Chemoprevention of hereditary colon cancers: time for new strategies

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Abstract | Colorectal cancer (CRC) is potentially preventable. Chemoprevention, a focus of research for the past three decades, aims to prevent or delay the onset of cancer through the regression or prevention of colonic adenomas. Ideal pharmacological agents for chemoprevention should be cheap and nontoxic. Although data indicate that aspirin can reduce the risk of CRC in the general population, the highest return from chemopreventive strategies would be expected in patients with the highest risk of developing the disease, particularly those with a defined hereditary predisposition. Despite compelling data showing that a large number of chemopreventive agents show promise in preclinical CRC models, clinical studies have yielded conflicting results. This Review provides a historical and methodological perspective of chemoprevention in familial adenomatous polyposis and Lynch syndrome, and summarizes the current status of CRC chemoprevention in humans. Our goal is to critically focus on important issues of trial design, with particular attention on the choice of appropriate trial end points, how such end points should be measured, and which patients are the ideal candidates to be included in a chemopreventive trial.

The premise that most cancers are preceded by premalignant lesions, whose removal or suppression would be expected to stop the carcinogenic process, has framed the modern approach to cancer prevention. Adenomatous polyps usually precede colorectal cancer (CRC), so this approach has had a particular effect on its prevention, in which both primary and secondary prevention has taken central stage. For sporadic cases, at least in the USA, aggressive colonoscopy screening programmes are leading to declines in cancer incidence, at least in part through the finding and removal of adenomatous polyps[1]. In addition, it is believed that colon cancer risk can be reduced through modification of factors such as diet[2]. Chemoprevention can be defined as the use of synthetic or natural compounds that prevent cancer development by either inhibiting cancer initiation, or suppressing further development into malignancy[3]. Epidemiological data clearly show that regular, long-term aspirin use is the most consistent example of a chemopreventive agent affording effective protection against colon cancer[4].

Although most colon cancer cases are sporadic, up to 5% are due to hereditary syndromes[5]. Patients with CRC syndromes are ideal candidates for chemoprevention trials because they are at increased, predisposed risk, with the implication being that the success of any CRC chemoprevention programme might be increased in individuals at high risk of the disease[6]. Familial adenomatous polyposis (FAP) and Lynch syndrome, the two most common hereditary CRC syndromes, account for a considerable proportion of all CRC cases[5]. They have been the targets of intensive preclinical and clinical research involving the use of several classes of potentially chemopreventive compounds. Both syndromes are associated with an exceedingly high risk of developing CRC and extraintestinal malignancies. Thus, it is not surprising that researchers have focused on efforts to develop chemopreventive strategies as an adjunct to endoscopic cancer surveillance. These efforts are of particular interest for two reasons. First, markedly higher cancer risks in these syndromes should enable smaller, shorter-term studies to address the efficacy of chemoprevention than would be required for average-risk populations. Secondly, CRCs in these syndromes are driven by two different molecular pathways[7,8], so the results of chemoprevention trials in these settings might provide insight into subsets of sporadic CRCs that might benefit from specific chemopreventive agents. However, despite substantial effort and many published trials, we are still lacking medications with approved indications for either syndrome. To provide direction for future interventions, the goal of this Review is to discuss critical issues related to previous chemopreventive trial designs and failures, including the choice and assessment of appropriate end points and target populations.

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Key points

- Colon cancer can be prevented through chemoprevention
- The highest return from chemopreventive strategies is in patients with a hereditary predisposition for developing colorectal cancer
- Clinical trials have yielded conflicting results and currently no chemopreventive agent is approved in hereditary colorectal cancer syndromes
- Chemoprevention should ultimately be aimed at delaying colectomy, reducing endoscopies and polypectomies, and preventing cancer development
- Future trials must be carefully designed and conducted to be successful, with an effort to avoid wasting resources and time

