# METHODS OF TREATING CANCER

## CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 61/413,275~~, which is incorporated herein by reference in its entirety~~.

## TECHNICAL FIELD

The invention relates to methods and compositions for the treatment of a cancer with a folate-vinca conjugate in combination with at least one additional chemotherapeutic agent. The invention includes methods and compositions for the treatment of tumors including ovarian, endometrial or non-small cell lung cancers.

## BACKGROUND AND SUMMARY

Despite the fact that there have been significant developments in anti-cancer technology, such as radiotherapy, chemotherapy and hormone therapy, cancer still remains the second leading cause of death following heart disease in the United States. Most often, cancer is treated with chemotherapy utilizing highly potent drugs, such as mitomycin, paclitaxel and camptothecin. In many cases, these chemotherapeutic agents show a dose responsive effect, and cell killing is proportional to drug dose. A highly aggressive style of dosing is thus necessary to eradicate neoplasms. However, high-dose chemotherapy is hindered by poor selectivity for cancer cells and severe toxicity to normal cells. This lack of tumor-specific treatment is one of the many hurdles that needs to be overcome by current chemotherapy.

One solution to current chemotherapy limitations would be to deliver a biologically effective concentration of anti-cancer agents to the tumor tissues with very high specificity. To reach this goal, much effort has been undertaken to develop tumor-selective drugs by conjugating anti-cancer drugs to such ligands as hormones, antibodies, or vitamins. For example, the low molecular weight vitamin compound, folate, is useful as a tumor-targeting agent.

Another approach to overcoming current chemotherapeutic limitations would be to deliver a combination of a tumor-targeted drug with one or more chemotherapeutic agents where the toxicity profile of the tumor-targeted drug and the chemotherapeutic agent are different. A further modification of this approach is to use the tumor-targeted drug and the chemotherapeutic agent in the combination treatment in amounts of each lower than typically used when the tumor-targeted drug or the chemotherapeutic agent is used alone for treatment.

may be present in solution or suspension in an ionized form, including a protonated form. EC145 can be synthesized, for example, by the method described in PCT/US11/037134, ~~incorporated herein by reference~~. EC145 is used interchangeably with the term "conjugate" herein.

In one embodiment, a method of treatment of a cancer is provided. The method comprises administering EC145 in combination with one or more chemotherapeutic agents having a mode of action selected from the group consisting of angiogenesis inhibition, microtubule assembly inhibition, DNA cross-linking, topoisomerase inhibition, apoptosis induction, DNA intercalation, DNA synthesis inhibition, tyrosine kinase inhibition, and mitosis inhibition.

In another embodiment, the preceding method wherein the one or more chemotherapeutic agents are selected from the group consisting of cisplatin (e.g., PLATINOL™), carboplatin (e.g., PARAPLATIN™), topotecan (e.g., HYCAMTIN™), irinotecan (e.g., CAMTOSAR™), bevacizumab (e.g., AVASTIN™), erlotinib (e.g., TARCEVA™), lapatinib (e.g., TYKERB™), and pemetrexed (e.g., ALIMTA™) is described.

In another embodiment, use of EC145 in combination with one or more chemotherapeutic agents having a mode of action selected from the group consisting of angiogenesis inhibition, microtubule assembly inhibition, apoptosis induction, DNA cross-linking, topoisomerase inhibition, DNA intercalation, DNA synthesis inhibition, tyrosine kinase inhibition, and mitosis inhibition for the treatment of folate receptor expressing cancer cells is described.

In another embodiment, the use described above is provided wherein the one or more chemotherapeutic agents are selected from the group consisting of cisplatin, carboplatin, topotecan, irinotecan, bevacizumab, erlotinib, lapatinib, and pemetrexed.

In another embodiment, the use is provided of EC145 for the manufacture of a medicament for the treatment of folate receptor expressing cancer cells in combination with one or more chemotherapeutic agents having a mode of action selected from the group consisting of angiogenesis inhibition, microtubule assembly inhibition, apoptosis induction, DNA cross-linking, topoisomerase inhibition, DNA intercalation, DNA synthesis inhibition, tyrosine kinase inhibition, and mitosis inhibition.

