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A Glimpse of the Future. New Directions in the Treatment of Colorectal Cancer

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This paper overviews new directions in colorectal cancer therapy, focusing on adjuvant therapy, new cytotoxic agents and novel approaches. Reduced mortality with adjuvant therapy is mainly achieved in resectable colorectal cancer, using regimens of 5-fluorouracil (5-FU) plus leucovorin, 5-FU plus levamisole and 5-FU plus radiation. Not all patients are suitable for adjuvant therapy, and a better definition of prognostic factors will improve selection of patients for therapy. Several thymidylate synthase inhibitors are in development, including 'Tomudex'™ (raltitrexed, previously known as ZD1694), which has reached phase III studies, LY 231514, AG 331, AG 337, BW 1843 U89 and ZD 9331. Topoisomerase I inhibitors (e.g. CPT II, topotecan), ethynyluracil, oxaliplatin and 5-FU prodrugs are also promising therapeutic agents. Novel approaches include angiogenesis inhibitors, drugs which can stimulate apoptosis, generation of cytotoxic drugs from non-toxic prodrugs at tumour sites and conjugation of anti-tumour agents to polymeric carriers. Various modalities of specific and non-specific immunotherapy are also under research. These advancements promise improvements in the prognosis of colorectal cancer patients. Copyright © 1996 Elsevier Science Ltd

Key words: colorectal cancer, adjuvant therapy, thymidylate synthase, topoisomerase, immunotherapy, apoptosis

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INTRODUCTION

DURING THE last decade, we have gained exciting new insights into the genetic and biochemical changes leading to malignancy. Of fundamental importance are the studies of the genetic aberrations that occur in colorectal cancer. Vogelstein and colleagues have provided extensive evidence that there is an accumulating series of specific chromosonal and genetic changes that accompany (and perhaps cause) the transition from normal colonic mucosa to adenoma, adenocarcinoma and finally to metastatic cancer [1]. This basic knowledge will certainly help in the development of new approaches for the prevention and treatment of colorectal cancer, hopefully leading to an improved prognosis and survival for patients.

This paper will give an overview of new directions in the treatment of colorectal cancer. The focus will be on three main topics: adjuvant therapy, new cytotoxic agents and novel approaches (Table 1).

ADJUVANT THERAPY

Among the most striking advances in oncology over the past 5 years has been the widespread application of adjuvant therapy in colorectal cancer. At present, reduced mortality is mainly achieved by applying adjuvant treatement strategies in completely resectable stages of colon and rectal cancer. Large trials have demonstrated that regimens of 5-fluorouracil (5-FU) and levamisole [2] or 5-FU and leucovorin in stage III colon cancer [3, 4] and 5-FU with postoperative radiation in stages II and III rectal cancer [5–7] can reduce mortality. The definition of risk factors predicting more precisely the risk of a relapse for an individual patient is also of major importance. The selection of patients who could benefit from adjuvant treatment is actually based on stages according to the TNM system. Since not all patients benefit from adjuvant treatment, the use of better

Table 1. New directions in the treatment of colorectal cancer

Adjuvant therapy	• better selection of patients by new prognostic parameters • new drugs: —monoclonal antibodies —thymidylate synthase inhibitors —topoisomerase I inhibitors
New cytotoxic agents	• thymidylate synthase inhibitors • topoisomerase I inhibitors • 5-FU prodrugs (capecitabine, uracil–ftorafur) • ethynyluracil • oxaliplatin
Novel approaches	• anti-angiogenic agents • stimulation of apoptosis • targeting genes involved in regulation of cell death • antibody directed enzyme prodrug therapy • targeting with polymer drug conjugates • immunotherapy

prognostic factors could identify patients who really need adjuvant treatment, leading to improvements in the efficacy of a treatment regimen. A better knowledge of prognostic factors could also avoid treatment of patients who are at relatively low risk of recurrence.

Several parameters have been suggested as candidates for prognostic factors, independent of the TNM stage: blood vessel invasion, perineural invasion, overexpression of p53 oncoprotein [8]; allelic loss on chromosone 18q (deleted in colorectal cancer suppressor-gene) [9]; mutation of *NM23H1* [10], thymidylate synthase (TS) expression, high DNA content of tumour cells and high proliferative index [11].

