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New directions in the treatment of colorectal cancer: a look to the future

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Abstract

Today, adjuvant 5-fluorouracil based therapy is known to significantly reduce the relapse rates and the risks of dying from resected colon cancer; chemotherapy approximately doubles overall survival of advanced colorectal cancer and second line treatment prolongs the survival of patients compared with best supportive care. At the molecular level a number of key genes are often mutated in cancer of the colon and some of these key regulators of apoptosis are discussed (p53 and bcl-2 family of proteins). Dihydropyrimidine dehydrogenase (DPD) activity may be a potential factor controlling fluorouracil (FU) responsiveness at the tumoral level and its importance is stressed. The rationale of combining FU with DPD inhibitors is fairly strong. Ethynyluracil, UFT and S1 pursue this strategy while capecitabine has another the rationale. Drug resistance should be at least partially overcome by combination chemotherapy (FU plus mitomycin, oxaliplatin, irinotecan) and combined modality (FU + RT) regimens. Improved surgical techniques and radiotherapy have substantially decreased local failure rates for rectal cancers. Finally, innovative treatment modalities such as anti-angiogenetic and antimetastatic agents, farnesyl transferase inhibitors, vaccine and gene therapy are in early clinical trials.

Keywords: 5-FU; Colorectal cancer; Thymidylate synthase; Dihydropyrimidine dehydrogenase; p53; Apoptosis; Mitomycin; Oxaliplatin; Irinotecan; Ethynyluracil; UFT; S1; Capecitabine

1. Introduction

Ten years ago the value of the adjuvant treatment of colon cancer was not proven, the patient benefit of palliative chemotherapy for advanced disease was under debate and no one would dare to talk about second-line chemotherapy in this disease. There was only one class of active drugs, the fluoropyrimidines, and the main focus of experimental and clinical research was whether biochemical modulation was effective or not. Local failure after rectal cancer surgery was very common and severely disabling. Today, 6 months of adjuvant 5-fluorouracil and leucovorin (5-FU + LV) is known to be better for the patient than 12 months of 5-FU/levamisole that in turn was proven to significantly reduce the relapse rates and the risks of dying from resected colon cancer; we know that chemotherapy approximately doubles overall survival of advanced colorectal cancer (CRC) and that second-line treatment prolongs the survival of patients compared with best supportive care; there are at least three classes of new, active agents and the main focus of ongoing clinical research is

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the combination of these new agents with the fluoropyrimidines. Improved surgical techniques with better lateral clearance and preoperative radiotherapy (RT) or postoperative RT+ chemotherapy have substantially decreased local failure rates for rectal cancers. Finally, innovative treatment modalities such as anti-angiogenic and antimetastatic agents, farnesyl transferase inhibitors, vaccine and gene therapy are in early clinical trials.

What follows is a concise review of some of the above achievements with an emphasis on the clinical aspects that are or should be under intense investigation in the near future.

2. A sharper focus on patient expectations

A patient with a recently diagnosed metastatic CRC discussing palliative chemotherapy may have several legitimate questions, as shown in Fig. 1. There will be some general questions that every patient will ask. Some other questions will depend upon whether the patient is symptomatic or not and whether he or she has been informed about the objective response rates.

Unfortunately the questions related to 'well being', 'feeling better' and 'benefit' (Fig. 1) have not been addressed as extensively as those related to objective response rates, time to disease progression and overall survival.

The Nordic Gastrointestinal Tumour Study Group has had a longstanding interest in these patient-related questions. A rather consistent pattern emerges that can guide the clinicians in discussing whether to initiate palliative chemotherapy or not. The evidence from the trials is that patients receiving chemotherapy have a median survival 5–6 months longer than that of patients who receive only best supportive care. This gain appears to hold whether treatment is given systemically [1–3] or via hepatic arterial infusion in case of liver metastases [4,5]. The magnitude of survival benefit is still rather uncertain since some of the trials are very small and since chemotherapy has also been given to some control patients upon request.