Chemoprevention does matter in hereditary CRC

High risk and early onset

FAP is an autosomal dominant syndrome caused by germline mutations in the *APC* gene and is characterized by the development of hundreds to thousands of adenomas[9–12]. This syndrome represents up to 1% of all CRC cases, but mutation carriers have an almost 100% lifetime risk of developing CRC[5] (BOX 1). Classic FAP has a very early onset; the mean age of polyp occurrence is 15.9 years[13]. Attenuated FAP (AFAP) is characterized by the presence of 10–100 polyps, usually distributed in the right colon[14]. Additionally, upper gastrointestinal tract polyposis is frequently found in FAP and AFAP; adenomatous polyps of the duodenum are observed in 50–80% of patients and patients have a lifetime risk of developing duodenal cancer of up to 12%[5].

Lynch syndrome is also autosomal dominant, caused by a germline mutation in one of the DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) leading to microsatellite instability in associated cancers, when they occur[15]. This syndrome is characterized by the early development of colon cancer and other extraintestinal malignancies, particularly genitourinary cancers. The lifetime risk of developing colorectal and endometrial cancers in Lynch syndrome can be as high as 50% and 40%, respectively, and the average age of colon cancer diagnosis is around 44 years old[16]. Importantly, Lynch syndrome seems to involve an accelerated progression from polyp to cancer, thus chemoprevention would be an attractive addition to colonoscopic surveillance.

Box 1 | **Why chemoprevention matters for FAP and Lynch syndrome**

Familial adenomatous polyposis (FAP)

- Early onset of polyps and colorectal cancer (CRC)
- 100% risk of developing CRC
- Surgery is the only standard of care treatment
- High frequency of endoscopic procedures
- Negative psychophysical effect of surgery performed in young adulthood

Lynch syndrome

- Early onset of CRC
- High risk of developing CRC and malignancies associated with Lynch syndrome such as genitourinary cancers
- Negative psychophysical effect from possible multiple surgeries
- Interval cancer possible even with strict follow-up due to accelerated carcinogenesis

Frequent invasive procedures

Endoscopic management of FAP and Lynch syndrome can be challenging as the risk of developing interval cancer (tumours that occur between otherwise planned medical examinations or which are clearly present at a follow-up examination) cannot be completely avoided, even with the most careful procedures and the most advanced technologies[17–19].

FAP. For *APC* mutation carriers, screening is invariably endoscopic and typically starts in the pre-teenage years (10–12 years of age) when polyps first appear[20]. Colonoscopic surveillance frequency is tailored to the polyp burden, but is often once or twice per year. Over time, patients with FAP have a substantial risk of postpolypectomy-related and procedure-related complications[21,22]. Furthermore, endoscopic surveillance and polypectomy is frequently insufficient and surgery remains the standard of care for prevention of CRC in profuse FAP. On the basis of the rectal polyp burden, surgical options include total abdominal colectomy with ileorectal anastomosis or total proctocolectomy, with or without ileal pouch formation. Even after colectomy, lifelong annual or biannual surveillance of the residual rectum or ileal pouch is required, and repeated surgical procedures can greatly affect quality of life[23–25].

Surveillance of teenagers whose polyp burden is not yet severe enough to warrant colectomy can enable patients to reach an age when they can more actively participate in decision-making. A longer period of preoperative surveillance could also enable a clearer estimate of the ultimate rectal adenoma burden. In the case of AFAP, many patients have such a modest adenoma burden that indefinite deferral of colectomy altogether is not an unreasonable goal. Thus, an effective chemopreventive agent could make the difference between surgery and its avoidance. Furthermore, chemoprevention could represent an important strategy for duodenal polyposis[26], in which management of duodenal adenomas can pose a substantial challenge and pancreaticoduodenectomy carries notable morbidity and mortality[27,28]. The possible scenarios in which chemoprevention might have a critical effect on FAP are shown in TABLE 1.

