The impact of anti-inflammatory agents on the outcome of patients with colorectal cancer

Short Title:
Manipulating colorectal cancer-related inflammation

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Abstract:

Although there is increasing appreciation of the role of the host inflammatory response in determining outcome in patients in colorectal cancer, there has been little concerted effort to favourably manipulate cancer-associated inflammation, either alone or in combination with current oncological treatment. Epidemiological and cardiovascular disease studies have identified aspirin, other nonsteroidal anti-inflammatory drugs and statins as potential chemotherapeutic agents which may manipulate the host inflammatory response to the benefit of the patient with cancer. Similarly, evidence of a chemotherapeutic effect of histamine-2 receptor antagonists, again mediated by an immunomodulatory effect, has previously led to increased interest in their use in gastrointestinal cancer. Extensive pre-clinical data and a limited number of clinical investigations have proposed a direct effect of these agents on tumour biology, with an anti-tumour effect on several of the hallmarks of cancer, including proliferative capacity, evasion from apoptosis and cell cycle regulation, and invasive capability of tumour cells. Furthermore, clinical evidence has suggested a pertinent role in down-regulating the systemic inflammatory response whilst favourably influencing the local inflammatory response within the tumour microenvironment. Despite such compelling results, the clinical applicability of nonsteroidal anti-inflammatory drugs, statins and histamine-2 receptor antagonists has not been fully realised, particularly in patients identified at high risk on the basis of inflammatory parameters. In the present review, we examine the potential role that these agents may play in improving survival and reducing recurrence in patients with potentially curative colorectal cancer, and in particular focus on their effects on the local and systemic inflammatory response.

Introduction

Colorectal cancer (CRC) is the second most common cause of cancer-related death in Western Europe and North America. In the UK, 41 000 new cases are diagnosed each year with over 16 000 deaths(1). Despite advances in surgical and adjuvant treatment over the past two decades, survival remains poor, with a five-year survival of approximately 50% in patients undergoing resection with curative intent(2). Since the establishment of 5-fluorouracil and platinum-based regimes, few new chemotherapeutic agents have shown any significant survival benefit(3). Similarly, biological agents, such as bevacizumab and cetuximab have proven to be of only modest benefit, and only in the palliation of metastatic disease(4). As such, there remains a need to identify potential adjuvant and neo-adjuvant agents in patients with CRC.

Inflammation has been implicated in the pathogenesis of many adult malignancies and is now recognised as the seventh "hallmark" of cancer(5). Furthermore, the host inflammatory response to CRC influences disease recurrence and survival. A pronounced local inflammatory response with intra- and peri-tumoural lymphocytic infiltration is a stage-independent predictor of increased survival(6). Conversely, up-regulation of the systemic inflammatory response has been shown to be a predictor of recurrence and reduced survival in several cancers including CRC (7).

Impaired cell-mediated immunity is common in cancer patients(8). Particularly in patients undergoing surgical resection of CRC, that is recognised to attenuate post-operative cell-mediated immunity(9), this may be an important mechanism by which disseminated or shed tumour cells evade effective immunosurveillance and establish *de novo* metastases(10-12). Furthermore, the presence of a systemic inflammatory response has been associated with a poorer response to chemotherapeutic agents and an increased risk of toxicity(13).

It is clear that manipulation of the host inflammatory response, particularly in those patients with an "unfavourable" inflammatory profile, presents an intriguing concept. Despite this, few agents have been examined in the clinical setting for their potential effects on CRC-associated inflammation, particularly in the context of contemporary surgical and oncological treatment of high-risk disease.

Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), including the cyclooxygenase-2 inhibitors (COXIBs), have been identified as potential chemotherapeutic drugs which may favourably manipulate the inflammatory response in CRC. Despite convincing evidence from epidemiological studies and cardiovascular secondary prevention trials of a chemoprophylactic effect in reducing CRC incidence and mortality(14, 15), it is relatively recently that a potential benefit in patients with established CRC has been realised, with NSAID users less likely to present with advanced or metastatic disease at diagnosis or follow-up(16, 17). Indeed, emerging evidence of as much as a 40% reduction in mortality in patients undergoing curative treatment makes the concept of the use of

NSAIDs as adjuvant treatment in high risk disease more compelling(13, 18-23), where potential survival benefits may outweigh the risks which have so far abrogated their use in CRC prevention(24).

Similarly, statins and histamine-2 receptor antagonists (H2RAs) have also been identified as drugs with a potential benefit in improving survival and reducing risk of recurrence in patients with established CRC. A direct effect on tumour biology has been proposed through manipulation of several key signalling pathways, with a resultant effect on several of the key hallmarks of carcinogenesis, including proliferative and anti-apoptotic capacity as well tumour-mediated angiogenesis and invasiveness (25). Furthermore, these drugs have also been identified as potential agents capable of manipulating the host systemic and local inflammatory response to CRC[Table 1]. Although the use of such agents to manipulate the tumoural and inflammatory microenvironment in CRC as well as the systemic inflammatory response presents an attractive concept, most evidence to date arises from *in vitro* and *in vivo* investigations, with little confirmation from clinical studies. In particular, there has been no attempt to stratify the use of anti-inflammatory agents and subsequent benefit in CRC patients according to the presence of a systemic inflammatory response.The present review examines the clinical evidence supporting the use of NSAIDs, statins and H2RAs in influencing the tumour microenvironment and host inflammatory response in CRC and focuses on their utility in improving survival in patients with potentially curative disease.

Aspirin, NSAIDs and COX-2 inhibitors

Early evidence of a prophylactic effect of aspirin and NSAIDs in CRC originally arose out of studies of hereditary cancer syndromes. The use of NSAIDs decreases the number and size of colonic polyps in patients with familial adenomatous polyposis; similarly, aspirin has also been found to confer a protective effect on the colorectum in patients with Lynch syndrome(26, 27). Over the past two decades, increasing evidence from epidemiological studies has identified a potential role in the prophylaxis of sporadic CRC, with an approximate 30% risk reduction with aspirin and non-aspirin NSAIDS and a potentially greater reduction with COXIB use(28, 29). In general, a duration-dependent increase in risk reduction has been observed, with the greatest benefit seen after at least 10 years of continuous use. Similarly, cessation of regular use results in a return to normal population risk for subsequent CRC development. Furthermore, secondary analyses of cardiovascular secondary prevention trials have found a significant benefit with aspirin doses commonly employed for cardiovascular disease prevention, rather than doses commonly associated with analgesic use (19). Despite such convincing evidence, concerns regarding the safety profile of NSAIDs have discouraged their use as prophylactic agents in the general population, at least until the optimal target population is identified(24).

Direct Tumoural Effects

The direct cellular effects of aspirin and other NSAIDs have been under close scrutiny since their anti-tumour effects were first appreciated, and have been reviewed extensively elsewhere. In general, pre-clinical investigations have found an increase in tumour cell apoptosis in association with a decrease in cell proliferation, angiogenesis and metastatic potential(30, 31). Although limited, mechanistic studies in patients with CRC have again suggested similar effects, with an NSAID-mediated decrease in primary and metastatic tumour blood flow and microvessel density even with short courses of NSAIDs(32, 33). Of further interest, NSAID administration has also been shown to facilitate tumour cell differentiation, with a loss of cancer cell stemness and down-regulation of gene expression associated with increased metabolic turnover and resistance to oxidative stress(34, 35).

