

Advances **in the** treatment **of colorectal** cancer: **role of target expression**

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Introduction

For the last three decades, fluorouracil (5-FU)-based chemotherapy has been the mainstay of treatment in advanced colorectal cancer [1-3]. When given alone as intravenous (IV) bolus once weekly or for 5 consecutive days every 4 to 5 weeks, 5-FU produces response rates from 11% to 17% and a median survival time of approximately 1-year [4-6]. Although increased therapeutic efficacy of 5-FU in terms of higher response rates by the biomodulation with leucovorin (LV) has been well established, a meta-analysis of clinical studies failed to demonstrate a clear survival benefit [6,7]. Recently, evidence has accumulated that a pro-longed infusion of 5-FU may improve the tumor response rate and survival time when compared with 5-FU bolus regimens [8]. DeGramont et al [9] reported results of a randomized study involving 448 patients with advanced colorectal cancer and comparing high-dose LV in combination with bolus plus infusional 5-FU to the standard low-dose LV-5-FU bolus schedule given according to the North Central Cancer Treatment Group (NCCTG) regimen. The response rate (348 patients; 32.6% v 14.4%, $P = .0004$) and an improved progression-free survival time (27.6 v 22.0 weeks, $P = .0012$). Furthermore, a significant in-

crease in the median survival time was achieved in patients with measurable disease. Additionally, the German Association of Medical Oncology (AIO), in a randomized multicenter trial in metastatic colorectal cancer demonstrated an overall response rate of 44% and a median survival time of 16 months using a weekly-times-six schedule of high-dose LV (500 mg/m²/2-hour IV infusion) followed by infusional 5-FU (2.6 g/m² given as a 24-hour IV infusion) (5-FU_{24h}/LV) [10]. These results again indicate the superiority of LV-modulated infusional 5-FU over LV-5-FU bolus regimens given on a weekly or daily-times-five schedule. In contrast to these data, no significant differences in overall survival time were found in a multicenter trial of the Southwest Oncology Group, which compared low-dose LV-modulated bolus 5-FU, administered according to the NCCTG regimen, with several 5-FU regimens including single-agent infusional 5-FU_{24h} at a dose of 2.6 g/m² on a weekly schedule. Thus, significant emphasis has been placed on designing more effective 5-FU-based combination protocols.

Irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin) is a new semisynthetic derivative of a plant alkaloid, camp-

tothecin, with significant clinical efficacy against colorectal cancer [11,12]. CPT-11 (SN-38) appears to exert its cytotoxic mechanism by binding to DNA-associated topoisomerase I, a nuclear enzyme that facilitates DNA replication and transcription by causing single strand protein-bridged DNA breaks. The collision of these drug-stabilized protein-bridged DNA breaks, referred to as cleavable complexes, with moving replication forks leads to cell death during replication [13,14].

As first-line chemotherapy in metastatic colorectal cancer, CPT-11 produced a cumulative response rate of 26% (95% confidence interval, 20% to 32%), a median remission duration of 8 to 9 months, and a median survival time of 12 months [15-18]. Thus, CPT-11 demonstrates antitumor efficacy comparable to that achievable with LV-modulated 5-FU-based standard regimens. Furthermore, CPT-11 showed considerable antitumor activity in 5-FU-refractory colon cancer, producing response rates of 13% to 23% and median remission duration of 6 to 8 months [19-21]. These results suggest that CPT-11 is one of the most active single agents for chemotherapy in colorectal cancer [20-22].

On the basis of promising single-agent activity of CPT-11 in colorectal cancer, different schedules of 5-FU-based regimens (with or without LV) combined with CPT-11 have been evaluated or are currently under investigation [23-26]. Preclinical data on human colon tumor cell lines and tumor xenografts suggested additive to synergistic antitumor activity for the combination of both drugs if CPT-11 preceded 5-FU [27-30]. Although it was reported earlier that 5-FU may decrease the carboxylesterase-mediated conversion of CPT-11 to its active metabolite SN-38 (7-ethyl-10-hydroxy-camptothecin) [31], recent data on human hepatic microsomes showed no substantial alterations of the metabolism of CPT-11 to SN-38 by 5-FU [32]. Furthermore, no