In patients with advanced but not yet symptomatic cancer, a Nordic study has shown that early chemotherapy significantly prolongs symptom-free survival and overall survival without reducing the quality of life [1,6]. It is not yet properly known whether one can safely start a 'wait and see' policy in an asymptomatic patient with very limited disease without compromising survival. This will require close monitoring with repeated tumour examinations and/or serum marker measurements with the intent to initiate chemotherapy prior to symptom development.

The results from the Nordic studies also strongly suggest that treatment improves survival by 8–12 months in patients who achieve a PR or CR and by at least 4 months in those with stable disease [7]. That a response to chemotherapy prolongs survival in an individual patient was also recently suggested by a trial using CPT-11 [8].

An objective response or disease stabilisation with a minimum duration of 4 months not only improves survival, but also substantially improves quality of life [9–11]. In fact, using modulated 5-FU-regimens, improved subjective response/improved quality-of-life occurs twice as frequently as objective responses. Improved response rates and duration of response continue, therefore, to be major endpoints of clinical trials in advanced CRC. However, since this will probably be reached only at the expense of higher toxicity and higher costs, two additional features are needed in research trials and in clinical routine: some measurement of patients' overall well-being, along with economical analyses and the development of response and resistance predictors working at an individual patient level. Alternatively, another way to reduce toxicity and costs could be to give shorter or intermittent treatments. It is, however, not known whether this will have the same favourable effects as continued cycles of chemotherapy until progression.

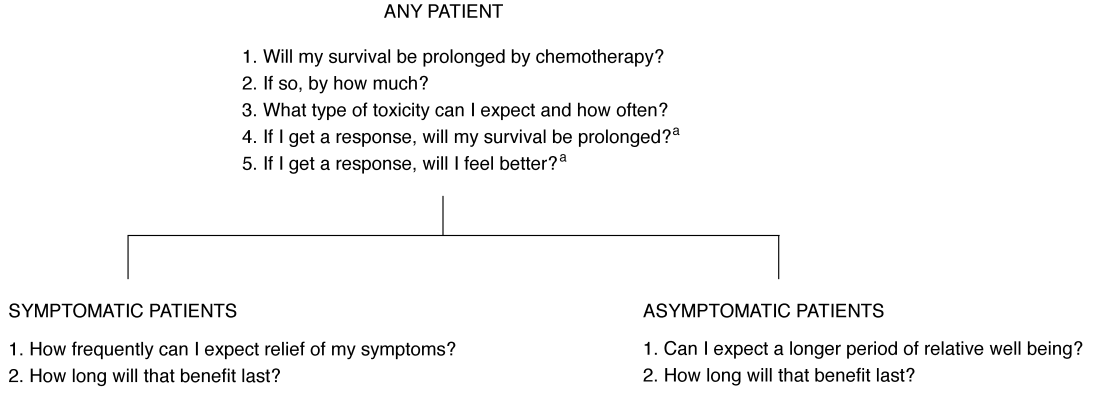

Fig. 1. Key questions by advanced colorectal cancer patients as a function of their condition. [a] Only in patients informed about objective responses.

3. Without forgetting cancer-related endpoints

Whilst clinical research on traditional antineoplastic agents must emphasise patient-related endpoints, the novel, so-called target-based antineoplastic therapies should initially be proved to have clinical anticancer activity. Demonstrating the proof of principle, i.e. that the novel agent is actually doing what it is supposed to do is not enough *per se* to pursue the clinical development of these new agents. Objective response is not a realistic endpoint for the majority of these compounds, given their proposed mechanism of action. Rather, duration of response, disease stabilisation and its duration and progression-free survival seem more appropriate primary endpoints for clinical trials using these drugs. These concepts have been integrated into the 'growth modulatory index' [12], and specific innovative trial designs are under active debate in this respect [13].