Lynch syndrome. The challenges associated with risk-reducing surgery and endoscopic polypectomy in Lynch syndrome are substantial, but not as great as those in FAP. For Lynch syndrome mutation carriers, endoscopic screening should start at the age of 20–25 years, or 10 years earlier than the earliest cancer in the family. Examinations should be annual or biennial, with the aim of identifying and removing high-risk lesions if possible, thereby reducing the risk of developing cancer[20]. Lynch syndrome mutation carriers are very aware and potentially anxious about their risk of developing cancer, and can be advised on the possibility of undergoing prophylactic surgeries (for example, hysterectomy and/or oophorectomy)[29]. However, because of the nature of the disease, many individuals undergo multiple surgical procedures with

Table 1 | **Potential role of chemoprevention in different FAP settings**

FAP setting	Role
Children with classic FAP	Delaying surgery
AFAP	Delaying or avoiding colectomy
Ileorectal anastomosis	Preventing new surgeries and reducing morbidity of polypectomy
Duodenal polyposis	Preventing duodenectomy and reducing morbidity from ampullectomy or polypectomies

AFAP, attenuated familial adenomatous polyposis; FAP, familial adenomatous polyposis.

the potential of serious negative psychophysical effects. Furthermore, patients undergoing surgeries are still at risk of developing subsequent cancers and will need to undergo lifelong surveillances.

The greatest concern in Lynch syndrome mutation carriers undergoing surveillance colonoscopy is the need for adenoma examination at intervals as short as 1–2 years, owing to the well-known propensity for accelerated cancer development[20,30]. In the USA, where colonoscopy is the accepted screening method of choice, the goal of surveillance is to prevent cancer altogether, with the need for an operation generally considered a screening failure. Even examinations conducted every 1–2 years carry an unacceptable rate of interval cancer[17,18].

With these risks in mind it is quite understandable that, for the past 30 years, there has been a growing emphasis on disease control and cancer prevention through chemopreventive strategies in patients with FAP or Lynch syndrome. This approach is particularly true for FAP due to the presence of adenomas that call for some intervention at any given examination. For Lynch syndrome, chemoprevention is attractive because of the limitations of endoscopic identification of precursor lesions, and the frequency of interval cancers during surveillance. However, despite promising data from preclinical and clinical studies, no single medication has been approved by the FDA or the European Medicines Agency (EMA) for cancer chemoprevention in either FAP or Lynch syndrome.

Chemopreventive trials in FAP

FAP has been considered more approachable in chemoprevention trials because changes in number and size of rectal polyps are considered a straightforward clinical end point. Essentially, all chemopreventive trials performed in FAP thus far have used anti-inflammatory agents such as sulindac, aspirin and selective cyclooxygenase (COX)-2 inhibitors and natural agents with potent anti-COX and anti-inflammatory activities, such as eicosapentaenoic acid (EPA) and curcumin[31–46] (FIG. 1).

Sulindac

Non-placebo-controlled clinical trials and observational studies have shown evidence of a reduction in polyp number and size with sulindac lasting up to 98 months[47]. However, these studies were particularly limited in terms of patient numbers and by the fact that most of the treated patients had evaluable disease limited to the rectum. Furthermore, development of cancer was seen in some patients over time, indicating the possible development of drug resistance[48,49].

Several important randomized placebo-controlled trials of sulindac in FAP have been performed, but these have produced mixed results. Although short-term trials have demonstrated a reduction in rectal polyp number and size[50,51], in some trials, either reappearance or increases in polyp number were reported after stopping the agent[34,35]. A study designed for primary prevention of polyps in *APC* mutation carriers aged 8–25 years showed a non-statistically significant trend toward a benefit from sulindac compared with placebo[38], so a modest benefit in prolonging time to progression cannot be excluded (see Supplementary information S1 (table)).

Sulindac sulfone, a metabolite of sulindac, was also tested following promising *in vitro* data. In a phase I trial in 18 patients with FAP who underwent ileorectal anastomosis, the drug was found to have no clinically significant effect on polyp number after 6 months of treatment[37].

