THE CAPP2 STUDY
Handbook

A randomised controlled trial of colorectal polyp and cancer prevention using aspirin and resistant starch in carriers of Hereditary Non-Polyposis Colon Cancer (Lynch syndrome)

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CONTENTS **PAGE**

THE CAPP2 STUDY:
COLORECTAL **A**DENOMA/CARCINOMA **P**REVENTION **P**ROGRAMME

1. BACKGROUND

1.1 Introduction

Colorectal cancer is the second leading cause of cancer death. It shows marked variation in prevalence, being very common in Western societies and rare among rural populations in economically underdeveloped countries. Strategies which explore approaches to prevention are needed since survival after development of a symptomatic cancer remains poor.

There is epidemiological and experimental evidence demonstrating a protective effect of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Separate epidemiological and experimental evidence support a similar effect for starches resistant to digestion which are fermented in the bowel to form short chain fatty acids. Progressive accumulation of gene changes underlying the adenoma/ carcinoma sequence is now widely accepted. It has provided an explanation for the large minority in whom a germline gene defect predisposes to early and frequent colorectal cancers. The recording of adenoma size and number is a surrogate endpoint in the study of colorectal cancer prevention when systematic removal of adenomata at routine endoscopy is employed.

1.2 Hereditary Non-Polyposis Colon Cancer (Lynch syndrome)

Five to ten percent of colorectal cancer is associated with a high risk of colon cancer in an affected individual's first degree relatives due to its hereditary etiology. Multiple colonic polyps characterise familial adenomatous polyposis coli (FAP) and its milder variant known as atypical familial adenomatous polyposis, both due to a mutated form of the *APC* gene. Lynch syndrome usually results from a mutation in one of the mismatch repair gene family; *MSH2, MLH1, PMS2* or MSH6 (Peltomäki and De la Chapelle, 1997; Marra and Boland, 1995). If a somatic mutation inactivates the wild-type allele in a person with an inherited defect, the affected cell will begin to accumulate new mutations at a very high rate, presumably enhancing the potential for malignant transformation. The concept of ineffective DNA mismatch repair had a major impact on our understanding by offering a model of carcinogenesis which complements and expands the multistep adenoma carcinoma sequence.

Lynch syndrome is characterized by early age of cancer onset (≈44 years), proximal predominance of colorectal cancer (≈70% proximal to splenic flexure), multiple synchronous and metachronous CRCs (≈45% within 10 years after incomplete colonic resection), and also by excess extra-colonic cancers. These include endometrial, ovarian, stomach, small bowel, and uroepithelial transitional cell carcinomas (Lynch and Smyrk, 1996). Studies in large Lynch syndrome families indicate that males who inherit a germline mutation in the *MLH1* or *MSH2* genes are at an 85 to 90% lifetime risk of developing colorectal cancer with a lower risk in women. Dunlop et al (1997) traced all the relatives of individuals who developed colon cancer under 35. In those families with a

mismatch repair gene defect, the lifetime risk for all cancers in women was 69% approximately equally divided between colorectal and endometrial cancer (Lynch and Lynch, 1995). It is likely that aberrant colon crypts, considered the precursor of colon cancer, can progress to malignancy at an accelerated rate.

Further refinement of genotype/phenotype relationships are ongoing, but is now believed that mutations in MSH6 have a later age of onset of CRC and a higher risk of endometrial cancer than in MLH1 and MSH2. This may be due to the fact that MSH6 mutations result in the failure to correct single base changes in replicating DNA, whereas MSH2 and MLH1 mutations lead to a failure in correcting a variety of changes.

Colonoscopy screening in Lynch syndrome patients has been shown to reduce colorectal cancer incidence, colorectal cancer mortality, and overall mortality and identify more polyps in those screened regularly compared to those not screened (Järvinen et al., 1995). In most centres, colonoscopy commences in the third decade and continues one to three yearly thereafter (Lynch and Lynch, 1995).

Carriers of mismatch repair gene defects represent an ideal population for evaluation of chemoprevention strategies; molecular genetic testing is adding to the large number of carriers identified on clinical grounds who still have an intact colon and are undergoing regular surveillance. Uncertainty about the utility of surgical intervention has resulted in a large cohort available to test chemoprevention strategies. Many are known to clinical registries which are, in turn, linked through the International Collaborative Group. Gene carriers have the additional motivation to comply in that they are helping to develop strategies of value to their close relatives. Long term surveillance beyond the end of the initial trial is relatively easy through existing registers.

A single nucleotide polymorphism in *APC* in an Ashkenazim population, T3920A, has recently been associated with a familial cancer phenotype attributable to the rarer allele having a poly-A tract which predisposes to somatic mutation (Laken et al., 1997). Proven carriers of this variant are eligible for enrolment in CAPP2 and will be distributed equally in the four limbs to allow subgroup analysis.

1.3 Molecular Pathology of Colorectal Cancer in Lynch syndrome

Tumours in Lynch syndrome gene carriers display unusual histological features; an excess of mucinous carcinoma has been reported in series from Finland, New Zealand and the USA (Lynch et al., 1993; Mecklin et al., 1986; Jass et al., 1994).

Microsatellite Instability MSI

Malignant colonic tumours in Lynch syndrome accumulate genetic defects as a result of a breakdown of mismatch repair. The term RER, (abbreviation of Replication Error) was applied. The diagnostic feature of somatic changes in the length of microsatellite markers can develop in the absence of cell division, so the term RER is now replaced with a division into high and low levels of microsatellite instability (MSI). MSI is a measure of incorrect DNA replication and is seen in 13% of sporadic colorectal cancers and > 90% of Lynch syndrome cases. A threshold of

2 or more unstable mono- or dinucleotide markers out of the panel of 5 has a high predictive value for Lynch syndrome and should lead to consideration of mutation detection in the mismatch repair genes.

It is possible that the biology of cancer progression is different in these individuals. For example, Konishi (Konishi et al., 1996) found mutation in the Transforming Growth Factor β Receptor II (TGF-βRII) gene to be much more frequent in colorectal adenomas and malignancies from Lynch syndrome patients than similar tissue specimens from patients with FAP and sporadic colorectal cancer. In addition, detectable mutations in the *APC, p53*, and *K-ras-2* genes were more common in specimens from FAP and sporadic cases than Lynch syndrome cases. The colon cancers may show unusual histology, including an excess of poorly differentiated carcinoma, mucinous carcinoma, signet cell carcinoma and undifferentiated histology (Jass et al., 1994; Mecklin et al., 1986; Lynch et al., 1993).

1.4 The Study Population: Carriers of Lynch syndrome

Henry Lynch was the first to identify the natural history of Lynch syndrome and to define the autosomal dominant mode of inheritance. He originally used the term "Cancer Family Syndrome" (Lynch et al., 1966) subsequently referred to as Hereditary Non-Polyposis Colorectal Cancer (HNPCC), and then termed as the Lynch syndromes by Boland (Boland and Troncale, 1984).

At least half of families with "Amsterdam criteria positive" Lynch syndrome have mutations in MSH2 or MLH1, two members of the mismatch repair gene family. In the early stages of research into the genetic basis of Lynch syndrome, the Amsterdam criteria were developed to increase the chance of finding genetic linkage. They required;

- 3 cases of colorectal cancer in the family
- 1 is a first degree relative of the other two
- 1 under 50 years
- at least two generations affected.

In 1997 the ICG agreed that one of the cases could be endometrial cancer or one of the other recognised cancers in Lynch syndrome: gastric cancer, uroepithelial cancer, multiple basal cell carcinomas or ovarian cancer. In practice, the majority of Lynch syndrome families do not fulfil these criteria but the criteria remain valuable as a means of identifying the families most likely to yield results in mutation detection. Other useful approaches to targeting include, multiple relevant tumours in a relatively young person, colorectal cancer under the age of 35 in the absence of familial adenomatous polyposis and high levels of MSI in a colonic tumour.

The primary objective of CAPP2 is to determine, by randomised controlled trial, whether daily ingestion of aspirin and/or resistant starch will reduce adenoma initiation and progression in this genetically predisposed population. In general,

those with a family history are motivated and accessible and many are under regular surveillance. They are likely to represent a group in which environmental influences relevant to the general population can be tested more easily. In practice, those at familial risk are most likely to heed advice on lifestyle modification and should be investigated in their own right, whether or not general population studies are undertaken. A randomised trial is justified in view of the recognised side effects of non--steroidal anti-inflammatory drugs and the need to be sure that an effect on polyp formation does not conceal an increased rate of malignancy in "flat lesions" (Lynch et al., 1996).

1.5 CAPP1 & CAPP2

The original trial involving FAP gene carriers was initiated in 1993 with funding from the European Union Biomed 1 programme as CAPP (Concerted Action Polyposis Prevention) (Burn et al., 1995), and was supported by a grant from the UK Medical Research Council, and subsequently by Cancer Research UK. This trial was delayed by limited funds, European ethics committee delays and by complex logistics requiring collection of video recordings of endoscopies.

By May 2002 CAPP1 had recruited 230 gene carriers from registries in 13 European countries and it was reported on during 2003. It showed evidence of the beneficial effects of aspirin and resistant starch but not sufficient to change the need to complete CAPP2. This conclusion was supported by the CAPP2 Steering Committee.

Funding of CAPP2 as a Reinforced Concerted Action commenced on May 1st 1998. Cancer Research UK will support data management and central administration. The two interventions have been provided by the Bayer Corporation and by National Starch. The National Starch and Chemical Company have covered packaging, storage and shipping costs for the nutritional supplements. Bayer has contributed to recruitment, administration and mutation detection costs.

Participants will be randomised in a factorial design to 600mg aspirin[1] and/or 30g "resistant" corn starch with placebo controls. The primary endpoint will be the incidence of bowel neoplasia. A secondary endpoint will be the incidence of associated extracolonic malignancies (Lynch et al., 1993). The number, size and histological status of all colonic adenomas and carcinomas will be recorded and compared with treatment and placebo groups. Crypt cell proliferation and apoptosis will be measured in colorectal biopsies from a sub-set of participants (Mills et al., 2001). Sub-group analyses will include genotype, on the basis that hMLH1 mutations cause a milder phenotype (Sankila et al., 1996), sex, in view of recent evidence of a lower colon cancer risk in female carriers (Dunlop et al., 1997), and dietary habit.

[1] "AspirinR" is a registered trademark of Bayer AG in more than 70 countries world-wide

Aspirin is already in widespread use as a protective agent against cardiovascular disease. It is cheap, widely available, well understood and represents a credible population therapy if this study shows it to be protective against cancer. If resistant starch is shown to have an effect either independent of aspirin or in concert, it will be possible to promote a change in food processing to incorporate a larger proportion of indigestible starch. Our reason for including the starch recently developed by National Starch is that it remains resistant to digestion after cooking and is, therefore, a potential ingredient for a wide spectrum of foodstuffs. The need for CAPP2 is driven by the urgency to evaluate the protective effects of aspirin before more widespread use occurs. If it can be shown that aspirin has a protective effect in Lynch syndrome, we can recommend aspirin to those with a family history with greater confidence.

1.6 Study Design Background

Professor Burn, CAPP Project Leader, was a member of the international steering group of the successful UK MRC vitamin trial. He led recruitment in Northern England and the trial proved the preventative effect of taking folic acid in pregnancy to prevent neural tube defects in babies. The current trial is based on that successful factorial design which operated on the basis of an independent data monitoring group whose role was to review the results of intervention using the randomisation code and interrupt the trial when a statistically significant result was obtained. The current trial lends itself to a similar approach. Professor Tim Bishop and his team at the Cancer Research UK Genetic Epidemiology Unit, Leeds, will be responsible for data management, randomisation and presentation to the MRC Data Monitoring Committee, chaired by Professor Doug Altman.

1.7 Aspirin

There is strong epidemiological evidence in favour of aspirin and other NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) being protective against colorectal cancer (Giovannucci et al., 1995) which is summarised in Figures 1 and 2, adapted and updated from Burn (1995). All studies to date have pointed to a relative risk of colorectal cancer in regular users of NSAIDs of about 0.6. The one exceptional study involved a group with a mean age of 73 years (Paganini-Hill et al., 1989) (figure 1). This latter work also failed to support a protective effect of aspirin in cardiovascular disease.