4. Specific predictors of response to chemotherapy

As long as chemotherapy of CRC was confined to 5-FU, the benefit of determining the chances of response in individual patients was limited to sparing resistant patients from experiencing unnecessary toxicity. The value of this is even more relevant now when several potentially effective drugs are available on the market. This means having something to offer to patients who are predicted to be resistant to 5-FU. One would be tempted to investigate the major determinants of activity and resistance to 5-FU, raltitrexed, CPT-11 and oxaliplatin and then make the selection of the drug/drugs most likely to be effective against that particular tumour.

There are three problems with this paradigm. The first is the complexity of the mechanisms of action of the drugs mentioned. High thymidylate synthase (TS) levels predict for resistance to 5-FU and raltitrexed; however, the biochemical determinants of these drugs' activity may be at many different sites from the key enzyme.

The second is the intrinsic difficulty in obtaining reliable results from the specific assays; TS levels in the primary tumour may not correlate well with those in the corresponding metastases and the same may be true from one metastasis to another in the same patient; in addition, which feature of p53 to measure and by which assay is a challenge.

The third are the ethical problems connected with obtaining specimens for such determinations. Measuring a single biochemical or molecular parameter may thus not lead to meaningful predictions. For TS, both gene expression and protein levels correlate with clinical outcome of patients treated with specific 5-FU based regimens, albeit weakly [14,15]. Should most of the above problems be solved say, for example, by a microarray technology, the short duration of responses should still be taken into account when advising a patient whether to have an additional biopsy to determine drug sensitivity.

At the molecular level, a number of key genes are often mutated in colon cancer and some of these are key regulators of apoptosis (p53 and the Bcl-2 family of proteins). Apoptosis is responsible for the programmed death of cells with mutant DNA, therefore, it has been proposed that the ability of a patient's cells to undergo apoptosis following the administration of chemotherapeutic drugs will influence the overall effects of treatment [16]. *TP53* status of colonic tumours is predictive of response to modulated 5-FU in some studies [17], but not in others. However, there are a number of problems in determining such parameters.

The measurement of an apoptotic index in tissue sections is not easy and relies on a number of assumptions [18]. Also, the TdT in the *in situ* nick-end labelling method to detect and quantitate apoptosis is suboptimal in the gastrointestinal tract [19]. Moreover, immunohistochemical staining intensity may vary considerably depending on the length of time the slides have been stored prior to staining [20] and the quality of the antibody. Thus, slides may be difficult to interpret.

Dihydropyrimidine dehydrogenase (DPD) activity may be a potential factor controlling 5-FU responsiveness at the tumoral level. This hypothesis was first tested in experimental tumour models [21] and subsequently extended to the clinic [22]; the lower the enzyme activity, the greater the cytotoxicity. This parameter, along with TS and thymidine phosphorylase determination has also recently been studied in advanced CRC patients within a Southwest Oncology Group (SWOG) trial showing a 100% response rate to infusional 5-FU plus weekly LV, in patients having low TS, low DPD and low thymidine phosphorylase [23].

5. Teaching old drugs new tricks

Despite the host of new cytotoxics and novel treatment strategies to be mentioned below, standard treatment of advanced CRC is still 5-FU-based chemotherapy. A few concepts are widely accepted: unmodulated bolus 5-FU is in general no longer recommendable; biochemical modulation is needed when using bolus 5-FU; whether infusional 5-FU needs biochemical modulation is uncertain; and hybrid or alternating bolus infusional regimens are probably more active than either schedule alone.

For the near future, the following fluoropyrimidine-targeted programmes can be considered.

5.1. Interfering with 5-FU catabolism

More than 80% of an administered dose of 5-FU is eliminated by catabolism through plasma DPD [24]. In a prospective study on 185 cancer patients, DPD activity showed a unimodal distribution fitting a Gaussian distribution [25]. One may thus wonder if individual dose tailoring should be extensively pursued in this way; however, since approximately 3% of these patients are located below the threshold for increased risk of developing severe 5-FU toxicity, the practical interest of determining plasma DPD before 5-FU treatment must be carefully weighed in terms of the cost–benefit balance.