Cyclooxygenase-dependent effects

Several potential mechanistic pathways have been implicated in the anti-tumour effects of aspirin and other NSAIDs. The most studied mechanism is their inhibitory effect on cyclooxygenase (COX)-mediated synthesis of prostanoids, and in particular prostaglandin E_2 (PGE_2)(30, 31, 36, 37). Increased synthesis of PGE_2 by COX-2, the inducible form of the enzyme has been shown to have several pro-tumour and immunosuppressant effects *in vitro* and *in vivo*, including an increase in tumour cell proliferation, decreased apoptosis, increased angiogenesis and increased chemo- and radio-resistance. Indeed, COX-2 is overexpressed in some but not all colorectal neoplasia, particularly those arising in the distal colon and rectum (38, 39), where its expression is associated with increased differentiation, tumour invasiveness, metastatic potential and poorer survival(30, 36, 40). Furthermore, epidemiological evidence suggests a prominent role for COX-2 inhibition, with a reduced risk of COX-2 overexpressing tumours in long-term aspirin users and a modification of their anti-tumour effects observed in patients with common COX-2 gene polymorphisms(41, 42). Similarly, an increase in tumour cell apoptosis and decrease in tumour vascularity has also been confirmed in human subjects in response to NSAID administration, mediated by a reduction in COX-2 expression and tissue PGE_2(32, 43).

Aspirin, particularly at low doses employed in cardiovascular disease, is a weak inhibitor of COX-2 whereas it remains a strong inhibitor of the constitutive enzyme COX-1, particularly in anucleated cells such as platelets(44). As such, inhibition of COX-1 has also been suggested as another potential mechanism for the anti-tumour effects of NSAIDs by inhibiting platelet activation, facilitating immunosurveillance and preventing haematogenous spread. Indeed, aspirin can abrogate the increase in platelet activation demonstrated in CRC patients, even after only five days (45).

Cyclooxygenase-independent effects

Although many of the anti-proliferative effects of NSAIDs may be explained by their inhibitory effects on PGE_2 synthesis, several COX-independent actions have also been identified (46). Similarly, many of

the effects of NSAIDs on proliferation and apoptosis have also been identified in cancer cell lines known not to express COX-2 (47). Several signal transduction pathways, including Wnt/β-catenin, nuclear factor-kappa B (NF-κB) and the phosphatidylinositide 3-kinase/AKT/mammalian target of rapamycin pathway have been identified as potential targets for the non-COX mediated effects of NSAIDs, with limited clinical evidence suggesting an NSAID-mediated effect on associated signalling and transcription pathways(47-49). Furthermore, epidemiological data again suggests these as valid targets of NSAID therapy in CRC, with increased survival with aspirin use in patients with *PIK3CA* mutated cancers(49), and a reduced risk of cancer with NSAIDs in patients with mutations within the NF-κB pathway(50).

Effects on cancer-related inflammation

The anti-inflammatory properties of aspirin and non-aspirin NSAIDs have identified them as likely candidates in the manipulation of CRC-related inflammation; indeed evidence of a NSAID-mediated attenuation of the acute phase response and weight loss in advanced cancer suggests a potential role in the management of the cancer cachexia syndrome(51). Furthermore, the chemoprophylactic effects of NSAIDs appear to be greater in patients with evidence of a systemic inflammatory response(52), although unfortunately, so do the cardiovascular risks of long-term COXIB use(53).

Local inflammation

The presence of a pronounced inflammatory infiltrate at the invasive margin and within the tumour stroma is recognised as an indicator of reduced recurrence and superior survival(6). The effects of aspirin, non-selective NSAIDs and COXIBs on the tumoural inflammatory response have been investigated in a number of solid cancers, with significant anti-tumour responses identified in gastrointestinal, breast, bladder and head and neck cancers(54). A decrease in the levels of pro-tumour, immune-suppressing cytokines including PGE_2, has been identified in the colorectum and in colorectal hepatic metastases, likely mediated at a gene transcription level.(32, 34, 43). Furthermore, NSAIDs have been shown to induce expression of MHC class II molecules on the surface of CRC cells(55). Such changes within the tumour milieu may in turn allow for the recruitment and propagation of a co-ordinated, effective anti-tumour lymphocytic response [Table 2]. Indeed, Lönnroth and colleagues have shown an increase in tumour infiltration of activated T-lymphocytes and a decrease in immunosuppressive regulatory T-lymphocytes (T_{reg}) following a short course of pre-operative indomethacin or celecoxib in patients with CRC(55). Similarly, indomethacin augmented the anti-carcinoembryonic antigen (CEA) immune response in CRC patients *ex vivo* through inhibition of COX-2 and T_{reg} activity(56). The authors concluded that COX-2 inhibition could attenuate the inhibitory activity of T_{reg} cells identified in tumour tissue and regional lymph nodes, promoting an effective anti-tumour inflammatory response. The oncological benefits of NSAID-mediated manipulation of the local inflammatory response remain to be elicited.

Systemic inflammation

Suppression of the innate and adaptive immune response has been identified in patients with CRC(57, 58), with further attenuation of systemic immunity identified following exposure to surgical stress(59, 60). Indeed, cancer-related immune suppression is thought to contribute to the risk of recurrence through failure of immunosurveillance and the ability to clear micrometastatic deposits, residual microscopic disease and tumour cells shed at the time of surgery(10, 11). The administration of NSAIDs has been shown to abrogate suppression of systemic lymphocyte and natural killer (NK) cell activity in patients undergoing major surgery(59, 60) and in patients with CRC(57, 58) [Table 3].

Nonsteroidal anti-inflammatory drugs attenuate the acute phase response in patients with advanced cancer, with a decrease in several serum markers of inflammation including C-reactive protein (CRP), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) identified in tandem with an improvement in weight and functional status(51). Furthermore, the effect of NSAIDs on reducing risk of CRC appears to be greatest in patients with evidence of systemic inflammation as measured by soluble TNF receptor-2 (sTNFR-2) but not CRP(52). Interestingly however, in a polyp prevention study utilising low dose aspirin with or without folic acid, aspirin 325mg daily did not decrease CRP but did stabilise it over a three year period whereas patients receiving placebo experienced a significant increase(61). Regardless, CRP did not predict the chemoprophylactic effects of aspirin use. Despite this, the role of NSAIDs in patients with CRC-related systemic inflammation undergoing potentially curative surgical resection remains largely unknown [Table 3]. In patients with rectal cancer, the use of celecoxib has been shown to decrease elevated circulating levels of TNFα and IL-8, potentially through a direct effect on tumour cells and NFκB activity(62). Similarly, in CRC patients with an elevated CRP, ibuprofen decreases circulating CRP, cortisol and IL-6(63). Whether attenuation of the systemic inflammatory response by NSAIDs in CRC patients undergoing curative surgery translates into a benefit in recurrence rates and survival however remains unknown, and must be addressed by future trials of neoadjuvant and adjuvant NSAID use.

Disease progression and survival

Recent evidence has suggested a potential beneficial effect of NSAIDs on CRC progression, with as much as a 40% reduction in CRC-specific mortality with regular aspirin and NSAID use(19-23). Rothwell and co-workers suggested that the observed reduction in mortality apparent on secondary analysis of cardiovascular disease prevention trials was greater than what would be expected as a result of an NSAID-mediated decrease in cancer incidence alone(19). In addition, evidence that NSAID users are less likely to present with advanced or metastatic disease at diagnosis or follow-up further supports a direct effect on disease progression(16, 17).