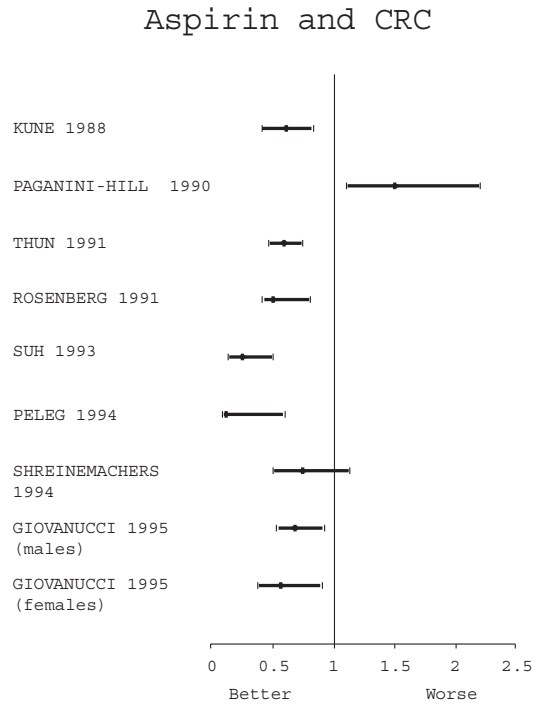


Figure 1. Relative risk of colorectal cancer in regular aspirin users.

(modified and updated from Burn et al 1995)

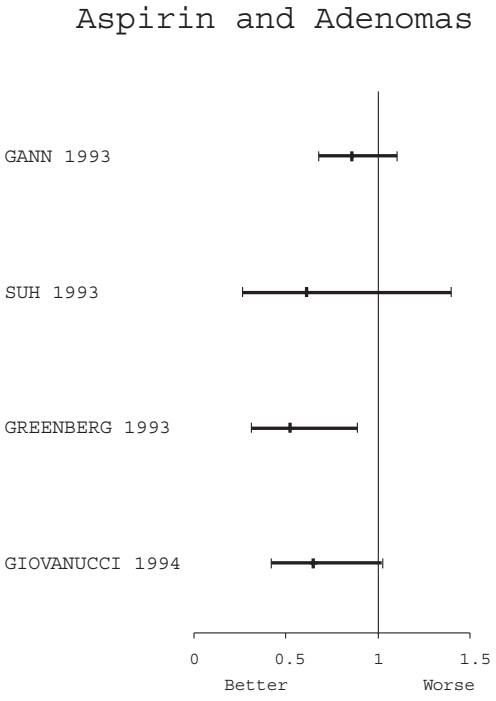


Figure 2. Relative risk of colorectal adenomas in regular aspirin users

There has been one randomised trial of aspirin that did not show an effect. Gann et al (Gann et al., 1993) reviewed the prevalence of colonic tumours in over 22,000 physicians who had been randomised to alternate day 325mg aspirin for 5 years and found a relative risk of 1.15 for colon cancer and a non-significant reduction in the number of colonic adenomata (RR 0.86). This study was not designed to test the colon cancer prevention hypothesis but it does emphasise the need for formal trials to exclude confounding among the observational studies.

As well as the accumulating epidemiological evidence for a protective effect of aspirin in CRC, there is also support from animal studies. For example, Davis and Patterson (Davis and Patterson, 1994) reported that aspirin reduces the incidence of colonic carcinoma in the dimethylhydrazine rat animal model. Aspirin inhibits cyclo-oxygenases, COX 1 & 2. Oshima et al. (Oshima et al., 1996) have shown that COX2, prevalent in the colonic mucosa, plays an early role in adenoma development in the *APC*^{716} knockout mouse, reinforcing the biological case for the hypothesis that aspirin will suppress adenoma development. A selective COX2 inhibitor, SC-58125, has been shown to suppress growth of H-ras-transformed rat intestinal epithelial cells.

Of equal interest is the evidence from plant studies. Aspirin is produced by acetylation of salicylate, a naturally occurring anti-inflammatory found originally in willow bark (Leutwyler, 1994). Recent studies have shown salicylate to be an

important mediator of plant resistance (Zhu et al., 1996) and to be present in substantial amounts in a wide range of green vegetables. Salicylate assists in plant resistance by induction of apoptosis at the site of infection. Given the relationship of salicylate levels to plant infection, it is likely that the modern Western diet will have relatively little salicylate as a result of conventional farming methods. Ingestion of currently available green vegetables does not elevate blood salicylate levels (Janssen et al., 1996). It is possible that salicylate will prove to be a conditionally "essential dietary component" present in only limited amounts in Western diets as a result of use of agrochemicals for plant protection. (Burn et al., 1998).

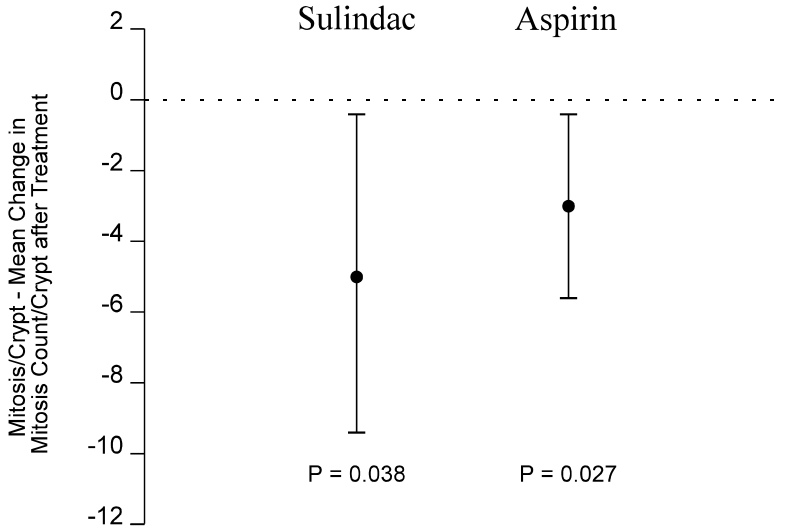
The group led by Paraskeva has investigated the effect of salicylate on colorectal tumour cell lines (Elder et al., 1996). They found a dose dependent inhibitory effect on all lines (50% inhibitory concentration 1.65mM ($\pm$0.36) to 7.38mM($\pm$ 1.08). Carcinoma and *in vitro* transformed adenoma cells were more sensitive than adenoma cell lines. Cells accumulated in G_o-G_1 phase of the cell cycle. Apoptosis was induced in the carcinoma and in vitro transformed adenoma cells but was less consistent in adenoma cell lines. These findings are in keeping with the known effects of sulindac. This NSAID is metabolised to a more active form in the gut and has been shown to cause adenoma regression in controlled studies with FAP patients (Piazza et al., 1995; Debinski et al., 1995). In vitro studies have shown that this agent also inhibits cell growth by the induction of apoptosis (Shiff et al., 1996; Boolbol et al., 1996). It is noteworthy that this effect is not considered to occur via inhibition of prostaglandin synthesis. Shiff et al (Shiff et al., 1995) found sulindac sulphide to be an inhibitor of proliferation, to induce cell quiescence and induce apoptosis in HT-29 colon adenocarcinoma cells. COX2 is not overexpressed in HNPCC cancers. Whether aspirin has its effects through COX inhibition or other mechanisms remains controversial. Genomic stabilisation and apoptotic enhancement may well be independent of COX inhibition.

In 1998 Ruschoff et al reported that aspirin caused mismatch repair deficient cells to become apoptotic. While some laboratories failed to replicate Ruschoff's findings Wallinger et al, 1999, reproduced Ruschoff's data in studies of the effects of aspirin on mismatch repair deficient cell lines (MLH1, MSH2 and MSH6, but not PMS2): microsatellite instability was stabilised.

While the case for NSAID prevention of colon cancer is powerful it is not proven. Most of the epidemiology is observational, the apparently long time delay between use and effect is not explained, there is no clear evidence of an optimum dose and the in vitro studies have used concentrations which could not be achieved in the context of low dose preventive therapy. The International Agency on Research in Cancer (IARC) agreed there was a need for randomised controlled trials and that use of aspirin in a genetically predisposed population was an attractive approach (IARC., 1997).

During the course of CAPP1, Professors' Mathers and Burn and colleagues have investigated the effect of aspirin and sulindac on the rectal mucosa of FAP

patients who have previously undergone colectomy with ileorectal anastomosis (Mills et al., 1998). In a pilot study based on 6 carriers of FAP, treatment for three months using either sulindac or aspirin with a cross over design and one month washout period, three independent observers recorded a non-significant reduction in polyp counts. The study also showed a significant inhibitory effect on the number of mitoses per crypt in normal mucosa *in vivo* (Figure 4).



Mitosis/Crypt - means and 95% confidence intervals -
Changes after treatment with both sulindac and aspirin (Paired T-test)

Figure 3. A significant reduction in the number of mitoses per crypt in rectal mucosa after treatment for three months with oral sulindac or aspirin (Mills et al., 1998)

1.8 Aspirin safety

The adverse effects of aspirin are real and well documented. The primary focus is on haemorrhagic complications due to gastric erosion and anti platelet effects. The use of enteric coated aspirin should reduce to a modest degree the direct adverse effects on the gastric mucosa. The platelet inhibition is irreversible and is apparent at very low doses. The absolute risk of serious complications is small and is made clear in patient information. The population under study is relatively young, which should reduce effects as these are, to some extent, age dependent. Contrary to popular belief, the randomised trials of various “low dose” regimes up to 1200mg per day did not demonstrate a significant difference between 300 and 600mg dosage (Figure 4) though there was a trend to more symptoms and complications. The overall figure in both groups was an approximate doubling of gastrointestinal symptoms and signs.

There is evidence of tolerance with continued use and symptoms may be countered with traditional antacids. The modest increase in side effects must be set against the importance of the trial and the beneficial effect of aspirin in reducing thrombotic events. There is a risk of exacerbation of asthma in a small

percentage of sufferers so this will be an exclusion criterion as will pregnancy though there have been few reports of presumed adverse effects in human pregnancy (de Swiet and Fryers, 1990).

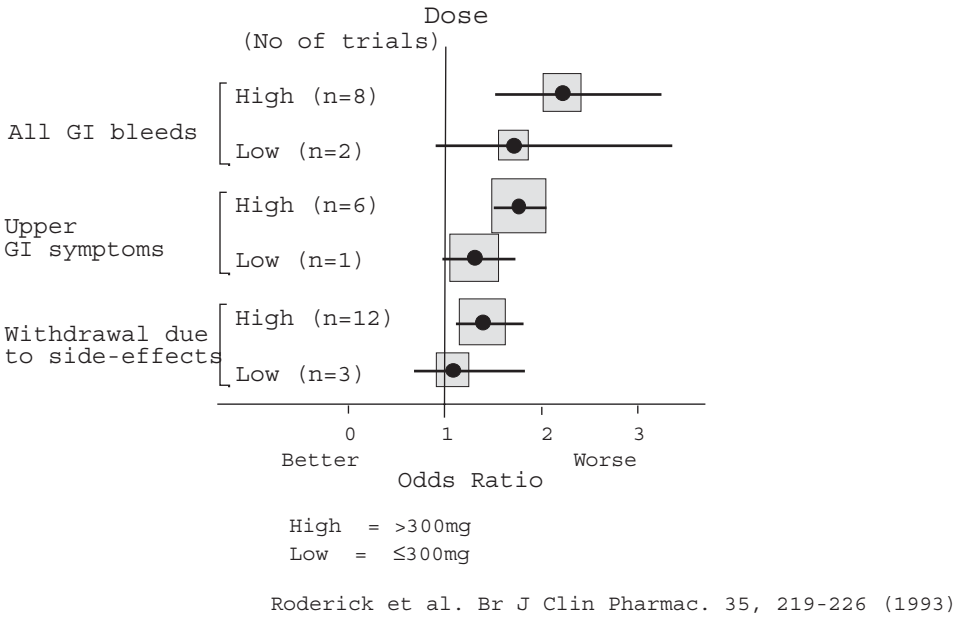


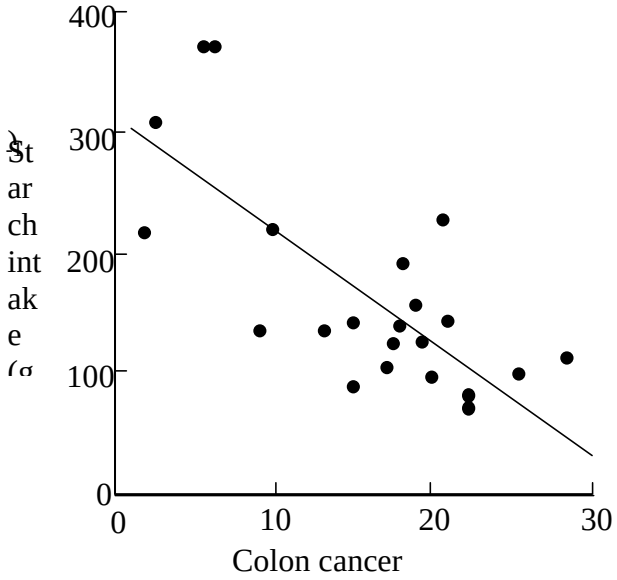
Figure 4 Relative risk of gastrointestinal side effects in placebo controlled trials of aspirin in doses above and below 300mg