Aspirin

Considering the amount of attention devoted to aspirin as a potential chemopreventive agent in CRC in general, it is surprising that it has not received more attention in FAP. In the largest trial involving patients with FAP to date, the CAPP-1 study[44], 227 young patients (average age of 18 years) with intact (not yet operated on) colons were randomly allocated, using a 2×2 factorial design, to receive aspirin and/or resistant starch (placebo, aspirin, resistant starch, aspirin plus resistant starch). All patients on aspirin received 600 mg daily but, after a median treatment of 17 months, no differences were observed between the arms in terms of polyp number in the rectosigmoid colon, whereas a marginally significant ($P = 0.02$) reduction in the diameter of the largest polyp was found for patients taking aspirin for more than 1 year. A small placebo-controlled trial conducted in Japan on 34 patients with ileorectal anastomosis who received either daily 100 mg doses of aspirin, or placebo, for 6–10 months found no differences in the mean diameter of all colorectal polyps (the prespecified primary end point) between active treatment and placebo[45].

Coxibs

In 1996, Oshima and colleagues demonstrated a significant reduction in polyp number and size in the $APC^{\Delta 716}$ mouse model of FAP, by either knocking out the COX-2 gene ($P < 0.0001$) or by treatment with a selective COX-2 inhibitor ($P < 0.0001$ and $P = 0.0037$ fed with 14 or 3.5 mg/kg per day of COX-2 inhibitor, respectively)[52]. In a placebo-controlled trial of 77 patients with FAP, assessed in an area of dense polyps marked with a tattoo before and after treatment, 6 months of twice daily 400 mg celecoxib led to a 28% reduction in mean polyp number and 30% reduction of polyp burden[36]. This study used a more global measure of adenoma burden, in which pretreatment and post-treatment videos were blind compared by a panel of expert reviewers on a scale