Given such compelling evidence of an NSAID-mediated effect on established CRC, it is not surprising that their potential utility as adjuvant agents is currently being considered(13). Certainly, analysis of pre- and post-diagnosis NSAID usage further confirms a potential role for aspirin in addition to potentially curative surgery and adjuvant therapy, with an almost 50% reduction in cancer mortality in patients who commence regular aspirin use following diagnosis(64). Interestingly, no significant survival benefit was seen in patients continuing pre-diagnosis aspirin use, suggesting that cancers arising in these circumstances may be aspirin-resistant (64, 65).

Surprisingly, there have been few trials of aspirin or NSAIDs as adjuvant agents in CRC. Sub-analysis of a randomised trial of 5-fluorouracil and leucovorin with or without irinotecan in patients with stage III colon cancer examined the effect of aspirin and COXIBs on recurrence and survival(66). Even after controlling for treatment arm, NSAID use was associated with a 50% reduction in disease recurrence or death. Two further clinical trials of adjuvant COXIB following curative resection in patients with stage II/III disease ceased recruitment early following concerns regarding the cardiovascular safety profile of prolonged COXIBs(67, 68). The VICTOR trial, which randomised patients who had undergone surgery and adjuvant treatment for stage II/III disease to daily rofecoxib or placebo, was terminated early with only 33% of patients receiving active treatment for at least one year(67). Interestingly however, despite no significant difference in cancer-specific mortality and recurrence-free survival, a statistically significant reduction in recurrence within the first year was found with regular COXIB use. Given that most adenoma prevention trials exposed patients to at least two years of regular COXIB use, the early termination of VICTOR likely precluded the investigators from finding any significant survival benefit.

Given the observed effects on tumour biology and micro-environment, the use of NSAIDs prior to surgery in addition to standard neoadjuvant chemoradiotherapy has also been investigated. Indeed, decreased synthesis of protective prostaglandins via inhibition of COX-2 has been shown to increase tumour radiosensitivity(69). To date however, only phase II feasibility studies have shown a potential increase in tumour response and clinicopathological downstaging with the addition of COXIBs to neoadjuvant chemoradiotherapy(70). Certainly such time-restricted use may be promising and favour the risk-benefit ratio of COXIB use. Regardless, although trials of adjuvant aspirin use are currently recruiting(4), it is clear that further, adequately powered trials are required to fully ascertain the benefit of aspirin, NSAIDs and COXIBS, both in the adjuvant and neoadjuvant setting.

Statins

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, commonly known as statins, are primarily used in the treatment of hypercholesterolaemia and atherosclerotic cardiovascular disease and are known to have a number of pleiotropic effects on cell proliferation, angiogenesis, inflammation and endothelial cell function(71, 72). Although a reduction in the risk of several cancers has been found in epidemiological studies(73-75), the results of meta-analyses

suggest only a modest effect if any of statins on reducing the incidence of CRC in the general population(72, 76). Despite this, the results of *in* vivo studies and evidence of an increased expression of HMG-CoA reductase in colon cancer, particularly tumours arising in the left colon, suggests a potential role for statins in the treatment of CRC(77).

Direct tumoural effects

Mevalonate, the end product of HMG-CoA reductase metabolism and its isoprenoid metabolites are required for the activation of the Ras superfamily of small GTPases by prenylation(78). In turn, these GTPases are crucial for downstream activity of several signal transduction pathways(79); inhibition of mevalonate synthesis by statins subsequently has indirect and direct effects on cell survival and growth. Such inhibition has been shown to have a pleiotropy of effects, including a reduction in cell proliferation(77, 80), induction of apoptosis(77, 80), increased susceptibility to oxidative stress(81) and inhibition of metastatic transformation and angiogenesis(82). A role for non HMG-CoA reductase-mediated pathways has also been suggested, particularly in tumours exhibiting the CpG island methylator phenotype (CIMP). CIMP-associated tumours exhibit hypermethylation of tumour suppressor gene promoter regions, including those implicated in the bone morphogenic protein (BMP) pathway(83). Statin-mediated demethylation of the *BMP2* promoter region and subsequent activation of the BMP pathway has previously been shown to increase apoptosis and promote cell differentiation in cell line studies(84); indeed such an effect may suggest a pertinent role for statins in patients with CIMP-associated tumours.

Of further interest, statin therapy has been shown to augment the activity of a number of chemotherapeutic agents, even in resistant cell lines(78, 85, 86). The activity of epidermal growth factor receptor inhibitors, including cetuximab, also appears to be potentiated *in vitro* and *in vivo*, even in cell lines with known *KRAS* mutations and resistance(87). Furthermore, statin therapy may also increase the likelihood of pathological complete response following neoadjuvant chemoradiotherapy(85, 88).

Effects on cancer-related inflammation

Cardiovascular disease prevention trials have identified a clear anti-inflammatory effect of statins, with down-regulation of pro-inflammatory cytokines and increased cardiovascular risk reduction in patients with elevated serum inflammatory markers(89). Furthermore, favourable effects on organ rejection following heart and renal transplant suggest a potent immunomodulatory effect, potentially through a direct effect on MHC class II expression and subsequent T-cell activation(90). Similar effects on the inflammatory response may also be expected in patients with CRC, and certainly evidence from clinical trials of a 90% reduction in risk of inflammatory bowel disease-related CRC is compelling(91).

Local inflammation

To date, no clinical evidence exists to support the role of statins in influencing the local inflammatory response in CRC, although pre-clinical data suggests a direct inhibitory effect on NF-kB activation, with subsequent down-regulation of COX-2 and pro-inflammatory cytokine expression(92-94). A cohort study of patients undergoing radical prostatectomy found that statin use was associated with a reduced tumour inflammatory infiltrate(95); in contrast to CRC, however, a minimal local inflammatory response is associated with reduced recurrence and improved survival. Whether similar effects on the tumour inflammatory infiltrate in CRC can be expected remains to be seen.

Systemic inflammation

Despite a clear benefit on the systemic inflammatory response in cardiovascular disease and in patients following transplant, the clinical application of these effects in CRC is less clear [Table 3]. In an interventional study of patients undergoing curative CRC resection, Malicki and co-workers found a significant reduction in pre-operative serum IL-6 in patients receiving statins(96). In contrast however, a recent study of the systemic inflammatory response to neoadjuvant chemoradiotherapy in patients with oesophageal and rectal cancer found that concomitant statin use did not attenuate the serum inflammatory response or treatment-associated symptoms(97). Further clarification of the effects of statins on cancer-related systemic inflammation is required, and such measures should be incorporated in to future studies of the chemotherapeutic benefits of statins.

Disease progression and survival

Despite an unclear effect on the incidence of CRC, statins may influence the progression of established disease, with regular statin use being associated with earlier stage at diagnosis in three case-control studies(73, 98, 99). Siddiqui and co-workers, in a case-control study of 326 male users with CRC and regular statin use of at least three years, found a lower mean stage and lower frequency of metastases (28.4% vs. 38.8%, $p<0.01$) at presentation, with a higher prevalence of right-sided tumours in statin users(99). Furthermore, statin users had superior five-year survival (37% vs. 33%, $p=0.03$). Coogan and colleagues also found a significant reduction in the risk of stage IV CRC (odds ratio 0.18, 95% CI 0.05-0.62) with regular use of statins for at least 3 months(98). Similarly, a modest reduction of stage III/IV CRC was also observed by Poynter et al, however this failed to reach statistical significance (odds ratio 0.90, 95% CI 0.54 to 1.50). In contrast however, despite finding a reduced risk of CRC with statin use, a recent case-control study with prescription data linkage from Scotland found no difference in stage at diagnosis or survival(75), although the study was underpowered to identify any significant survival benefit. Of more interest, a prospective observational study of statin use within a randomised trial of adjuvant chemotherapy in stage III colon cancer found no survival benefit with statin use, irrespective of duration of use or presence of *KRAS* mutations(100). These conflicting results may in part be explained by population-based genetic

variation in HMG-CoA reductase, as the presence of single nucleotide polymorphisms have previously been shown to modify the protective effect of statins on risk of CRC(101).

