2018

First-in-human phase I study of infusional and bolus schedules of Deflexifol, a novel 5-fluorouracil and leucovorin formulation, after failure of standard treatment

Phillip R. Clingan
University of Wollongong, *Southern Medical Day Care Centre*, philipc@uow.edu.au

Stephen Ackland
Calvary Mater Newcastle Hospital

Daniel Brungs
University of Wollongong, dbrungs@uow.edu.au

Paul de Souza
Western Sydney University, p.desouza@uws.edu.au

Morteza Aghmesheh
University of Wollongong, *Illawarra Cancer Care Centre*, morteza@uow.edu.au

See next page for additional authors

Publication Details

Clingan, P. R., Ackland, S. P., Brungs, D., de Souza, P., Aghmesheh, M., Garg, M. B., Ranson, R. D., Parker, S., Jokela, R. & Ranson, M. (2019). First-in-human phase I study of infusional and bolus schedules of Deflexifol, a novel 5-fluorouracil and leucovorin formulation, after failure of standard treatment. Asia-Pacific Journal of Clinical Oncology, 15 (3), 151-157.

First-in-human phase I study of infusional and bolus schedules of Deflexifol, a novel 5-fluorouracil and leucovorin formulation, after failure of standard treatment

Abstract

Background: 5-Fluorouracil (5-FU) is administered with leucovorin (LV) to enhance clinical activity. However, simultaneous administration is not feasible due to their chemical incompatibility, so conditions for the maximum possible beneficial interaction cannot be met. To overcome this, we developed a novel all-in-one, pH neutral stable solution of 5-FU plus LV with β-cyclodextrin (termed Deflexifol) and assessed its safety and tolerability in a first-in-human phase I trial.

Methods: Patients with advanced solid malignancy received Deflexifol as weekly bolus (375–575 mg/m^2) or two-weekly 46 h infusion (1200–3600 mg/m^2) for six cycles in a 3+3 dose escalation design. Adverse events, pharmacokinetics and tumor response rates were assessed by standard methods.

Results: Forty patients were treated (19 bolus, 21 infusional, median age 67) with no grade 4 adverse events reported. Dose-limiting toxicities of grade 3 diarrhea and myelosuppression were reported for the bolus schedule at 575 mg/m^2 (maximum tolerated dose 525 mg/m^2), whereas none were reported for the infusional schedule. The recommended phase II infusional dose was declared as 3,000 mg/m^2, >25% that of 5-FU used in standard-of-care regimens. Pharmacokinetic analyses showed evidence of inter-patient variability, with no evidence of saturation in clearance, and a trend to linear increase in AUC with dose. Disease control rate was 64% despite most patients having failed previous 5-FU regimens.

Conclusion: Deflexifol is safer and effective in bolus and infusion schedules at higher doses than that permitted by separate infusion of 5-FU and LV. A phase II study evaluating Deflexifol is planned.

Disciplines

Medicine and Health Sciences

Publication Details

Clingan, P. R., Ackland, S. P., Brungs, D., de Souza, P., Aghmesheh, M., Garg, M. B., Ranson, R. D., Parker, S., Jokela, R. & Ranson, M. (2019). First-in-human phase I study of infusional and bolus schedules of Deflexifol, a novel 5-fluorouracil and leucovorin formulation, after failure of standard treatment. Asia-Pacific Journal of Clinical Oncology, 15 (3), 151-157.

Authors

Phillip R. Clingan, Stephen Ackland, Daniel Brungs, Paul de Souza, Morteza Aghmesheh, Madhu Garg, Richard Ranson, Suzanne Parker, Rebecca Jokela, and Marie Ranson

First-in-human phase I study of infusional and bolus schedules of Deflexifol, a novel 5-fluorouracil and leucovorin formulation, after failure of standard treatment

Philip R. Clingan,[1,2*] Stephen P. Ackland,[3] Daniel Brungs,[2,4] Paul de Souza,[5] Morteza Aghmesheh,[2,6] Madhu B. Garg,[3] Richard D. Ranson,[7] Suzanne Parker,[1] Rebecca Jokela,[1] Marie Ranson[2,4]

[1]Southern Medical Day Care Centre, Wollongong, Australia; [2]Illawarra Health and Medical Research Institute, Wollongong, 2522 Australia; [3]Calvary Mater Hospital/ HMRI, Newcastle Australia; [4]School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, 2522 Australia; [5]Western Sydney University School of Medicine, Sydney Australia, [6]Illawarra Cancer Care Centre, Wollongong Australia, [7]FivepHusion Pty Ltd, Austinmer, NSW, 2515 Australia.

Running title: Phase I trial of 5-FU formulation

Corresponding Author: Dr Marie Ranson

Mailing Address: Illawarra Health and Medical Research Institute, University of Wollongong, NSW 2522

Phone: +612 4221 3291

Fax: +612 4221 8130

Email: mranson@uow.edu.au

Acknowledgements: Funding for the trial came from FivepHusion Pty Ltd and local philanthropic sources including the Illawarra Cancer Carers. D Brungs is supported by the Centre for Oncology Education and Research Translation (CONCERT) Translational Cancer Centre (Cancer Institute New South Wales Grant 13/TRC/1-01). P Clingan is a patent holder for Deflexifol (US 8372834 B2), and along with M Ranson and R Ranson, owns shares in Fivephusion Pty Ltd.

This research was approved by the Bellberry Human Research Ethics Committee (TGA HREC Code EC00419), and all patients gave their informed consent prior to their inclusion in this study.

ABSTRACT

BACKGROUND

5-Fluorouracil (5-FU) is administered with leucovorin (LV) to enhance clinical activity. However, simultaneous administration is not feasible due to their chemical incompatibility, so conditions for the maximum possible beneficial interaction cannot be met. To overcome this, we developed a novel all-in-one, pH neutral stable solution of 5-FU plus LV with β-cyclodextrin (termed Deflexifol) and assessed its safety and tolerability in a first-in-human phase I trial.