<ul style="list-style-type: none"> • TS Inhibitor 5-FU fluoropyrimidine prodrugs <ul style="list-style-type: none"> • S-1 • Capecitabine; approved • UFT; approved – Japan 5-FU modulators <ul style="list-style-type: none"> • Leucovorin; approved • MTX • Ethynyluracil Antifolate antimetabolites <ul style="list-style-type: none"> • ZD1694 (Tomudex); approved • ZD9331 • BW1843U89 • AG337 • LY231514 n DHFR Inhibitors <ul style="list-style-type: none"> • TMTQ • MDAM n GARFT Inhibitors <ul style="list-style-type: none"> • AG2034 • Lemetrexol • LY309887 	<ul style="list-style-type: none"> • Molecular targets • Topoisomerase I Inhibitors <ul style="list-style-type: none"> • CPT-11; approved • BNPI-1350 • TAS-1 • iAlkylators <ul style="list-style-type: none"> • JM216 • Oxaliplatin; approved • Mechanism-based combination <ul style="list-style-type: none"> • ZD1694/ CPT-11 • 5-FU/LV/ CPT-11; approved • FU/LV/Oxaliplatin; approved • JM216/ CPT-11 • DHFR/ GARFT inhibitors • TMTQ/ AG2034 • TMTQ/5-FU/ LV or ZD1694 • EGFR <ul style="list-style-type: none"> • Eribitux (C225) • Iressa • VEGF • Combination of target-directed drugs
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Abbreviations: MTX, methotrexate; TMTQ, trimetrexate; MDAM, g-methylene-10-deazaaminopterin; CPT-11, irinotecan; DHFR, dihydrofolate reductase; GARFT, glycylamide ribonucleotide formyltransferase; TS, thymidylate synthase; DPD, dihydropyrimidine dehydrogenase; Tpsathymidine phosphorylase; EGFR, epidermal growth factor; VEGF, vascular epidermal growth factor.

Table 1 - New drugs in colorectal cancer

pharmacokinetic interactions between 5-FU and CPT-11 were observed.

Recently, Saltz et al reported the results of an extended phase I study in patients with metastatic colon cancer using a weekly schedule of bolus 5-FU (500 mg/m²) plus low-dose LV (20 mg/m²), together with CPT-11. The maximum-tolerated dose (MTD) for CPT-11 in this combination was 125 mg/m² given on a weekly-times-four schedule, with neutropenia being the dose-limiting toxicity (DLT). Furthermore, the incidences of grade 3 or higher diarrhea and neutropenia for weekly bolus LV/5-FU regimens are similar to those observed with 5-FU_{24h}/LV, as reported previously [33,34]; however, the antitumor efficacy of the 5-FU_{24h}/LV regimen appears to be higher [10].

The concept of this regimen was further supported by preclinical data demonstrated a lack of cross-resistance between CPT-11 and 5-FU based on different molecular mechanisms of cytotoxic action and the potential for syner-

gistic antitumor activity between both drugs in vitro and in vivo [27-30].

Investigational and Approved Drugs in the Treatment of Patients with Advanced Colorectal Cancer

Table 1 is a brief outline of new investigational and FDA approved drugs in colorectal cancer. While FU/LV, irinotecan, oxaliplatin, Avastin, Eribitux and Iressa are drug approved alone and in various combinations; other drugs and combinations are now being pursued experimentally and it is likely that some may be considered for approval in the future.

Mechanism-Based Selective Metabolism of 5-FU Prodrug

One direction vigorously pursued is the development of orally administered fluoropyrimidine prodrugs with greater therapeutic efficacy and selectivity than 5-FU. Because the oral bioavailability of 5-FU is low, several 5-FU prodrugs

with bioavailability close to 100% have been synthesized and are at various stages of preclinical and clinical development. This includes UFT/LV, FU/EU, S-1 and capecitabine (Xeloda).

