instead of leucovorin, would be advantageous due to lower inter- and intrapatient variability regarding both clinical safety and efficacy.

Indeed, in a Phase II Trial in previously untreated metastatic colorectal cancer, the response rate for CoFactor® was found to be 35% (Saif, M.W, Merritt, J, Robbins J, Stewart J., Schupp, J, 2006. Phase III Multicenter Randomized Clinical Trial to Evaluate the Safety and Efficacy of CoFactor®/5-Fluorouracil/Bevacizumab Versus Leucovorin/5-Fluorouracil/ Bevacizumab as Initial Treatment for Metastatic Colorectal Carcinoma Clinical Colorectal Cancer, Vol. 6, No. 3, 229-234, 2006), and in another phase I/II clinical trial it was demonstrated that CoFactor® combined with 5-FU showed clinical benefit in pancreas cancer, defined as stable disease or tumor response, in 40% of patients (Saif, M.W., Makrilia N., Syrigos K., 2010. CoFactor: Folate Requirement for Optimization of 5-Fluouracil Activity in Anticancer Chemotherapy. Journal of Oncology Vol. 1-5). However, apart from presenting an unnecessary hepatic detoxification burden, the unnatural (6S)-isomer is a partial competitive inhibitor of the natural [6R]-isomer regarding its effect as co-substrate for TS (Leary, R.P., Gaumont, Y., Kisliuk, R.L., 1974. Effects of the diastereoisomers of methylenetetrahydrofolate on the reaction catalyzed by thymidylate synthetase. Biochem. Biophys. Res. Commun. 56, 484–488). Furthermore, in a Phase IIb study CoFactor® in colorectal cancer was not demonstrated to be more efficacious than leucovorin as no significant differences between the study arms with regard to either efficacy or safety could be found, and a planned Phase III study colorectal cancer was discontinued before completion (Press release: ADVENTRX Provides Update on Cofactor Program. Nov 2, 2007).

There remains a great need for compositions and methods for stabilizing the ternary complex and enhancing the inhibition of TS. The inventors have surprisingly discovered that administration of 6R-MTHF increases plasma levels of 2′-deoxyuridine (dUrd) compared to the administration of equimolar concentrations of LV. Surprisingly, the inventors have discovered that administration of 6R-MTHF increases the inhibition of TS compared to the administration of equimolar concentrations of LV.

SUMMARY

In one aspect, the present disclosure refers to a pharmaceutical composition comprising [6R]-5,10-methylene tetrahydrofolate (6R-MTHF) for use in increasing plasma concentration of 2'-deoxyuridine (dUrd) in a human subject, wherein:

a. 5-fluorouracil (5-FU) is to be administered intravenously at a dose of 400 mg/m^2 or 500 mg/m^2 as a bolus injection in said human subject;

b. followed by a dose of 30, 60, 120, or 240 mg/m^2 of said pharmaceutical composition to be administered intravenously as one or more bolus injections to said human subject; and

c. optionally said 5-FU is to be administered as a continuous infusion at a dose of 2400 mg/m^2 either simultaneously with or following (b).

In another aspect, the present disclosure refers to use of 6R-MTHF and 5-FU in the manufacture of a medicament for combination therapy of cancer in a human subject, wherein

a) said 5-FU is to be administered intravenously at a dose of 400 mg/m^2 or 500 mg/m^2 as a bolus injection to said human subject;

b) followed by a dose of 30, 60, 120, or 240 mg/m^2 of said pharmaceutical composition to be administered intravenously as one or more bolus injections to said human subject; and

c) optionally said 5-FU is to be administered as a continuous infusion at a dose of 2400 mg/m^2 either simultaneously with or following (b).

The inventors have discovered that equimolar doses of 6R-MTHF surprisingly produces significantly higher levels of dUrd in blood plasma compared to with LV when co-administered with 5-FU. The elevation of plasma 2'-deoxyuridine (dUrd) is a marker of TS inhibition. (Ford et al (2002) *Clinical Cancer Research*, 8(1): 103-109). The present disclosure surprisingly provides methods for increasing plasma dUrd levels comprising the administration of 6R-MTHF. The methods of increasing plasma dUrd increase levels compared to equimolar concentrations of LV. The present disclosure also provides methods for increasing TS inhibition comprising the administration of 6R-MTHF.

Plasma dUrd elevation as a surrogate marker of TS-inhibition has progressively become the biomarker of choice. The analysis is also relatively simple to perform, by blood sampling and LC-MS/MS analysis. Treatment with TS inhibitors, like 5-FU, causes a rise in intracellular pools of the TS substrate dUMP, which is reflected in raised levels of the corresponding nucleoside dUrd, which is largely extracellular and can be measured in the plasma. Furthermore, elevations of plasma dUrd have been shown to follow administration of TS inhibitors, and is thus a surrogate marker of TS inhibition (Ford et al (2002)).