METHODS

Patients with advanced solid malignancy received Deflexifol as weekly bolus (375 mg/m² to 575 mg/m²) or two-weekly 46 h infusion (1200 mg/m² to 3600 mg/m²) for 6 cycles in a 3+3 dose escalation design. Adverse events, pharmacokinetics, and tumor response rates were assessed by standard methods.

RESULTS

40 patients were treated (19 bolus, 21 infusional, median age 67) with no grade 4 adverse events reported. Dose-limiting toxicities of grade 3 diarrhea and myelosuppression were reported for the bolus schedule at 575 mg/m^2 (maximum tolerated dose 525 mg/m²), whereas none were reported for the infusional schedule. The recommended Phase II infusional dose was declared as 3,000 mg/m², > 25% that of 5-FU used in standard-of-care regimens. Pharmacokinetic analyses showed evidence of inter-patient variability, with no evidence of saturation in clearance, and a trend to linear increase in AUC with dose. Disease control rate was 64% despite most patients having failed previous 5-FU regimens.

CONCLUSION

Deflexifol is safer and effective in bolus and infusion schedules at higher doses than that permitted by separate infusion of 5-FU and LV. A phase II study evaluating Deflexifol is planned.

Keywords: Antimetabolites; Chemotherapy; Clinical trial; Colorectal cancer; Phase I.

INTRODUCTION

5-Fluorouracil (5-FU) in combination with its biomodulator leucovorin (LV; 5-formyl tetrahydrofolate, folinic acid or calcium folinate) remains a fundamental component of many efficacious chemotherapy regimens used in patients with colorectal, gastrointestinal, head and neck, and breast carcinoma.[1] Most schedules of 5-FU/LV administration involve a bolus or short infusion (2 h) of LV followed by a bolus and/or an infusion of 5-FU. The requirement for separate administration of these drugs is due to the physical incompatibility of 5-FU (formulated as a highly alkaline solution to improve aqueous solubility) and the acidic LV, which otherwise results in precipitation of 5-FU and/or calcium carbonate.[2-6] The incompatibility issues of 5-FU and LV dosing are not fully resolved by the use of sequential administration through central ports, as blockages are observed in these lines after repeated treatment cycles resulting in treatment interruption and discontinuation[3,5] and/or reduced quality of life for patients.

The rationale for co-administration of 5-FU and LV is based on sound pharmacological principles. Leucovorin acts to increase the intracellular pool of 5,10-methylene tetrahydrofolate (THF), leading to stabilization of the ternary complex (TS-fluorodeoxyuridine monophosphate-THF) thereby enhancing thymidylate synthase (TS) inhibition.[7] Thus, concomitant delivery of 5-FU and LV should maximize anti-tumor activity. After LV administration, free folates reach peak serum levels within 10 min of injection and are cleared within ~ 6-8 h, with the half-life of the active l-isomer of LV only 48 min;[8] intratumoral THF levels fall rapidly after LV infusion.[9] Given that most 5-FU infusional regimens run over 22–46 h, the lack of a pharmacokinetic overlap with LV does not allow for optimal formation of a stable ternary complex and subsequent TS inhibition.

Attempts at simultaneous rather than sequential administration of LV and 5-FU have suggested possible benefits, but confirmed the chemical incompatibilities between the two solutions. In a phase II study by Ardalan et al[10], co-administration of 5-FU and LV into a dual lumen catheter increased the mean Overall Survival (OS) to 22 months in advanced colorectal cancer, a figure substantially higher than the 11.7 versus 10.5 months cited for LV/5-FU and 5-FU alone, respectively.[11] However, 50% of patients experienced catheter blockages due to calcium carbonate formation and could not complete their treatment.[2] In a more recent study of 29 patients with gastro-esophageal cancer who received 24 h infusions of 5-FU (variable dose) concomitantly with sodium folinate (to avoid calcium carbonate crystallization) catheter complications were still reported in 50% of patients, including thrombosis (17%) and line blockage (10%).[12]

To address the potential for simultaneous administration of LV and 5-FU without adverse chemical interaction, we developed an all-in-one injectable reformulation of active ingredients 5-FU and LV at physiological pH, termed Deflexifol, using hydroxypropyl β-cyclodextrin as an FDA approved excipient.[4] Incorporation of β-cyclodextrin improves the water solubility of 5-FU, negating the need for a strongly alkaline solution, thus preventing calcium carbonate precipitation and enabling a stable 5-FU/LV solution.[4] The ratio of 5-FU:LV in Deflexifol is 15:1, which is similar to standard sequential low dose LV/5-FU regimens.[1] In preclinical studies, Deflexifol showed equivalent tissue distribution and pharmacokinetics (PK) to LV followed by 5-FU, and was efficacious against colorectal and breast tumor models but with significantly reduced toxicity, including a complete absence of phlebitis.[13]

Advances in chemotherapy use and design are important given that 5-FU remains a key treatment component for many cancer patients. Deflexifol was developed to maximize the clinical activity and safety profile of 5-FU and thus has the capacity to become a new standard fluoropyrimidine in chemotherapy regimens for a range of solid tumors. Herein we report the phase I results of Deflexifol in both bolus and infusional schedules.

PATIENTS AND METHODS

Patient eligibility

Patients over the age of 18 with advanced or metastatic malignancies who had exhausted all standard treatments with an Eastern Cooperation Oncology Group performance status 0-2 were enrolled. A life expectancy of ≥12 weeks, and satisfactory renal, hepatic, and hematological function were required. Exclusion criteria included patients with known deficiency of dihydropyrimidine dehydrogenase or a history of severe reactions to 5-FU or fluoropyrimidines, untreated brain metastases, patients who had completed chemotherapy or radiotherapy $\leq$ 4 weeks prior, or who had severe comorbidities. Pregnant or breastfeeding women were also excluded.