In the adjuvant setting, the toxicity profile of a treatment regimen, the quality of life of treated patients and health economic aspects are even more crucial than in patients with advanced disease. Therefore, in addition to expanding upon traditional 5-FU based regimens, new opportunities have arisen, including the potential role for monoclonal antibody therapy (e.g. monoclonal antibody against the epitope 17-1A) and the development of new cytotoxic drugs. TS inhibitors (e.g. 'Tomudex'™*) and topoisomerase I inhibitors [e.g. CPT 11 (irinotecan)] represent alternative drugs which may be either more efficacious or better tolerated. These drugs must be urgently evaluated as adjuvant therapy in colorectal cancer.

NEW CYTOTOXIC AGENTS

A number of novel drugs are under investigation and are expected to significantly improve the treatment of colorectal cancer. The antiproliferative agents, 'Tomudex' and CPT 11, will soon be available to clinicians for the treatment of patients with metastatic disease.

TS inhibitors

TS catalyses the methylation of dUMP (deoxyuridine monophosphate) to produce TMP (thymidine monophosphate), which, after metabolism to TTP (thymidine triphosphate), is exclusively incorporated into DNA. This makes TS an attractive target for the development of new anticancer agents. More effective inhibition of TS may offer an improvement in therapy. This has led to the development of new TS inhibitors which bind to the reduced folate binding site of TS and act independently of any cofactor. Two approaches have been taken in the development of these drugs. Firstly, modifications have been made to the folate chemical structure at the pteridine ring, *para*-aminobenzoic moeity and glutamate region. Secondly, the X-ray crystallography structure of TS has been used to design a molecule which would bind to the reduced folate binding site. These two approaches have led to the synthesis of several compounds which are now undergoing preclinical and clinical evaluation.

The TS inhibitors currently being evaluated ('Tomudex'™, LY 231514, AG 331, AG 337, BW 1843 U89 and ZD 9331) have different spectra of activity against cell lines and different pharmacological characteristics, implying they may vary in their effectiveness against human cancers. 'Tomudex'™ is a water-soluble TS inhibitor which is transported by the reduced folate carrier system and undergoes intracellular metabolism to polyglutamate forms by folylpolyglutamate synthetase (FPGS). Polyglutamation serves as a mechanism for drug retention. 'Tomudex'™ polyglutamates are up to 100-fold more potent inhibitors of TS compared with the parent drug [12]. Phase II and III studies on 'Tomudex'™ have been carried out, with promising results [13, 14].

LY 231514, a pyrrolopyrimidine folate-based TS inhibitor, has biochemical properties similar to those of 'Tomudex'™. Although less active than 'Tomudex'™ as an inhibitor of isolated TS, it is only marginally less active as an inhibitor of cell growth. The drug has shown activity *in vitro* in colon cancer, renal cancer, hepatoma and lung cancer [15]. It has activity against two human xenografted colon tumours [16]. Phase I studies have been performed with this drug [17].

AG 331 and AG 337 are lipophilic TS inhibitors designed on the basis of the crystal structure of the drug target. These compounds cross cell membranes by passive diffusion and the potential advantages of such inhibitors are thus that they do not require active uptake into cells or polyglutamation. While these two processes may contribute to the antitumour selectivity of classical anti-folates in tumours expressing high levels of the relevant proteins, they are also implicated in mechanisms of resistance [18].

BW 1843 U89, a benzaquinazoline, is a very potent 'specific' non-competitive inhibitor of TS *in vitro* [19]. It does not appear to require polyglutamation for effective enzyme inhibition; the FPGS substrate activity of the diglutamate is very poor, so that further polyglutamate chain elongation does not occur to a significant extent. Antitumour effects have been seen in human tumour xenografts against colon, breast, ovarian and osteosarcoma tumours [19].

ZD 9331 is a water-soluble, potent, non-polyglutamatable TS inhibitor in preclinical development. It is thought that a compound which is active without needing to undergo metabolism to polyglutamates would exhibit a different spectrum of antitumour activity compared with 'Tomudex', particularly against tumours expressing low levels of FPGS. ZD 9331 has been shown to retain activity in a 'Tomudex'-resistant cell line with a polyglutamation defect [20].