1.9 Dietary Factors

Of all cancer sites, the strongest evidence for a link with sustained patterns of food choice is for the colon and rectum (Willett 1995). Evidence is also accumulating that those with a family history of colon cancer may be at higher risk from adverse dietary patterns (Slattery et al. 1997 a and b). If Lynch syndrome is a paradigm for the part played by disabled DNA mismatch repair systems in the development of neoplasia, then diet-delivered environmental factors could affect outcomes in this study. Efforts will, therefore, be made to monitor diet as a variable (see section 2.10).

Although a dietary contribution to colorectal cancer is accepted, the relevance of individual dietary components is unclear. The early suggestions that dietary fibre was pivotal failed to recognise the distinction between non-starch polysaccharides and undigested carbohydrates. It is the latter which show a clear negative correlation (Cassidy et al., 1994); the negative correlation between cancer incidence and non-starch polysaccharide ("true fibre") intake was only 0.29 whereas that between starch intake and cancer rates was 0.76, the most highly correlated of all food components (figure 5). Much of the literature on "fibre" is flawed by old methods of analysis which failed to take into account unabsorbed or resistant starch.

Resistant starch is that part of dietary intake which is resistant to the action of alpha amylase in the small gut. In many foods, some of the starch has crystalline components which are resistant to the action of amylase. Some starch also reaches the large bowel because it is physically inaccessible, in seeds for example. Some starch is retrograded; cooking disrupts starch granules but on cooling the gelatinised starch crystallises. The degree of hydrolysis, the temperature and the speed of cooling influence the subsequent digestibility (Bingham, 1988).



The association between starch intake (g day^{-1}) and colon cancer incidence (males and females combined, n=20) (cases per 100,000 age standardised world poulation year^{-1})

Figure 5 Starch intake and CRC (Cassidy et al., 1994)

Starches which reach the colon undigested are fermented by bacteria to form short chain fatty acids. Of these, butyrate in particular appears to have a beneficial anti-neoplastic effect. The literature supporting a major beneficial role for RS and butyrate continues to grow. Butyrate has a protective effect in the rat model (McIntyre et al., 1993) while in humans, RS has been shown to reduce mucosal proliferation, the level of secondary bile acids and the mutagenicity of faecal water (van Munster et al., 1994). Butyrate produces many changes in a wide variety of cell types but, in general, in transformed cells terminal differentiation is induced resulting in programmed cell death or apoptosis (Hague et al., 1993).

An early event in colorectal carcinogenesis appears to be the upward expansion of the proliferative compartment within the crypt and, indeed, this may form a useful pre-neoplastic biomarker. In vitro, butyrate reduced upper crypt cell proliferation in normal cells when the latter is induced by the co-carcinogenic secondary bile acid deoxycholate (Scheppach, 1994). Butyrate may also influence tumorigenesis at a much later stage by inhibiting colonocyte secretion

of urokinase which appears to be involved in control of cell migration and invasiveness (Kruh et al., 1994).

The most compelling evidence for the beneficial effects of butyrate in preventing colon tumorigenesis comes from a study in which butyrate, given daily by rectal enema to rats treated with carcinogens, reduced both tumour number and size (D'Argenio et al., 1996). A review of the likely mechanisms by which butyrate may prevent colorectal neoplasia has been recently published by Williams et al., (2003).

Secondary bile acids are thought to play a role in the progression of colonic cancer. Their production in the colon from primary bile acids is pH sensitive and the reduced pH accompanying starch fermentation inhibits this conversion. Whatever the mechanism, starch malabsorption reduces secondary bile acid excretion in rats and in humans (McIntyre et al., 1993).

Supplementing a normal Dutch diet with resistant starch was found to reduce the concentration of co-carcinogenic secondary bile acids in faecal water, increase faecal short chain fatty acid output and decrease colonic mucosal proliferation rate. (Van Munster et al.,1994)

Evidence has been published for a specific effect of butyrate on mismatch repair. Smith (1986) showed that butyrate alters chromatin accessibility to DNA repair enzymes - perhaps maximising repair function of any residual enzyme available. This has also been shown in fibroblasts (Dresler et al 1985).

There is thus strong support for a role for resistant starch both from epidemiological studies and experimental studies in laboratory animals and Man. It is a natural dietary component which is cheap and non-toxic and which is amenable to testing in a double blind study. As a dietary supplement it is more likely to attract adequate compliance than more stringent dietary manipulation. Starch may be given to children without reservation and, if a controlled trial were to demonstrate a protective effect, it is possible to influence resistant starch content by altered food processing techniques without significant effects on cost and organoleptic properties.

1.10 Resistant starch safety

There are no known major adverse effects of resistant starch in humans apart from the possibility of mild symptoms of increased stool frequency and distension. These rapidly subside with continued use and can be ameliorated by a gradual introduction of the starch. The high amylose resistant corn starch (RCS) to be used in CAPP2 has the advantage of being tolerant of heating without becoming gelatinised, which would make it digestible.

Studies by the Newcastle group (Burn et al., 1996) of high dose resistant starch in the *APC* 1638N (Fodde et al., 1994) mouse have revealed a statistically significant increase in small bowel tumours, an effect reversed by aspirin. Given the significant differences between the mouse and human gut, this effect is

unlikely to be of relevance to humans. The dose used in this mouse study was the equivalent of a dose in humans over three times bigger than the 30g daily to be used in CAPP2. The APC 1638N mouse is characterised by its propensity to upper GI tumours, a rare feature in human gene carriers of Lynch syndrome. Population studies do not show an excess of upper GI tumours in human populations with a high starch intake.

It should be remembered that the daily dose of RCS used in CAPP2 is analogous to eating one banana each day. The calorie content of 2 sachets of starch is 84Kcals.

1.11 Synergistic effects of aspirin and RS

The mechanism of action of butyrate may be *COX2* dependent in which case an enhanced effect may be seen in the study group receiving aspirin and starch (Scheppach, 1994). In 2000 Mariadason et al in a microarray analysis of 8063 gene sequences, showed synergy between butyrate and sulindac, but also displayed subsets of genes exclusively expressed by each, suggesting predominantly independent pathways to suppression, including genes involved with cell cycle, G0-G1 arrest, beta catenin Tcf signalling and apoptotic cascading. Crew et al (2000) showed synergism between butyrate and a COX2 selective agent (NS398) in inducing apoptosis in HT-29 cell line, though this is a mismatch repair proficient cell line. The possibility of synergy reinforces the case for a factorial design that combines two interventions.

1.12 Relevance to Sporadic Colon Cancer

Carriers of the Lynch syndrome gene and their families together with those responsible for family cancer registers are highly motivated to develop treatment strategies which may, ultimately delay or even avoid the need for surgical intervention. Such studies are of general relevance because analysis of sporadic colon cancer tissue reveals that the genetic events leading to formation and progression to cancer are comparable to those in Lynch syndrome. The main distinction between the groups lies in the faster speed of progression in Lynch syndrome.

If pharmaceutical interventions or dietary supplements can be shown to slow the progression of adenoma formation and delay the need for surgery in Lynch syndrome gene carriers, this will add to the evidence in favour of intervention in the general population. If, as is likely, the course of Lynch syndrome neoplasia shows significant differences at the genetic and cellular levels, the results of this study has relevance to those with a history of early onset CRC or with a strong family history. It also, probably has significance to the prevention in those with presumed somatic mismatch repair gene defects leading to RER positive tumours.

These findings call into question the relevance of Lynch syndrome as a model for the general population. They are, however, an important subset in their own right where there is a need to evaluate the effects of chemoprevention. If

chemoprevention is shown to work, those with a strong family history are most likely to make use of this treatment.

Furthermore, the primary assumption in chemoprevention is that the effect will predate the development of colonic adenomata. The Newcastle group have studied crypt cell proliferation in carriers of Lynch syndrome in flat mucosa using the MIB1 antibody and the crypt squash and microdissection techniques. There was no significant difference between proliferation rates in carriers and controls, (Green et al 1998), supporting the belief that the biological divergence becomes apparent only after the initiation of tumour formation.

2. STUDY PROTOCOL

2.1 Interventions

Lynch syndrome gene carriers will receive either 30 grams of treatment starch, equivalent to 13.2 grams of resistant starch, or 600mg of aspirin, neither, or both.

The relatively large dose of aspirin is still in the sub-analgesic "low dose" range but will have a greater capacity to demonstrate efficacy. Existing sporadic adenoma studies are using 75 and 325mg doses making this study complimentary by being able to test a larger dose. Enteric coated tablets will be used to facilitate randomisation and reduce side effects. Bayer Corporation has provided the tablets in blister packs, each pack containing 14 x 300mg tablets, or 1 week's supply. This company will provide an inert calcium salt placebo providing 15mg of calcium daily, which is insignificant, compared to a normal dietary intake of 600-800mg. The dose of starch will be sufficient to have a biological effect, based on volunteer studies, and will be amenable to packaging and dispensing in two sachets to be mixed with any meal of the day. The control will be fully digestible cornstarch. Each will be mixed with para-aminobenzoic acid (PABA) (Bingham et al., 1997) as a compliance marker (see section 2.10).

2.2 Design

This will be a double blind randomised placebo controlled trial applied to gene carriers of Lynch Syndrome. The trial will evaluate two interventions in a factorial design.

2.3 Duration of treatment

Recruits will be treated for a minimum of two years, and for up to 4 years.

A minimum interval of two yearly colonoscopies will be required (plus or minus three months). The precise duration will depend on local practice and the timing and interval of routine clinical colonoscopy for each individual. However, it is anticipated that most patients will fit into one of the following two follow-up categories:

- yearly colonoscopy where neoplasms are detected or have been recorded in the past
- two yearly colonoscopy for patients with no neoplasia

All enrolees will be **treated for a minimum of two years** whether they have annual or biennial colonoscopy. Those screened annually may have a higher

rate of diagnosed polyps so randomisation will be stratified on the intended screening interval. If a participant develops symptomatic neoplasia necessitating earlier endoscopy, this will be taken to be the end of that treatment period.

2.4 Eligibility

Gene carriers of Lynch syndrome are the target population.

This group may be ascertained by

A) GENETIC DIAGNOSIS OR B) CLINICAL DIAGNOSIS:

A) GENETIC DIAGNOSIS

Proven carriers of pathological mutations in mismatch repair genes

B) CLINICAL DIAGNOSIS

Belong to a recognised Lynch Syndrome family based on the modified Amsterdam criteria (see 1.4)

<u>AND</u> have had at least one of the following events:

- **a colorectal cancer**
- **a related carcinoma (endometrial carcinoma is particularly predictive of gene carrier status but others include small bowel, uroepithelial or stomach)**
- **an adenoma of over 5mm diameter**
- **an adenoma under 40 years of age**
- **a confirmed adenoma (of any size) at more than one endoscopy**

- **All enrolees should also:**
  Be over 25 years old. There is no upper age limit.
- Have an intact colon, or if had a segmental resection have normal (non-medicated) bowel actions equal to 3 or fewer formed bowel actions per day.

