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Point of View

Irinotecan and 5-Fluorouracil in Colorectal Cancer: Time for a Pause?

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INTRODUCTION

COLORECTAL CANCER is one of the three major causes of cancer-related deaths in the Western hemisphere. For over 40 years, 5-fluorouracil (5-FU) has been the mainstay of systemic and regional chemotherapy, both in the adjuvant and advanced settings of the disease. The response rates of single agent 5-FU are relatively low at 10–15% [1]. Various attempts have been made to improve the objective response rates of 5-FU, the most successful of which has been biochemical modulation by folinic acid (FA). A meta-analysis of the 5-FU/FA combination in colorectal cancer showed a 23% response rate compared with 11% with single agent 5-FU [2]. 5-FU/FA combination chemotherapy is currently the standard for metastatic colorectal cancer. New active drugs which have been approved for the treatment of colorectal cancer include novel thymidylate synthetase inhibitor, ralitrexed and topoisomerase I inhibitor, irinotecan. Other newer agents which have shown promise include oxaliplatin and 5-FU prodrugs (capecitabine, uracil-ftorafur).

Irinotecan has been shown to be an active agent in colorectal cancer. Single-agent chemotherapy with irinotecan in metastatic colorectal cancer produced response rates of 15–32% in chemonaive patients and 18–27% in 5-FU pretreated patients [3–7]. In view of its definite activity in colorectal cancer, there has been considerable enthusiasm for combining irinotecan with the established agent 5-FU. This enthusiasm stems from the following observations.

(1) 5-FU and irinotecan act against different intracellular targets, 5-FU inhibiting thymidylate synthetase and irinotecan targeting the DNA topoisomerase I enzyme.
(2) Irinotecan has demonstrated *in vivo* activity against both chemonaïve and 5-FU refractory colorectal cancer patients.
(3) Topoisomerase I levels are reported to be substantially higher in colorectal cancer cells than in normal tissues [8, 9]. Also, the topoisomerase I enzyme is expressed in both proliferating and quiescent cells. Therefore, it is likely to be active against slowly proliferating and actively dividing cancer cells.
(4) No significant adverse pharmacokinetic interaction has been demonstrated between the two drugs.
(5) *In vitro* data on combination therapy using 5-FU and irinotecan showed some additive effect (see below).

In spite of what seems to be a reasonable and sound rationale for combining these two otherwise active drugs in colorectal cancer, one has to ask whether this combination has a mechanism-based logic for its recommendation. If there is an *in vitro* additive effect, does this translate into clinically relevant synergism? These questions will be addressed in the following sections with an emphasis on the mechanism-based interactions between the two drugs.

IN VITRO DATA ON 5-FU AND IRINOTECAN COMBINATION

Results from limited *in vitro* experiments using 5-FU and irinotecan have showed some additive effect. The cytotoxic effects of irinotecan in combination with other anticancer drugs have been studied in the human T-cell leukaemia cell line MOLT-3 in culture. Both irinotecan and SN38 (an active metabolite of irinotecan) were used and cytotoxic effects determined by MTT assay. The effects on ID_{50} were evaluated using an isobologram method. No obvious synergistic or supra-additive effect was observed, but an additive effect was demonstrated in favour of the combination [10]. In another *in vitro* study in the human SUIT-2 cell line, combination treatment with SN38 and 5-FU yielded a better cell kill compared with single treatment with SN38 alone [11]. Similar results were reported using SN38 and 5-FU in the human Burkitt's lymphoma (Dauji) cell line [12].

Thus, *in vitro* data suggest that there may be an additive effect with the combined treatment. Whether this additive effect is adequate to produce an *in vivo* improvement in clinically relevant response rates is open for debate. What is interesting is that no definite supra-additive or synergistic effects were demonstrated when 5-FU was combined with irinotecan.

MECHANISM OF ACTION OF 5-FU AND IRINOTECAN

5-FU exerts its cytotoxic effects by inhibiting the thymidylate synthetase (TS) enzyme. The active 5-FU metabolite

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5dUMP, in the presence of reduced folate, forms a stable covalent complex with TS resulting in inhibition of DNA synthesis. 5-FU is also incorporated into RNA and DNA. The principal mechanism central to the clinical activity of 5-FU is its ability to inhibit TS [13]. Thus, 5-FU can essentially be regarded as a DNA synthesis inhibitor with some additional effects on RNA and protein synthesis (Figure 1).