In another embodiment, the use for manufacture of a medicament described above wherein the one or more chemotherapeutic agents are selected from the group consisting

with at least one chemotherapeutic agent selected from the group consisting of cisplatin, carboplatin, topotecan, irinotecan, bevacizumab, erlotinib, lapatinib, and pemetrexed.

In other embodiments of the compositions, methods, uses, pharmaceutical compositions, combinations, or kits described herein, pharmaceutically acceptable salts of the conjugates described herein are described. Pharmaceutically acceptable salts of the conjugates described herein include the acid addition and base salts thereof.

Suitable acid addition salts are formed from acids which form non-toxic salts. Illustrative examples include the acetate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, camsylate, citrate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulphate, naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, saccharate, stearate, succinate, tartrate, tosylate and trifluoroacetate salts.

Suitable base salts of the conjugates described herein are formed from bases which form non-toxic salts. Illustrative examples include the arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts. Hemi-salts of acids and bases may also be formed, for example, hemi-sulphate and hemi-calcium salts.

In one embodiment, the conjugates or additional chemotherapeutic agents described herein may be administered as a formulation in association with one or more pharmaceutically acceptable carriers. The carriers can be excipients. The choice of carrier will to a large extent depend on factors such as the particular mode of administration, the effect of the carrier on solubility and stability, and the nature of the dosage form. Pharmaceutical compositions suitable for the delivery of conjugates or additional chemotherapeutic agents described herein and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in Remington: The Science & Practice of Pharmacy, 21st Edition (Lippincott Williams & Wilkins, 2005)~~, incorporated herein by reference~~.

In one illustrative aspect, a pharmaceutically acceptable carrier includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, and combinations thereof, that are physiologically compatible. In some embodiments, the carrier is suitable for parenteral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile

In one aspect, a conjugate or additional chemotherapeutic agent as described herein may be administered directly into the blood stream, into muscle, or into an internal organ. Suitable routes for such parenteral administration include intravenous, intraarterial, intraperitoneal, intrathecal, epidural, intracerebroventricular, intraurethral, intrasternal, intracranial, intratumoral, intramuscular and subcutaneous delivery. Suitable means for parenteral administration include needle (including microneedle) injectors, needle-free injectors and infusion techniques.

In one illustrative aspect, parenteral formulations are typically aqueous solutions which may contain carriers or excipients such as salts, carbohydrates and buffering agents (preferably at a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. In other embodiments, any of the liquid formulations described herein may be adapted for parenteral administration of the conjugates or additional chemotherapeutic agents described herein. The preparation of parenteral formulations under sterile conditions, for example, by lyophilization under sterile conditions, may readily be accomplished using standard pharmaceutical techniques well-known to those skilled in the art. EC145, for example, can be lyophilized by the method described in U.S. Appl. Ser. No. 61/474,428~~, incorporated herein by reference~~. In another embodiment, the EC145 can be present as a reconstitutable lyophilizate. In one embodiment, the solubility of a conjugate or additional chemotherapeutic agent used in the preparation of a parenteral formulation may be increased by the use of appropriate formulation techniques, such as the incorporation of solubility-enhancing agents. EC145 formulations are described in U.S. Appl. Ser. No. 61/474,428, PCT/US11/037134, and WO 2011/014821~~, each incorporated herein by reference in its entirety~~.

In various embodiments, formulations for parenteral administration may be formulated for immediate and/or modified release. In one illustrative aspect, active agents of the invention may be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulations, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PGLA). Methods for the preparation of such formulations are generally known to those skilled in the art. In another embodiment, the conjugates or additional chemotherapeutic agents described herein

additional chemotherapeutic agent. In another embodiment, there is provided use of EC145 in combination with the additional chemotherapeutic agent for the treatment of platinum-resistant ovarian cancer in a patient. In another embodiment, there is provided the use of EC145 for the manufacture of a medicament for the treatment in combination with the additional chemotherapeutic agent of platinum-resistant ovarian cancer in a patient.