2.4.1 Eligibility criteria for patients who have had surgery

Eligible patients who have also had any bowel cancer will not be recruited immediately after surgery. We will use the table below to determine an enrolment date.

TABLE OF COMPARISON OF CLINICAL STAGING

ENGLISH/USA	TNM SYSTEM	UICC	CAPP RECRUITMENT
DUKES A	Tis, N0, M0 T1, N0, M0 T2, N0, M0	Stage 0 Stage I Stage I	I YEAR POST SURGERY
DUKES B	T3, N0, M0 T4, N0, M0	Stage II	2 YEARS POST SURGERY (Evidence that the patient is disease free to be judged on a case by case basis)
DUKES C	Any T, N1,2 or 3, M0	Stage III	5 YEARS POST SURGERY
DUKES D	Any T, Any N, M1	Stage IV	5 YEARS POST SURGERY on condition that patient has been disease free on scan for 2 years

NOTES:

T = primary tumour **N** = regional lymph nodes **M** = distant metastasis	TNMx cannot be assessed TNM0 no evidence Tis in situ

(Any Tx, Nx, Mx where the tumour cannot be specifically assessed we would anticipate making an individual assessment on eligibility based on available clinical history).

This table is based on information from the UICC (International Union against Cancer) TNM Classification of Malignant Tumours, fourth edition, 2nd revision, 1992.

Potential enrolees who have suffered other cancers in the recent past will be assessed on an individual basis.

2.4.2 Exclusion criteria

- Pregnancy
  (note: there have been few reports of adverse effects associated with aspirin use in pregnancy and aspirin is not regarded as a teratogen so women of child bearing age may be recruited. However, women should temporarily withdraw from the trial if they become pregnant. They can restart immediately after delivery if they are not breast feeding. If mothers are breast feeding they should not re-enter the trial until they have completed breast feeding.
- Medical contraindications for aspirin e.g aspirin induced asthma, previous aspirin/ NSAID induced peptic ulcer, renal impairment beyond creatinine of 0.15 mmol/l, or haemorrhagic diathesis**.**
- Already taking NSAIDs or steroids.
  (note: If, during participation in the trial, a participant needs to take a course of NSAID's they should be temporarily withdrawn from all limbs of the trial)
- Severe intercurrent disease.
- Known to be HIV positive (routine testing not required).

2.4.3 Long Term Follow-up

The study consent form (**Form 1**) includes explicit consent to contact the recruit and/or their doctor on a regular basis after completing their study participation. This consent will be used to collect information about the recruit's general health status on an annual basis for ten years. A Long Term Follow up Yearly Review form (**Form 17A** and **17 B**) will be used to collect this information. It will be sent to the recruit and another health care professional by the recruiting centre for completion. If a recruit does not wish the CAPP team to monitor their health in subsequent years, no contact will be made.
Under normal circumstances, a 10 year follow-up would be a considerable undertaking, but for those people recruited to CAPP2 it is a small matter since they are all under life long surveillance.

The data collected will be used to test the hypothesis that, regardless of the immediate outcome of CAPP2, there will be a long term effect on cancer development and progression related to the interventions.

2.5 Recruitment

In Europe, North America and Australia there are over a third of a million cases of colorectal cancer each year (Parker et al., 1997). If current estimates of Lynch syndrome's frequency (1 to 5%) proves accurate, as many as 16,000 of those cancers will represent new patients with Lynch syndrome. It must be noted that all attempts to investigate the frequency of Lynch syndrome have been handicapped by the fact that until very recently the diagnosis of Lynch syndrome rested on descriptive criteria. In an effort to standardise reporting of putative Lynch syndrome families, an international panel, meeting in Amsterdam, put forward a list of criteria that must be satisfied for a diagnosis of Lynch syndrome (Vasen et al., 1991). The "Amsterdam criteria" were restrictive as they were designed to aid linkage mapping (see 1.4).

Small families are not likely to meet the criteria for diagnosis, and extra-colonic malignancies, clearly an important component of the syndrome, were not given diagnostic weight. A recent review (ICG meeting Noordwijk, 1997) allows one of the tumours to be another relevant cancer e.g. endometrial or gastric. Current estimates of Lynch syndrome's frequency based on the Amsterdam criteria, then, are likely to be low.

In order to ensure rapid recruitment from "Amsterdam positive" families, CAPP2 will provide a mutation detection service (see section 2.7.1).

2. 6 Enrolment

The intention is to enrol at least 1000 participants who are, or are highly likely to be, carriers of mismatch repair gene defects.

The study requirements should be discussed with each eligible person and a CAPP2 Patient Information leaflet left with them to give them an opportunity to think about joining and to discuss this with others. It is important to ensure that, before enrolment, an individual has given considerable thought to the need for regular consumption of the diet supplement and tablet treatment over a prolonged period, even though the daily tablet and diet supplement should not be difficult to take. A sample pack can be given to the potential recruit. This contains one day's supply of starch powder and aspirin so they know what is expected of them.
A leaflet explaining the treatments will also be sent to each person who enrols in the study. This includes illustrations showing different ways of adding the starch to food and drink.
It must be emphasised that;

Involvement is entirely voluntary and clinical care will not be altered in any way, whether or not they take part.

Anyone eligible for the study should be invited to consent and participate (**Form 1**). Even if the patient does not wish to join, this form should be returned to the Newcastle office giving the reasons for refusal if possible. In addition to agreeing to join the study, Form 1 also requests consent for the collection of biopsies at colonoscopy, the collection of DNA and tissue samples and to annual long term follow up. Consent can be given or withheld to each of the above requests. **Form 2** (CAPP Study Enrolment) should also be completed now.

2.6.1 Requirements at entry

Once consent is given to join the study (**Form 1**), and after an entry colonoscopy date has been determined, a randomisation number should be obtained, either by the recruiting centre themselves or by requesting Newcastle centre to do so, from the randomisation office in Leeds, see section 2.6.2 for more details. This randomisation number, together with a brief medical, drug, and DNA testing

history, will allow completion of **Form 2.** The randomisation number must be quoted on all subsequent correspondence with the CAPP office.

A colonoscopy history is required **(Form 2A)** in order to ascertain how many polyps the patient has had in the past, and to know how many colonoscopic examinations have been performed, as this affects the number of polyps expected during the study. Completion of form 2A should be done at enrolment.

At the entry colonoscopy, and at all subsequent colonoscopies, **Forms 13** and **6** (form 6 only if biopsies are taken) must be completed. The data on these forms are crucial for analysis of the trial results. At entry, or sometime during the study, a pedigree should be sent to the CAPP office (**Form 8**).

PLEASE NOTE: All Subjects must start taking the treatment within 3 months of their entry colonoscopy.

2.6.2 Randomisation

Once an individual has agreed to take part, and form 2 has been completed, contact the randomisation office to be allocated a patient identifying number. Contact can be made by:

E-mail: j.gascoyne@cancer.org.uk
Telephone: [44](0)113 2065038
Fax: [44](0)113 2340183

The randomisation office will ask a few questions before a number is allocated:
1) Name of Centre
2) Surname, Forename and date of birth of the recruit
3) Are there any other family member taking part in the Study?
4) Does the recruit have any contraindications to taking the starch or aspirin?
5) Are the treatment packs to be sent direct to recruit or to the centre?

Once a randomisation/study number has been received it should be used on all correspondence and samples from the patient thereafter. At the same time as the recruiting centre is advised, the Newcastle Pharmacy will independently receive a copy of the randomisation number containing additional information on which treatment category should be assigned to the recruit.

CAPP2 will use the same Randomisation Centre as CAPP1, i.e. the Cancer Research UK Genetic Epidemiology Unit in Leeds UK, under the supervision of Professor Tim Bishop. Randomisation numbers will be allocated by phone, e-mail or fax, and the Randomisation Centre will generate a prescription relevant to the allocated treatment group. The prescribed treatment will be dispensed in Newcastle and sent to the Recruiting Centre or direct to the participant, according to preference.

The recruiting centre and the central team will have no knowledge of the randomisation code, this will be kept by Professor Tim Bishop in Leeds. Annual

surveillance results will be made available to a separate MRC Data Monitoring Committee chaired by Professor Doug Altman. This group will be responsible for formal interruption of the trial if, and when, statistically significant results are obtained.

2.7 Blood Collection

2.7.1 Mutation Analysis

Families eligible for mutation analysis via the Newcastle Molecular Genetics Department must fulfil the following criteria:

- "Amsterdam criteria" positive
  OR
- Positive for modified "Amsterdam criteria" where related cancers are considered as a predictive factor (see section 2.4).
  OR
- Show positive microsatellite instability (MSI) at 2 of the following 5 loci; BAT26, BAT40, D2S123, D5S346, D17S250. This working definition of MSI was agreed at the National Cancer Research Workshop, December 1997 (National Cancer Institute Workshop, 1998).
  AND
- There should be family members eligible for the CAPP study.

If mutation analysis is required then:
1. A pedigree must be sent to Newcastle to confirm eligibility.
2. The family with eligible individuals should be invited to send blood or a DNA sample from an affected individual to the Study Centre in Newcastle **(Form 10).**
Following the detection of a mutation, predictive testing for family members should be undertaken by their local centre.

2.7.2 Modifier Genes

It is likely that ongoing research will lead to the identification of modifier genes which may allow refined analysis. If blood samples are sent, DNA and plasma will be stored for potential future use in such studies. Participation in this part of the study will be optional, and should not influence a centre's decision to participate in CAPP2. Testing of samples will depend on the agreement of the contributing team and, where required by national rules, additional consent from the subject. If the original DNA analysis is performed locally, DNA and plasma samples for future analysis should be stored in a retrievable form, or sent to Newcastle for banking.

2.7.3 DNA Collection

DNA will be collected and stored from recruits where pathology samples are available. This will be used to compare microsatellite instability in blood and tumour cells. These samples will be used, with appropriate consent, to further understanding of intermediate biomarkers relevant to prediction, management and/or treatment of Lynch syndrome and related disorders.

2.8 Requirements at all colonoscopies

The examination should ideally examine the whole colon as far as the caecum. If the caecum is not visualised this should be recorded on the colonoscopy report form **(Form 13)**, together with a statement of the quality of the views achieved. It is assumed that excellent bowel preparation is a prerequisite of the effective clinical care of Lynch syndrome gene carriers. The colonoscopist will be asked to confirm that a full view of the colon was achieved. If a poor preparation is recorded and the colonoscopy is repeated then a second report should be provided for CAPP2.

If the caecum cannot be visualised, a double contract barium enema is used in many centres to complete the examination. A copy of the barium enema films will be requested for the purpose of the CAPP2 study assessment. If originals are sent they will be copied and returned. If normal colonoscopy fails other visual imaging techniques e.g. virtual colonoscopy will be accepted.

A clear colonoscopy (i.e. no adenomas or all adenomas removed, good preparation and caecum visualised) performed in the previous three months is acceptable for immediate enrolment. If the latest colonoscopy was done more than three months before recruitment, enrolment and treatment must wait until after the next colonoscopy. Where possible, this should be brought forward. Any unusual cases should be referred to the CAPP office in Newcastle where a member of the central team will be able to discuss issues raised.
At entry to the study, the recruiting centre should complete a Colonoscopy Review (**Form 2A**) see 2.6.1.

Other details to be collected at colonoscopy and recorded on Form 13 will be:

- Current size and number of polyps
- Location of polyps (marked on table provided).
- Histology of polyps removed (see 2.12), including villosity and level of dysplasia.
- Removal Total, Partial or Failed
- Description of other pathology/ neoplasia
- Past number of adenomas

2.8.1 Mucosal Biopsies

Where possible, we will also request mucosal biopsies to allow investigation of the effects of the interventions on apparently normal tissues **(Form 6, Biopsy Identification).** Mucosal biopsies are usually less than 3mm diameter and cause only transient local bleeding.