Tumoral DPD inversely correlates with objective response [23]. The rationale of combining 5-FU with DPD inhibitors is thus fairly strong. 5-Ethynyluracil [26,27] and the new oral 5-FU prodrugs UFT and S1 are examples of such a strategy in the search for potentially more selective targeting [28]. It is worth remembering that the other oral fluoropyrimidine in a phase III clinical trial, capecitabine, does not contain DPD inhibitory activity. Rather, the rationale for its development has been the need for thymidine phosphorylase for activation. The high level of this enzyme in tumours compared with normal tissues may leave hope for an enhanced selectivity [29]. However, at the same time, high levels of this enzyme predict for resistance to 5-FU, the active metabolite of capecitabine [23], making the rationale a bit shaky. The presence or absence of DPD inhibitory activities associated with oral fluoropyrimidines may explain their different spectrum of toxicity [30,31] and may justify the classification of oral fluoropyrimidines into those containing and those not containing DPD inhibitory activity (Fig. 2).

5.2. 5-FU and apoptosis

5-FU induces acute apoptosis in the crypt compartments of murine small and large intestine following bolus intraperitoneal administration. This response is greatly diminished in *TP53* null mice [32]. An analysis of the relationship between this acute apoptosis and the long-term intestinal toxicity induced by multiple high doses of 5-FU has revealed that apoptosis is only one factor determining toxicity and that the effects of the drug on cell cycle progression and the ability of cells to proliferate and repair the damaged tissue are also important. None the less, the intestinal toxicity induced by 5-FU is also reduced in *TP53* null mice, demonstrating that the expression of a single gene in otherwise identical mice can alter drug response [33]. The implications of these findings are that drug resistance should be at least partially overcome by combination chemotherapy (5-FU plus mitomycin, oxaliplatin, CPT-11) and combined modality (5-FU + RT) regimens.

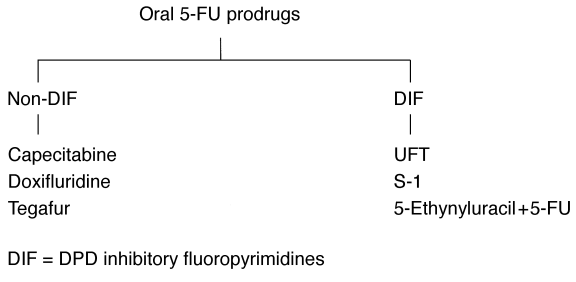

Fig. 2. Suggested 5-fluorouracil (5-FU) prodrug classification.

5.3. The future of infusional 5-FU

Whether given for 24, 48, 72 h or for protracted periods of times (months), continuous infusion (CI) 5-FU behaves very similarly in terms of spectrum of toxicity, its severity, maximum tolerated dose and activity. There are two open questions on the prospects of CI 5-FU: first does LV modulation of CI 5-FU enhance its efficacy? And will the oral 5-FU prodrugs make CI 5-FU outdated? The most promising reports are those where LV is also given for protracted periods of times, but phase III studies with any infusional 5-FU regimen ± LV are still ongoing. The same is true for comparative studies with oral 5-FU prodrugs.