S-1

S-1, an oral 5-FU prodrug, is a combination of FT, CDHP, and potassium oxonate (Oxo) in a fixed molar ratio of 1.0:0.4:1.0, predetermined for optimal selective in vivo tissue distribution. CDHP is a potent inhibitor of DPD. Oxo is an inhibitor of phosphoribosylpyrophosphate transferase (PRPPT), an enzyme that metabolizes 5-FU to 5-FUMP. Inhibition of PRPPT in gastrointestinal tissues was reported as a possible mechanism for reduction of diarrhea, generally associated with 5-FU regimens. Daily oral administration of S-1 at the MTD or below to rats bearing advanced colorectal carcinoma showed a greater antitumor efficacy with less toxicity than 5-FU or FT. An improved therapeutic index for S-1 compared with 5-FU and FT was observed in sarcoma. The antitumor activity of S-1 has been evaluated in mice bearing C-26, which is relatively resistant to 5-FU, and in rats bearing sensitive Ward tumors. The antitumor activity was compared with FT (oral) and with continuous IV infusion of 5-FU using 7- and 28-day schedules at the MTDs. In addition, a 100% complete tumor regression (CR) rate was achieved at doses lower than the MTD with no detectable host toxicity, suggesting a significant safety margin with S-1 over FT or FU as optimal responses with these agents were only achieved at the MTD. Of interest is that the 50% CR rate was achieved with the 7-day continuous IV infusion of 5-FU, but not the 28-day protracted infusion. The therapeutic indices for S-1, FT, and 5-FU were 5.3, 1.0, and 1.0, respectively. In mice bearing C-26, or nude mice bearing human HCT-8 xenografts, both of which are relatively resistant to 5-FU, lower CR rates were achieved with S-1 treatment than was

observed in rats bearing Ward tumors.

The therapeutic selectivity of S-1 is based on the following premises: (1) high oral bioavailability; (2) differential tumor expression of the key activation enzyme, namely, TPase; (3) a lower level of the 5-FU degradation enzyme, DPD, in tumor tissues than in normal tissues; and (4) a lower level of the target enzyme, TS, in tumor tissues.

The data demonstrated that S-1 is not another formulation mimicking 5-FU infusion. This conclusion is based on the following observations. (1) The high therapeutic index and efficacy profile of S-1 in model systems could

selective tumor tissue activation of S-1 by TPase, together with inhibition of DPD by CDHP and inhibition of PRPPT by Oxo, are predictive for curative therapy by S-1. Measurement of the plasma pharmacokinetics parameter of 5-FU either derived from 5-FU or its prodrugs is, therefore, unlikely to be a useful predictor of response, but may be predictive for toxicity. (4) Results from our laboratory indicate that the cure rate achieved with S-1 is directly associated with higher and sustained levels of treatment-induced apoptosis, which could not be produced with 5-FU treatment.

UFT/LV

UFT, another oral 5-FU prodrug, is a combination of FT and uracil in a molar ratio of 1:4; this was determined to be the optimal therapeutic ratio by in vitro and in vivo studies. Uracil inhibits the degradation of 5-FU formed from FT by inhibiting DPD. Co-administration of uracil and FT enhanced the concentration of 5-FU in tumor and potentiated the antitumor activity of FT. The antitumor efficacy of UFT and FT was evaluated in rats bearing advanced colorectal cancer using 7- and 28-day oral administration schedules. The MTDs of UFT and FT were 80 and 200 mg/kg/d for 7 days and 60 and 150 mg/kg/d for 28 days, respectively. At the MTD, the antitumor efficacy of UFT was superior to that of FT. The sustained CR rates were 75% for UFT on both schedules, and 19% on the 7-day and 0% on the 28-day schedule for FT. In this model, LV was shown to further increase the antitumor activity of UFT.

The antitumor activity of UFT has been demonstrated in patients with colorectal and gastric cancer. Clinical trials of 28-day oral administration of UFT in combination with low- or high-dose LV demonstrated significant antitumor activity with manageable toxicity among patients with metastatic colorectal cancer. Phase III trials of oral UFT plus LV

Of interest is that the 50% CR rate was achieved with the 7-day continuous IV infusion of 5-FU, but not the 28-day protracted infusion

not be reproduced by 5-FU administered by various schedules, including a 28-day protracted continuous IV infusion. In a preclinical model system, a 28-day protracted continuous IV infusion of 5-FU at the MTD (12.5 mg/kg/d x 28) yielded a response rate significantly less than that of oral daily administration of S-1 (0% v 100%). (2) Based on the results generated in this laboratory and by other groups, the observed decrease in diarrhea and neurotoxicity with S-1, but not with 5-FU is likely due to selective inhibition of PRPPT by Oxo and DPD by CDHP, respectively. (3) Pharmacokinetic studies demonstrated that, although the plasma concentrations of 5-FU derived from continuous IV infusion and from oral S-1 at the MTD were similar, differences in response rates appear to be due to cellular factors. It is likely that

in the treatment of patients with advanced colorectal cancer are currently underway.