Camptothecin and its analogues, including irinotecan, specifically inhibit the eukaryocytic DNA topoisomerase I enzyme by trapping a covalent enzyme–DNA intermediate. This covalent intermediate, termed the cleavable complex, is a reversible topoisomerase I–camptothecin–DNA ternary complex. Collisions between the advancing replication forks and the trapped topoisomerase I cleavable complexes result in DNA fork damage and formation of highly toxic DNA double strand breaks. This DNA damaging effect explains the relatively high S-phase toxicity of camptothecin derivatives (at least in some cell types), despite relatively constant topoisomerase I protein levels throughout the cell cycle. In addition to the topoisomerase I enzyme levels and the rate and level of formation of the cleavable complexes, other equally (if not more) important events contribute to the cytotoxicity of the topoisomerase I inhibitors. DNA damage induced by topoisomerase I inhibitors, such as irinotecan, has to be converted into a series of events that lead to cell cycle arrest and/or apoptosis. Thus, intracellular events occurring downstream to the formation of cleavable complexes have an important bearing in determining the cytotoxicity of topoisomerase I inhibitors [14–18] (Figure 2).

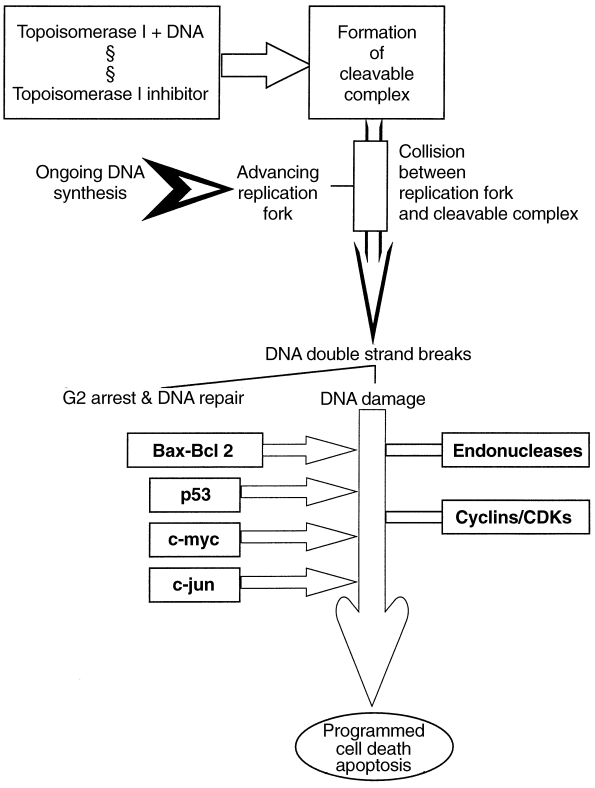

Figure 2. Topoisomerase I inhibition and probable apoptotic pathways. CDKs, cyclin dependent kinases; Bax, anti-apoptotic protein; Bcl-2, pro-apoptotic protein; c-myc and c-jun, oncogenes; p53, tumour suppressor gene.

IS THERE A MECHANISM-BASED SYNERGY BETWEEN 5-FU AND IRINOTECAN?

As outlined above, 5-FU and irinotecan seem to have fairly distinct unrelated mechanisms of action targeting different intracellular targets. Also, there seems to be some additive cell kill effect when both drugs are combined *in vitro*. Based on the known intracellular targets and mechanisms of action discussed above, is it sensible to combine these two drugs? Before one makes a statement either in favour or against, it may be worthwhile examining the intracellular events required for the cytotoxic effects of the individual drugs and explore whether there is any molecular mechanism-based interaction between the two drugs.

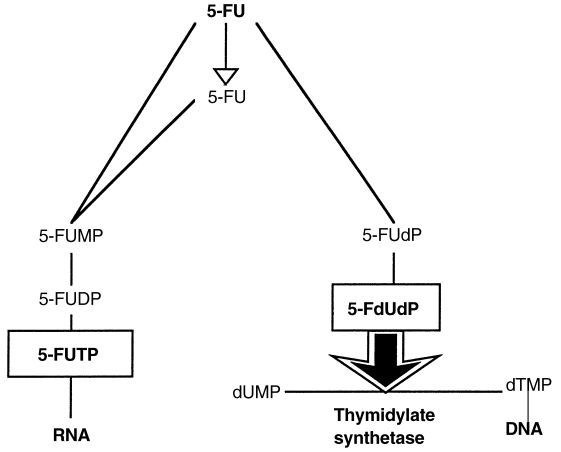

Figure 1. Mechanism of action of 5-fluorouracil. 5-FU, 5-fluorouracil; 5-FUMP, 5-fluorouridine monophosphate; 5-FUDP, 5-fluorouridine diphosphate; 5-FUTP, 5-fluorouridine triphosphate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate; 5-FdUdP, 5-fluorodeoxyuridine diphosphate; RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