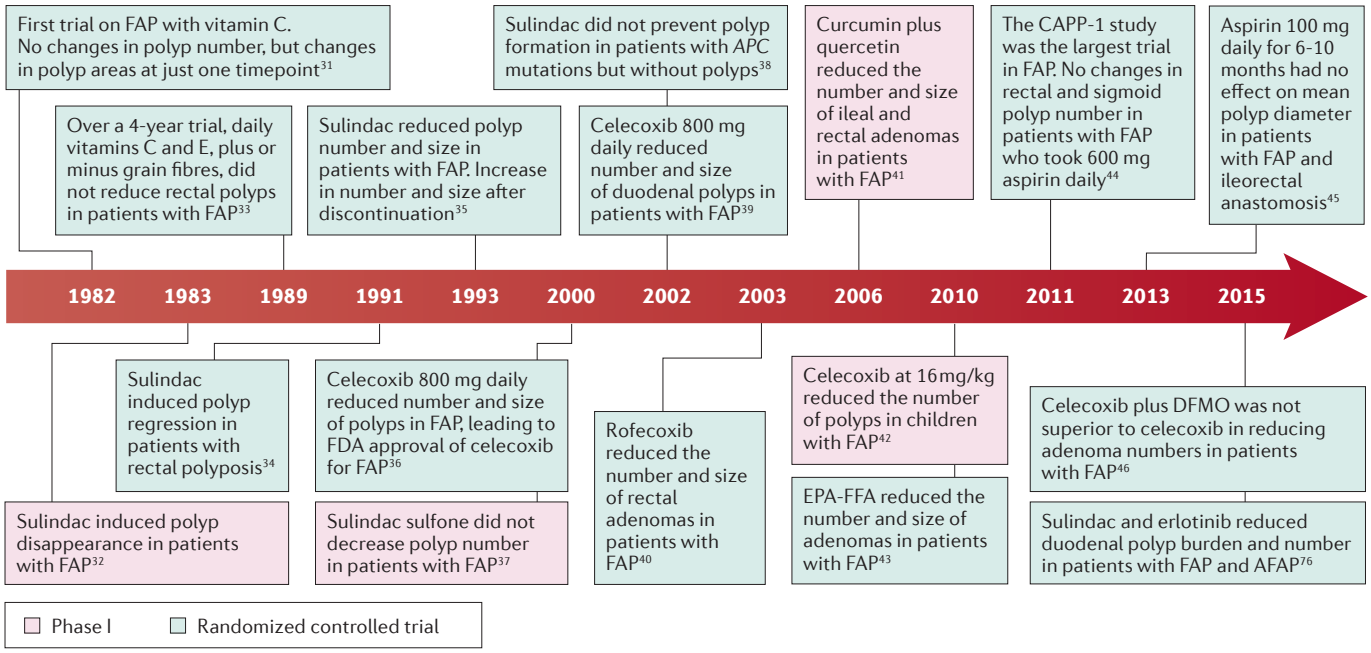

Figure 1 | **Timeline of selected published chemopreventive clinical trials for FAP.** AFAP, attenuated familial adenomatous polyposis; DFMO, difluoromethylornithine; EPA-FFA, eicosapentaenoic acid-free fatty acid; FAP, familial adenomatous polyposis.

of 'same', 'better' or 'worse'. This measure corresponded better with the magnitude of benefit seen than the more quantitative measure within a very limited field of view. A similar, but less substantial chemoregressive, effect was seen in the duodenum of patients enrolled in this trial[39]. Following this study, celecoxib received approval under "exceptional circumstances" by the EMA and "accelerated approval" by the FDA, for the reduction of the number of adenomatous intestinal polyps in FAP, as an adjunct to surgery and further endoscopic surveillance.

Celecoxib was also tested in a phase I, dose escalation trial, involving 18 children aged 10–14 years with *APC* gene mutations, a family history of FAP, and/or clinical polyposis[42]. The effects of various doses of celecoxib (4, 8 or 16 mg/kg per day, in which the highest dose was comparable to the twice daily 400 mg dose in the adult trial described earlier[36]) versus placebo were tested by colonoscopy performed at baseline and after 3 months. A significant reduction in polyp number ($P = 0.003$) was seen with the 16 mg/kg daily dose of celecoxib and no adverse effects were observed. A second paediatric trial called the CHIP study was a phase III, double-blind, randomized, placebo-controlled, multicentre trial in patients with FAP aged 10–17 years old[53]. The study was designed to compare the efficacy and safety of celecoxib versus placebo over a 5-year treatment period. The study was terminated due to slow recruitment and low event rate on interim analysis, although a nonsignificant trend toward benefit was seen. No serious drug-related toxicities were encountered, though insufficient long-term safety data are available. Perhaps more relevant than the mixed findings from the COX-2 trials has been the effect of safety signals from unrelated studies. Data from long-term (3 years) placebo-controlled trials for the prevention of sporadic colonic adenomas demonstrated a dose-related increase in risk of cardiovascular death, nonfatal myocardial infarction, or stroke[54,55]. Rofecoxib, which had shown promise in two small FAP trials[40,56], was removed from the market altogether owing to safety concerns of an increased risk of cardiovascular events and, in 2011, the marketing authorization of celecoxib for FAP was withdrawn both in Europe and the USA because the postmarketing study intended to verify clinical benefit, and required as a condition of approval, was never completed.

Interest in combinations of NSAIDs with other drugs was raised by the report that found the combination of difluoromethylornithine (DFMO) plus sulindac led to a marked reduction in sporadic metachronous adenomas[57]. DFMO is an irreversible inhibitor of ornithine decarboxylase that reduces the production of the essential growth factors, polyamines; however, its use at higher doses has been limited by potentially irreversible hearing loss[58]. In 2016, celecoxib was tested in combination with DFMO in 112 adults with FAP[46]. Patients with a clinical diagnosis of FAP and an evaluable colon and/or rectal segment, were randomly allocated to receive either celecoxib plus DFMO, or celecoxib alone, for a period of 6 months. The study found no statistically significant difference in percentage change in adenoma count in a defined field of view between celecoxib alone and celecoxib plus DFMO. Notably, when a more comprehensive video assessment was performed, a reduction in polyp burden was seen in the celecoxib plus DFMO arm. An ongoing multicentre FAP trial is currently enrolling and is randomly allocating patients to sulindac alone, DFMO alone, or to a combination[59].