Study Design and Treatment

This open-label single-center phase I study used a standard 3+3 dose escalation scheme to explore two treatment regimens. The primary objectives were to evaluate the safety and tolerability of Deflexifol in subjects with relapsed or refractory malignancy and to determine the maximum tolerated dose (MTD). Secondary objectives were 5-FU pharmacokinetic profile and anti-tumor activity. This study was approved by a local Human Investigations Committee (Bellberry Limited Approval #2014-05-259; TGA CTN 2014/0737)) and written informed consent was obtained from all patients.

Patients were treated with Deflexifol (5-FU 15mg/ml/ LV 1mg/ml/ HP-β-CD 100mg/ml, pH 7.4 +/- 0.1; formulated as ready-to-use solution) either as a bolus [based on the colorectal adjuvant Roswell Park (modified) fluorouracil and leucovorin weekly regimen; ID: 1271 v.4] or continuous infusion [based on the colorectal adjuvant de Gramont (modified) regimen; ID: 76 v.4].[1] Treatment regimens were allocated based on clinical factors such as the presence of an existing central line. Bolus injections were administered within approximately 5 min via a peripheral cannula or central line weekly for 6 consecutive weeks every 8 weeks. Infusional injections were administered continuously over approximately 46 h via a central line, portacath or PICC line using a CADD Pump every 2 weeks for 12 weeks, followed by a 2 week break before patients were eligible for a repeat cycle of treatment. Patients continued Deflexifol while tolerated until disease progression. The dose escalation levels and number of patients enrolled in the bolus and infusion regimens are summarized in Supplementary Table 1.

Safety Evaluations

All adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version V4.03. AE monitoring continued for 4 weeks after the final treatment, and monitoring of AEs related to Deflexifol was continued until stabilization or resolution. Dose limiting toxicities (DLTs) were assessed as AEs that were possibly related to study treatment, including any grade 3 or 4 non-hematologic toxicity with the exception of grade 3 nausea, vomiting, alopecia or diarrhea that resolved to a lower grade with supportive treatment within 7 days; febrile neutropenia, grade 4 neutropenia without fever lasting >7 days; grade 4 thrombocytopenia lasting > 7 days; any grade of thrombocytopenia associated with bleeding.

In both schedules, MTD was declared as the dose level previous to the one at which two or more patients (out of 6) experienced DLTs.

Pharmacokinetic Evaluations

Blood samples were taken from patients on both the bolus and infusional regimens during the first and sixth dose of Deflexifol (bolus: pre, 10, 20, 60, 120 min and 24 h; infusion: pre and 2 h). Blood plasma levels of 5-FU and its metabolite 5-fluoro-5,6-dihydrouracil (5-FUH_2) were measured by HPLC method[14] with minor modifications. Area under the curve (AUC), clearance (CLR) and plasma half-lives ($t_{1/2}$) were estimated for each patient to assess PK variability and adequacy of dosing in comparison to historical data.[15,16]

Clinical Response and follow-up

Responses were evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria[17], after approximately 6 – 8 weeks from baseline treatment.

Statistical Analysis

Descriptive statistics on patient characteristics, analysis of toxicities, and outcomes were performed for all patients. The Kaplan and Meier method was used to calculate progression-free and overall survival from the treatment start date to the date of death, or last review. PK values were calculated by program ‘PK Functions for Microsoft Excel’ using add-ins of PK1 and PK2 functions to excel data analysis files, and Statistica (V12) was used for simple descriptive statistics to summarize PK data within each dose level and, where appropriate, across dose levels.

RESULTS

Patient characteristics

40 patients (19 bolus, 21 infusion) were entered into the study. Patient characteristics are summarised in Table 1. Patients were heavily pre-treated, with 13/40 (33%) of patients having previously undergone more than 5 lines of treatment and 34/40 (85%) had failed prior fluoropyrimidine treatment. The most common tumour types were colorectal (60%) and breast cancer (18%).

Safety

No grade 4 adverse events were observed in any patients (Table 2). Only eight patients (20 percent) reported treatment-related adverse events with a severity of grade 3. The DLT in the bolus schedule was grade 3 diarrhea and myelosuppression (pancytopenia) at 575 mg/m^2 (dose of 5-FU). The bolus regimen MTD is thus 525 mg/m^2, which exceeds that of current standard colorectal cancer adjuvant or metastatic weekly schedules (e.g., AIO, Roswell Park; 375 - 500 mg/m^2)[1] and with DLTs consistent with that reported in various weekly bolus 5-FU regimens.[18,19] No DLT was observed in the infusion schedule up to dose level 5 (3600 mg/m^2) (Table 2). However, it should be noted that no patients at this dose level completed a full treatment cycle due to disease progression, and it was decided to halt the trial at this point and consolidate the dose at 3000 mg/m^2.

Overall, grade 1 - 2 fatigue and nausea were the most common toxicities observed among patients in both bolus and infusional regimens (Supplementary Table 2). No > grade 2 toxicity was noted at 375 - 475 mg/m^2 bolus (dose levels 1- 3), or at 1200-1800 mg/m^2 infusion (dose levels 1 - 2). No cardiac toxicity was observed. Grade 1 - 2 myelosuppression was only observed in the bolus

regimen. Three grade 3 adverse events were observed but these were related to disease progression and not to the study drug.

Pharmacokinetics

Of the 40 patients available for assessment of PK variability and adequacy of dosing, 38/40 patients treated at dose 1 and 24/32 patients treated at dose 6 had plasma levels assessed. All patients had measurable plasma concentrations of 5-FU and FUH_2, with the FUH_2 levels consistently greater than 5-FU (Supplementary Figures 1 and 2), as expected for patients with normal dihydropyrimidine dehydrogenase activity and 5-FU catabolism.[14] PK showed evidence of inter-patient variability consistent with known pharmacology of 5-FU. In the weekly bolus schedule 5-FU CLR was 21-900 L/h, $t_{1/2}$ 0.11-0.52 h, with the intra-patient dose 6 CLR equal to 54-117% of dose 1 (Table 3) and there was a trend to increased AUC (mg.h/L) with dose (Supplementary Figure 1). The first 4 dose levels gave median AUCs that are well below the median AUC for toxicity in a study using a weekly bolus schedule of 500-864 mg/m^2.[15] With the infusion schedule, 5-FU CLR (13 – 700 L/h) and AUC estimates were somewhat variable due to 3 patient outliers (>10-fold AUC values compared to the median; Supplementary Figure 1C, D) and some cases having insufficient data to thoroughly analyze, especially at dose 6 (Table 4). However, compared to historical PK data of 5-FU alone[15,16], AUC was likely sub-therapeutic until > 525 mg/m^2 in the bolus schedule, and for some patients with infusion at all dose cohorts.