Capecitabine

Capecitabine (N4-pentoxy-carbonyl-5'-deoxy-5-fluoro-cytidine; Xeloda, Hoffman-La Roche, Nutley, NJ) is a new oral fluoropyrimidine carbamate, converted to 5-FU by three enzymatic steps (Figure 1). Capecitabine is absorbed intact in the gastrointestinal tract and initially metabolized in liver by carboxylesterase to 5'-deoxy-5-fluorocytidine (5'-dFCR). It is subsequently metabolized to 5'-deoxy-5-fluorouridine (5-d5-FUR) and doxifluoridine by cytidine deaminase, is expressed in higher concentrations in tumor tissues than normal tissues, and is subsequently converted to 5-FU by

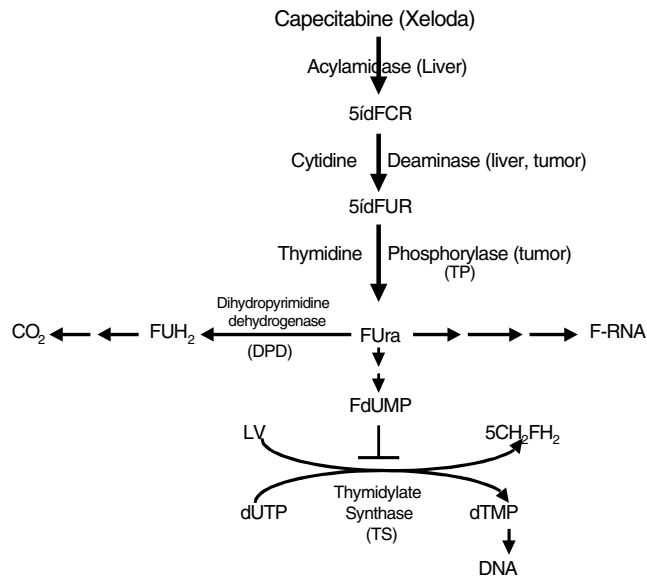


Figure 1

Preclinical studies in model systems demonstrated the selectivity of capecitabine in tumor tissue

TPase, which is present at higher levels in the tumor. Since the conversion to doxifluoridine does not take place in the gut, the therapeutic selectivity of capecitabine should be higher than that of doxifluoridine.

Preclinical studies in model systems demonstrated the selectivity of capecitabine in tumor tissue. Capecitabine was effective against 5-FU-resistant human colon cancer xenografts. The 5-FU concentrations in tumors was more than 30 times higher with capecitabine than with 5-FU compared at the MTD. In addition, tumor concentrations of 5-FU were higher than in plasma (127-fold) or muscle (22-fold). The antitumor activity and selectivity of capecitabine (oral administration) was evaluated and compared

with 5-FU (IV push) on a schedule of 5- days a week, 2-day rest, for 3 weeks in several model systems, including highly 5-FU-resistant murine C-26, nude mice bearing human colon carcinoma, and 5-FU-sensitive and -resistant HCT-8. The results, summarized in Table 3, indicate that capecitabine is highly active against 5-FU-resistant tumors. Capecitabine was also effective against CSF280, HCT116, and COLO205 human colon xenograft models, whereas 5-FU was effective only in CFX280 tumors. In three models, 5-FU concentrations in tumor were 23- to 25-fold higher after capecitabine administration than after 5-FU, with both drugs administered at their MTD. In addition, capecitabine-treated animals had higher levels of 5-FU in tumor than in plasma (114- to 209-fold higher) and muscle (22-fold higher), whereas 5-FU was not selectively distributed to tumors. The therapeutic index of capecitabine was also superior to that of 5-FU or UFT. Furthermore, efficacy of capecitabine consequently correlated well with the ratio of TPase and DPD in tumor. Preclinical studies suggest that efficacy of capecitabine can be optimized by selecting patients

with a high ratio of PTAs to DPD.