Natural compounds

Curcumin. One of the main components of the Asian spice turmeric, curcumin is known for its anti-inflammatory properties[60]. Curcumin has been widely studied in preclinical models of FAP and has shown protective effects toward polyp growth and progression toward cancer[60]. In a small uncontrolled series, the effects of curcumin were tested in five patients with FAP with prior colectomy. All patients were also given quercetin, a polyphenol that increases curcumin absorption[41]. After 6 months of treatment, all patients had a significant reduction in mean percent polyp size (60.4%, $P<0.05$) and number (50.9%, $P<0.05$) compared with baseline without adverse effects. A dual-centre phase III trial of adults with FAP is currently ongoing[61]. The duration of treatment is 12 months and the primary end point will be the changes in number and size of polyps.

Omega-3 polyunsaturated fatty acids. EPA-free fatty acid (EPA-FFA), an ultrapure formulation of an omega-3 polyunsaturated fatty acid, led to a dramatic reduction in the number and burden of intestinal adenomas in the *Apc*$^{Min+/-}$ mouse[62]. In a double-blind placebo-controlled trial, 58 patients with FAP post-colectomy and with ileorectal anastomosis, were randomly allocated to receive either EPA-FFA or placebo for 6 months. A significant reduction in polyp numbers (based on a previously tattooed area) ($P=0.012$) and sum of polyp diameters ($P=0.027$) was demonstrated in patients receiving EPA-FFA[43]. No differences in adverse effects were observed between the two groups.

Chemopreventive trials in Lynch syndrome

Several preclinical models of Lynch syndrome have been tested with multiple compounds, but with mixed results[63–66]. To date, only a single, randomized placebo-controlled trial has been performed in patients with Lynch syndrome. In the CAPP-2 trial, 937 patients with either a genetic (proven germline mutation in one of the DNA MMR genes) or clinical (meeting the Amsterdam criteria) diagnosis of Lynch syndrome were randomly allocated to receive resistant starch, placebo (cornstarch), 600 mg aspirin plus placebo or 600 mg aspirin plus resistant starch[67]. No differences in terms of colonic adenoma or carcinoma incidence were found among the 695 patients who took either aspirin or placebo for up to 4 years. Thus, the pre-specified primary end point was not met at the end of the trial. However, longer-term follow-up analyses revealed that patients who took aspirin for ≥2 years had a lower incidence of colon cancer and Lynch-syndrome-related cancers than those who took placebo at an average follow-up of 55.7 months[68]. This secondary analysis might be in line with the epidemiological data on long-term use of aspirin in the general population[4], indicating that its protective effect might require 5–10 years to be seen. This finding highlights the importance of incorporating a plan for post-intervention follow-up in the trial design, regardless of the chosen end points.

The ongoing CAPP-3 trial is a double-blind randomized trial designed to compare the degree of cancer prevention resulting from three daily doses of enteric coated aspirin (600 mg, 300 mg and 100 mg) taken for 5 years. Up to 3,000 Lynch syndrome mutation carriers are expected to be enrolled in this trial. Regular review and cancer registry data will be combined to assess the effect of the aspirin doses on new cancers, as well as adverse events related to the medication[69].

Pitfalls and new strategies for trials

After almost 30 years, it is rather disappointing that there are only a handful of clinical chemoprevention trials in hereditary CRC syndromes and no approved drugs for these indications. Economic considerations have complicated the situation as pharmaceutical companies are less likely to commit to large chemoprevention trials of existing drugs due to the risk of uncovering unexpected adverse effects, such as those seen in the coxib trials. Additionally, development of new drugs for rare hereditary conditions can raise concerns about limited revenues (a drug approved to treat a rare disease is considered less profitable). It is a pity that many compounds have not seen the clinical stage, but why is this the case? Do we need a new class of medications or better agent selection? Are preclinical data reliable? Or should we modify our trial design?