5-FU, irinotecan and nucleic acid synthesis

Irinotecan forms cleavable complexes with topoisomerase I. As the DNA replication continues, this leads to the collision of the cleavable complexes with the replication fork, thus resulting in lethal DNA double strand breaks. For this to happen there should be ongoing DNA synthesis subsequent to the formation of the cleavable complex. This enzyme–DNA complex by itself is relatively non-toxic to the cells, as, when the drug is removed, the DNA nick is repaired successfully. Therefore, as mentioned earlier, it is reasonable to hypothesise that subsequent cellular events, of which DNA synthesis is probably most crucial, may determine the degree of cytotoxicity of topoisomerase I inhibitors. The corollary to this statement is that if DNA synthesis is inhibited, one might expect decreased cytotoxicity, as the cell will have more time to repair the DNA damage.

Is there any evidence to show that inhibiting DNA synthesis would result in decreased topoisomerase I inhibitor toxicity? The involvement of nucleic acid synthesis in the cell killing mechanisms of topoisomerase I inhibitors has been investigated in various cell lines. In V79, chinese hamster lung fibroblast cells, cotreatment with aphidicolin, a DNA polymerase inhibitor, decreased the cell kill effects of the topoisomerase I inhibitor, camptothecin. It was observed that the best protection against the cell kill was when aphidicolin was present at the time of exposure to the topoisomerase I poison. Post-camptothecin treatment with aphidicolin failed

to inhibit the topoisomerase I-mediated cell kill. RNA synthesis inhibitors, such as DRB, had no effect on the cell kill by topoisomerase I inhibitors on V79 cells [19]. In chinese hamster DC3F cells, it was noted that there was a good correlation between the degree of DNA synthesis inhibition by aphidicolin and the reduction of camptothecin cytotoxicity [20]. Similar results were obtained in mammalian cells and S-phase HeLa cells [21, 22]. The frequency of sister chromatid exchanges induced by DNA topoisomerase poisons in CHO cells was significantly reduced in the presence of aphidicolin [23].

Therefore, at least based on the *in vitro* data, there seems to be fairly conclusive evidence to support the statement that ongoing DNA synthesis is a prerequisite for the expression of topoisomerase I-mediated cytotoxicity and that DNA synthesis inhibitors may negate or decrease camptothecin-mediated cell kill. One might be allowed to assume that as 5-FU is a DNA synthesis inhibitor (through TS inhibition), combined treatment with irinotecan may not result in any significant synergistic effect. On the contrary, there is every reason to suspect that such a combination may have sub-additive, if not a frank antagonistic effect. One hopes that this potential problem, if it does exist, may be circumvented to some extent by proper scheduling/sequencing of the two drugs.

5-FU is extensively incorporated into both nuclear and cytoplasmic RNA, thus affecting RNA processing and function. Camptothecin analogues are potent RNA synthesis inhibitors, especially synthesis of high molecular weight RNAs, such as 45S or gamma RNA precursors and heterogeneous nuclear RNAs [19, 24]. This action of the topoisomerase I inhibitor is reversible on withdrawal of the drug. Although both drugs seem to have some inhibitory effect on RNA synthesis, it is not entirely clear to what degree this action contributes to their cytotoxicity. This interaction may be to some extent, dependent on the schedule and sequence of the two drugs. If RNA synthesis/transcription are actively inhibited by topoisomerase I inhibitors, this might prevent incorporation of 5-UdTP into RNA, thus negating the potential effect of RNA synthesis inhibition caused by 5-FU.