Preliminary clinical results of capecitabine with or without LV in patients with solid tumors have been reported. Recently, capecitabine was approved by the FDA as a second-line therapy for patients with breast cancer.

5-Ethynyluracil

5-Ethynyluracil (EU; 776C85) is a potent mechanism-based irreversible inactivator of DPD, the enzyme that rapidly degrades 5-FU. EU markedly increases systemic exposure to 5-FU in laboratory animals and in cancer patients. For example, EU increases the plasma half-life of 5-FU in patients from approximately 10 minutes to 5 hours. To evaluate further the role of inhibition of DPD in correlation with the therapeutic efficacy of 5-FU, the antitumor activity and toxicity of 5-FU alone and in combination with EU in rats bearing advanced colon carcinoma were evaluated. Two schedules were studied: (1) 5-FU administered by IV push daily for 4 days (daily x 4); and (2) 5-FU by IV push once a week for 3 weeks (weekly x 3). EU as administered at 1 mg/kg 1 hour

before 5-FU and for 2 additional days post-5-FU therapy. The MTDs of 5-FU alone were 35 and 100 mg/kg, and for 5-FU plus EU were 10 and 15 mg/kg for the daily x 4 and weekly x 3 schedules, respectively. The dose limiting toxicities were diarrhea and stomatitis both for 5-FU alone and for 5-FU in combination with EU. Although EU itself was neither toxic nor active, it markedly improved the efficacy and therapeutic index of 5-FU. The combination of 5-FU and EU produced 100% sustained CRS on both schedules. The therapeutic index was ≤ 1 for 5-FU alone and 6 for 5-FU plus EU. EU also increased the efficacy and therapeutic index of 5-FU in tumor-bearing mice. These results are similar or better than those achieved with UFT, demonstrating further the important role of inhibition of DPD in reversal of resistance to 5-FU. These preclinical results provided in part the basis for the design and implementation of clinical protocols. The results of phase I clinical and pharmacologic studies of 5-FU with EU have been reported. Phase III clinical trials of 5-FU with EU in patients with advanced colorectal cancer are underway in multiple cancer centers.

The antitumor activity and pharmacokinetics of EU in combination with FT administered orally for 7 days to rats bearing advanced colon carcinoma was also evaluated

Since preclinical results demonstrated that the therapeutic efficacy of 5-FU and EU can be further modulated by LTV in vitro, and in vivo, the use of

double modulation of 5-FU by EU and LV should be evaluated clinically.

The antitumor activity and pharmacokinetics of EU in combination with FT administered orally for 7 days to rats bearing advanced colon carcinoma was also evaluated. The MTDs of FT alone and FT plus EU were 200 and 5 mg/kg/d, respectively. At the MTD, while FT alone achieved only 19% sustained CRS, 100% sustained CRS were achieved with FT plus EU without inducing significant host toxicity. Pharmacokinetic studies show that the area under the plasma concentration-versus-time curve of 5-FU generated from FT and FT/EU at their MTD was 50 and 27 $\mu\text{mol/L/h}$, respectively. The results from preclinical studies provided the basis for EU with 5-FU or FT as once-daily oral therapy for patients with colorectal cancer.

Markers Associated with Response to Chemotherapy in Advanced Colorectal Cancer

Many biochemical and molecular markers evaluated to date have been found useful in the design of new and more selective therapy for advanced colorectal cancer. Approved drugs for the treatment of colorectal cancer include: the so-called Mayo Clinic regimen of 5-FU + LV daily x 5; weekly and monthly CPT-11; and the triple combination irinotecan + 5-FU +LV.