Candidate compounds and their targets

One issue worthy of consideration is that the implementation of chemoprevention and the strategies adopted in the various clinical trials might be hampered by the magnitude of the underlying genetic risk; that is, an otherwise effective agent in the non-syndromic population simply does not work in a high-risk group. Thus, a change of strategy and new possibilities should be explored.

The selection of candidate chemopreventive agents has always relied on testing compounds on colon cancer cells, and then on specific animal models (FIG. 2a). However, in both of these preclinical situations, testing does not reflect clinical scenarios, and many compounds have not advanced to further clinical analysis. In fact, in most *in vitro* models candidate chemopreventive agents are used at concentrations that lead to cytotoxicity and/or apoptosis, which is considered a successful result. However, this finding might not be indicative of chemopreventive activity as killing cancer cells is not the same as chemoprevention. Furthermore, animal studies have also used doses that, compared to possible human intakes, are substantially higher and for shorter periods of time, and which therefore can only indicate possible protective effect in humans[52,62].

Importantly, the most commonly used animal model for FAP, the *Apc*$^{Min/+}$ mouse, develops polyps mostly in the small intestine, thus not accurately reflecting the human disease. Advances in preclinical models might aid the more accurate assessment of chemopreventive agents. For example, organoid

cultures might be more useful as *in vitro* models than standard cancer cell cultures as they probably reflect the effects at the tissue level more closely[70]. For FAP, it might also be useful to use the polyposis in the rat colon (PIRC) model, which is based on a mutagen-induced nonsense allele of the rat *Apc* gene[71,72]. This model develops multiple colonic adenomas over a longer period of time, thus mimicking patients with FAP more accurately (FIG. 2b).

The situation for Lynch syndrome is more complicated. Although we have multiple colon cancer cell lines derived from patients with Lynch syndrome that are frequently used to test agents (including HCT116, DLD1/HCT15), the development of suitable animal models has yielded disappointing results as most Lynch syndrome models do not develop early-onset cancers, and homozygous knockouts tend to die prematurely (reviewed elsewhere[73]). In 2010, a conditional knockout mouse model for the tissue-specific inactivation of *Msh2* was shown to develop small intestine cancers displaying microsatellite instability[74]. This model is probably the closest to Lynch syndrome in humans, yet the development primarily of small intestinal tumours is far from ideal.

Finally, there are also issues regarding suitable candidate targets. Should we look for target genes or effectors (including DNA methylation and microRNAs), rather than investigating major upstream molecular pathways (COX-2 inhibition for example)? Should we aim at different outputs with multiple compounds? Or should we target the predisposing genes? Although patients with FAP or Lynch syndrome are genetically predisposed to develop CRC, multiple genes and mechanisms contribute to CRC development and spread[75]. For this reason, the use of multiple agents might be needed for a substantial clinical effect. The combination of multiple agents targeting different genes is already being tested in preclinical models, and compelling clinical data on combination treatments exist (for example, sulindac plus DFMO, or curcumin plus quercetin[41,57]). In 2016, results were reported from a randomized trial testing the combination of sulindac (150 mg twice daily) and the anti-epidermal growth factor receptor erlotinib (75 mg daily) on 72 patients with AFAP or FAP who completed the study[76]. After 6 months of treatment, when compared with baseline measurements, sulindac plus erlotinib led to a median 37.9% decrease in polyp burden whereas a median 30.6% increase was observed in the placebo group (net difference of –71.2% between the groups). Differences were also observed in terms of polyp count, with the placebo group showing a median increase of 4.3 polyps from baseline numbers and the therapy group a median decrease of 2.8 polyps (net difference of –8 polyps between the groups). By relying on synergistic effects, combination treatments could potentially use lower concentrations of pharmacological agents, reducing the chances of toxicity.