5-FU, irinotecan and cell cycle regulation

The cytotoxicity of anticancer drugs largely depends on their ability to induce cell death by apoptosis. The intracellular signalling pathways leading to apoptotic cell death following a cytotoxic drug insult are not yet clearly elucidated. The presence or absence of various crucial check points throughout the cell cycle may determine the ultimate fate of the cancer cell which has been subjected to a potentially serious assault. S-phase cells are up to 1000-fold more sensitive to killing by pulse treatment with camptothecin than cells in other phases of the cell cycle [25]. This is related to its dependence on ongoing DNA synthesis for optimal cell kill effect. 5-FU, likewise, is S-phase specific, toxic mainly against actively cycling cells. It is known that topoisomerase I inhibitors induce synchronisation of the cell cycle to S-phase after the removal of the drug. In the absence of a functional p53, G1 checkpoint arrest is lost and so, following camptothecin-induced DNA damage, cancer cells progress through G1. In this situation, G2 arrest becomes crucial for the survival of the cell. A key regulator of eukaryocytic cell division is p34 cdc-2/cyclin B, activation of which triggers the G2–M transition. Topoisomerase I-mediated DNA damage may inhibit activation of this kinase, thus resulting in G2 arrest [22]. There is also some evidence that topoisomerase I-inhibitors may activate c-jun-mediated kinases and ICE/CED-3-like proteases which may induce apoptotic cell death [26]. Also, long-term *in vivo* exposure to 5-FU or its metabolites may induce apoptotic cell death with G2 arrest [27]. Whether a similar effect on cell cycle progression is seen with short-term infusion or bolus is yet to be studied.

CLINICAL EXPERIENCE WITH 5-FU AND IRINOTECAN IN COLORECTAL CANCER

Irinotecan and 5-FU combination has been investigated in various phase I/II studies in Europe, U.S.A. and Japan. Different schedules and sequences have been evaluated in chemonaive and 5-FU resistant tumours. So far, from the published data, the overall objective response rates with the 5-FU and irinotecan combination are in the region of 11–26% [28–31]. This is in comparison with the responses with single agent irinotecan, of 15–32% in chemonaïve patients and 18–27% in pretreated patients. The combination of 5-FU and irinotecan, although it seems to be feasible, at least on the surface, does not appear to produce significantly higher response rates. There is some suggestion from clinical data that sequential therapy with irinotecan followed by 5-FU may be an optimal schedule for comparative studies. Irinotecan has also been combined with the 5-FU and folinic acid regimen in both chemonaïve and 5-FU pretreated colorectal cancer patients. Early results show that the irinotecan+5-FU/FA combination is feasible and reasonably well tolerated. However, the objective response rates are not significantly higher when compared with the single agent irinotecan and 5-FU/FA data [32–36]. Patient selection, tumour heterogeneity, schedule/sequence dependent cytotoxicity are probably some of the important variables affecting the clinical responses. Alternative schedules and sequences should be explored before comparative studies are initiated.

DISCUSSION

Both irinotecan and 5-FU are active against colorectal cancer. The strategy of combining these two drugs is a very attractive one and has already been evaluated in some phase I/II studies. The enthusiasm for the combined treatment arises from the two distinctly different mechanisms of action, the relative lack of cross-resistance and the absence of adverse pharmacokinetic interactions between the two drugs. In spite of all these attractive attributes, one has to be cautious in evaluating the irinotecan and 5-FU combination, because of the problems outlined above. If ongoing DNA synthesis is essential for the expression of the cytotoxic effect of topoisomerase poisons, then cotreatment with 5-FU is probably not likely to result in any significant clinical synergy. There are some very important issues which need to be addressed before this combination can be taken into comparative trials. Schedule and sequence dependent interactions probably play a significant role in contributing to the cytotoxicity of these two drugs when used in combination. Future studies using this combination should take into account the molecular and cell cycle interactions between the two drugs.

As observed *in vitro*, if the cells do synchronise into S-phase in the post-topoisomerase I inhibition period, then this may be the most appropriate time to challenge the already damaged DNA replication machinery with a DNA synthesis inhibitor, 5-FU. The most optimal schedule/sequence may be the one where the tumour is exposed to the topoisomerase I

inhibitor followed by a delayed challenge with 5-FU. In view of the known wide variations in the efficiency of DNA repair mechanisms within the cancer cells and the tumour heterogeneity with regards to its cell cycle machinery, a universal schedule/sequence may at best be regarded as a compromise. A reverse sequence, i.e. 5-FU followed by irinotecan, has not been evaluated and it is difficult to speculate the comparative efficacy of such a schedule.