It is clear that the benefit of statins in the treatment of CRC has not yet been defined and that further clinical trials are required. Recruitment for the National Surgical Adjuvant Breast and Bowel Project: Statin Polyp Prevention Trial is currently underway with the aim of investigating the effects of rosuvastatin on polyp/cancer recurrence and metachronous cancer development in patients who have undergone resection for stage I/II colon cancer(102). This and further trials may in time define the role statins may play in treatment of CRC.

H2RAs

Since early reports of a survival advantage in patients with gastric cancer(103), there has been interest in the potential use of H2RAs in the treatment of CRC. Aside from potentially beneficial effects on the local and immune responses, pre-clinical data suggests direct anti-tumour effects, including inhibition of histamine as a growth factor and inhibition of tumour-endothelial cell adhesion and motility. Furthermore, prolonged H2RA use has been shown to increase the systemic bioavailability of 5-fluourouracil(104).

Direct tumoural effects

Histamine acts as an autocrine tumour growth factor and has been shown to increase CRC cell proliferation and growth *in vitro* and *in vivo*(105). Indeed, expression of histamine and histidine decarboxylase, the enzyme responsible for histamine synthesis, is increased in CRC when compared to normal colorectal mucosa(106, 107); increasing expression has been associated with the presence of nodal and distant metastases as well as increased microvessel density, suggesting a potential role in the transformation to invasive and metastatic disease. Furthermore, histamine has also been shown to increase expression of COX-2 and PGE2 as well as vascular endothelial growth factor in cell lines constitutively expressing COX-2(106). Celecoxib has been shown to abrogate the histamine-induced increase in vascular endothelial growth factor expression, suggesting that at least some of the pro-tumour effects of histamine may be mediated by COX-2 and prostaglandin activity(106).

Although several histamine receptors have been identified with H2 and H4 receptor stimulation both being implicated in tumour growth(106), only H2 receptors appear to be preserved in CRC tissue with loss of H1 and H4 receptors when compared to normal mucosa(108). The use of H2RAs in both cell line and animal studies has been associated with a decrease in histamine-induced tumour growth, proliferation and increase in apoptosis *in vitro*(105, 109). The use of H2RAs may also reduce the metastatic potential of colorectal tumour cells by inhibition of E-selectin expression, endothelial cell adhesion and a decrease in tumour microvessel density(106, 110).

Effects on cancer-related inflammation

Local inflammation

Activation of histamine receptor-2 on regulatory T-lymphocytes inhibits the cell-mediated immune response(111). Amelioration of this immunosuppressant effect by H2RA use has been shown to subsequently increase tumour infiltration of activated lymphocytes [Table 2]. Adams and co-workers, using quantitative assessments of peri-tumoural lymphocytic infiltration such as the presence of a Crohn's-like reaction or Jass criteria, found an increased conspicuous lymphocytic infiltration with peri-operative cimetidine use(112, 113). Qualitative assessment of the lymphocytic infiltrate using immunohistochemistry have been equivocal, with one study suggesting that H2RA use increases tumour infiltration of CD3+ T-lymphocytes, particularly in patients with late stage disease(9), whereas another study examining the dose-response of cimetidine suggested that H2RAs may exert their effects through other, non-CD3+ cellular components(114). Interestingly, Kapoor et al. found that pre-operative use of the H2RA famotidine led to a significant increase in tumour lymphocyte infiltration in colon cancer rather than rectal cancer, with the largest effect seen in those patients with a normal pre-operative CEA(115).

Systemic inflammation

Histamine attenuates the systemic immune response in patients with CRC. Similarly, the exaggerated post-operative immune suppression experienced in patients with CRC is in part mediated by histamine release(9). The use of H2RAs has been shown to abrogate tumour-associated systemic immune suppression [Table 3], with restoration of circulating levels and activity of T-lymphocyte and NK cell subsets(116), potentially via augmentation of IL-2 and interferon activity. Furthermore, peri-operative H2RA use restores normal cell-mediated immunity following surgery(9, 117). Although shown to decrease post-operative CRP in patients without cancer (118), the effects of H2RA use on systemic cytokine profiles and biomarkers of the systemic inflammatory response in patients with CRC remains unknown.

Survival

The first reports of a survival advantage for H2RAs in patients with CRC were in the early 1990s, when Adams and co-workers reported a non-significant increase in 3-year survival with peri-operative cimetidine in patients with Dukes A to C CRC (3-year survival 93% vs. 59%, p=0.17)(112). In 1995, Matsumoto and co-workers reported the survival analysis of a multicentre, randomised controlled trial of the effects of cimetidine on adjuvant 5-fluorouracil-induced appetite loss and oesophagitis(119). Interestingly, they found a significant increase in survival for both colonic and rectal cancers at almost 4 years. A 10-year analysis from the same patient cohort further confirmed

increased survival and reduced risk of recurrence with cimetidine, with greatest benefit seen in Dukes C patients(110).

Further studies of differing doses and types of H2RAs given either prior to surgery or as adjuvant treatment have only shown a non-significant trend towards improved survival(114, 115, 120, 121), particularly in patients with Dukes C cancers(120). Subgroup analyses have identified potential patient groups who may be more likely to benefit from H2RA treatment, such as those with microsatellite instability (MSI) low tumours or tumours with a low peritumoural lymphocytic infiltrate(114). MSI-low tumours are less likely to have a pronounced lymphocytic infiltrate(122). As such patients with MSI-low tumours may represent a subgroup of CRC patients likely to benefit from H2RA use, however no large scale studies have examined these relationships and therefore this area merits further investigation. In addition, patients who did not receive peri-operative blood transfusion or develop post-operative infectious complications have similarly been identified as groups who may benefit oncologically(121). Differences in type and dose of drug used as well as inclusion of patients with metastatic disease at enrolment may have precluded finding significant results in these studies. The consistency of trend towards improved survival however does suggest that further, standardised studies are required. A recent Cochrane Collaboration review of H2RAs as adjuvant treatment for resected CRC found overall a significant improvement in survival for cimetidine only (combined hazard ratio (HR) 0.53; 95% confidence interval (CI) 0.32 to 0.87)(123). Given that most of the included trials were performed before the routine use of diagnostic cross-sectional imaging, total mesenteric excision surgery and contemporary chemoradiotherapy regimes, the authors advised caution regarding the applicability of these trials and advised the need for further studies incorporating current “best practice” treatment.

Conclusion

Increasing appreciation of the role of host-tumour factors has allowed for better identification and prognostication of patients deemed at high risk, regardless of pathological staging. Indeed, assessment of the local and systemic inflammatory responses should be incorporated in to the routine staging of patients with CRC(7, 124).

Even though measurement of the host inflammatory response allows for greater risk stratification, the appropriate management of such patients remains unknown. Although more intense surveillance may be beneficial, oncological management is impaired by the systemic inflammatory response(13). Certainly it is clear that optimal management should attempt to manipulate the inflammatory response.