Combination of TS inhibitors with other chemotherapeutic agents, such as 5-FU and CPT 11, will probably improve their therapeutic efficacy. It has been shown that 'Tomudex' and 5-FU exhibit only partial cross resistance in cell lines selected by repeated exposure to each drug alone [21]. Colorectal carcinoma cell lines made resistant to 'Tomudex' by repeated exposure to the drug do not show significant cross resistance to 5-FU [21]. Design of further clinical trials for the combination of these chemotherapeutic agents should be based on these *in vitro* experiments [21].

Topoisomerase I inhibitors

The topoisomerase I inhibitors continue to move rapidly through clinical development, having activity against a variety of advanced malignancies [22]. Topoisomerase I relaxes torsionally strained (supercoiled) duplex DNA so that replication and transcription can proceed. This is achieved by the formation of a covalent adduct between topoisomerase I and DNA. Topoisomerase I inhibitors are S-phase specific drugs that stabilise this cleavable complex, resulting in single strand DNA breaks that cannot be re-ligated in the presence of the drug [22, 23]. This in turn results in an inhibition of RNA synthesis. Several topoisomerase I inhibitors are in various stages of preclinical or clinical development: CPT 11, topotecan, GI 147211, 9-aminocamptothecin and its prodrug 9-*N*-camptothecin, and DX 8951.

Large phase II studies with CPT 11 have demonstrated activity against colorectal cancer in both chemotherapy-naive

* 'Tomudex' is a trademark, the property of Zeneca Limited.

and pretreated patients (response rates of 15–32%), even those with clinical evidence of resistance to 5-FU [24–26]. The efficacy of CPT 11 as second-line therapy for colorectal cancer is particularly interesting, as patients resistant to 5-FU do not usually respond to any type of treatment [24].

Topotecan has been studied in a variety of dosing schedules and it appears that activity against certain tumour types may depend on the schedule of administration [23]. The most promising activity has been noted in ovarian cancer and small-cell lung cancer [27–29]. Small phase II studies have demonstrated some activity against colon cancer [23].

The unique mechanism of action of topoisomerase I inhibitors offers a strong scientific rationale for the development of combination therapy. These drugs show *in vitro* synergistic cytotoxicity with other anticancer agents such as cisplatin and etoposide [23, 29]. Early data from clinical studies appear to support this hypothesis [29]. In view of their activity in colon cancer, the combination of topoisomerase inhibitors with 5-FU and TS inhibitors should be examined.

Ethynyluracil

Ethynyluracil is a potent inactivator of dihydropyrimidine dehydrogenase, the first enzyme in the degradative pathway of 5-FU. In preclinical and early clinical trials, doses of ethynyluracil sufficient to inactivate more than 99% of endogenous dihydropyrimidine dehydrogenase proved to be non-toxic and exhibited no antiproliferative activity [30, 31]. Treatment with ethynyluracil in combination with 5-FU improved the antitumour efficacy of 5-FU and increased its therapeutic index by up to six-fold in three rodent tumour models [30, 31].

5-FU prodrugs

Uracil–ftorafur modulation. Ftorafur is a 5-FU prodrug, which is rapidly converted to 5-FU by thymidine/uridine phosphorylase. To optimise the therapeutic selectivity of the 5-FU generated from ftorafur, uracil has been added in the molar ratio of four uracil to one ftorafur. By inhibiting 5-FU degradation, co-administration of uracil enhances the concentration of 5-FU in tumours, and hence the antitumour efficacy of ftorafur. In animal models, modulation of ftorafur produced greater efficacy and selectivity than modulation of 5-FU [32], and a phase II clinical study demonstrated considerable activity with the combination of ftorafur, uracil and leucovorin [32, 33].