Response Rate

A total of 36 of 40 patients were evaluable for response. Four patients withdrew from the study prior to imaging, due to toxicity, thus response could not be assessed. Of these 36 patients, there was 1 (3%) partial response, 22 (61%) stable disease, and 13 (36%) patients with progressive disease, with a disease control rate (partial response plus stable disease) of 23/36 (64%). Figure 1

shows response rate (RECIST 1.1) in the 28 patients with measurable disease. Seven patients clinically progressed prior to cycle six and were withdrawn from the trial prior to imaging, and one patient did not have measurable disease as per RECIST 1.1. Duration of clinical benefit for patients with a partial response or stable disease ranged from 1.6 – 13.1 months (median 3.8 months). Of the 23 patients with disease control 11 (47%) were stable for at least 4 months.

Based on all 40 patients, the median progression free survival was 2.6 months (95% CI, range 0.5 – 23.0 mo). The median OS was 4.65 months (95% CI, range 0.8 – 23.0 mo).

DISCUSSION

Deflexifol is a rationally designed novel formulation of 5-FU with LV with potential to increase the clinical value of 5-FU. The co-administration of these two agents, rather than the currently employed sequential administration, has capacity for more sustained inhibition of TS as a consequence of longer duration of presence of both LV and 5-FU in tumor cells, with more profound and prolonged stability of the ternary complex.[7]. Indeed, the promising efficacy signals seen in this phase I study in heavily pretreated patients, including many with prior 5-FU exposure, implies that Deflexifol may display superior anti-tumor efficacy than sequential LV and 5-FU.

Deflexifol also offers a more favorable toxicity profile than sequential LV and 5-FU, based on historical comparisons,[20] and no catheter blockages or phlebitis were observed. The reduced toxicity of Deflexifol may be due in part to the pH neutral formulation in contrast to the alkaline pH of standard 5-FU. Preclinical rabbit models showed a marked reduction in phlebitis, a common and unreported problem, with Deflexifol compared to 5-FU.[13] Similarly, the basic pH of standard 5-FU was proposed to be a contributor to 5-FU cardiotoxicity through the presence of fluoroacetaldehyde impurities which are metabolized to the highly cardiotoxic compound fluoroacetate.[21] We did not observe any cardiotoxicity in either the preclinical or this phase I study, although we acknowledge the limited sample size and absence of comprehensive cardiac investigations. On a practical level, Deflexifol also reduces nursing and drug administration time, since the need to administer LV separately is avoided which may have cost-saving implications.

The current PK studies support the view that the pharmacology of 5-FU within Deflexifol is not substantially different to native 5-FU,[15,22] with mean half-life (10-12 min), mean clearance (130-

170 L/h), and FUH_2 levels being similar to our own data with 5-FU alone.[23,24] We acknowledge PK variability in this trial due to both inter-patient variability, which is consistent with historical data for 5-FU[16], and limited sampling frequency from the infusional schedule patients. However, the similar PK suggests that 5-FU is not bound by β-cyclodextrin to any extent, and that 5-FU metabolism is not impeded by the presence of β-cyclodextrin or LV. In addition, the toxicity profile, generally consistent with the clinical experience of 5-FU alone, suggests that the additional components in Deflexifol, necessary to facilitate co-administration of 5-FU and LV, do not create any adverse interactions.

The MTD for Deflexifol in the weekly bolus regimen was 525 mg/m^2 which exceeds the current standard of care dose for bolus LV and 5-FU regimens (AIO, Roswell Park)[1]. Given that DLT for the 46 h infusional regimen was not reached, even at the highest dose of 3600 mg/m^2, for practical reasons the recommended phase II dose for further study is 3000 mg/m^2 by 46 h infusion. This dose exceeds that used in current standard of care regimens with infusional 5-FU (e.g., modified de Gramont; 400 mg/m^2 bolus + 2400 mg/m^2 infusion).[1]

The higher doses achieved with Deflexifol are more congruent with doses in schedules using 5-FU alone,[20] which suggests that the availability of LV in Deflexifol could possibly be compromised. Deflexifol was formulated to emulate low dose LV since several studies have shown no therapeutic advantage in using standard high (200 or 500 mg/m^2) compared to standard low doses (20 mg/m^2),[25-27] and large inter-patient variability of tissue folate levels in colorectal cancer patients, regardless of LV dosing levels, has been reported[28] (suggesting that assessment of plasma and tissue folates may not yield meaningful data). Further, low dose LV confers lower financial

cost and is reported to reduce hospitalization to manage chemotherapeutic toxicity.[27] It is possible, however, to formulate Deflexifol with higher amounts of LV.[4]

This phase I clinical trial has the following limitations. A limited number of dose levels were evaluated for pragmatic purposes; we did not expect that substantially higher doses of 5-FU could be tolerated when given as infusional Deflexifol compared to the sequential administration of LV and infusional 5-FU. Therefore, we could not identify a DLT or a MTD with precision for infusional Deflexifol. The PK limited sampling strategy was clearly not ideal to comfortably confirm each patients 5-FU metabolism parameters, and in future studies we recommend collection of plasma at more timepoints during (infusion) and after (bolus) administration. Despite these limitations, this study confirmed that LV and 5-FU can be mixed together and administered without unexpected side-effects, and with a 5-FU PK profile consistent with previous studies of 5-FU alone.