The elevation of plasma dUrd levels is understood by the inventors to directly mirror the current integrated tumor TS-inhibitory state in the body during 5-FU treatment, reflecting all existing tumor sites including metastases.

The above is strongly supported by the findings that the basic activity of TS in tumors is much higher than in mucosa and that both binding of the metabolite 5-fluorodeoxyuridine monophosphate (FdUMP) to TS and TS-inhibitory effect during 5-FU treatment is very much higher in tumors than in mucosa and other cells of the body. It is notable that nearly no binding of FdUMP is observed in tumors without the addition of exogenous folate (MTHF)(Peters et al. (1991) *Eur J Cancer*, 27(3): 263-267).

Thus, the plasma dUrd level as a surrogate marker for TS inhibition clearly stands out against older assays based on e.g. ^{3}H-FdUMP TS-binding, which are only confined to in vitro studies of e.g. biopsies of tumors or mucosa, or of isolated TS-enzyme. These radiolabel-based binding assays are today rarely if ever used and are strongly associated with very large degrees of variation (Peters et al. (1991) *Eur J Cancer*, 27(3): 263-267) as well as difficulties to get hold of necessary materials for the assays.

It is believed by the inventors that a degree of TS inhibition above 90 % is necessary for the tumors to enter into the apoptosis state which is required for tumor necrosis to take place. It is further believed that 6R-MTHF has the ability to stabilize the above inhibitory ternary complex up to the level required for obtaining a TS inhibition of the 90% level and above.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising [6R]-5,10-methylene tetrahydrofolate (6R-MTHF) for use in increasing plasma concentration of 2'-deoxyuridine (dUrd) in a human subject, wherein:

    a. 5-fluorouracil (5-FU) is to be administered intravenously at a dose of 400 mg/m^2 or 500 mg/m^2 as a bolus injection in said human subject;

    b. followed by a dose of 30, 60, 120, or 240 mg/m^2 of said pharmaceutical composition to be administered intravenously as one or more bolus injections to said human subject; and

    c. optionally said 5-FU is to be administered as a continuous infusion at a dose of 2400 mg/m^2 either simultaneously with or following (b).

2. The pharmaceutical composition for use according to claim 1, wherein the 6R-MTHF is provided as a pharmaceutically acceptable salt.

3. The pharmaceutical composition for use according to claim 2, wherein the pharmaceutically acceptable salt is provided as a lyophilisate.

4. The pharmaceutical composition for use according to claim 1, wherein the pharmaceutical composition produces an increased plasma concentration of dUrd in said human subject relative to dUrd plasma concentration in a human subject receiving administration of an equimolar dose of 5-formyl tetrahydrofolate (LV).

5. The pharmaceutical composition for use according to claim 1, wherein the pharmaceutical composition is to be administered intravenously as more than one bolus injections in said human subject.

6. The pharmaceutical composition for use according to claim 1, wherein the pharmaceutical composition is to be administered intravenously as two bolus injections to said human subject.

7. The pharmaceutical composition for use according to claim 1, wherein the pharmaceutical composition is to be administered intravenously as a bolus injection to said human subject approximately 30 minutes following (a).

8. The pharmaceutical composition for use according to claim 1, wherein the pharmaceutical composition is to be administered intravenously as a bolus injection to said human subject approximately 30 and 60 minutes following (a).

9. The pharmaceutical composition for use according to claim 1, wherein said human subject suffers from cancer.

10. The pharmaceutical composition for use according to claim 9, wherein said cancer is colorectal cancer.

11. The pharmaceutical composition for use according to claim 1, wherein (d) at least one anticancer agent selected from the group consisting of oxaliplatin, irinotecan and bevacizumab is to be administered separately.

12. The pharmaceutical composition for use according to claim 1, wherein the pharmaceutical composition produces an increased inhibition of thymidylate synthase (TS) in said human subject relative to TS inhibition in a human subject receiving administration of an equimolar dose of LV.

13. The pharmaceutical composition for use according to claim 1, wherein the pharmaceutical composition has a chemical purity greater than 90% and in 95% isomeric excess over [6S]-5,10-methylene tetrahydrofolate (6S-MTHF).

14. Use of 6R-MTHF and 5-FU in the manufacture of a medicament for combination therapy of cancer in a human subject, wherein

a) said 5-FU is to be administered intravenously at a dose of 400 mg/m^2 or 500 mg/m^2 as a bolus injection to said human subject;

b) followed by a dose of 30, 60, 120, or 240 mg/m^2 of said pharmaceutical composition to be administered intravenously as one or more bolus injections to said human subject; and

c) optionally said 5-FU is to be administered as a continuous infusion at a dose of 2400 mg/m^2 either simultaneously with or following (b).