Based on polypectomy in over 1000 individuals, 320 of whom had taken NSAID's, Shiffman (Shiffman et al., 1994) concluded that the risk of significant gastrointestinal bleeding after endoscopic biopsy or polyp removal was small (<1%); although the use of NSAID's did increase the incidence of minor self-limited bleeding, an increase in the rate of major bleeding was not observed.

Details of the collection procedure for biopsies are given on **Form 14**. Information on how to send the biopsies to Newcastle is included in the biopsy pack. It should be noted that **biopsies should arrive in Newcastle within 3 days** for the optimal assessment of cell proliferation and apoptosis.

2.8.2 Sample Collections

Study centres are asked to send to the CAPP office, representative histology sections and/or paraffin block fixed tissue from any and all polyps removed during the study. These can be sent after the local reporting has been completed. The collected samples will be reviewed by an expert panel of histopathologists.

2.8.3 MSI Analysis

MSI analysis will be carried out on colorectal polyp and cancer tissue removed at entry and during participation in CAPP2. Tumour cells will be separated from surrounding tissue using laser capture micro-dissection, from which DNA will be extracted. The precise method used to analyse MSI will be guided by the latest technical developments. The current plan (2002) is to analyse six markers in tumour cells and normal cells at 6 loci (Boland et al., 1998). Comparison of MSI in polyps prior to and after treatment will allow assessment at the molecular level of effects of the intervention treatments on mismatch repair. As is other data analyses, comparisons can be made between treatment groups, but changes in MSI status in polyps from a single recruit may also be detected and related to their treatment. The samples will also be used for biomarker development relevant to the CAPP2 project. Other research analysis of stored samples will be anonymised and will be assessed individually by the local research ethics committee.

2.9 Dietary Analysis

Diet plays a large role in the aetiology of colorectal cancer, so it is important that efforts are made to assess its impact on the outcomes of this trial. At the European conference on Nutrition and Cancer, Lyon, June 2001, the first results of the EPIC study revealed a 40% reduction in CRC among the top quintile of “plant polysaccharide” intake. This food category tends to include resistant starches (RS). The current hypothesis is that RS is an important protective component of high starch diets and acts as a pro-drug delivering the polysaccharide to the large bowel to stimulate butyrate production.

UK recruits will be invited to keep a diary describing all food and drink ingested over 4 sequential days. This will take place once during a recruit’s study participation. A sample of urine will be collected at this time to measure treatment compliance. A dietician will visit the recruit and explain how to complete the diary and how to collect the urine. A return visit will be made to collect the completed food diaries and urine sample.

The dietary analysis will be conducted by Prof John Mathers and his team at the Human Nutrition Centre in Newcastle, UK.

2.10 Compliance

Carriers of mismatch repair genes are likely to be highly motivated to participate in an intervention trial for both personal and altruistic reasons. We have found family members to be particularly motivated to develop medical interventions that may be of value to other relatives, now or in the future.

CAPP2 is a long-term trial and assurance is needed that subjects comply with the allocated treatment. Non- compliance, whether permanent or temporary, should be recorded on **Form 4** and the study centre notified. We will try to maximise compliance by providing the treatments in acceptable forms and by providing the subjects with regular updates on the progress of the study to maintain their interest in and enthusiasm for the study.

It will be necessary to have an independent assessment of compliance every 6 months **(Form 11),** and the recruiting centre will be reminded by the study centre when this is due. Centres will then be required to contact/visit recruits to count and record on Form 11 all unused tablets and sachets from the current treatment pack. A new pack of treatment will be received every 6 months. Each treatment pack contains details of the dates on which to start and end that box of treatment.

It is expected that a few participants will feel unable to complete the trial and we would like to know the reasons for non-compliance (**Form 4**). However, brief episodes 'off treatment' for up to 2 weeks for holidays or business trips are quite acceptable. Longer periods should always be recorded and the study centre notified. Permanent or temporary withdrawal from the trial should always be recorded on **Forms 4 and 12.** In the case of permanent withdrawals, the study centre should be notified immediately so that the dispensing of all treatment can be cancelled.

Any medical problems should be reported to the study centre on **Form 5** so that any side-effects from the treatments can be monitored. This information will be made available to the Data Monitoring Group. Building on our experience in the years up to 2000, when it was observed that some recruits withdrew early because of difficulties with integrating the starch into daily diet habits, it was decided that a "trial pack" would be given to potential recruits. This gives the recruits an opportunity to try the interventions for one day prior to randomisation.

The starch supplements will contain a small amount (50mg/d) of 4-amino benzoic acid (PABA), a B complex vitamin which is readily absorbed and excreted in urine. In selected subjects, with ethical committee approval, a measure of compliance may be obtained by analysing random urine samples for PABA.

2.10.1 Disposal of unused treatment

Once a new pack of treatment has been received and started, any remaining tablets and starch in the previous box should be disposed of (after counting and recording see 2.10). Unused tablets can be taken to any pharmacy for disposal

(the recruiting nurse may like to do this). The starch can be disposed of as normal household waste as it is a foodstuff.

2.11 Outcome measures

2.11.1 Primary endpoint

The primary endpoint will be the number, size and histological stage of colorectal carcinomas found after a minimum of two years treatment.

We will ask local pathologists to send representative material for central review. Histology will take account of particular features seen in Lynch syndrome tumours i.e. whether mucinous, displacing Crohns-like reactions are present and the degree of lymphocyte infiltration. All histology will be reviewed by the study pathologists Professor Neil Shepherd and Professor Jeremy Jass. In addition, MSI analysis will be undertaken in Newcastle (2.8.3)

Colonoscopy interval will be determined by local practice. Given reports of early interval cancers (Vasen et al., 1995), a reasonable case can be made for annual colonoscopy in this high risk group though many centres offer two or three yearly surveillance. The speed of progression of tumours in Lynch syndrome makes a 3 year interval unwise. For the purpose of CAPP2 we will ask that 2 years is the longest colonoscopy interval.

The standard preference will be:
2 year colonoscopy interval where there have been no neoplasms OR
1 year colonoscopy where neoplasms have been reported.

2.11.2 Secondary endpoints

- **Adenoma size and number**

Elective removal of polyps will make fully developed cancers rare. The main outcome measure will be the number, size, location, villosity and dysplasia of adenomatous polyps. The open jaws of standard biopsy forceps will be used to assess polyp size in vivo.

- **Apoptosis in adenomata**

A recent observation in the histology of an adenoma from a participant in CAPP1 has led us to consider that the pattern of apoptosis within adenomata is worthy of study. This is in keeping with the evidence in vivo and in vitro for an effect of aspirin on apoptosis. We will therefore request histopathological assessment of adenomas snared at colonoscopy, with special interest in signet cells and undifferentiated medullary carcinoma.

- **Cell proliferation and apoptosis in flat mucosa**

In a sub-set of participants, biopsies of flat rectal mucosa will be collected before and after treatment to test the hypothesis that altered cell proliferation (see Mills et al 2001) and/or apoptosis is a reliable biomarker of tumorigenesis.

- **Other cancers**

Gene carriers of Lynch syndrome are at increased risk of many extracolonic cancers, and these will be systematically reported in the study group **(Form 5**). In particular, there is a 42% lifetime risk of endometrial cancers in female gene carriers (Dunlop et al., 1997; Watson et al., 1994). These data are important in monitoring any favourable or unfavourable change in all cancers within the different study groups. In particular, it will be important to ascertain if the interventions might reduce colonic tumours while at the same time increasing upper GI or non GI tumours. In mouse studies parallel to CAPP1, we have found a significant increase in small bowel polyps in *APC* knockout mice fed excess resistant starch (Burn et al., 1996). Aspirin reversed the effect. Regular aspirin use is associated with a reduced incidence of gastric cancer, a malignancy reported with increased frequency in Lynch syndrome families.

2.12 Power analysis

Power analysis has been performed on the basis of the long term study performed in Finland (Järvinen et al., 1995). Follow-up colonoscopy in Lynch syndrome families revealed an annual rate of pathology development of 2.5% in an at risk population with a mean age of 37 years. In the present study most participants will be followed for two years of treatment between colonoscopies. During that time proven gene carriers would be expected to have developed polyps or cancer in 10% of cases (double the annual rate of the Finnish population who were at 50% risk).

If aspirin treatment reduced polyp development by 40%, it would require 1000 recruits to have a 90% probability of achieving a result significant at the 95% level. This takes account of the present level of withdrawals for any reasons.

2.13 Data analysis

As two treatments are being tested, it has been decided to adopt a factorial design. There will be four treatment combinations with participants being randomly allocated to one of these. All participants will receive aspirin or placebo AND a starch supplement which will be either resistant or digestible starch, i.e.:

A	600mg aspirin + 30g treatment starch
B	Placebo tablets + 30g treatment starch
C	600mg aspirin + 30g placebo starch
D	Placebo tablets + 30g placebo starch

(A + B) compared to (C + D) will test the efficacy of resistant starch.
(A + C) compared to (B + D) will test the effect of aspirin.

This approach means only one quarter of the study group receive only placebo (group D). It allows an assessment of possible interaction between aspirin and starch by comparing groups A and D to groups B and C.

If there is reason to believe that certain variables such as the type of mutation might influence the phenotype, the randomisation centre will have the capacity to weight one or more treatment categories in order to achieve a balance between treatment and placebo categories for these variables.

Each enrolled patient will receive 6 months supply of tablets and starch. The tablets will be either enteric-coated aspirin or placebo. These have been supplied and packed by Bayer into 14 tablet blister packs. Each day, 2 tablets are to be taken together, with a meal, providing a daily dose of 600mg. The resistant starch will be provided as a new product produced by National Starch. This product is unflavoured and can be added to hot food, so there is the potential for it to be added to a variety of food and drink. The digestible starch will be corn starch. Two sachets, each containing 15grams of starch, should be taken daily providing a total of 30 grams of treatment starch.

Table 1 Groups for analysis

	Number/size/worst histological grade of adenomas	
	Aspirin	**Placebo**
Resistant starch	**a**	**b**
Placebo	**c**	**d**

A generalised linear statistical model will be applied to the above groups.
No interim analysis of major endpoints is planned. The size of the study has been computed on the assumption of no interim analyses and performing such an analysis would require more recruits. However, an interim analysis would be conducted if proposed by the steering committee. The only circumstances in which such a proposal could be made would be the publication of information deemed to compromise the successful completion of this project to its end date. An example of such a publication would be a study of reasonable size and scientific credibility which reported a significant favourable or unfavourable effect using aspirin or resistant starch.

2.14 Subgroup analyses

- Age; There is a suggestion in some epidemiological studies that the protective effects of aspirin may be age related (Weiss and Forman, 1996).

- Sex; There is growing evidence to suggest that male Lynch syndrome gene carriers have a higher lifetime risk of colorectal cancer that women (Dunlop et al., 1997).

- Genotype; There are reported differences in the severity of cancer predisposition between the two main predisposing genes, with *MLH1* producing a milder phenotype than *MSH2* (Sankila et al., 1996). Genotype will therefore be examined as a variable in this study.

- Diet and lifestyle; A dietary and lifestyle questionnaire will be obtained from each recruit. This questionnaire is based on the EPIC study questionnaire. The EPIC study is an on-going European cohort study of dietary habits in relation to cancer.

- Number of previous neoplasms at or before enrolment; a strong predictor for metachronous tumours.

2.15 Exit from Study

2.15.1 Full participation

All enrolees will be treated for a minimum of two years and must have an entry and exit colonoscopy. In some centres, annual colonoscopies are offered to gene carriers. This should be made clear at enrolment to ensure even distribution throughout the randomisation groups. The 1 year and 2 year colonoscopy report from these enrolees will be evaluated.

Note: surgery should not be delayed as a result of participation in trial.

2.15.2 Loss to follow up.

The recruiting centres all have active genetic registers, and are therefore ideally placed to ensure regular follow up, which is already an established role of these registries. Participation in CAPP2 will add another layer of reminders to the system, through regular contact between the co-ordinating and participating centres.