In conclusion, this phase I trial demonstrated that Deflexifol, a novel formulation of 5-FU together with LV managed by chemical manipulation using β-cyclodextrin, is safe and tolerable, and can be administered to cancer patients at 5-FU doses higher than those used in current clinical practice. In both a bolus and an infusion schedule, the toxicity spectrum of Deflexifol is minimal, with no unexpected adverse effects. Pharmacokinetic studies suggest that 5-FU in Deflexifol is distributed and metabolized similarly to native 5-FU, and indicate the potential for greater antitumor efficacy as a consequence of considerably longer duration of ternary complex formation. Furthermore, given that a positive 5-FU dose intensity versus tumor response relationship has been

demonstrated,[20] the higher doses of 5-FU deliverable via Deflexifol also suggests that improved efficacy is possible.

REFERENCES

1. Cancer Institute NSW: EviQ Cancer Treatments Online: Medical Oncology. https://www.eviq.org.au/
2. Ardalan B, Flores MR: A new complication of chemotherapy administered via permanent indwelling central venous catheter. Cancer 75:2165-8, 1995
3. Bruch HR, Esser M: Catheter occlusion by calcium carbonate during simultaneous infusion of 5-FU and calcium folinate. Onkologie 26:469-72, 2003
4. Locke JM, Stutchbury TK, Vine KL, et al: Development and assessment of novel all-in-one parenteral formulations with integrated anticoagulant properties for the concomitant delivery of 5-fluorouracil and calcium folinate. Anticancer Drugs 20:822-31, 2009
5. Stiles ML, Allen LV, Jr., Tu YH: Stability of fluorouracil administered through four portable infusion pumps. Am J Hosp Pharm 46:2036-40, 1989
6. Trissel LA, Martinez JF, Xu QA: Incompatibility of fluorouracil with leucovorin calcium or levoleucovorin calcium. Am J Health Syst Pharm 52:710-5, 1995
7. Danenberg PV, Gustavsson B, Johnston P, et al: Folates as adjuvants to anticancer agents: Chemical rationale and mechanism of action. Crit Rev Oncol Hematol 106:118-31, 2016
8. Trave F, Rustum YM, Petrelli NJ, et al: Plasma and tumor tissue pharmacology of high-dose intravenous leucovorin calcium in combination with fluorouracil in patients with advanced colorectal carcinoma. J Clin Oncol 6:1184-91, 1988
9. Houghton JA, Williams LG, de Graaf SSN, et al: Relationship between Dose Rate of [6RS]Leucovorin Administration, Plasma Concentrations of Reduced Folates, and Pools of 5,10-Methylenetetrahydrofolates and Tetrahydrofolates in Human Colon Adenocarcinoma Xenografts. Cancer Res 50:3493-3502, 1990
10. Ardalan B, Chua L, Tian EM, et al: A phase II study of weekly 24-hour infusion with high-dose fluorouracil with leucovorin in colorectal carcinoma. J Clin Oncol 9:625-30, 1991
11. Thirion P, Michiels S, Pignon JP, et al: Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: an updated meta-analysis. J Clin Oncol 22:3766-75, 2004
12. Karapetis CS, Cheong KA, Yip D, et al: A phase I and II trial of epirubicin, cisplatin, 24-hour infusion 5 fluorouracil and sodium folinate in patients with advanced esophagogastric carcinomas. Asia Pac J Clin Oncol 6:298-305, 2010
13. Stutchbury TK, Vine KL, Locke JM, et al: Preclinical evaluation of novel, all-in-one formulations of 5-fluorouracil and folinic acid with reduced toxicity profiles. Anticancer Drugs 22:24-34, 2011
14. Ackland SP, Garg MB, Dunstan RH: Simultaneous determination of dihydrofluorouracil and 5-fluorouracil in plasma by high-performance liquid chromatography. Anal Biochem 246:79-85, 1997
15. van Groeningen CJ, Pinedo HM, Heddes J, et al: Pharmacokinetics of 5-fluorouracil assessed with a sensitive mass spectrometric method in patients on a dose escalation schedule. Cancer Res 48:6956-61, 1988
16. Saif MW, Choma A, Salamone SJ, et al: Pharmacokinetically guided dose adjustment of 5-fluorouracil: a rational approach to improving therapeutic outcomes. J Natl Cancer Inst 101:1543-52, 2009
17. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228-47, 2009

18. de Gramont A, Bosset JF, Milan C, et al: Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer: a French intergroup study. J Clin Oncol 15:808-15, 1997

19. Poon MA, O'Connell MJ, Moertel CG, et al: Biochemical modulation of fluorouracil: evidence of significant improvement of survival and quality of life in patients with advanced colorectal carcinoma. J Clin Oncol 7:1407-18, 1989

20. Arbuck SG: Overview of clinical trials using 5-fluorouracil and leucovorin for the treatment of colorectal cancer. Cancer 63:1036-44, 1989

21. Lemaire L, Malet-Martino MC, de Forni M, et al: Cardiotoxicity of commercial 5-fluorouracil vials stems from the alkaline hydrolysis of this drug. Br J Cancer 66:119-27, 1992

22. Hillcoat BL, McCulloch PB, Figueredo AT, et al: Clinical response and plasma levels of 5-fluorouracil in patients with colonic cancer treated by drug infusion. Br J Cancer 38:719-24, 1978

23. Garg MB, Sevester JC, Sakoff JA, et al: Simple liquid chromatographic method for the determination of uracil and dihydrouracil plasma levels: a potential pretreatment predictor of 5-fluorouracil toxicity. J Chromatogr B Analyt Technol Biomed Life Sci 774:223-30, 2002

24. Garg MB, Lincz LF, Adler K, et al: Predicting 5-fluorouracil toxicity in colorectal cancer patients from peripheral blood cell telomere length: a multivariate analysis. Br J Cancer 107:1525-33, 2012

25. QUASAR: Comparison of flourouracil with additional levamisole, higher-dose folinic acid, or both, as adjuvant chemotherapy for colorectal cancer: a randomised trial. QUASAR Collaborative Group. Lancet 355:1588-96, 2000