5.4. Further development of combined bolus-infusional regimens

It is known that approximately 15% of patients progressing during bolus 5-FU treatment still respond to infusional 5-FU. Preclinical evidence is strongly supportive of the concept that bolus 5-FU predominantly kills cells via the RNA-directed mechanism, whilst CI 5-FU is cytotoxic via TS inhibition. Further elaboration of this hypothesis has provided experimental evidence that CI 5-FU eradicates resistance to bolus 5-FU, that the two schedules of 5-FU administration synergise and that, given the schedule-specific mechanism of action of the fluoropyrimidine, its modulation should also be schedule-specific [34–36]. Hence a number of clinical strategies are derived. (1) Bolus and infusional 5-FU can be given back to back in order to exploit the described *in vitro* synergy. Indeed the De Gramont schedule reflects this strategy [37]. (2) Schedule-specific biochemical modulation has been optimised in an alternating regimen of sequential methotrexate (MTX)→bolus 5-FU alternated with CI 5-FU plus LV [38] (Fig. 3). This regimen produced higher response rates and longer progression-free survivals than modulated bolus 5-FU in a recently completed randomised study [39]. It is now being refined in both components with a number of substitutions/additions: mitomycin C has been added to the infusional part, oxaliplatin will be added to the

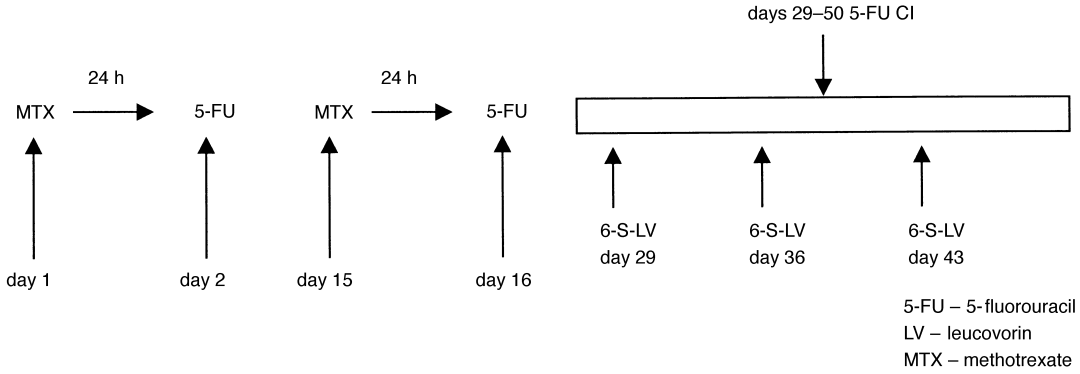

Fig. 3. Schedule-specific biochemical modulation of 5-FU.

bolus part, methotrexate will be substituted by trimetrexate in the bolus part as well. (3) The two strategies could be combined, i.e. sequential MTX or trimetrexate→ bolus 5-FU could be immediately followed by CI 5-FU + LV.

6. Preoperative and postoperative chemotherapy in the metastatic setting

Two new features make the idea of using surgery after chemotherapy appealing in certain settings of advanced CRC. The response rate to the most sophisticated regimens (chronomodulated 5-FU plus oxaliplatin) can be as high as that of hepatic arterial chemotherapy (50–60%) [40,41]. For fit patients with liver or lung metastases recent reports indicate very encouraging mid-term results with surgical eradication in patients who were not surgical candidates because of unresectable disease [42]. This sequential treatment may no longer be exceptional once greater than 50% response rates are routinely obtained. None the less this approach must still be regarded as highly experimental. Similarly, postoperative intra-arterial fluoropyrimidines may become standard adjuvant treatment in the rare cases of radically resected hepatic metastases, if the promising results reported recently in this setting [43,44] are confirmed after an appropriate follow-up time.

7. New cytotoxic agents

The topoisomerase I inhibitor CPT-11 prolongs survival as a second-line treatment in patients resistant to 5-FU [45,46]. Despite the side-effects of this agent, the quality of life is maintained during treatment and CPT-11 is now regarded as standard second-line treatment after 5-FU failure [47,48].

Moving CPT-11 to front-line chemotherapy is a logical step in its further clinical development, however, since its activity as single agent is not higher than that of 5-FU therapy, its role as single agent may be limited. Phase I trials suggest that the combination of CPT-11 and 5-FU/LV is feasible and has a high degree of activity and the preliminary analysis of the results of two phase III studies in first-line treatment suggest more efficacy compared with modulated 5-FU alone [49,50]. These data are the basis for the recently launched trial of 5-FU + CPT-11 in the adjuvant setting of this disease. Another area of CPT-11 development is the possibility to interfere with its activation to SN-38 by agents such as cyclosporin, phenobarbital and valproate.