Capecitabine. Capecitabine is a 5′-deoxy-5-fluocytidine derivative which requires several step activations to 5-FU. It is a successor to another 5-FU prodrug, 5′-deoxy-5-fluoridine, with less toxicity and greater therapeutic efficacy in several preclinical tumour models (including colorectal) [32, 34]. Furthermore, capecitabine has demonstrated activity in 5-FU resistant cell lines [34]. Therapeutic indices are superior to those seen with 5-FU or 5′-deoxy-5-fluoridine [34]. Although phase I trials are not designed to study clinical efficacy, there have been reports of response in some of the patients with various tumour types, including colorectal cancer [35]. A phase II programme has started.

Oxaliplatin

Because oxaliplatin, a new third generation platinum complex, is not associated with renal toxicity and has minimal haematological toxicity, it is considererd a good candidate for platinum modulation of 5-FU and leucovorin. The combination of all three drugs is synergistic against murine L1210 leukaemia. Oxaliplatin also displays activity against cisplatin-resistant human colorectal cell lines and its addition to 5-FU and leucovorin induces responses in 5-FU resistant colorectal cancer, especially when adminstered as a chronomodulated regimen [36, 37].

NOVEL APPROACHES

The past few years have seen the development and, in some cases, the clinical trials of chemicals with quite different mechanisms of action to antiproliferative agents [38]. Among these are anti-angiogenic agents, which may interfere with the formation of new blood vessels and inhibit tumour growth [39]. A wealth of biological evidence supports the hypothesis that tumour growth is dependent on the formation of new blood vessels [40, 41]. Furthermore, in a number of tumours, correlations have been established between the level of tumour angiogenesis, as measured by capillary density in the primary tumour, and the likelihood of metastatic disease and even survival [40]. Anti-angiogenic agents downregulate neovascularisation by inhibiting the proliferation and migration of endothelial cells, rather than by killing the cells. The regression or involution of a vigorously growing capillary bed is a slower process than the lysis of tumour cells. The generally low toxicity of anti-angiogenic therapy, and the fact that drug resistance has not been a significant problem in long-term animal studies, suggest that long-term anti-angiogenic therapy may be well tolerated in the clinic [40].

It has been suggested that some of the antitumour effects of alpha-interferon may be related to its inhibitory effects on angiogenesis [39]. Pentosan polysulphate is a sulphated semi-synthetic polysaccharide which *in vitro* inhibits the activity of basic fibroblast growth factor, an important angiogenesis-inducing factor [42]. It can also inhibit Kaposi's sarcoma-derived spindle cells *in vitro* and the growth of a number of tumours in athymic mice [42]. It has now entered early clinical trials [40]. Analogues of the fungally derived antibiotic, fumagillin (TNP-470), which inhibit angiogenesis through incompletely defined mechanisms are being clinically evaluated [43]. Other inhibitors of angiogenesis under investigation include interleukin-12 and the metalloproteinase inhibitors, BB-94 and BB-2516 [44].

Understanding how programmed cell death is initiated in response to genotoxic events such as TS inhibition and the mechanism by which other cellular factors (such as overexpression of p53, c-myc and bcl-2) influence the ability of cells to undergo apoptosis may provide insights into the molecular basis of intrinsic insensitivity to cytotoxic agents. The main importance of apoptosis in intestinal disease lies in its role in carcinogenesis. Colorectal cancer is now believed to result from a series of mutations of specific oncogenes and tumour suppressor genes [1]. One of the functions of some of these genes (*C-MYC*, *TP53*, and the anti-apoptosis gene, *BCL-2*) is the regulation of apoptosis; for example, overexpression of wild-type p53 induces apoptosis in human tumour colonic epithelial cells [45]. It is now realised that anticancer agents do not kill by necrosis, but rather by causing sensitive cancer cells to commit suicide by the induction of apoptosis [46]. Specific genes and proteins must be expressed before apoptosis can occur. Some cancers are not able to undergo apoptosis because the genes required have mutated or their protein products are incorrectly expressed [47]. This is one reason for therapeutic resistance.

Therefore, new approaches may include stimulation of apoptosis, interference with the synthesis of agents opposing apoptosis or altered signalling. Studies are underway which aim to

alter tumour cell susceptibility to cytotoxic chemotherapies by targeting genes involved in the regulation of cell death. Likely targets for such strategies are drugs which inactivate bcl-2 or which restore wild-type p53 function or mimic its effects. Limonene, which has recognised anticarcinogenic properties, is able to inhibit the signalling of the *RAS* gene [38]. Since 50% of colon tumours express mutated *RAS*, they may prove to be susceptible to treatment with limonene or other more potent inhibitors of the mevalonate pathway [38].

