the first composition comprises a plurality of bacterially derived intact minicells or intact killed bacterial cells, wherein each minicell or killed bacterial cell: (a) comprises an anti-neoplastic agent, wherein the antineoplastic agent is selected from the group consisting of a radionuclide, a chemotherapy drug, a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme, and a polynucleotide from which a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme is transcribed, and (b) carries a ligand on the surface of the minicell or killed bacterial cell, wherein the ligand has specificity to a non-phagocytic

mammalian cell surface receptor, wherein said subject is to be administered concurrently with or sequentially to the first composition, a second composition comprising interferon-gamma (IFN-gamma) or an agent that increases the expression of IFN-gamma wherein the agent that increases the expression of IFN-gamma is a viral vaccine that induces IFN-gamma expression or a nucleic acid encoding IFN-gamma.

[0006] In embodiments, the present disclosure provides a method for treating a tumor in a subject. The method entails administering to the subject (A) a first composition comprising a plurality of bacterially derived intact minicells and/or killed bacterial cells, each of which minicells and killed cells encompasses an anti-neoplastic agent and are targeted to a tumor cell surface receptor via a ligand attached to the minicell surface, and (B) a second composition comprising IFN-gamma or an agent that increases the expression or activity of IFN-gamma in the subject.

[0007] The second composition may comprise IFN-gamma protein, in particular a pharmaceutically suitably purified IFN-gamma protein. The second composition may

comprise a viral vaccine. In some embodiments, the second composition comprises a nucleic acid encoding IFN-gamma.

[0008] In some embodiments, the first composition comprises from about 10^9 to about 10^{10} minicells or killed bacterial cells.

[0009] In some embodiments, the anti-neoplastic agent is a radionuclide. In some embodiments, the anti-neoplastic agent is a chemotherapy drug. In some aspects, the anti-neoplastic agent is a functional nucleic acid or a polynucleotide encoding a functional nucleic acid. In some aspects, the functional nucleic acid inhibits a gene that promotes tumor cell proliferation, angiogenesis or resistance to chemotherapy and/or that inhibits apoptosis or cell cycle arrest. In some aspects, the functional nucleic acid is selected from siRNA, miRNA, shRNA, lincRNA, antisense RNA, or ribozyme.

[0010] Also provided are packages, products or kits comprising a first composition comprising a plurality of bacterially derived intact minicells or intact killed bacterial cells, wherein each minicell or killed bacterial cell comprises:

(1) an anti-neoplastic agent, wherein the antineoplastic agent is selected from the group consisting of a radionuclide, a chemotherapy drug, a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme, and a polynucleotide from which a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme is transcribed, and

(2) carries a ligand on the surface of the minicell or killed bacterial cell wherein the ligand has specificity to a non-phagocytic mammalian cell surface receptor, and a second composition comprising interferon-gamma (IFN-gamma) or an agent that increases the expression of IFN-gamma in the subject, wherein the agent that increases the expression of IFN-gamma is a viral vaccine that induces IFN-gamma expression or a nucleic acid encoding IFN-gamma.

[0011] In another embodiment, provided is a composition comprising (a) a plurality of bacterially derived intact minicells or intact killed bacterial cells, wherein each minicell or killed bacterial cell comprises:

(1) an anti-neoplastic agent wherein the antineoplastic agent is selected from the group consisting of a radionuclide, a chemotherapy drug, a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme, and a polynucleotide from which a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme is transcribed,and

(2) carries a ligand on the surface wherein the ligand has specificity to a non-phagocytic mammalian cell surface receptor, and (b) IFN-gamma or an agent that increases the expression of IFN-gamma in the subject, wherein the agent that increases the expression of IFN-gamma is a viral vaccine that induces IFN-gamma expression or a nucleic acid encoding IFN-gamma.

[0012] Other objects, features, and advantages are apparent from the following description. The detailed description and specific examples are given for illustration only, since various changes and modifications within the spirit and scope of the particular embodiments are apparent from this description.