CONCLUSION

Several studies on irinotecan-based combinations are in progress in colorectal cancer. Mature data from these studies are eagerly awaited. Further work is needed to define the optimal schedule and sequence of these otherwise active drugs in colorectal cancer. Also, any synergistic effect demonstrated should ideally result in clinically measurable benefit to the patient with colorectal cancer. In addition, any new combination has to be assessed against the most successful of the 5-FU combination regimens, 5-FU/FA. Such comparative trials should incorporate validated quality of life and clinical benefit response measuring instruments so that one can ascertain the 'real benefit' to the patient.

1. Devita Jr VT, Hellman S, Rosenberg SA. *Cancer: Principles and Practice of Oncology*. Philadelphia, JB Lippincott, 1996.
2. Advanced Colorectal Cancer Meta Analysis Project: modulation of fluorouracil by leukovorin in patients with advanced colorectal cancer: evidence in terms of response rate. Advanced Colorectal Meta Analysis Project. *J Clin Oncol* 1992, **10**, 896–903.
3. Rougier P, Culine S, Bugat R, *et al.* A phase II study of CPT-11 (irinotecan) in the treatment of advanced colorectal cancer in chemotherapy naive patients and patients pre-treated with 5-FU based chemotherapy. *J Clin Oncol* 1997, **15**, 251-260.
4. Conti JA, Kemeny NE, Salts LB, *et al.* Irinotecan is an active agent in untreated patients with metastatic colorectal cancer. *J Clin Oncol* 1996, **14**, 709–715.
5. Armand JP, Ducreux M, Mahjoubi M, *et al.* CPT-11 (irinotecan) in the treatment of colorectal cancer. *Eur J Cancer* 1995, **31A**, 1283–1287.
6. Rothenberg ML, Eckardt JR, Burris III HA, *et al.* Irinotecan (CPT-11) as second line therapy for patients with 5FU-refractory colorectal cancer. 30th Annual Meeting of the American Society of Clinical Oncology, 14–17 May 1994, Dallas, Texas, abstract 578.
7. Shimada M, Yashino M, Wakui A, *et al.* Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. *J Clin Oncol* 1993, **11**, 909–913.
8. Giovanella BC, Stehlin JS, Wall ME, *et al.* DNA topoisomerase I targeted chemotherapy of human colon cancer in xenografts. *Science* 1989, **246**, 1046-1048.
9. Hirabayashi N, Kim R, Nishiyama M, *et al.* Tissue expression of topoisomerase I and II in digestive tract cancers and adjacent normal tissues. *Proc Am Assoc Cancer Res* 1992, **33**, 436.
10. Kano Y, Suzuki K, Akutsu M, *et al.* Effects of CPT-11 in combination with other anticancer agents in culture. *Int J Cancer* 1992, **50**, 604–610.
11. Matsuoka-H, Yano K, Takiguchi S, *et al.* Advantge of combined treatment with CPT-II and 5-fluorouracil. *Anti Cancer Res* 1995, **15**, 1447–1452.
12. Akutsu M, Suzuki K, Tsunoda S, *et al.* Effects of SN-38 in combination with other anticancer agents against Dauji cells. *Gan-To-Kagaku-Ryoho* 1994, **21**, 1607–1611.
13. Allegra CJ, Grem JL. Antimetabolites. In Devita Jr VT, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. Philadelphia, JB Lippincott, 1996, pp. 432–452.
14. Hsiang YH, Hertzberg R, Hecht S, *et al.* Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. *J Biol Chem* 1985, **260**, 14873–14878.
15. Liu LF. DNA topoisomerase poisons as anticancer drugs. *Annu Rev Biochem* 1989, **58**, 351–375.
16. Avemann K, Knippers R, Koller T, Sogo JM. Camptothecin, a specific inhibitor of type I DNA topoisomerase induces DNA breakage at replication forks. *Mol Cell Biol* 1988, **8**, 3026–3034.
17. Ryan AJ, Squires S, Strutt HL, Johnson RT. Camptothecin cytotoxicity in mammalian cells is associated with the induction of persistent double strand breaks in replicating DNA. *Nucleic Acid Res* 1991, **19**, 3295–3300.
18. Pommier Y, Leteurtre F, Fesen M, *et al.* Cellular determinants of sensitivity and resistance to DNA topoisomerase inhibitors. *Cancer Invest* 1994, **12**, 530–542.