The new platinum compound oxaliplatin has low activity as a single agent. But there is evidence that the combination of 5-FU/LV/oxaliplatin is active as second-line treatment after 5-FU failure [51] and that the combination is more active than 5-FU/LV in first-line treatment [52]. Comparative studies with CPT-11 are in progress. The clinical development of oxaliplatin has been linked to the concept of chronomodulation [40,41]. In many of these trials it is not clear whether the very high activity reported is due to the chronomodulation or to the cisplatin analogue. However, since (a) oxaliplatin added to the De Gramont schedule (non-chronomodulated) produced excellent results; and (b) chronomodulated 5-FU was not superior to flat continuous infusion 5-FU in a recent large randomised trial [53], it is likely that oxaliplatin is the major contributor to the high efficacy reported in the chronomodulated trials. This also provides a rationale for the recently launched adjuvant trial of 5-FU (De Gramont regimen) plus or minus oxaliplatin.

The activity of several other agents in first-line treatment has been shown in phase II and/or large phase III trials. Such is the case for the TS inhibitor, raltitrexed, the previously mentioned oral 5-FU prodrugs and trimetrexate, a lipophylic folate analogue that inhibits dihydrofolate reductase. Although these drugs have interesting and promising activities, it is unlikely that significantly higher response rates and longer survival will be obtained with any of these drugs as single agents. Rather, their benefit will likely rely upon a

favourable toxicity profile and convenient schedules of administration.

The combination of 5-FU and raltitrexed, raltitrexed and CPT-11, raltitrexed and oxaliplatin and oxaliplatin and CPT-11 could also show high activity. All these combinations are at present being investigated in phase I and II trials [54,55]. The oral 5-FU prodrugs ± DPD inhibitory activity could possibly replace 5-FU in the different combination regimens.

The greatest challenge will probably not be to show a drug combination that is superior to the 'standard' combination 5-FU and folinic acid, but to determine the exact sequence of the different drug combinations and the place and indications of all the different drug combinations. Another important question regarding the sequence of the different treatment options is whether it is preferable to the patient starting with a relatively non-toxic regimen and using the newer, more toxic agents after disease progression or to start with one of the above new drug combinations right from the beginning. The outcome of ongoing large multicentre studies looking at progression-free survival, quality of life and clinical benefit will answer these questions.

8. Novel approaches to adjuvant treatment

One of the most interesting challenges facing the clinical community is the design of clinical trials to test the worth of non-cytotoxic therapeutic agents for colorectal cancer. Traditional phase II studies seeking to demonstrate conventional efficacy in advanced disease, followed by comparative phase III trials against standard therapy cannot be appropriate for, say immunological agents, which are most likely to be effective against minimal residual disease. Rather than using conventional endpoints (response, progression-free and overall survival, etc.) which are likely to consistently undervalue these novel antineoplastic agents, it would be more logical to design trials with hypothesis-led endpoints so that scientific proof of principle could be established. An example could be to demonstrate activation of antigen-specific cytotoxic T-lymphocytes following vaccination against a relatively tumour-specific antigen like carcinoembryonic antigen. Once safety and tolerability has been described in phase I trials and proof of principle established in relatively advanced disease, then serious consideration should be given to advancing to a randomised trial in an adjuvant or minimal residual disease setting. Still, in order to take that step, some proof of classic anticancer activity has to be demonstrated (see above). It may be possible to allay the anxiety that some clinicians might have about taking a relatively 'untried' agent into a trial involving thousands of patients by linking it, using a factorial or 2×2 trial design, to a randomised study comparing more mainstream chemotherapeutic options (assuming that there was no interaction between the different agents).

There are several promising classes of non-cytotoxic agents in various stages of development which are likely to be tested in the adjuvant arena.