In spite of convincing epidemiological evidence, the role of statins, H2RAs and particularly NSAIDs in the management of patients with CRC has yet to be defined. Although shown to have a direct effect not only on tumour biology but also on the host systemic and local inflammatory response, most

evidence has arisen from pre-clinical investigations of CRC *in vitro* and *in vivo*. The few clinical investigations reviewed above have been limited in their clinical applicability, and the long-term oncological outcomes have not yet been fully explored.

The use of these agents is an attractive option not only because of their low cost, but also due to their relatively well-defined long-term safety profiles. Clinical trials of adjuvant aspirin and statins in CRC are currently recruiting. It is clear however, that further studies are required to identify the role of anti-inflammatory agents in the management of patients with CRC, and particularly those patients identified at high risk due to the presence of an "unfavourable" inflammatory profile.

Conflict of interest statement

All authors disclose no conflict of interest.

References

1. UK CR. Bowel cancer statistics: Cancer Research UK. Available from: http://info.cancerresearchuk.org/cancerstats/types/bowel/mortality.
2. McArdle CS, McMillan DC, Hole DJ. Male gender adversely affects survival following surgery for colorectal cancer. Br J Surg. 2003 Jun;90(6):711-5.
3. Andre T, Boni C, Navarro M, Tabernero J, Hickish T, Topham C, et al. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol. 2009 Jul 1;27(19):3109-16.
4. Ali R, Toh HC, Chia WK. The utility of Aspirin in Dukes C and High Risk Dukes B Colorectal cancer--the ASCOLT study: study protocol for a randomized controlled trial. Trials. 2011;12:261.
5. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009 Jul 30;30(7):1073-81.
6. Roxburgh CSD, McMillan DC. The role of the in situ local inflammatory response in predicting recurrence and survival in patients with primary operable colorectal cancer. Cancer Treat Rev. 2012 Aug;38(5):451-66.
7. Roxburgh CS, McMillan DC. Role of systemic inflammatory response in predicting survival in patients with primary operable cancer. Future Oncology. 2010 Feb;6(1):149-63.
8. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999 Feb;340(6):448-54.
9. Lin CY, Bai DJ, Yuan HY, Wang K, Yang GL, Hu MB, et al. Perioperative cimetidine administration promotes peripheral blood lymphocytes and tumor infiltrating lymphocytes in patients with gastrointestinal cancer: Results of a randomized controlled clinical trial. World J Gastroenterol. 2004 Jan;10(1):136-42.
10. Ueda T, Shimada E, Urakawa T. Serum levels of cytokines in patients with colorectal cancer: possible involvement of interleukin-6 and interleukin-8 in hematogenous metastasis. J Gastroenterol. 1994 Aug;29(4):423-9.
11. Hanna N, Fidler IJ. Role of natural killer cells in the destruction of circulating tumor emboli. J Natl Cancer Inst. 1980 Oct;65(4):801-9.
12. McMillan DC, Fyffe GD, Wotherspoon HA, Cooke TG, McArdle CS. Prospective study of circulating T-lymphocyte subpopulations and disease progression in colorectal cancer. Dis Colon Rectum. 1997 Sep;40(9):1068-71.
13. Chia WK, Ali R, Toh HC. Aspirin as adjuvant therapy for colorectal cancer-reinterpreting paradigms. Nat Rev Clin Oncol. 2012 Aug 21;9(10):561-70.
14. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann Intern Med. 1994 Aug 15;121(4):241-6.
15. Giovannucci E, Egan KM, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC, et al. Aspirin and the risk of colorectal cancer in women. N Engl J Med. 1995 Sep 7;333(10):609-14.
16. Benedetti AL, Collet JP, Boivin JF, Hanley JA. Effect of nonsteroidal anti-inflammatory drugs on stage of colon cancer at diagnosis. J Clin Epidemiol. 2003 Aug;56(8):782-7.
17. Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. Lancet. 2012 Apr 28;379(9826):1591-601.
18. Flossmann E, Rothwell PM. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet. 2007 May 12;369(9573):1603-13.
19. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet. 2010 Nov 20;376(9754):1741-50.
20. Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW, Jr. Aspirin use and risk of fatal cancer. Cancer Res. 1993 Mar 15;53(6):1322-7.
21. Thun MJM, Namboodiri MMM, Heath CWC. Aspirin use and reduced risk of fatal colon cancer. The New England journal of medicine. 1991 Dec 05;325(23):1593-6.
22. Zell JA, Ziogas A, Bernstein L, Clarke CA, Deapen D, Largent JA, et al. Nonsteroidal anti-inflammatory drugs: effects on mortality after colorectal cancer diagnosis. Cancer. 2009 Dec 15;115(24):5662-71.

23. Coghill AE, Newcomb PA, Campbell PT, Burnett-Hartman AN, Adams SV, Poole EM, et al. Prediagnostic non-steroidal anti-inflammatory drug use and survival after diagnosis of colorectal cancer. Gut. 2011 Apr;60(4):491-8.
24. Routine aspirin or nonsteroidal anti-inflammatory drugs for the primary prevention of colorectal cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2007 Mar 6;146(5):361-4.
25. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646-74.
26. Giardiello FMF, Hamilton SRS, Krush AJA, Piantadosi SS, Hylind LML, Celano PP, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. The New England journal of medicine. 1993 Jun 05;328(18):1313-6.
27. Burn J, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet. 2011 Dec 17;378(9809):2081-7.
28. Vinogradova Y, Hippisley-Cox J, Coupland C, Logan RF. Risk of colorectal cancer in patients prescribed statins, nonsteroidal anti-inflammatory drugs, and cyclooxygenase-2 inhibitors: nested case-control study. Gastroenterology. 2007 Aug;133(2):393-402.
29. Harris RE, Beebe-Donk J, Alshafie GA. Similar reductions in the risk of human colon cancer by selective and nonselective cyclooxygenase-2 (COX-2) inhibitors. BMC Cancer. 2008;8:237.
30. Church RD, Fleshman JW, McLeod HL. Cyclo-oxygenase 2 inhibition in colorectal cancer therapy. Br J Surg. 2003 Sep;90(9):1055-67.
31. Cha YI, DuBois RN. NSAIDs and cancer prevention: targets downstream of COX-2. Annu Rev Med. 2007;58:239-52.
32. Fenwick SW, Toogood GJ, Lodge JP, Hull MA. The effect of the selective cyclooxygenase-2 inhibitor rofecoxib on human colorectal cancer liver metastases. Gastroenterology. 2003 Sep;125(3):716-29.
33. Chalmers CR, Wilson DJ, Ward J, Robinson PJ, Toogood GJ, Hull MA. Antiangiogenic activity of the selective cyclooxygenase 2 inhibitor rofecoxib in human colorectal cancer liver metastases. Gut. 2006 Jul;55(7):1058-9.
34. Auman JT, Church R, Lee SY, Watson MA, Fleshman JW, McLeod HL. Celecoxib pre-treatment in human colorectal adenocarcinoma patients is associated with gene expression alterations suggestive of diminished cellular proliferation. Eur J Cancer. 2008 Aug;44(12):1754-60.
35. Lonnroth C, Andersson M, Nordgren S, Lundholm K. Downregulation of Prominin 1/CD133 expression in colorectal cancer by NSAIDs following short-term preoperative treatment. Int J Oncol. 2012 Jul;41(1):15-23.
36. Tuynman JB, Peppelenbosch MP, Richel DJ. COX-2 inhibition as a tool to treat and prevent colorectal cancer. Crit Rev Oncol Hematol. 2004 Nov;52(2):81-101.
37. Schror K. Pharmacology and cellular/molecular mechanisms of action of aspirin and non-aspirin NSAIDs in colorectal cancer. Best Pract Res Clin Gastroenterol. 2011 Aug;25(4-5):473-84.
38. Dimberg J, Samuelsson A, Hugander A, Soderkvist P. Differential expression of cyclooxygenase 2 in human colorectal cancer. Gut. 1999 Nov;45(5):730-2.
39. Nasir A, Kaiser HE, Boulware D, Hakam A, Zhao H, Yeatman T, et al. Cyclooxygenase-2 expression in right- and left-sided colon cancer: a rationale for optimization of cyclooxygenase-2 inhibitor therapy. Clin Colorectal Cancer. 2004 Feb;3(4):243-7.
40. Gustafsson A, Hansson E, Kressner U, Nordgren S, Andersson M, Wang W, et al. EP1-4 subtype, COX and PPAR gamma receptor expression in colorectal cancer in prediction of disease-specific mortality. Int J Cancer. 2007 Jul 15;121(2):232-40.
41. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med. 2007 May 24;356(21):2131-42.
42. Barry EL, Sansbury LB, Grau MV, Ali IU, Tsang S, Munroe DJ, et al. Cyclooxygenase-2 polymorphisms, aspirin treatment, and risk for colorectal adenoma recurrence--data from a randomized clinical trial. Cancer Epidemiol Biomarkers Prev. 2009 Oct;18(10):2726-33.
43. Krishnan K, Ruffin MT, Normolle D, Shureiqi I, Burney K, Bailey J, et al. Colonic mucosal prostaglandin E2 and cyclooxygenase expression before and after low aspirin doses in subjects at high risk or at normal risk for colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2001 May;10(5):447-53.

44. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nat Rev Clin Oncol. 2012 May;9(5):259-67.
45. Sciulli MG, Filabozzi P, Tacconelli S, Padovano R, Ricciotti E, Capone ML, et al. Platelet activation in patients with colorectal cancer. Prostaglandins Leukot Essent Fatty Acids. 2005 Feb;72(2):79-83.
46. Grosch S, Maier TJ, Schiffmann S, Geisslinger G. Cyclooxygenase-2 (COX-2)-independent anticarcinogenic effects of selective COX-2 inhibitors. J Natl Cancer Inst. 2006 Jun 7;98(11):736-47.
47. Boon EM, Keller JJ, Wormhoudt TA, Giardiello FM, Offerhaus GJ, van der Neut R, et al. Sulindac targets nuclear beta-catenin accumulation and Wnt signalling in adenomas of patients with familial adenomatous polyposis and in human colorectal cancer cell lines. Br J Cancer. 2004 Jan 12;90(1):224-9.
48. Greenspan EJ, Madigan JP, Boardman LA, Rosenberg DW. Ibuprofen inhibits activation of nuclear {beta}-catenin in human colon adenomas and induces the phosphorylation of GSK-3{beta}. Cancer Prev Res (Phila). 2011 Jan;4(1):161-71.
49. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med. 2012 Oct 25;367(17):1596-606.
50. Seufert BL, Poole EM, Whitton J, Xiao L, Makar KW, Campbell PT, et al. IkappaBKbeta and NFkappaB1, NSAID use and risk of colorectal cancer in the Colon Cancer Family Registry. Carcinogenesis. 2013 Jan;34(1):79-85.
51. Solheim TS, Fearon KC, Blum D, Kaasa S. Non-steroidal anti-inflammatory treatment in cancer cachexia: a systematic literature review. Acta Oncol. 2013 Jan;52(1):6-17.
52. Chan AT, Ogino S, Giovannucci EL, Fuchs CS. Inflammatory markers are associated with risk of colorectal cancer and chemopreventive response to anti-inflammatory drugs. Gastroenterology. 2011 Mar;140(3):799-808, quiz e11.
53. Chan AT, Sima CS, Zauber AG, Ridker PM, Hawk ET, Bertagnolli MM. C-reactive protein and risk of colorectal adenoma according to celecoxib treatment. Cancer Prev Res (Phila). 2011 Aug;4(8):1172-80.
54. Hussain M, Javeed A, Ashraf M, Al-Zaubai N, Stewart A, Mukhtar MM. Non-steroidal anti-inflammatory drugs, tumour immunity and immunotherapy. Pharmacol Res. 2012 Jul;66(1):7-18.
55. Lonnroth C, Andersson M, Arvidsson A, Nordgren S, Brevinge H, Lagerstedt K, et al. Preoperative treatment with a non-steroidal anti-inflammatory drug (NSAID) increases tumor tissue infiltration of seemingly activated immune cells in colorectal cancer. Cancer Immun. 2008;8:5.
56. Yaqub S, Henjum K, Mahic M, Jahnsen FL, Aandahl EM, Bjornbeth BA, et al. Regulatory T cells in colorectal cancer patients suppress anti-tumor immune activity in a COX-2 dependent manner. Cancer Immunol Immunother. 2008 Jun;57(6):813-21.
57. Han T, Nemoto T, Ledesma EJ, Bruno S. Enhancement of T lymphocyte proliferative response to mitogens by indomethacin in breast and colorectal cancer patients. Int J Immunopharmacol. 1983;5(1):11-5.
58. Balch CM, Dougherty PA, Cloud GA, Tilden AB. Prostaglandin E2-mediated suppression of cellular immunity in colon cancer patients. Surgery. 1984 Jan;95(1):71-7.
59. Markewitz A, Faist E, Lang S, Endres S, Fuchs D, Reichart B. Successful restoration of cell-mediated immune response after cardiopulmonary bypass by immunomodulation. J Thorac Cardiovasc Surg. 1993 Jan;105(1):15-24.
60. Gogos CA, Maroulis J, Zoumbos NC, Salsa B, Kalfarentzos F. Effect of Parenteral Indomethacin on T-Lymphocyte Subpopulations and Cytokine Production in Patients under Major Surgical Operations. Res Exp Med (Berl). 1995 Apr;195(2):85-92.
61. Ho GY, Xue X, Cushman M, McKeown-Eyssen G, Sandler RS, Ahnen DJ, et al. Antagonistic effects of aspirin and folic acid on inflammation markers and subsequent risk of recurrent colorectal adenomas. J Natl Cancer Inst. 2009 Dec 2;101(23):1650-4.
62. Konturek PC, Rembiasz K, Burnat G, Konturek SJ, Tusinela M, Bielanski W, et al. Effects of cyclooxygenase-2 inhibition on serum and tumor gastrins and expression of apoptosis-related proteins in colorectal cancer. Dig Dis Sci. 2006 Apr;51(4):779-87.
63. McMillan DC, Leen E, Smith J, Sturgeon C, Preston T, Cooke TG, et al. Effect of extended ibuprofen administration on the acute phase protein response in colorectal cancer patients. Eur J Surg Oncol. 1995 Oct;21(5):531-4.
64. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA. 2009 Aug 12;302(6):649-58.