For any method, use, pharmaceutical composition, composition, combination, or kit described above concerning the treatment of platinum-resistant ovarian cancer using EC145 in combination with the additional chemotherapeutic agent, one embodiment is one wherein the purity of EC145 or the additional drug is at least 90%. Another embodiment is one wherein the EC145 or the additional chemotherapeutic agent is provided in an aqueous sterile liquid formulation.

In another embodiment, a method of selecting a patient for treatment is described wherein the method comprises the step of administering to the patient a composition comprising EC20 combined with an isotope of technetium as described in PCT/US2010/043992 (the EC20 structure is described in PCT/US2010/043992, now published as WO 2011/014821), the disclosure of which is incorporated by reference herein in its entirety, wherein patients are selected for therapy if they express the folate receptor to levels of at least FR+ (i.e., at least one lesion expresses the folate receptor) or FR++ (i.e., all lesions express the folate receptor). In one embodiment of this imaging procedure, the patient is injected with 0.5 mg of folic acid, followed within 1 to 3 minutes by a 1 to 2 mL injection of 0.1 mg of EC20 labeled with 20 to 25 mCi of $^{99m}$Tc and imaging is performed 1 to 2 hours later. In one embodiment, the imaging methods are selected from the group consisting of planar, SPECT, and SPECT/CT imaging.

In another embodiment, the methods, uses, compositions, pharmaceutical compositions, combinations, or kits described herein include the following examples. The examples further illustrate additional features of the various embodiments of the invention described herein. However, it is to be understood that the examples are illustrative and are not to be construed as limiting other embodiments of the invention described herein. In addition, it is appreciated that other variations of the examples are included in the various embodiments of the invention described herein.

## EXAMPLES

### MATERIALS

**Claims**

1. Use of EC145 in the manufacture of a medicament for the treatment of a cancer in a patient, wherein

the medicament is to be administered with one or more additional chemotherapeutic agents having a mode of action selected from the group consisting of angiogenesis inhibition, microtubule assembly inhibition, DNA cross-linking, topoisomerase inhibition, DNA intercalation, DNA synthesis inhibition, tyrosine kinase inhibition, and mitosis inhibition; and wherein the EC145 and the additional chemotherapeutic agent are in sterile containers or packages.

2. The use of claim 1 wherein the additional chemotherapeutic agent is selected from the group consisting of cisplatin, carboplatin, topotecan, irinotecan, bevacizumab, erlotinib, lapatinib, and pemetrexed.

3. The use of claim 2 wherein the additional chemotherapeutic agent is selected from the group consisting of carboplatin and bevacizumab.

4. The use of claim 3 wherein the additional chemotherapeutic agent is carboplatin.

5. The use of claim 3 wherein the additional chemotherapeutic agent is bevacizumab.

6. The use of claim 1 wherein the cancer is an epithelial cancer.

7. The use of claim 6 wherein the epithelial cancer is an ovarian, an endometrial, or a non-small cell lung cancer.

8. The use of claim 7 wherein the epithelial cancer is an ovarian cancer.

9. The use of claim 7 wherein the epithelial cancer is a non-small cell lung cancer.

10. The use of claim 1 wherein the additional chemotherapeutic agent is to be administered at a dose that is 50 to 80% of the maximum tolerated dose for the chemotherapeutic agent.

11. The use of claim 1 wherein the EC145 and the chemotherapeutic agent are to be administered in therapeutically effective amounts.

12. The use of claim 11 wherein the effective amounts range from about 1 $\mu g/m^2$ to about 500 $mg/m^2$ of body surface area.

13. The use of claim 11 wherein the effective amounts range from about 1 $\mu g/m^2$ to about 300 $mg/m^2$ of body surface area.

14. The use of claim 11 wherein the effective amounts range from about 10 $\mu g/kg$ to about 100 $\mu g/kg$ of patient body weight.

15. The use of claim 1 wherein the cancer is a platinum-resistant ovarian cancer.

16. The use of claim 1 wherein the EC145 and the additional chemotherapeutic agent have a purity of at least 90% based on weight percentage.

17. The use of claim 1 wherein the EC145 is in the form of a reconstitutable lyophilizate.

18. The use of claim 1 wherein the EC145 and the additional chemotherapeutic agent are in sterile, pyrogen-free aqueous solutions.