2.15.3 Side Effects

Any medical problems of any kind should be reported to the study centre using **Form 5**. Major life threatening side effects such as stroke or gastrointestinal haemorrhage should be recorded without delay on the Serious Adverse Event Form 7 (April 2004) and these will be immediately reported to the Data Monitoring and Ethical committees. All medical reports will be analysed by the randomisation centre annually and a report of side effects in different treatment groups assessed and made available to Doug Altman.

2.16 Ethical Considerations

All information relating to patients enrolled in the CAPP study will be stored in a dedicated confidential computer within the CAPP Office in Newcastle in accordance with the UK Data Protection Act.

Aspirin is available as an "over the counter" preparation. Its prescription for this study is unlikely to cause problems other than the well recognised complications of rare gastrointestinal haemorrhage and even rarer haemorrhagic stroke (see section 1.8). Recruitment to this trial requires a clinician to take responsibility for this prescription.

2.16.1 Declaration of Helsinki

The study will be performed in accordance with the principles stated in the Declaration of Helsinki. The study protocol, including the final version of the patient information and informed consent form to be used, must be approved by an ethics committee before enrolment of any patients in the study. The opinion of the ethics committee should be dated and given in writing. The principal investigator of each participating centre is responsible for informing the regional/national ethics committee of any serious adverse events or major amendments to the protocol as per local requirements.

2.16.2 Patient Information and Consent

The investigators in each centre will ensure that patients are given full and adequate verbal and written information about the nature, purpose, possible risks and benefits of the study. Patients must also be notified that they are free to discontinue their participation in the study at any time and without any negative effect on their usual treatment or surveillance. The principal investigator in each centre is responsible for ensuring that signed informed consent is obtained from all patients before enrolment.

Following changes to clinical trial consent regulations (COREC guidelines) and in order to simplify our consenting procedure the following patient information and consent forms will be used from April 2004:

- CAPP2 Patient Information Leaflet (April 2004)
- CAPP2 Consent **Form 1** (April 2004)
  (This form now contains ALL the consents required for the study. It combines the previous form 1; the additional consent leaflet of January 2002 and the consent for long term follow up).
- CAPP2 Reply Slip for continuing/completing the study **Form 16** (April 2004)
- CAPP2: Food Diary. A letter asking if a recruit wishes to take part in the UK food diary analysis will be sent to all recruits during their time on the study. Participation is entirely voluntary.

All collected tissue and samples are stored in strict confidence by the Institute of Human Genetics which is a combined University/NHS facility. Any analysis will be solely for the purpose of understanding the effectiveness of the trial

treatments used in relation to the different types of genetic predisposition. The samples will not be released or used for any other purpose.

2.16.3 Insurance Issues

Liability for National Starch and Chemical Company, and Bayer Corporation is limited to issues relating to manufacturing of the supplement or treatment. The University of Newcastle has a general indemnity for research trials carried out under its auspices but, given the international nature of this trial, a named clinician should be involved in provision of the aspirin supplements to minimise the prospects of legal action. Given the fact that side effects of aspirin are well understood and are addressed in the trial literature it is very unlikely that any legal action would result from this trial.

2.17 Education and training

Training courses for recruiting nurses are organised by the Newcastle Study centre. A wide range of topics has been covered including ethics and psychosocial issues as well as recruitment strategies. An annual CAPP Delegates Meeting is also organised by the Newcastle Study centre. This meeting brings together everyone involved in the study and provides an opportunity to discuss new scientific developments as well as discuss issues surrounding the study development and recruitment.

2.18 Financial Support

The project will be funded by the UK MRC from January 2002 and Cancer Research UK, to completion in 2007.

The project was designed by members of the Institute of Human Genetics and Human Nutrition Research Centre, University of Newcastle upon Tyne and by members of the Imperial Cancer Research Fund Genetic Epidemiology Unit, St James's Hospital, Leeds.

A project application to the EU was successful as a Concerted Action under the Biomed 2 programme and funded the trial for 36 months from 1 May 1998 to 31 April 2001. Concerted Actions were designed "to co-ordinate the individual research activities carried out in the Member States" and did NOT attempt to provide all the research funding needed to carry out the work. In 1998, the infrastructural support of the Imperial Cancer Research Fund (now Cancer Research UK) became an integral part of the UK Clinical Cancer Genetics Network.

From 1998 to date, Bayer Corporation donated matching funds to support recruitment, administration and mutation detection costs as well as providing the aspirin and placebo in blister packaging. They continue to supply the aspirin/placebo free of charge to the study.

From 1998 to date, National Starch have provided the starch and also covered attendant packaging, storage and shipping costs and they have pledged support through to the end of the study.

From January 1999, initially for 3 years, the Cancer Council of Victoria in Australia has provided generous financial support to cover staff salaries, travel and office costs for the CAPP Study in Australia. This support has enabled increasing recruitment numbers in Australia.

From 2000 to date, SIAK have financially supported the CAPP recruiting centres in Switzerland.

In 2001 the special trustees of the Newcastle upon Tyne Hospitals NHS Trust donated bridging funds to support CAPP2.

From 2002 a successful application for MRC funding provided support for the Newcastle study office, staff salaries, and conference costs.
Continuing Cancer Research UK funding will support data management and central administration.

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CAPP2 List of forms and consents

The recruiting centre must complete those forms shown in bold for every recruit. Others are only required in certain circumstances.

FORM	FORM NAME	WHEN COMPLETED
1	**CONSENT**	**Before enrolment**
2	**Study Enrolment**	**Enrolment**
2A	**Colonoscopy Review**	**Enrolment**
3	Surgical notification	After any Surgery
4	Contact with non-complier	Subject not taking either intervention
5	Medical problem report	Any medical problems at all during study
6	Biopsy Identification	At colonoscopy if biopsies are taken
7	Serious Adverse Event	For any serious adverse event
8	Enrolee pedigree	Any time
9	Travelling expenses	Expenses for attending CAPP meetings
10	Request for mutation detection	If eligible for mutation; check with CAPP office
11	**Compliance**	**At 6 monthly intervals throughout trial**
12	**Withdrawal from study**	**Temporary or permanent withdrawal**
13	**Colonoscopy report**	**At each colonoscopy: on entry, during and at end of study**
14	Method of rectal biopsy collection	For information only
15	Randomisation	For randomisation office use only
16	**Consent for continuing for additional time on study**	**After 2 years on study**
17A (recruit) **17B (clinician)**	**Long term follow up yearly review**	**Yearly for 10 years after patient has completed trial**

A5 Leaflets and Consent	WHEN COMPLETED
Patient Information Sheet	**Prior to recruitment**
Treatment pack Information Leaflet	**Inside every pack of treatment**
Food Diary	**UK recruits only.** **Diary completed by Nutritionist and recruit.**

THE CAPP2 STUDY
COLORECTAL ADENOMA/CARCINOMA PREVENTION PROGRAMME

SHORT PROTOCOL FOR CLINICIANS

Colorectal cancer is the second leading cause of cancer death. There is epidemiological and experimental evidence demonstrating a protective effect of aspirin, and for starches resistant to digestion which are fermented in the bowel to short chain fatty acids.

Hereditary Non-Polyposis Colon Cancer (Lynch Syndrome)

Family studies suggest that at least five to ten percent of colorectal cancer is the result of a genetic predisposition. Hereditary non-polyposis colorectal cancer (Lynch Syndrome) accounts for about half of this group and usually results from a mutation in one of the mismatch repair gene family; *hMSH2, hMLH1, hPMS1 or hPMS2* . Colonoscopy screening in HNPCC patients has been shown to reduce colorectal cancer incidence, colorectal cancer mortality, and overall mortality. In most centres, colonoscopy commences in the third decade and continues one to three yearly thereafter. Carriers of mismatch repair gene defects represent an ideal population for evaluation of chemoprevention strategies. Molecular genetic testing is adding to the large number of carriers identified on clinical grounds who still have an intact colon and are undergoing regular surveillance. Gene carriers have the additional motivation to comply in that they are helping to develop strategies of value to their close relatives.

CAPP2

The primary objective of this study is to determine by randomised controlled trial whether daily ingestion of aspirin and/or resistant starch reduces adenoma initiation and progression in this genetically predisposed population. Funding of CAPP2 as a Reinforced Concerted Action commenced on 1st May 1998 and was supported by ICRF, Bayer Corporation and National Starch & Chemical Company. The trial is currently funded by the MRC and Cancer Research UK; Bayer and National Starch continue to support the trial.

Carriers of HNPCC (Lynch Syndrome) are the target population.

This is a double blind randomised placebo controlled trial, which will evaluate two interventions in a factorial design. This group may be ascertained in the following ways:

EITHER Proven carriers of pathological mutations in mismatch repair genes.

OR Belong to a recognised HNPCC family **AND** have had at least one of the following events:

- a colorectal cancer
- a related carcinoma (endometrial carcinoma is particularly predictive of gene carrier status but others include small bowel, uroepithelial, or stomach).
- an adenoma of over 5mm diameter
- an adenoma under 40 years of age
- a confirmed adenoma of any size at more than 1 endoscopy.

All recruits should also;

+ be over 25 years old . There is no upper age limit.
+ have an intact colon or have had only a local or segmental resection and have normal (non medicated) bowel actions - 3 or fewer formed bowel actions per day.

Recruits will receive either 30grams of treatment starch, equivalent to 13.2grams of resistant starch, or 600mg of aspirin, neither, or both. Treatment will be for a minimum of 2 years and for up to four years.

Safety

The adverse effects of aspirin are real and well documented. The relatively large dose of aspirin is still in the sub-analgesic "low dose" range but will have a greater capacity to demonstrate efficacy. Enteric coated tablets will be used to facilitate randomisation and reduce side effects. The absolute risk of serious complications is small and is made clear in patient information. Contrary to popular belief, the randomised trials of various "low dose" regimes up to 1200mg per day did not demonstrate a significant difference in side effects between 300 and 600mg dosage. There is evidence of tolerance with continued use and symptoms may be countered with traditional antacids. There are no known major adverse effects of resistant starch in humans apart from the possibility of mild symptoms of increased stool frequency and distension.

Exclusion criteria

- Pregnancy
  note: there have been few reports of adverse effects associated with aspirin use in pregnancy and aspirin is not regarded as a teratogen so women of child bearing age may be recruited. However, women should temporarily withdraw from the trial if they become pregnant. They can restart immediately after delivery if they are not breast-feeding. If mothers are breast-feeding, they should not re-enter the trial until they have completed breast-feeding.
- Medical contraindications for aspirin e.g. aspirin induced asthma, previous aspirin/ NSAID induced peptic ulcer, renal impairment beyond creatinine of 0. 150 umol/l, or haemorrhagic diathesis**.**
- Already taking NSAIDs or steroids.
- Severe intercurrent disease.
- Known to be HIV positive (routine testing not required).

Requirements at all colonoscopies.

A minimum of two yearly colonoscopy will be required (plus or minus 3 months). The precise duration will depend on local practice and the timing and interval of routine clinical colonoscopy for each individual. However, it is anticipated that most patients will fit into one of these follow-up categories:

- yearly colonoscopy where neoplasms are detected or have been recorded in the past.
- two yearly colonoscopy for patients with no neoplasia.

Note: surgery should not be delayed as a result of participation in trial.

The examination should examine the whole colon as far as the caecum. If the caecum is not visualised this should be recorded on the colonoscopy report from, together with a statement of the quality of views achieved. It is assumed that excellent bowel preparation is a prerequisite of the effective clinical care of Lynch Syndrome gene carriers. The colonoscopist will be asked to confirm that a full view of the colon was achieved. If a poor preparation is recorded and the colonoscopy is repeated then a second report should be provided. If the caecum cannot be visualised, a double contract barium enema is used in many centres to complete the examination. A copy of the barium enema films will be requested for the purpose of the CAPP2 study assessment**.** If originals are sent they will be copied and returned.

The standard preference will be a 2 year colonoscopy interval where there have been no neoplasms or a 1 year colonoscopy where neoplasms have been reported.