65. Bastiaannet E, Sampieri K, Dekkers OM, de Craen AJ, van Herk-Sukel MP, Lemmens V, et al. Use of aspirin postdiagnosis improves survival for colon cancer patients. Br J Cancer. 2012 Apr 24;106(9):1564-70.
66. Fuchs CS, Meyerhardt JA, Heseltine DL, Niedzwiecki D, Hollis D, Chan AT, et al. Influence of regular aspirin use on survival for patients with stage III colon cancer: Findings from Intergroup trial CALGB 89803. J Clin Oncol. 2005 2005;23(16S):3530.
67. Midgley RS, McConkey CC, Johnstone EC, Dunn JA, Smith JL, Grumett SA, et al. Phase III randomized trial assessing rofecoxib in the adjuvant setting of colorectal cancer: final results of the VICTOR trial. J Clin Oncol. 2010 Oct 20;28(30):4575-80.
68. Phase III Randomized Study of Adjuvant Celecoxib With Fluorouracil and Leucovorin Calcium in Patients With Curatively Resected Stage III Adenocarcinoma of the Colon National Cancer Institute: National Institutes of Health; 2004 [updated 22/6/2006; cited 2012 October]. Available from: http://cancer.gov/clinicaltrials/search/view?cdrid=367335&version=healthprofessional.
69. Davis TW, Hunter N, Trifan OC, Milas L, Masferrer JL. COX-2 inhibitors as radiosensitizing agents for cancer therapy. Am J Clin Oncol. 2003 Aug;26(4):S58-61.
70. Debucquoy A, Roels S, Goethals L, Libbrecht L, Van Cutsem E, Geboes K, et al. Double blind randomized phase II study with radiation+5-fluorouracil+/-celecoxib for resectable rectal cancer. Radiother Oncol. 2009 Nov;93(2):273-8.
71. Hindler K, Cleeland CS, Rivera E, Collard CD. The role of statins in cancer therapy. Oncologist. 2006 Mar;11(3):306-15.
72. Bardou M, Barkun A, Martel M. Effect of statin therapy on colorectal cancer. Gut. 2010 Nov;59(11):1572-85.
73. Poynter JN, Gruber SB, Higgins PD, Almog R, Bonner JD, Rennert HS, et al. Statins and the risk of colorectal cancer. N Engl J Med. 2005 May 26;352(21):2184-92.
74. Hachem C, Morgan R, Johnson M, Kuebeler M, El-Serag H. Statins and the risk of colorectal carcinoma: a nested case-control study in veterans with diabetes. Am J Gastroenterol. 2009 May;104(5):1241-8.
75. Lakha F, Theodoratou E, Farrington SM, Tenesa A, Cetnarskyj R, Din FV, et al. Statin use and association with colorectal cancer survival and risk: case control study with prescription data linkage. BMC Cancer. 2012 Oct 22;12(1):487.
76. Bonovas S, Filioussi K, Flordellis CS, Sitaras NM. Statins and the risk of colorectal cancer: a meta-analysis of 18 studies involving more than 1.5 million patients. J Clin Oncol. 2007 Aug 10;25(23):3462-8.
77. Notarnicola M, Messa C, Pricci M, Guerra V, Altomare DF, Montemurro S, et al. Up-regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in left-sided human colon cancer. Anticancer Res. 2004 Nov-Dec;24(6):3837-42.
78. Agarwal B, Bhendwal S, Halmos B, Moss SF, Ramey WG, Holt PR. Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cancer cells. Clin Cancer Res. 1999 Aug;5(8):2223-9.
79. Luo J, Manning BD, Cantley LC. Targeting the PI3K-Akt pathway in human cancer: rationale and promise. Cancer Cell. 2003 Oct;4(4):257-62.
80. Wachtershauser A, Akoglu B, Stein J. HMG-CoA reductase inhibitor mevastatin enhances the growth inhibitory effect of butyrate in the colorectal carcinoma cell line Caco-2. Carcinogenesis. 2001 Jul;22(7):1061-7.
81. Qi XF, Kim DH, Yoon YS, Kim SK, Cai DQ, Teng YC, et al. Involvement of oxidative stress in simvastatin-induced apoptosis of murine CT26 colon carcinoma cells. Toxicol Lett. 2010 Dec 15;199(3):277-87.
82. Skaletz-Rorowski A, Walsh K. Statin therapy and angiogenesis. Curr Opin Lipidol. 2003 Dec;14(6):599-603.
83. Hinoue T, Weisenberger DJ, Pan F, Campan M, Kim M, Young J, et al. Analysis of the association between CIMP and BRAF in colorectal cancer by DNA methylation profiling. PLoS One. 2009;4(12):e8357.
84. Kodach LL, Jacobs RJ, Voorneveld PW, Wildenberg ME, Verspaget HW, van Wezel T, et al. Statins augment the chemosensitivity of colorectal cancer cells inducing epigenetic reprogramming

and reducing colorectal cancer cell 'stemness' via the bone morphogenetic protein pathway. Gut. 2011 Nov;60(11):1544-53.
85. Wang W, Collie-Duguid E, Cassidy J. Cerivastatin enhances the cytotoxicity of 5-fluorouracil on chemosensitive and resistant colorectal cancer cell lines. FEBS Lett. 2002 Nov 20;531(3):415-20.
86. Riganti C, Doublier S, Costamagna C, Aldieri E, Pescarmona G, Ghigo D, et al. Activation of nuclear factor-kappa B pathway by simvastatin and RhoA silencing increases doxorubicin cytotoxicity in human colon cancer HT29 cells. Mol Pharmacol. 2008 Aug;74(2):476-84.
87. Lee J, Lee I, Han B, Park JO, Jang J, Park C, et al. Effect of simvastatin on cetuximab resistance in human colorectal cancer with KRAS mutations. J Natl Cancer Inst. 2011 Apr 20;103(8):674-88.
88. Katz MS, Minsky BD, Saltz LB, Riedel E, Chessin DB, Guillem JG. Association of statin use with a pathologic complete response to neoadjuvant chemoradiation for rectal cancer. Int J Radiat Oncol Biol Phys. 2005 Aug 1;62(5):1363-70.
89. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat Rev Drug Discov. 2005 Dec;4(12):977-87.
90. Kwak B, Mulhaupt F, Myit S, Mach F. Statins as a newly recognized type of immunomodulator. Nat Med. 2000 Dec;6(12):1399-402.
91. Samadder NJ, Mukherjee B, Huang SC, Ahn J, Rennert HS, Greenson JK, et al. Risk of colorectal cancer in self-reported inflammatory bowel disease and modification of risk by statin and NSAID use. Cancer. 2011 Apr 15;117(8):1640-8.
92. Lee JY, Kim JS, Kim JM, Kim N, Jung HC, Song IS. Simvastatin inhibits NF-kappaB signaling in intestinal epithelial cells and ameliorates acute murine colitis. Int Immunopharmacol. 2007 Feb;7(2):241-8.
93. Cho SJ, Kim JS, Kim JM, Lee JY, Jung HC, Song IS. Simvastatin induces apoptosis in human colon cancer cells and in tumor xenografts, and attenuates colitis-associated colon cancer in mice. Int J Cancer. 2008 Aug 15;123(4):951-7.
94. Suh N, Reddy BS, DeCastro A, Paul S, Lee HJ, Smolarek AK, et al. Combination of atorvastatin with sulindac or naproxen profoundly inhibits colonic adenocarcinomas by suppressing the p65/beta-catenin/cyclin D1 signaling pathway in rats. Cancer Prev Res (Phila). 2011 Nov;4(11):1895-902.
95. Banez LL, Klink JC, Jayachandran J, Lark AL, Gerber L, Hamilton RJ, et al. Association between statins and prostate tumor inflammatory infiltrate in men undergoing radical prostatectomy. Cancer Epidemiol Biomarkers Prev. 2010 Mar;19(3):722-8.
96. Malicki S, Winiarski M, Matlok M, Kostarczyk W, Guzdek A, Konturek PC. IL-6 and IL-8 responses of colorectal cancer in vivo and in vitro cancer cells subjected to simvastatin. J Physiol Pharmacol. 2009 Dec;60(4):141-6.
97. Wang XS, Williams LA, Krishnan S, Liao Z, Liu P, Mao L, et al. Serum sTNF-R1, IL-6, and the development of fatigue in patients with gastrointestinal cancer undergoing chemoradiation therapy. Brain Behav Immun. 2012 Jul;26(5):699-705.
98. Coogan PF, Smith J, Rosenberg L. Statin use and risk of colorectal cancer. J Natl Cancer Inst. 2007 Jan 3;99(1):32-40.
99. Siddiqui AA, Nazario H, Mahgoub A, Patel M, Cipher D, Spechler SJ. For patients with colorectal cancer, the long-term use of statins is associated with better clinical outcomes. Dig Dis Sci. 2009 Jun;54(6):1307-11.
100. Ng K, Ogino S, Meyerhardt JA, Chan JA, Chan AT, Niedzwiecki D, et al. Relationship between statin use and colon cancer recurrence and survival: results from CALGB 89803. J Natl Cancer Inst. 2011 Oct 19;103(20):1540-51.
101. Lipkin SM, Chao EC, Moreno V, Rozek LS, Rennert H, Pinchev M, et al. Genetic variation in 3-hydroxy-3-methylglutaryl CoA reductase modifies the chemopreventive activity of statins for colorectal cancer. Cancer Prev Res (Phila). 2010 May;3(5):597-603.
102. Rosuvastatin in Treating Patients With Stage I or Stage II Colon Cancer That Was Removed By Surgery 2009 [updated 09/02/2012; cited 2012 05/12/2012]. Available from: http://clinicaltrials.gov/show/NCT01011478.
103. Tønnesen H, Knigge U, Bülow S, Damm P, Fischerman K, Hesselfeldt P, et al. Effect of cimetidine on survival after gastric cancer. Lancet. 1988 Oct;2(8618):990-2.
104. Harvey VJ, Slevin ML, Dilloway MR, Clark PI, Johnston A, Lant AF. The influence of cimetidine on the pharmacokinetics of 5-fluorouracil. Br J Clin Pharmacol. 1984 Sep;18(3):421-30.