There is an increasingly well recognised series of steps involved in the process of metastasis. The earliest is degradation of connective tissue and cell basement membrane as a component of tumour invasion. The cancer cell can synthesise and secrete different members of the family of matrix metalloproteinases, including collagenase, matrilysin and elastase. The activity of these enzymes is controlled by tissue-specific inhibitors, and potent and specific inhibitors of the metalloproteinases have been developed, with marimastat being the most clinically advanced. Early clinical trials have defined a tolerable oral dose of 10 mg b.i.d. (twice a day) associated with shoulder girdle pain and stiffness and a reduction in the rate of rise of serum carcinoembryonic antigen (CEA) concentration in patients with advanced CRC. Although marimastat is not a cytotoxic, it is possible that it could induce a state of tumour dormancy and extend relapse-free and perhaps overall survival if administered in an adjuvant setting [56].

A direct immunological approach to CEA-bearing tumours has been developed by the laboratory of Tumour Immunology and Biology at NCI using inoculation with a recombinant vaccinia virus that expresses the human *CEA* gene (rV-CEA). Since vaccinia elicits both humoral and cell-mediated immune responses, co-presentation of protein products with vaccinia may enhance immunogenicity and increase the possibility of tumour rejection. Based on preclinical evidence of efficacy, a phase I study in patients with metastatic adenocarcinoma using an escalating dose administration of the rV-CEA vaccine was completed having recruited 26 patients [57]. The vaccine was well tolerated and it was possible to demonstrate specific cytotoxic T-lymphocyte activity against CEA-expressing tumour cells in 5 out of 5 patients tested. It is likely that a vaccination strategy of this sort will be tested in the adjuvant setting, probably in combination with 5-FU and LV.

Virus Directed Enzyme Prodrug Therapy (VDEPT) is based on the introduction into a cell (using viral vectors) of a gene encoding a foreign enzyme which converts a relatively non-toxic agent (the prodrug) into an active cytotoxic compound [58]. Several enzymes have been used including the *Escherichia coli*-derived cytosine deaminase (CD) which converts the non-toxic prodrug 5-fluorocytosine (5-FC) to the cytotoxic agent 5-FU (Fig. 4). A benefit of the enzyme-prodrug is the so-called bystander effect in which active drugs can diffuse from transduced cells expressing the activating enzyme, causing the death of untransduced cells in the vicinity. This is a very important phenomenon, as it is

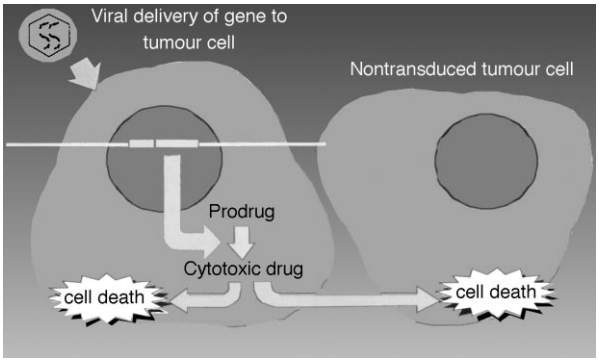

Fig. 4. VDEPT with killing of 'bystander cell'.

unlikely that every tumour cell will be transduced with the prodrug activating enzyme using viral delivery. In addition, transcriptional control of the CD enzyme by the CEA promoter means that even if a virus carrying this gene cassette infected all cells, the enzyme would only be transcribed in CEA-positive cancer cells, imposing a significant degree of specificity [59]. It is possible to envisage locoregional administration of adenovirus carrying the enzyme genes perioperatively, via the portal vein to patients who have undergone apparently curative resection of their primary colorectal cancer. Two to three days after adenoviral administration, the prodrug would be administered systemically. Given the favourable kinetics of microscopic metastatic growth following resection of the primary CRC, VDEPT could provide a useful adjunct to systemic adjuvant chemotherapy.

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