TS, TP and DPD

Biochemical and molecular markers associated with the response to 5-FU or its prodrug capecitabine include the enzymes thymidylate synthase (TS), thymidine phosphorylase (TP), and dihydropyrimidine dehydrogenase (DPD). Capecitabine is an example of a 5-FU prodrug which requires three-step activation before it gets to 5-FU. The final activating enzyme is thymidine phosphorylase (TP), which is expressed in tumor tissues over the normal adjacent tissue [35]. Once 5-FU is

generated from capecitabine, there are three metabolic pathways available to it: (1) It can be metabolized and incorporated into RNA; (2) it can be metabolized to the active metabolite of 5-FU, FdUMP. FdUMP acts as a very potent

Data from Japan shows the distribution of thymidine phosphorylase in normal and counterpart tumor tissues

inhibitor of the enzyme thymidylate synthase (TS), which is necessary for DNA synthesis. (3) It can degrade into CO₂. Eighty-five percent to 90% of the injected dose of 5-FU converts to CO₂ within five to ten minutes. Therefore, it provides a target for developing a compound for the ablation of tumor tissue that has a high level of DPD. (Figure 1)

Data from Japan shows the distribution of thymidine phosphorylase in normal and counterpart tumor tissues [35]. A significantly higher level of thymidine phosphorylase is expressed in tumor tissues as compared to the normal tissue counterpart. Is this an expression in all tumors with colorectal cancer or in selected patients with this disease? It is likely that only a small percentage of patients with advanced colorectal cancer have this profile activity for thymidine phosphorylase; therefore, one would suspect that patients with high levels of thymidine phosphorylase are likely to respond to a drug like capecitabine.

The second question that arises based on this finding is whether in patients with tumors that have a low level of thymidine phosphorylase, the level of this enzyme can be increased to a level comparable to what is seen in patients with higher levels. CPT-11, taxanes and

radiation can induce increased levels of thymidine phosphorylase, so the idea of increasing the level of thymidine phosphorylase is promising.

What is the relationship between thymidine phosphorylase, dihydropyrimidine dehydrogenase and the response to

Not only can response be predicted; survival also can be predicted for the patient treated with 5-FU + LV

capecitabine? Patients with higher levels of thymidine phosphorylase are the sensitive patients; those who have lower levels of thymidine phosphorylase are those who do not respond to chemotherapy. Patients with low levels of DPD are the patients who have a better chance or likelihood of response to capecitabine versus the patient who has a significantly higher level of DPD.

However, not all patients with high levels of thymidine phosphorylase respond to therapy. Similar correlation was obtained in xenografts (Ishikawa et al) [36,37].

Danenberg, Leichman et al looked at the relationships between thymidylate synthase and the response to chemotherapy with 5-FU [38-40]. This data shows that the responders have a lower level of thymidylate synthase versus the patient who does not respond to chemotherapy with 5-FU + LV and has a higher level of this enzyme thymidylate synthase [38]. However, about 50% of patients who are not responsive to 5-FU have a low level of thymidylate synthase comparable to those patients who responded to chemotherapy. These data suggest that there are other factors involved not only thymidylate synthase.

Not only can response be predicted; survival also can be predicted for the patient treated with 5-FU + LV. Patients who have a high level of thymidylate synthase were studied versus patients who had a lower level of thymidylate synthase. Low levels of thymidylate synthase were found to impact on survival. In a study linking the three enzymes together, it was found that, in general, the responders that have a lower level of the three enzymes. Collectively, these are the patients who responded to 5-FU + LV with the Mayo Clinic regimen. The non-responders are the patients who have a varying level of DPD, TS and TP. Patients who have a high level of DPD are the patients who are not responding to chemotherapy with 5-FU based therapy [38].

When tumor tissue is treated with a DPD inhibitor like uracil to lower the level of DPD, the tumor that was highly resistant to 5-FU + LV can be converted to be highly sensitive to 5-FU therapy, so indeed modulation of the level of DPD in tumors could offer a therapeutic advantage. (Rustum, unpublished data)

Based on the combined data, a high level of TP would seem to predict for a response to capecitabine and low levels of this enzyme seemed to predict for a response to 5-FU + LV. DPD, the second marker, is a catabolic enzyme. If it

is low, it is a predictor for response for 5-FU + LV as well 5-FU prodrug capecitabine. Thymidylate synthase (TS) for target enzyme is low for both columns. These enzyme levels can and should be used to monitor patients and determine which patients should be treated with certain modalities.

Therapeutic Outcome in Advanced Colorectal Cancer Patients

The data in Figure 2 is a summary of the experience gained in previously untreated, metastatic colorectal cancer, namely FU/LV (weekly and bi-weekly) capecitabine bid x 14d q 3 and irinotecan (weekly) in combination with FU/LV (IFL) using the American and French schedule. The data indicate that while the overall response to FU/LV is approximately 10-15%, the combination yielded an overall response rate of approximately 40%.