26. Poon MA, O'Connell MJ, Wieand HS, et al: Biochemical modulation of fluorouracil with leucovorin: confirmatory evidence of improved therapeutic efficacy in advanced colorectal cancer. J Clin Oncol 9:1967-72, 1991

27. Buroker TR, O'Connell MJ, Wieand HS, et al: Randomized comparison of two schedules of fluorouracil and leucovorin in the treatment of advanced colorectal cancer. J Clin Oncol 12:14-20, 1994

28. Taflin H, Wettergren Y, Odin E, et al: Folate levels measured by LC-MS/MS in patients with colorectal cancer treated with different leucovorin dosages. Cancer Chemother Pharmacol 74:1167-74, 2014

FIGURE LEGENDS

Figure 1. Tumor response by patient and regimen, based on % change in sum of size of target lesions between baseline and after dose 6 (= Change from baseline) of treatment. PD = progressive disease. PR = Partial remission. Patients in between the dotted lines exhibit stable disease. 28 patients were evaluable by RECIST 1.1.

Figure 1

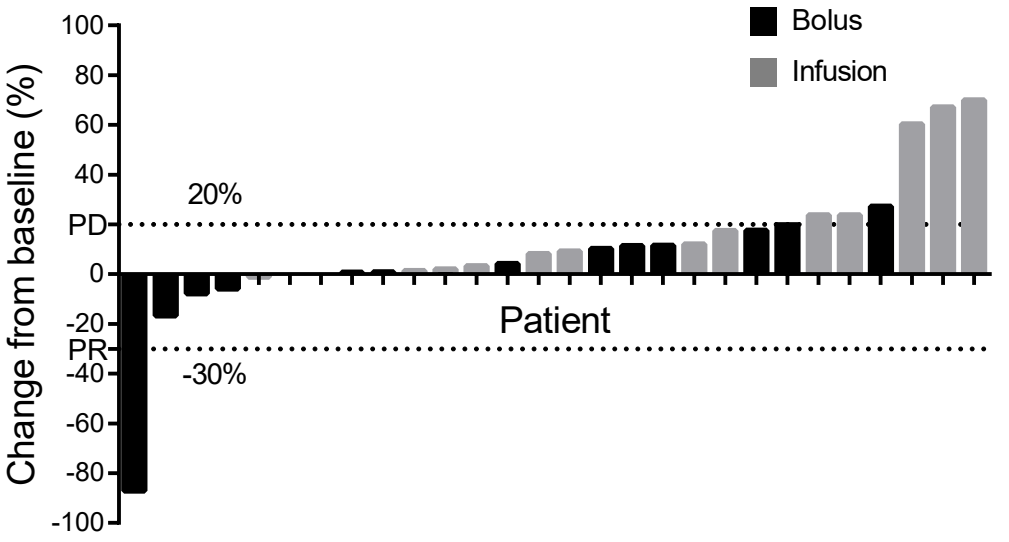

Bolus
Infusion
100
80
60
40
20%
PD
0
-20
PR
-40
-30%
-60
-80
-100
Change from baseline (%)
Patient

Table 1. Patient characteristics

Characteristic	**Bolus Regimen n=19 (%)**	**Infusion Regimen n=21 (%)**
Sex		
Male	7 (36)	12 (58)
Female	12 (63)	9 (42)
Age (y)		
Median (range)	64 (28 – 81)	67 (37 – 78)
Primary Tumour Location		
Breast	5 (26)	2 (10)
Colorectal	7 (37)	17 (80)
Other Gastrointestinal	4 (21)	2 (10)
Lung	3 (16)	0 (0)
Prior 5-FU Treatment		
Yes	14 (74)	20 (95)
No	5 (26)	1 (5)
Lines of Previous Treatment		
<5	13 (68)	14 (67)
≥5	6 (32)	7 (33)

Table 2. Treatment-related grade 3 or 4 adverse events by dose level and regimen

	Bolus Dose Level										**Infusion Dose Level**									
	1		2		3		4		5		1		2		3		4		5	
	Grade No. of events										Grade No. of events									
TOXICITY	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4
Diarrhea	0	0	0	0	0	0	**1**[*]	0	**2**	0	0	0	0	0	**1**[#]	0	0	0	0	0
Nausea & vomiting	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**[#]	0	0	0	**1**	0
Dyspnoea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Febrile neutropenia	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
Pancytopenia	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0
Venous thrombosis	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
Raised ALT/AST	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Patient No. 40. This AE occurred after the 5th dose of their first cycle and was resolved by changing to a 4 weeks on 2 weeks off schedule. The patient then continued through another 2 full cycles with no AEs, declining further treatment in their 5th cycle

\# Patient No. 17. These AEs occurred after the 3rd dose of their first cycle and were resolved by 25% dose reduction. The patient then continued on to another cycle with no AEs.

Table 3. Pharmacokinetic parameters of 5-FU in bolus Deflexifol (mean ± SEM)

	Dose 1 (cycle 1)				Dose 6 (cycle 1)			
Dose Levels (mg/m^2)	N	AUC (mg.h/L)	CLR (L/h)	$t_{1/2}$ (h)	N	AUC (mg.h/L)	CLR (L/h)	$t_{1/2}$ (h)
1 (375)	3	6.5 ± 1.1	97.1 ± 11.2		3	7.7 ± 1.2	84.7 ± 16.7	
2 (425)	3	5.5 ± 2.0	188.0 ± 76.8		3	12.4 ± 2.5	68.0 ± 19.4	
3 (475)	3	7.1 ± 3.8	201.8 ± 101.4		3	7.5 ± 2.5	129.8 ± 42.2	
4 (525)	5	17.7 ± 8.2	148.4 ± 85.0		3	8.5 ± 3.8	378.3 ± 306.9	
5 (575)	4	18.4 ± 3.7	59.4 ± 9.9		2	26.5± 8.1	37.8 ± 9.6	
All	**18**	**12.2 ± 2.7**	**135.6 ± 31.0**	**0.21± 0.02**	**14**	**11.5 ± 2.2**	**147.0 ± 66.1**	**0.22± 0.02**