Generation of cytotoxic agents from non-toxic prodrugs at tumour sites using enzymes vectored by antibodies introduces several opportunities for cancer therapy. The problem with this approach lies in the scarcity of tumours expressing a unique activating enzyme. However, this may be remedied by linking tumour-associated antibodies to non-mammalian enzymes and directing them to the metastasis. The metastasis would thus acquire an environment high in a unique enzyme capable of activating a prodrug. The first clinical trials with this procedure (ADEPT: antibody-directed enzyme prodrug therapy) have been performed against metastatic colon cancer using the bacterial enzyme carboxypeptidase G2 linked to an antibody fragment raised against a carcinoembryonic antigen [48]. Tumour responses have been observed in these early trials and further trials are planned using more appropriate prodrugs [48].

An alternative for the treatment of metastatic colon cancer is the relatively new concept of polymer drug conjugates [49]. Conjugation of an antitumour agent to a polymeric carrier creates a macromolecular prodrug. The molecule can incorporate drug–polymer linkers (e.g. peptides and pH-sensitive spacers) selected to promote preferential and prolonged intratumoural drug release. Polymer–cytotoxic drug conjugates are now in the early stages of drug development and are already showing considerable promise [38]. *N*-(2-Hydroxypropyl) methacrylamide (HPMA) copolymer–drug conjugates have been described, containing either anthracycline antibiotics or alkylating agents bound to polymers through peptide linkers designed for cleavage by thiol-dependent enzymes [50].

IMMUNOTHERAPY

Various modalities of immunotherapy are being developed. Immunotherapeutic approaches may be divided into two main categories: non-specific and specific. Non-specific immunotherapy may be defined as the use of immunomodulating agents which are administered with the aim of inducing general stimulation or suppression of the immune system, without attempting to direct the activity towards a specific antigen. Examples of this type of immunotherapy are the administration of BCG, levamisole, interferons and interleukins for patients with cancer [51].

Specific immunotherapy for treatment of malignancy is based upon several assumptions. The most important of these are that an antigenic difference exists between malignant and normal cells, that this difference is expressed by all malignant cells and that the host can recognise the difference and respond appropriately. Goals of immunotherapy are to increase the host response to the tumour (active immunotherapy) and/or to provide agents such as monoclonal antibodies or immune effector cells (passive immunotherapy) that are themselves immunologically active and theoretically depend less upon a host response.

The monoclonal antibody 17-1A has been studied in several clinical trials [52, 53]. It reacts against an antigen found on epithelial secretory surfaces and is released into serum. In an early clinical trial, disease-free survival was significantly increased in colonic cancer patients who received adjuvant immunotherapy with MAB 17-1A [52]. These data hold promise for immunotherapy, especially in patients presenting with minimal cancer burden or micrometastatic disease.

Tumour cell administration represents an active form of immunotherapy and is frequently described as a vaccination approach. The aim is to generate a host response to the administered tumour cells, which are usually altered *ex vivo* to increase their immunogenicity. Immunisation trials with an autologous colorectal cancer cell vaccine, anti-idiotype vaccines and several blood group-related carbohydrate antigen vaccines have been conducted in colorectal cancer patients [54]. These early efforts reveal both the promise of the approach and the difficulties in its implementation [54]. A prospective, randomised, controlled trial of active specific immunotherapy with an autologous tumour cell vaccine and BCG showed a significant improvement in disease-free and overall survival [55]. Although this trial had many deficiencies, these results are certainly encouraging.

CONCLUSIONS

The future of colorectal cancer treatment is promising. Many applications are in development, including the search for better prognostic factors for adjuvant treatment and new therapeutic possibilities in the adjuvant setting, the use of new drugs and the development of novel approaches. This should lead to better patient care and, hopefully, to an improved survival of colorectal cancer patients. An insight into the genetic and biochemical changes which occur in colorectal cancer will greatly contribute to this approach.

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