Overall Survival

The data in Figure 3 is an overall summary of overall survival of previously untreated patients and treated with various drug combinations since 1980 to present. It is clear that significant advances have been made with the use of drugs in combination. During the last four years, however, the 20-22 month in overall survival remains constant. It is hoped that

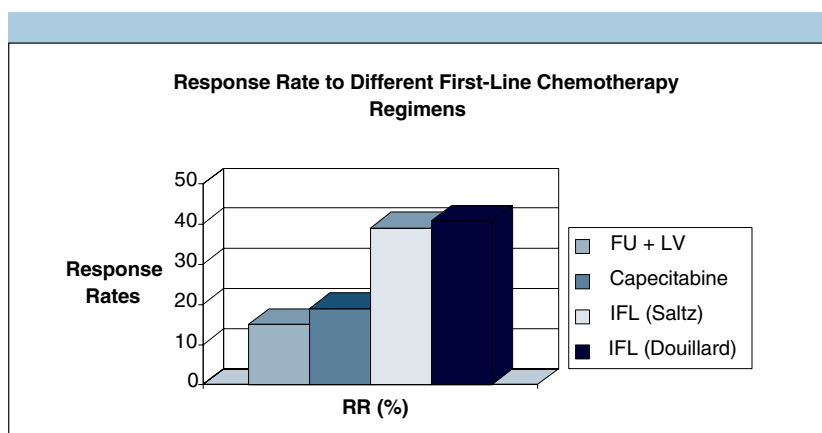


Figure 2 - Response Rate

newer drugs and better use of markers that improve on overall survival, quality of life and costs will continue.

Future Questions

Why do drugs with different mechanisms of action (e.g., CPT-11 and FU) yield a comparable response rate of around 20%-25% when used alone? Why, when these drugs are combined, are response rates increased and survival improved? With a response rate of between 50%-60%, why are a large number of patients still not benefiting from the combination?

There are hopeful signs and indications that the use of molecular markers can help identify individual patients with a unique biochemical and molecular profile, which can then be used to direct therapy and predict response. With this approach, it is hoped that toxicity and cost will be minimized and quality of life and curability of patients will be maximized.

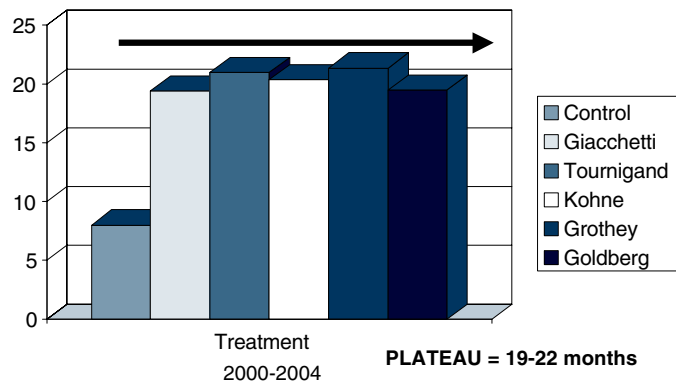


Figure 3 - Interventions incorporating 3 chemotherapeutic agents result in = survival

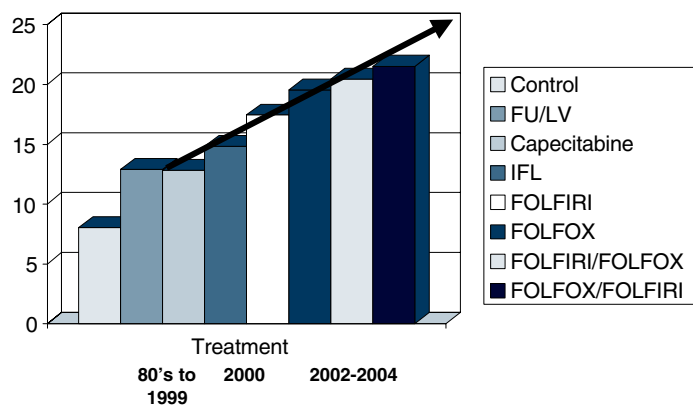


Figure 4 - Outcome of Patients with Metastatic Disease

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