Abbreviations: AUC = area under the curve; CLR = clearance; $t_{1/2}$ = terminal half-life

Table 4. Pharmacokinetic parameters of 5-FU in infusional Deflexifol (mean ± SEM)

	Dose 1 (cycle 1)			Dose 6 (cycle 1)		
Dose Levels (mg/m^2)	N	AUC (mg.h/L)	CLR (L/h)	N	AUC (mg.h/L)	CLR (L/h)
1 (1200)	3	75.2 ± 67.1	191.4 ± 89.2	1	8.56	287.5
2 (1800)	3	12.6 ± 1.04	229.3 ± 10.0	2	8.81 ± 0.1	260.1 ± 61.0
3 (2400)	6	54.7 ± 37.0	209.1 ± 40.4	5	92.4 ± 73.2	183.4 ± 46.5
4 (3000)	5	17.0 ± 2.2	355.3 ± 33.1	2	30.5 ± 17.6	322.2 ± 217.4
5 (3600)	3	15.7 ± 5.6	496.2 ± 131.0	0	-	-
All	**20**	**36.2 ± 14.5**	**289.1 ± 34.4**	**10**	**54.9 ± 36.9**	**236.9 ± 44.4**

Abbreviations: AUC = area under the curve; CLR = clearance

Supplementary Table 1. Number of patients and doses received in each treatment schedule (bolus weekly × 6, and 46 h infusion every 2 weeks × 6)

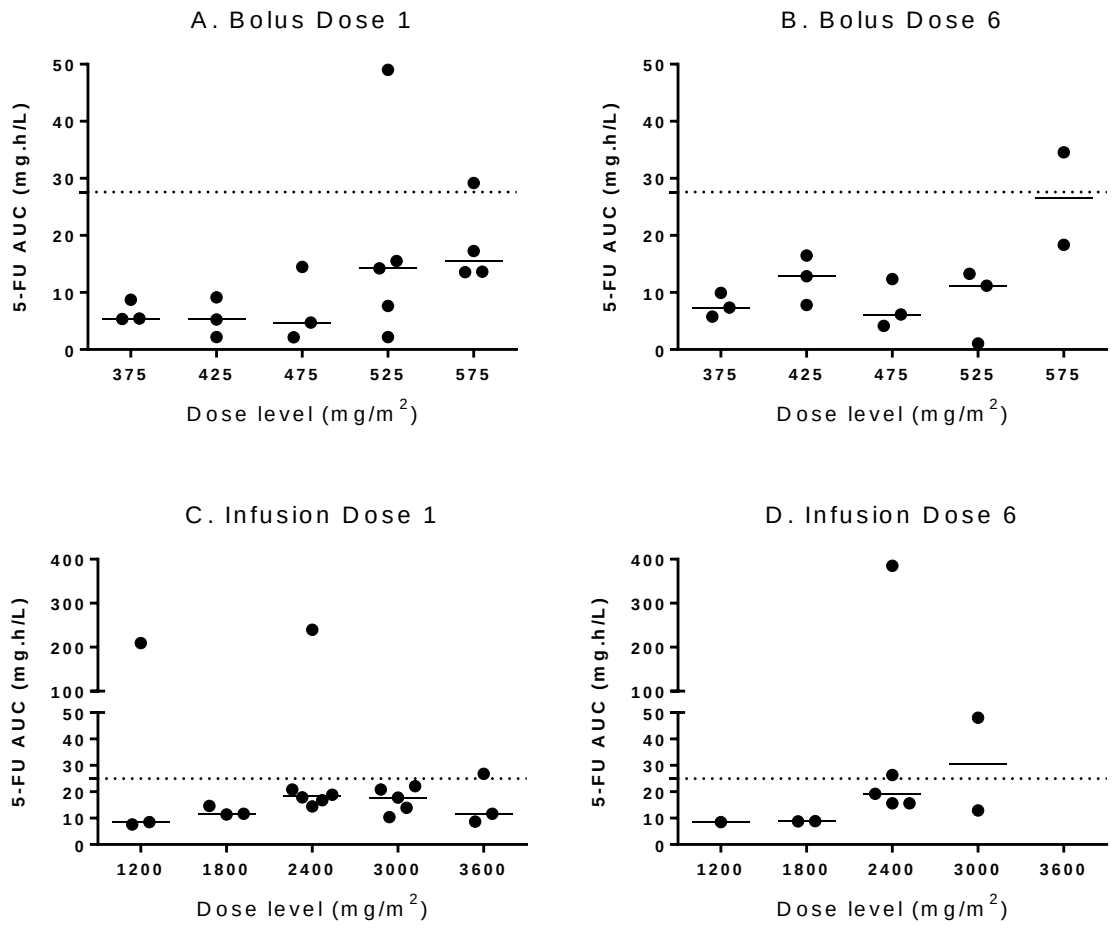
	Bolus					Infusion				
Dose level	Deflexifol dose (mg/m^2 5-FU equivalents)	No. patients treated	No. doses	Mean No. doses/ patient	Treatment related and non-related AEs	Deflexifol dose (mg/m^2 5-FU equivalents)	No. patients treated	No. doses	Mean No. doses/ patient	Treatment related and non-related AEs
1	375	3	35	11.67	no toxicity	1200	3	19	6.33	1 × incomplete bowel obstruction at dose 3 cycle 1, withdrew consent
2	425	3	34	11.33	no toxicity	1800	3	21	7	1 × ALT/AST increase due to unrelated infection, did not complete
3	475	3	30	10	1 × grade 3 Dyspnoea + compression fracture	2400	6	62	10.33	Patient #25 received > 4 cycles, 12 months treatment
4	525	6	41	6.83	1 × grade 3 diarrhea 1 patient with suspected DPDD, did not complete	3000	6	31.5	5.25	1 × SAE fall out of bed - attributed to rising Dysnopea, related to PD
5	575	4	19	3.8	1 × grade 3 diarrhea 1 × grade 3 diarrhea + myelosuppression	3600	3	10	3.33	No patients completed a full treatment cycle due to PD
	Total	19	159			Total	21	143.5		