CLAIMS:

1. Use of a first composition for the manufacture of a medicament for treating a tumor in a subject, wherein the first composition comprises a plurality of bacterially derived intact minicells or intact killed bacterial cells, wherein each minicell or killed bacterial cell: (a) comprises an anti-neoplastic agent, wherein the antineoplastic agent is selected from the group consisting of a radionuclide, a chemotherapy drug, a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme, and a polynucleotide from which a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme is transcribed, and

(b) carries a ligand on the surface of the minicell or killed bacterial cell wherein the ligand has specificity to a non-phagocytic mammalian cell surface receptor,

wherein said subject is to be administered concurrently with or sequentially to the first composition, a second composition and wherein the second composition comprises

interferon-gamma (IFN-gamma) or an agent that increases the expression of IFN-gamma, wherein the agent that increases the expression of IFN-gamma is a viral vaccine that induces IFN-gamma expression or a nucleic acid encoding IFN-gamma.

2. The use of claim 1, wherein the second composition comprises purified IFN-gamma protein.

3. The use of claim 1, wherein the second composition comprises a viral vaccine that induces IFN-gamma expression.

4. The use of claim 1, wherein the second composition comprises a nucleic acid encoding IFN-gamma.

5. The use of any one of claims 1-4, wherein the first composition comprises from about 10^9 to about 10^{10} minicells or killed bacterial cells.

6. The use of any one of claims 1-5, wherein the anti-neoplastic agent is a radionuclide.

7. The use of any one of claims 1-6, wherein the anti-neoplastic agent is a chemotherapy drug.

8. The use of claim 7, wherein the chemotherapy drug is a small molecule drug having a molecular weight of less than about 900 Dalton.

9. The use of claim 8, wherein the small molecule drug is cytotoxic.

10. The use of claim 9, wherein the small molecule drug is a morpholinyl anthracycline derivative.

11. The use of claim 10, wherein the small molecule drug is PNU-159682.

12. The use of any one of claims 1-7, wherein said anti-neoplastic agent is a functional nucleic acid or a polynucleotide encoding a functional nucleic acid.

13. The use of claim 12, wherein said functional nucleic acid inhibits a gene that promotes tumor cell proliferation, angiogenesis or resistance to chemotherapy and/or that inhibits apoptosis or cell cycle arrest.

14. The use of claim 12, wherein said functional nucleic acid is selected from siRNA, miRNA, shRNA, lincRNA, antisense RNA, or ribozyme.

15. A package, product, or kit comprising:

(a) a first composition comprising a plurality of bacterially derived intact minicells or intact killed bacterial cells, wherein each minicell or killed bacterial cell comprises

(1) an anti-neoplastic agent, wherein the antineoplastic agent is selected from the group consisting of a radionuclide, a chemotherapy drug, a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme, and a polynucleotide from which a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme is transcribed,

and

(2) carries a ligand on the surface of the minicell or killed bacterial cell, wherein the ligand has specificity to a non-phagocytic mammalian cell surface receptor, and

(b) a second composition comprising interferon-gamma (IFN-gamma) or an agent that increases the expression of IFN-gamma in the subject, wherein the agent that increases the

expression of IFN-gamma is a viral vaccine that induces IFN-gamma expression or a nucleic acid encoding IFN-gamma..

16. A composition comprising:

(a) a plurality of bacterially derived intact minicells or intact killed bacterial cells, wherein each minicell or killed bacterial cell comprises:

(1) an anti-neoplastic agent, wherein the antineoplastic agent is selected from the group consisting of a radionuclide, a chemotherapy drug, a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme, and a polynucleotide from which a functional nucleic acid selected from the group consisting of siRNA, miRNA, shRNA, lincRNA, antisense RNA, and ribozyme is transcribed, and

(2) carries a ligand on the surface wherein the ligand has specificity to a non-phagocytic mammalian cell surface receptor, and

(b) interferon-gamma (IFN-gamma) or an agent that increases the expression of IFN-gamma in the subject, wherein the agent that increases the expression of IFN-gamma is a viral vaccine that induces IFN-gamma expression or a nucleic acid encoding IFN-gamma.