19. The use of claim 1 wherein the EC145 and the additional chemotherapeutic agent are to be administered at doses lower than their maximum tolerable doses.

20. A kit comprising EC 145 and one or more additional chemotherapeutic agents having a mode of action selected from the group consisting of angiogenesis inhibition, microtubule assembly inhibition, DNA cross-linking, topoisomerase inhibition, DNA intercalation, DNA synthesis inhibition, tyrosine kinase inhibition, and mitosis inhibition.

21. The kit of claim 20 wherein the additional chemotherapeutic agent is selected from the group consisting of cisplatin, carboplatin, topotecan, irinotecan, bevacizumab, erlotinib, lapatinib, and pemetrexed.

22. The kit of claim 21 wherein the additional chemotherapeutic agent is selected from the group consisting of carboplatin and bevacizumab.

23. The kit of claim 22 wherein the additional chemotherapeutic agent is carboplatin.

24. The kit of claim 22 wherein the additional chemotherapeutic agent is bevacizumab.

25. The kit of claim 20 wherein the EC145 and the chemotherapeutic agent are in therapeutically effective amounts.

26. The kit of claim 20 wherein the EC 145 and the additional chemotherapeutic agent are in sterile containers or package.

27. The kit of claims 20 wherein the EC145 and the additional chemotherapeutic agent have a purity of at least 90% based on weight percentage.

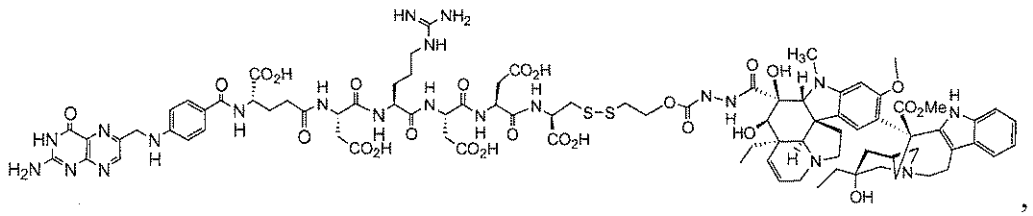
28. The kit of claim 20 wherein the EC145 and the additional chemotherapeutic agent have a purity of at least 95% based on weight percentage.

29. The kit of claim 20 wherein the EC145 is in the form of a reconstitutable lyophilizate.

30. The kit of claim 20 wherein the EC 145 and the additional chemotherapeutic agent are in sterile, pyrogen-free aqueous solutions.

**Claims**

1. Use of a compound of the formula



or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for the treatment of a cancer in a patient, wherein the medicament is to be administered with one or more additional chemotherapeutic agents having a mode of action selected from the group consisting of angiogenesis inhibition, microtubule assembly inhibition, DNA cross-linking, topoisomerase inhibition, DNA intercalation, DNA synthesis inhibition, and tyrosine kinase inhibition.

2. The use of claim 1 wherein the additional chemotherapeutic agent is selected from the group consisting of cisplatin, carboplatin, topotecan, irinotecan, bevacizumab, erlotinib, lapatinib, and pemetrexed.

3. The use of claim 2 wherein the additional chemotherapeutic agent is selected from the group consisting of carboplatin and bevacizumab.

4. The use of claim 3 wherein the additional chemotherapeutic agent is carboplatin.

5. The use of claim 3 wherein the additional chemotherapeutic agent is bevacizumab.

6. The use of any one of claims 1 to 5 wherein the cancer is an epithelial cancer.

7. The use of claim 6 wherein the epithelial cancer is an ovarian, an endometrial, or a non-small cell lung cancer.

8. The use of claim 7 wherein the epithelial cancer is an ovarian cancer.

9. The use of claim 7 wherein the epithelial cancer is a non-small cell lung cancer.

10. The use of any one of claims 1 to 9 wherein the additional chemotherapeutic agent is administered at a dose that is 50 to 80% of the maximum tolerated dose for the chemotherapeutic agent.

11. The use of any one of claims 1 to 10 wherein the compound, or the pharmaceutically acceptable salt thereof, and the chemotherapeutic agent are administered in therapeutically effective amounts.