Abbreviations: AE, adverse events; ALT/AST, aspartate transaminase/alanine transaminase; DPDD, dihydropyrimidine dehydrogenase deficiency; PD, Progressive disease; SAE, serious adverse event

Supplementary Table 2. Treatment-related grade 1 and 2 adverse events by regimen

	No. of events		
	Bolus	Infusion	Total
Abdominal pain	2	2	4
Diarrhea	6	2	8
Dyspnoea	4	5	9
Fatigue	15	11	26
Infection	2	4	6
Myelosuppression	7	0	7
Mucositis	4	5	9
Nausea	8	6	14
Raised ALT/AST	1	1	2
Vomiting	2	0	2

Supplementary Figure 1

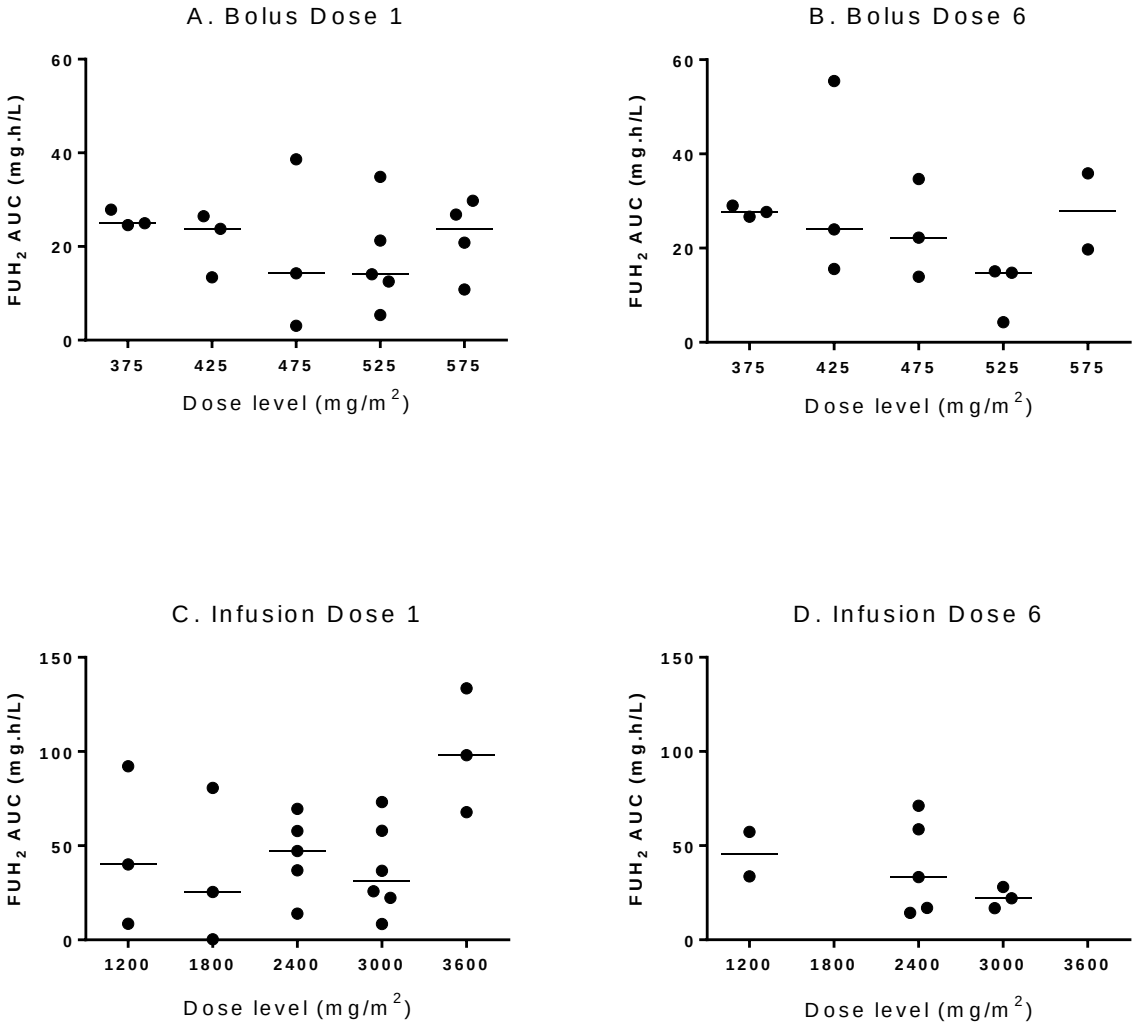


Supplementary Figure 1. Scatter dot plots showing relationship of area under the 5-FU plasma concentration × time curve (AUC) versus dose level (mg/m^2) measured after administration of (A. B.) bolus dose 1 and 6, or (C. D.) during infusion dose 1 and 6. • represent an individual patient with median values (−) for the cohort shown. For infusion dose 6, no samples were collected at the 3600 mg/m^2 dose level. Dashed lines indicate historical median AUC for toxicity using bolus[1] or infusion[2] regimens.

[1] van Groeningen CJ, Pinedo HM, Heddes J, et al: Pharmacokinetics of 5-fluorouracil assessed with a sensitive mass spectrometric method in patients on a dose escalation schedule. Cancer Res 48:6956-61, 1988

[2] Saif MW, Choma A, Salamone S J, Chu E. Pharmacokinetically guided dose adjustment of 5-fluorouracil: a rational approach to improving therapeutic outcomes. J Natl Cancer Inst 101: 1543-52, 2009.

Supplementary Figure 2



Supplementary Figure 2. Scatter dot plots showing relationship of area under the FUH_2 plasma concentration-×-time curve (AUC) versus dose level (mg/m^2) measured during administration of (A. B) bolus dose 1 and 6, or (C. D.) infusion dose 1 and 6. • represent an individual patient with median values (−) for the cohort shown. For infusion dose 6, no samples were collected at 1800 and 3600 mg/m^2 dose levels.