19. D'Arpa P, Beardsmore C, Liu LF. Involvement of nucleic acid synthesis in cell killing mechanisms of topoisomerase poisons. *Cancer Res* 1990, **50**, 6919–6924.
20. Holm C, Covey JM, Kerrigan D, Pommier Y. Differential requirement of DNA replication for the cytotoxicity of DNA topoisomerases I and II inhibitors in Chinese hamster DC3F cells. *Cancer Res* 1989, **49**, 6365–6368.
21. Ryan AJ, Squires S, Strutt HL, *et al.* Different rates of camptothecin induced replication fork associated double-strand breaks in mammalian cells. *Carcinogenesis* 1994, **15**, 823–828.
22. Tsao YP, D'Arpa P, Liu LF. The involvement of active DNA synthesis in camptothecin induced G2 arrest: altered regulation of p34cdc2/cyclin B. *Cancer Res* 1992, **52**, 1823–1829.
23. Pinero J, Lopez-Baena M, Ortiz T, Cortes F. Sister chromatid exchange induced by DNA topoisomerase poisons in late replicating heterochromatin. Influence of inhibition of replication and transcription. *Mutat Res* 1996, **354**, 195–201.
24. Zhang H, Wang JC, Liu LF. Involvement of DNA topoisomerase I in transcription of human ribosomal RNA genes. *Proc Natl Acad Sci USA* 1988, **85**, 1060–1064.
25. Del Bino G, Lassota P, Darzynkiewicz Z. The S phase cytotoxicity of camptothecin. *Exp Cell Res* 1991, **193**, 27–35.
26. Seimiya-H, Mashima-T, Toho M, *et al.* C-Jun NH2-terminal-kinase mediated activation of interleukin-1-beta converting enzyme/CED-3-like protease during anti cancer drug induced apoptosis. *J Biol Chem* 1997, **272**, 4631–4636.
27. Okamoto S, Sakai M, Uchida J, *et al.* 5-Fluorouracil induced apoptotic cell death with G2 phase arrest in human breast cancer grafted in nude mice. *Anti Cancer Res* 1996, **16**, 2699-2704.
28. Shimada Y, Sasaki Y, Sugano K, *et al.* Combination phase I study of CPT-11 (irinotecan) combined with continuous infusion of 5-fluorouracil in metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 1993, **12**, 196, abstract 575.
29. Saltz LB, Kanowitz J, Kemeny NE, *et al.* Phase I clinical and pharmaco kinetic study of irinotecan (CPT-11), 5-fluorouracil and leucovorin in patients with advanced solid tumours. *J Clin Oncol* 1996, **14**, 2959–2967.
30. Grossin F, Barbault H, Benhammoud A, *et al.* A phase I pharmacokinetic study of concomitant CPT-11(C) and 5FU(F) combination. Annual Meeting of the American Association of Cancer Research (AACR), 23 April 1996, Washington, USA.
31. Saltz L, Shimada Y, Khayat D. CPT-11 (irinotecan) and 5-fluorouracil; a promising combination therapy for colorectal cancer. *Eur J Cancer* 1996, **32A**, S24–S31.
32. Benhammouda A, Bastian G, Rixe O, *et al.* A phase I and pharmacokinetic (PK) study of CPT-11 (C) and 5-FU (F) combination. *Proc Am Soc Clin Oncol* 1997, **16**, abstract 710.
33. Ducreux M, Rougier P, Ychou M, *et al.* Phase I/II study of escalating dose of CPT-11 in combination with LV5-FU2 (DeGramont' regimen) every two weeks in the treatment of colorectal cancer (CRC) after 5-FU failure. *Proc Am Soc Clin Oncol* 1997, **16**, abstract 823.
34. Paz-Ares L, Sastre J, Diaz-Rubio E, *et al.* Phase I dose-finding study of irinotecan (CPT-11) over a short infusion combined with a fixed dose of 5-fluorouracil (5-FU) protracted continuous i.v. infusion in patients with advanced solid tumours. *Proc Am Soc Clin Oncol* 1997, **16**, abstract 874.
35. Rothenberg ML, Pazdur R, Rowinsky EK, *et al.* A phase II multicentre trial of alternating cycles of irinotecan (CPT-11) and 5-FU/LV in patients with previously treated metastatic colorectal cancer (CRC). *Proc Am Soc Clin Oncol* 1997, **16**, abstract 944.
36. Barone C, Pozzo V, Starkhammar H, *et al.* CTP-11 alternating with 5-fluorouracil (5-FU) folinic acid (FA): a multicentre phase II study in first line chemotherapy (CT) of metastatic colorectal cancer (CRC). *Proc Am Soc Clin Oncol* 1997, **16**, abstract 957.