105. Adams WJ, Lawson JA, Morris DL. Cimetidine inhibits in vivo growth of human colon cancer and reverses histamine stimulated in vitro and in vivo growth. Gut. 1994 Nov;35(11):1632-6.
106. Cianchi F, Cortesini C, Schiavone N, Perna F, Magnelli L, Fanti E, et al. The role of cyclooxygenase-2 in mediating the effects of histamine on cell proliferation and vascular endothelial growth factor production in colorectal cancer. Clin Cancer Res. 2005 Oct;11(19 Pt 1):6807-15.
107. Masini E, Fabbroni V, Giannini L, Vannacci A, Messerini L, Perna F, et al. Histamine and histidine decarboxylase up-regulation in colorectal cancer: correlation with tumor stage. Inflamm Res. 2005 Apr;54 Suppl 1:S80-1.
108. Boer K, Helinger E, Helinger A, Pocza P, Pos Z, Demeter P, et al. Decreased expression of histamine H1 and H4 receptors suggests disturbance of local regulation in human colorectal tumours by histamine. Eur J Cell Biol. 2008 Apr;87(4):227-36.
109. Chen JS, Lin SY, Tso WL, Yeh GC, Lee WS, Tseng H, et al. Checkpoint kinase 1-mediated phosphorylation of Cdc25C and bad proteins are involved in antitumor effects of loratadine-induced G2/M phase cell-cycle arrest and apoptosis. Mol Carcinog. 2006 Jul;45(7):461-78.
110. Matsumoto S, Imaeda Y, Umemoto S, Kobayashi K, Suzuki H, Okamoto T. Cimetidine increases survival of colorectal cancer patients with high levels of sialyl Lewis-X and sialyl Lewis-A epitope expression on tumour cells. Br J Cancer. 2002 Jan;86(2):161-7.
111. Siegel JN, Schwartz A, Askenase PW, Gershon RK. T-cell suppression and contrasuppression induced by histamine H2 and H1 receptor agonists, respectively. Proc Natl Acad Sci U S A. 1982 Aug;79(16):5052-6.
112. Adams WJ, Morris DL. Short-course cimetidine and survival with colorectal cancer. Lancet. 1994 1994 Dec 24-31;344(8939-8940):1768-9.
113. Adams WJ, Morris DL. Pilot study--cimetidine enhances lymphocyte infiltration of human colorectal carcinoma: results of a small randomized control trial. Cancer. 1997 Jul 1;80(1):15-21.
114. Kelly MD, King J, Cherian M, Dwerryhouse SJ, Finlay IG, Adams WJ, et al. Randomized trial of preoperative cimetidine in patients with colorectal carcinoma with quantitative assessment of tumor-associated lymphocytes. Cancer. 1999 Apr;85(8):1658-63.
115. Kapoor S, Pal S, Sahni P, Dattagupta S, Kanti Chattopadhyay T. Effect of pre-operative short course famotidine on tumor infiltrating lymphocytes in colorectal cancer: a double blind, placebo controlled, prospective randomized study. J Surg Res. 2005 Dec;129(2):172-5.
116. Nielsen HJ, Moesgaard F, Hammer JH. Effect of ranitidine and low-dose interleukin-2 in vitro on NK-cell activity in peripheral blood from patients with liver metastases from colorectal cancer. Eur J Surg Oncol. 1995 Oct;21(5):526-30.
117. Adams WJ, Morris DL, Ross WB, Lubowski DZ, King DW, Peters L. Cimetidine preserves non-specific immune function after colonic resection for cancer. Aust N Z J Surg. 1994 Dec;64(12):847-52.
118. Rasmussen LA, Nielsen HJ, Sorensen S, Sorensen C, Rasmussen R, Moesgaard F, et al. Ranitidine reduces postoperative interleukin-6 induced C-reactive protein synthesis. J Am Coll Surg. 1995 Aug;181(2):138-44.
119. Matsumoto S. Cimetidine and survival with colorectal cancer. Lancet. 1995 Jul;346(8967):115.
120. Svendsen LB, Ross C, Knigge U, Frederiksen HJ, Graversen P, Kjaergård J, et al. Cimetidine as an adjuvant treatment in colorectal cancer. A double-blind, randomized pilot study. Dis Colon Rectum. 1995 May;38(5):514-8.
121. Nielsen HJ, Christensen IJ, Moesgaard F, Kehlet H, Group DRCCS. Ranitidine as adjuvant treatment in colorectal cancer. Br J Surg. 2002 Nov;89(11):1416-22.
122. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010 Jun;138(6):2073-87 e3.
123. Deva S, Jameson M. Histamine type 2 receptor antagonists as adjuvant treatment for resected colorectal cancer. Cochrane Database Syst Rev. 2012;8:CD007814.
124. Galon J, Pages F, Marincola FM, Angell HK, Thurin M, Lugli A, et al. Cancer classification using the Immunoscore: a worldwide task force. J Transl Med. 2012;10:205.