12. The use of claim 11 wherein the effective amounts range from 1 $\mu g/m^2$ to 500 $mg/m^2$ of body surface area.

13. The use of claim 11 wherein the effective amounts range from 1 $\mu g/m^2$ to 300 $mg/m^2$ of body surface area.

14. The use of claim 11 wherein the effective amounts range from 10 μg/kg to 100 μg/kg of patient body weight.

15. The use of any one of claims 1 to 5 wherein the cancer is a platinum-resistant ovarian cancer.

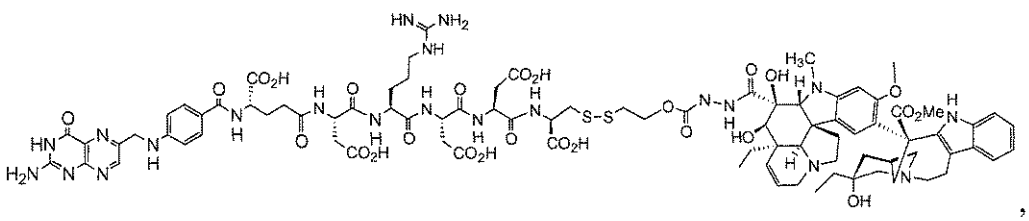
16. The use of any one of claims 1 to 15 wherein the compound, or the pharmaceutically acceptable salt thereof, and the additional chemotherapeutic agent have a purity of at least 90% based on weight percentage.

17. The use of any one of claims 1 to 16 wherein the compound, or the pharmaceutically acceptable salt thereof, is in the form of a reconstitutable lyophilizate.

18. The use of any one of claims 1 to 16 wherein the compound, or the pharmaceutically acceptable salt thereof, and the additional chemotherapeutic agent are in sterile, pyrogen-free aqueous solutions.

19. The use of any one of claims 1 to 18 wherein the compound, or the pharmaceutically acceptable salt thereof, and the additional chemotherapeutic agent are administered at doses lower than their maximum tolerable doses.

20. A kit comprising a compound of the formula



or a pharmaceutically acceptable salt thereof, and one or more additional chemotherapeutic agents having a mode of action selected from the group consisting of angiogenesis inhibition, microtubule assembly inhibition, DNA cross-linking, topoisomerase inhibition, DNA intercalation, DNA synthesis inhibition, and tyrosine kinase inhibition.

21. The kit of claim 20 wherein the additional chemotherapeutic agent is selected from the group consisting of cisplatin, carboplatin, topotecan, irinotecan, bevacizumab, erlotinib, lapatinib, and pemetrexed.

22. The kit of claim 21 wherein the additional chemotherapeutic agent is selected from the group consisting of carboplatin and bevacizumab.

23. The kit of claim 22 wherein the additional chemotherapeutic agent is carboplatin.

24. The kit of claim 22 wherein the additional chemotherapeutic agent is bevacizumab.

25. The kit of any one of claims 20 to 24 wherein the compound, or the pharmaceutically acceptable salt thereof, and the chemotherapeutic agent are in therapeutically effective amounts.

26. The kit of any one of claims 20 to 25 wherein the compound, or the pharmaceutically acceptable salt thereof, and the additional chemotherapeutic agent are in sterile containers or packages.

27. The kit of any one of claims 20 to 26 wherein the compound, or the pharmaceutically acceptable salt thereof, and the additional chemotherapeutic agent have a purity of at least 90% based on weight percentage.

28. The kit of any one of claims 20 to 27 wherein the compound, or the pharmaceutically acceptable salt thereof, and the additional chemotherapeutic agent have a purity of at least 95% based on weight percentage.

29. The kit of any one of claims 20 to 28 wherein the compound, or the pharmaceutically acceptable salt thereof, is in the form of a reconstitutable lyophilizate.

30. The kit of any one of claims 20 to 28 wherein the compound, or the pharmaceutically acceptable salt thereof, and the additional chemotherapeutic agent are in sterile, pyrogen-free aqueous solutions.

31. The use of any one of claims 1 to 19 wherein the compound, or the pharmaceutically acceptable salt thereof, and the additional chemotherapeutic agent are in sterile containers or packages before administration.