A clear colonoscopy performed in the previous three months would be acceptable for immediate enrolment. If the latest colonoscopy was done more than three months before recruitment, enrolment and treatment must wait until after the next colonoscopy. Where possible, this should be brought forward. Any unusual cases should be referred to the CAPP office in Newcastle where a staff member will be available to discuss issues raised. At entry to the study, the recruiting centre should complete a Colonoscopy Review (Form 2A).

Other details to be collected at colonoscopy will be: (Form 13)

- Current size and number of polyps
- Location of polyps (marked on table provided).
- Histology of polyps removed (see below), including villosity and level of dysplasia.
- Removal success: Total, Partial or Failed
- Description of other pathology/ neoplasia
- Past number of adenomas

Mucosal Biopsies

Where possible, we will also request mucosal biopsies to allow investigation of the effects of the interventions on apparently normal tissues (Form 6). Mucosal biopsies are usually less than 3mm diameter and cause only transient local bleeding. Details of the collection procedure for biopsies are given on form 14. Information on how to send the biopsies to Newcastle are included in the biopsy pack. It should be noted that **biopsies must arrive at Newcastle within 3 days** for the optimal assessment of cell proliferation and apoptosis.

Based on polypectomy in over 1000 individuals, 320 of whom had taken NSAID's, Shiffman (Shiffman et al., 1994) concluded that the risk of significant gastrointestinal bleeding after endoscopic biopsy or polyp removal was small (<1%); although the use of NSAID's did increase the incidence of minor self-limited bleeding, an increase in the rate of major bleeding was not observed.

The consent form will include a request for permission to contact the registry on a regular basis after the intervention study ends in order that long-term effects can be evaluated.

Other requirements from recruits

UK recruits will be invited to keep a diary describing all food and drink ingested over 4 sequential days. This will take place once during a recruit's study participation. A sample of urine will be collected at this time to measure treatment compliance. A dietician will visit the recruit and explain how to complete the diary and how to collect the urine. A return visit will be made to collect the completed food diaries and urine sample.

Outcomes

- Primary endpoints will be the number, size and histological stage of colorectal carcinomas found after two years treatment.
- Secondary endpoints will be the number, size, location, villosity, dysplasia,and apoptosis in adenomas. In a subset of patients, biopsies from normal rectal mucosa will be taken for histological study.
- Recruits are at increased risk of extracolonic cancers and these will be systematically recorded in the study group.

Data analysis

As two treatments are being tested, it has been decided to adopt a factorial design;

A	600mg aspirin + 30g treatment starch
B	Placebo tablets + 30g treatment starch
C	600mg aspirin + 30g placebo starch
D	Placebo tablets + 30g placebo starch

(A + B) compared to (C + D) will test the efficacy of resistant starch.
(A + C) compared to (B + D) will test the effect of aspirin.

Ethical Considerations

All information relating to patients enrolled in the CAPP study will be stored in a dedicated confidential computer within the CAPP Office in accordance with the Data Protection Act.

The study will be performed in accordance with the principles stated in the Declaration of Helsinki.

The investigators in each centre will ensure that patients are given full and adequate verbal and written information about the nature, purpose, possible risks and benefits of the study.

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SEQUENCE OF ENROLEE MANAGEMENT

Location	Sequence of Events	Relevant forms
Local Centre and Newcastle	ID high risk family	Form 8
Local Centre and Newcastle	Find mutation locally or centrally	Form 10
Local Centre	Invite to join Obtain consents	Form 1 Patient Leaflet
Local Centre and Leeds	Enrol and randomise	Form 2 Form 2A
Local Hospital and Newcastle	Enrolment colonoscopy and biopsies	Form 13 Form 6
Newcastle and Local Centre	Study treatment and management for 2-4 years. (6 monthly compliance contact)	Form 3 Form 4 Form 5 Form 11
Local Hospital	On study colonoscopies and biopsies	Form 13 Form 6
Local Hospital	Exit colonoscopy and biopsies	Form 13 Form 6
Local Centre and Newcastle	Long term follow up annual review	Form 17A and Form 17B

2 - 4 years

10 plus years

CAPP2 STUDY CHECKLIST
For consenting a recruit

Information to discuss with each patient prior to enrolment:

1. Purpose of the study:

- ❒ Chemoprevention trial, Diet
- ❒ Starch - food, undigested
- ❒ Aspirin
- ❒ Who can join?
- ❒ Information/data being collected
  biopsies, paraffin blocks, DNA, compliance, histology, past colonoscopy history, on study colonoscopy details, long term annual follow up

2. Treatment:

- ❒ Duration = 2 to 4 years
- ❒ Groups = 4
- ❒ Randomisation = double blind
- ❒ 3 out of 4 chance of receiving some treatment
- ❒ When to start (dates on treatment packs)
- ❒ How to take
- ❒ When to take
- ❒ Daily dose required
- ❒ Missed treatment/compliance
  Suggest keeping a diary of missed sachets/tablets

3. Benefits/Side effects:

- ❒ Overall benefits = less likely to have thrombosis, less likely to have heart attack
- ❒ Aspirin = rare allergic reactions (asthma), report all unusual symptoms especially bleeding
- ❒ Starch = build up to taking full dose gradually over 4 weeks to avoid bloating

4. Colonoscopy:

- ❒ Colonoscopy history
- ❒ Colonoscopy review form
- ❒ Date of next colonoscopy
- ❒ Where
- ❒ Who does it
- ❒ Endoscopy staff will inform, advise and support
- ❒ Stop treatment before bowel preparation

5. Liaison:

- ❒ 3/6 monthly by phone/letter/visit
- ❒ Study teams
- ❒ Clinical teams
- ❒ Genetic team
- ❒ GP notified of enrolment in study by letter (get contact details)

6. Other Information:

- ❒ Give study team contact details
- ❒ Delivery of treatment packs
- ❒ Attend GP as usual if unwell
- ❒ Inform study team of illness as well
- ❒ Dietary analysis (UK only) and random urine collection
- ❒ Study Results expected 2007-8. Recruits can be advised of their treatment group

RECRUITMENT SUMMARY

☐ Send introduction pack: Letter with reply slip plus patient information leaflet.

☐ Received reply slip with contact details

☐ Call to arrange a time for home visit/phone discussion

☐ Send / deliver 1 day treatment pack

☐ Consented

☐ Letter to Surgeon

☐ Letter to GP

☐ Request mutation report

☐ Request previous histology reports if applicable (important to get 1st ever)

☐ Entry colonoscopy. Date: _ _/ _ _ / _ _

☐ Randomisation number from Leeds

☐ Treatment pack sent

☐ Start date returned. 3 month deadline _ _/ _ _ / _ _

CAPP2 STUDY PLAN

This document is designed as a checklist that can be used by the local researcher/nurse at each study centre.
It is intended to provide a guide through the paperwork required by the CAPP 2 Study. A brief review of an individual patient's progress through the study period is also provided, see diary of events.

Enrolment Checks	**Form No.**	**Action at recruiting centre**
Identify patient at recruiting centre:	**8**	Complete enrolee pedigree Form 8 or attach a copy of the pedigree
If eligible, invite them to join the study:	**1**	Complete Consent Form 1, even if they decline to take part.
From patient/patient notes:	**2 & 2A**	Begin to fill in the Study Enrolment Form 2 and complete a colonoscopy review Form 2A
Randomise the patient onto the study: **Please note:** **DO NOT obtain a randomisation number unless you are sure that the patient is eligible for the study.** If in doubt check with the CAPP Office or see eligibility criteria in the protocol (page 15) before you randomise a patient.	**2** **6, 8, 13**	Obtain a randomisation number by contacting the randomisation office (details on form 2). The randomisation number should be added to forms 2, 6, 8, and 13. The randomisation number and patient name should also be added to the label on the biopsy bag, the two biopsy tubes and the biopsy specimen slip.
Advise the patients' GP and surgeon of their participation on this study:	**Letter to GP**	Send a copy of the appropriate letter and short protocol to the patient's GP and surgeon.
Advise CAPP Office of the new enrollee:	**1, 2,2A, 8**	Send a copy of completed forms 1, 2, 2A, and 8 to the CAPP Office. Keep forms 6 and 13 for the next step.

Enrolment Checks	Form no	Action at local centre
Check the date of the patient's most recent endoscopy**:** **Was it was less than 3 months ago?**		
If **YES** ➔ ➔ (and there were no polyps remaining and a good view was obtained see protocol for further details)	➔	**Try to arrange a procedure to collect biopsies e.g. proctoscopy** and follow points a) to c) below. (biopsy collection <u>is preferable</u> but not essential). ⬇
If **NO** ⬇ ⬇ ➔ ➔ ➔	**13**	**If a procedure can not be arranged** to collect the biopsies, complete form 13 only, using the information from the patient's most recent colonoscopy report. ⬇ ➔ **Arrange/check date of next colonoscopy and ensure:** a) Biopsies are taken and placed in the tubes. b) Biopsy specimen slip is completed and tubes labelled. c) Forms 6 and 13 are completed.
	biopsies **biopsy slip** **6, 13**	
PLEASE NOTE: If biopsies cannot be obtained recruitment should continue. Contact the CAPP Office for advice (0191 2331414).		
Immediately after the biopsy/collection procedure: a) **advise** the CAPP Study Office:	**13**	A copy of **completed form 13** to be sent to the CAPP Study Office.
b) **advise** the Department of Pathology, RVI, Newcastle:	**6** **biopsies** **biopsy slip**	A copy of completed **form 6, the labelled biopsies and biopsy specimen slip** to be sent to the Department of Pathology, RVI, (**NOT to the CAPP Office)** via the carrier DHL**.** Use the addressed label inside the biopsy pack.
c) if a tissue specimen was also sent for local histological reporting then please request a copy	**Histology report**	Send copy of histology report to CAPP Office

Dispensing of treatment packs from the CAPP Office*:*
Once all the above has been completed and the forms 1, 2, 2A, 6, 8, 13 are received by the CAPP Office in Newcastle, this will trigger the treatment packs being sent to the centre/patient.
Inside the first treatment pack is a letter asking the patient to fill in and return to the CAPP Office a slip advising us of the date they begin taking their treatment. This will become their internal START DATE on the CAPP2 Study. It is very important that we receive this date as all future treatment packs and correspondence will be sent automatically, based on this date. Each centre will be advised of this date on the recruits' first three month letter.
Patients will receive treatment packs at start date, and then at 6, 12, 18, 24,30, 36 and 42 months, if remaining on study for 4 years.

Exit Checks	Form no	ACTION by local centre
At the exit colonoscopy:	**biopsies** **6, 13**	a) Biopsies to be taken and placed in the labelled tubes. b) Biopsy specimen slip completed. c) **Forms 6** and **13** to be completed
Immediately after the colonoscopy: a) **advise** the CAPP Study Office:	**13** **11**	A copy of completed **form 13** also a copy of fully completed compliance **form 11** to be sent to the CAPP Study Office
b) **advise** the Department of Pathology, RVI, Newcastle:	**6 biopsies biopsy slip**	A copy of completed **form 6, the labelled biopsies and the completed biopsy specimen slip** to be sent to Department of Pathology, RVI, (**NOT to the CAPP Office) via the carrier DHL.** Use the addressed label inside the biopsy pack.
c) if a tissue specimen was also sent for local histological reporting then please request a copy of this report	**Histology report**	Send copy of histology report to CAPP Office.
This patient has now completed their two, three or four year participation on the CAPP2 Study.		
Long term follow up arrangements: *Following completion of the CAPP2 study, if a recruit gave their consent to us to contact them for long term follow up, we will contact them annually for 10 years. This contact will be from either the CAPP2 study office or their local recruiter and we will be requesting information regarding their general health over the past year. This information will be also be requested from the recruits' GP.*		

DIARY OF EVENTS for the initial TWO YEAR STUDY PERIOD

After enrolment onto the CAPP Study we hope to keep you in touch with the different stages your recruits are approaching by sending you a series of letters. The letters will be sent approximately three weeks before the following intervals and each will remind you of the action you should take:

TIME	**ACTION by local centre**
At 3 months	Telephone call to patient to learn if all is well.
At 6 months	Contact/meet the patient: a) Complete **form 11** with date of contact and number of treatments remaining. b) Check second treatment pack has arrived.
At 9 months	Telephone call to patient.
At 12 months	Contact/meet the patient: a) Complete contact date and number of treatments remaining on **form 11**. b) Check 3rd treatment pack has arrived.
At 15 months	Telephone call to patient.
At 18 months	Contact/meet the patient: a) Complete **form 11** as above. b) Check treatment pack has arrived. c) Check the date for exit or 2 year colonoscopy**.**
At 21 months	Contact letter will remind you to check if a recruit will consent for a further one or two years; complete Form 16 to inform CAPP Office.
At 24 months	Arrange to meet recruit before their colonoscopy and complete final **form 11** Collect exit colonoscopy details on form 13 and 6. If the recruit is exiting the study, send thank you card to recruit

At some point during a patient's study period you may be asked to collect a random urine sample for compliance estimation. Notice and equipment will be sent to you at the time.

A 4 day food diary will be collected from all UK recruits at some point during their 2 years on the study. Recruits will be contacted directly by the study nutritionist, who is based in Newcastle, to request all necessary consents, instructions and completion details.

DIARY OF EVENTS for recruits staying in study for a further ONE TO TWO YEARS	
We will continue to keep you in touch with the different stages your recruits are approaching during their extended time on the study. *Letters will be sent approximately three weeks before the following intervals and each will remind you of the appropriate action to take.*	
TIME	**ACTION BY LOCAL CENTRE**
At 24 months	Arrange to contact/meet recruit and a) Complete **form 11** b) Check 5th treatment pack has arrived c) Arrange collection of data from 2 yearly colonoscopy and biopsies
At 30 months	Arrange to contact/meet recruit and a) Complete **form 11** b) Check 6th treatment pack has arrived c) Confirm date for next annual colonoscopy
At 36 months	**Arrange to contact/meet recruit and** a) Complete **form 11** b) Check 7th treatment pack has arrived c) Arrange collection of biopsies if a colonoscopy has been arranged at this time d) If recruit is completing now arrange collection of biopsies and report from exit colonoscopy and send thank you card to recruit
At 42 months	Arrange to contact/meet recruit and a) Complete **form 11** b) Check final treatment pack has arrived c) Confirm date of exit colonoscopy
At 48 months	Arrange to contact/meet recruit and a) Complete the final **form 11** b) Arrange collection of biopsies and report from exit colonoscopy c) Send thank you card to recruit

SAMPLE LETTER TO RECRUIT

Date

Name
Address

Dear

Thank you for your interest in the **CAPP2 Study** .

Name, title and address of referring clinician has passed on your contact details to me as you have expressed an interest in learning more about this study.

I have enclosed a patient information sheet which I hope will answer many of the questions you may have.

After reading the leaflet I hope that you will wish to learn more about the work we are doing and to discuss the **CAPP2 Study** further. If you do, then please complete the attached reply slip and return it to me in the enclosed freepost envelope. I will then contact you on the number that you provide to discuss the study in more detail with you.

In the meantime, if you have any immediate questions or concerns I can be contacted on *insert local contact details.*

I look forward to hearing from you.

With best wishes

Name of sender
Job Title of sender

c.c. *referring clinician*

Reply slip for the CAPP2 Study

Name……..

Date of Birth…….

I am / am not* interested in being contacted to discuss the CAPP2 study.

* (please delete as appropriate)

Contact Telephone Number……
Please enter your preferred contact number (home, work or mobile)

Best time to call: …… **am**……**pm**

Signature…….....

Date…….....

SAMPLE LETTER TO SURGEON

Date

Name
Address

Dear

Re: ***Patient name, date of birth, hospital record number***

The above patient has consented to take part in an international trial called "The CAPP2 Study" (Colorectal Adenoma/carcinoma Prevention Programme).
His next colonoscopy date is …….. and we hope to start *patient name* on the trial, providing *he/she* has a clear colonoscopy, a few days later.

As part of this study we are collecting samples of normal mucosa at colonoscopy. A biopsy collection pack has been sent to *patient name* and he has been asked to bring it to the colonoscopy on *insert date*. I hope that you will be able to collect the samples that we require for the study and send them via DHL to the RVI in Newcastle for analysis.

I would also be grateful if you could provide me with copies of any pathology from this patient's previous investigations.

A short protocol for clinicians and full details of how to collect and send the biopsies to us has been included with this letter. I have also enclosed a copy of *patient name* signed consent form and a spare biopsy pack. The system for returning the biopsies to us is very simple and only requires a phone call to DHL.

If you have any further questions, please contact me on *insert telephone number*.

With best wishes

Yours sincerely

Name
Job Title

SAMPLE LETTER TO CLINICIAN

Date

Name
Address

Dear

Re:

The above patient has agreed to take part in an international trial called "The CAPP2 Study" (**C**olorectal **A**denoma/Carcinoma **P**revention **P**rogramme).

NAME has been enrolled as he/she fulfils the criteria for this study and has regular colonoscopic surveillance for adenomas.

The trial has a factorial design with two interventions; 30g of resistant starch or placebo or 600mg aspirin or placebo. As the trial is a randomised controlled trial we are unable to inform you which treatment group your patient has been allocated to, but we feel you should be aware that your patient may have been prescribed 600mg aspirin daily.

Please contact the trial centre at the above address if he/she has contraindications to the aspirin, or experiences side effects.

Yours sincerely

Name of sender
Job title of sender

SAMPLE TRAVEL LETTER

Date

TO WHOM IT MAY CONCERN

Re: The CAPP2 Study treatment

…….. is currently taking part in a clinical trial called **The CAPP2 study** and is carrying the trial medication consisting 15g sachets of resistant starch and 300mg aspirin (or placebo) tablets with them.

CAPP2 is an International study investigating the hypothesis that patients with HNPCC will benefit from taking resistant starch and/or aspirin in the prevention of colonic polyps. Participation in this trial requires recruits to take 2 tablets and 2 sachets every day for up to four years. This recruit is carrying their trial treatment with them in order to fulfil this undertaking.

The medication has no monetary or commercial value. It was dispensed and packed at the CAPP office in the University of Newcastle upon Tyne under the supervision of the pharmacist for this study, Mr John Gilroy.

If there are any problems please telephone me on [44] (0)191 233 1414.

Yours sincerely

Gail Barker
CAPP2 Trial Co ordinator

ASPIRIN ADVICE

Aspirin, prescribed for patients enrolled onto the CAPP 2 Study, should NOT be used in the following circumstances:

- Pregnancy
- Breast feeding mothers
- Patients with asthma
- Previous aspirin/NSAID induced peptic ulcer
- Renal impairment
- Patients with haemophilia or haemorrhagic diathesis
- Patients hypersensitive to aspirin or non-steroidal anti-inflammatory drugs

Ref: The CAPP2 Study: Protocol
British National Formulary No. 36, September 1998

Side effects of aspirin:

- Frequent side effects are gastro-intestinal complaints such as stomach pain and minor gastro-intestinal blood loss
- Occasionally nausea, vomiting and diarrhoea may occur
- Rarely gastric haemorrhage or gastric ulcers and primarily in asthmatics, hypersensitivity reactions may occur (affects are difficult breathing, skin reactions)
- Isolated cases of impaired hepatic and renal function, a reduction in blood glucose levels (hypoglycaemia) and particularly severe skin rashes (including erythema exudativum multiforme) have been reported
- In rare cases anaemia, as a result of occult gastro-intestinal blood loss. Black stools must be reported to your doctor immediately
- Dizziness and ringing in the ears

Ref: Aspirin Protect 300/03/ April 1997

Drug interactions with Aspirin:

Other Analgesics: avoid concomitant administration of other NSAID's (increased side effects) – see Information on NSAID's overleaf.

Antacids and Adsorbents: excretion of aspirin increased in alkaline urine; kaolin possibly reduces absorption

- Anticoagulants: increased risk of bleeding due to antiplatelet effect
  Antiepileptics: enhancement of effect of phenytoin and valproate
  Corticosteroids: increased risk of gastro-intestinal bleeding and ulceration; corticosteroids reduce plasma salicylate concentration

- Cytotoxics: reduced excretion of methotrexate (increased toxicity)
  Diuretics: antagonism of diuretic effect of spironolactone; reduced excretion of acetazolamide (risk of toxicity)
  Leukotriene antagonists – aspirin increases plasma concentration of Zafirlukast
  Metoclopramide and Domperidone : metoclopramide enhances effect of aspirin (increased rate of absorption)
  Mifepristone: manufacturer recommends avoid aspirin until 8-12 days after Mifepristone
  Uricosurics: effect of probenecid and sulphinpyrazone reduced

Key:
- potentially hazardous interaction

Others do not usually have serious consequences

Ref: British National Formulary No.36 September 1998

Aspirin Safety:

The adverse effects of aspirin are real and well documented. The primary focus is on haemorrhagic complications due to gastric erosion and antiplatelet effects. The use of enteric coated aspirin should reduce to a modest degree the direct adverse effects on the gastric mucosa. The platelet inhibition is irreversible and is apparent at very low doses. The absolute risk of serious complications is small and is made clear in the patient information.

The modest increase in side effects must be set against the importance of the trial and the beneficial effect of aspirin in reducing thrombotic events.

Ref: The CAPP2 Study: Background

INTERACTIONS OF ASPIRIN WITH NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS)

	RISK FACTORS	MECHANISM	MANAGEMENT
Diclofenac	No specific risk factors known.	Aspirin increase the plasma clearance of diclofenac, possibly by decreasing diclofenac plasma protein binding.	No specific action is required, but be alert for evidence of the interaction.
Diflunisal	No specific risk factors known.	Not established. It is likely that difunisal and salicylates compete for plasma protein binding sites.	No specific action is required, but be alert for evidence of the interaction.
Etodolac	Not stated.	Not established. Possibly due to decreased etodolac protein binding.	Concurrent therapy with etodolac and aspirin is not recommended due to increased risk of GI adverse effects.
Fenoprofen	No specific risk factors known.	Not established.	No specific action is required, but be alert for evidence of the interaction.
Fluribiprofen	No specific risk factors known.	Not established. Aspirin and fluribiprofen may compete for plasma protein binding sites.	No specific action is required, but be alert for evidence of the interaction.
Ibuprofen	No specific risk factors known.	Not established.	No specific action is required, but be alert for evidence of the interaction.
Indomethacin	No specific risk factors known	Not established. Some evidence suggests that aspirin inhibits the GI absorption of indomethacin, but aspirin-induced displacement of indomethacin from plasma protein binding sites would also account for the reduction in plasma indomethacin concentrations.	No specific action is required, but be alert for evidence of the interaction.
Ketoprofen	No specific risk factors known.	Not established.	No specific action is required, but

			be alert for evidence of the interaction.
Ketorolac	Not stated.	Additive GI irritation and reduced ketorolac plasma protein binding.	Concomitant use of ketorolac and aspirin is contra-indicated.
Meclofenamate	No specific risk factors known.	Not established. Aspirin probably displaces meclofenamate from plasma protein binding sites.	No specific action is required, but be alert for evidence of the interaction.
Naproxen	No specific risk factors known.	Aspirin may complete with naproxen for plasma protein binding sites thereby increasing the renal clearance of naproxen.	No specific action is required, but be alert for evidence of the interaction.
Piroxicam	No specific risk factors known.	No interaction.	No interaction.
Sulindac	No specific risk factors known.	Not established.	A possible increase in the incidence of adverse GI effects with the combination has prompted the manufacturers of Sulindac to recommend against combining it with aspirin.
Tenoxicam	No specific risk factors known.	Aspirin appears to displace tenoxicam from plasma protein binding sites.	No specific action is required, but be alert for evidence of the interaction.
Tolmetin	No specific risk factors known.	Aspirin appears to displace tolmetin from plasma protein binding sites.	No specific action is required, but be alert for evidence of the interaction.

References: Drug Interactions, Analysis and Management, Micromedex

NB: This list is not exhaustive for all NSAID's.