One attractive approach has been developed in the $Apc^{Min/+}$ mouse, using a combination of 9-*cis*-retinyl acetate (RAc) and TRAIL (a TNF family ligand) administered at intermittent dosing[77]. This dosing caused massive apoptosis in premalignant cells only and resulted in a dramatic reduction in the number and size of intestinal polyps. The discovery of the colonic stem cell could also be a game-changer in developing future treatments for both FAP and Lynch syndrome, utilizing restoration of WNT signalling in early adenomas and restoring DNA MMR proficiency in deficient cells of Lynch syndrome mutation carriers.

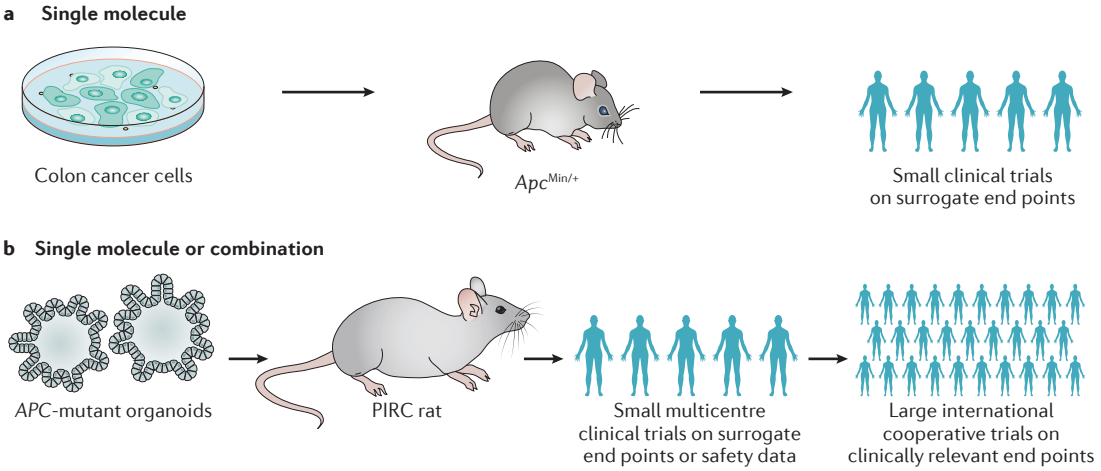

Figure 2 | **A new approach for drug development in chemopreventive trials for FAP. a** | The traditional approach. The testing of potential candidate chemopreventive agent has always relied on testing cancer cells first, then treating the $Apc^{Min/+}$ mouse or other animal models, and finally running small clinical trials for surrogate end points such as polyp size and number. **b** | A proposed new approach. The new strategy would start with testing of either single molecules, or a combination, on mutant *APC* organoids, followed by treatment in the PIRC model, which would enable easier disease monitoring of the colon. The next step would involve testing patients with FAP for surrogate end points and finally running large multicentre clinical trials for clinically relevant end points useful for marketing requests. FAP, familial adenomatous polyposis; PIRC, polyposis in the rat colon.

Table 2 | **Pros and cons of FAP, Lynch syndrome and sporadic adenoma trials**

Trial type	Pros	Cons
FAP	• Short trial duration, if polyp regression is the measure • Readily quantifiable end point • High-risk patients should be motivated to participate • Smaller sample size, high event rate • Can rationalize more toxic agent(s), due to high risk	• Rare disease so few patients • Adenoma burden highly variable • Requires an *APC* mutation • Attenuated FAP often excluded • Age <18 years typically excluded, though ideal target • Little information on duodenal polyposis • No data on desmoids • Polyp regression not deemed a "clinical benefit" by FDA and EMA • Trials often very expensive
Lynch syndrome	• More common than FAP • Higher event rate than general population • Existing collaboration network (CAPP)	• As with FAP, is still rare • Large sample size needed for relevant polyp incidence • Long trial duration • Trials often very expensive
Sporadic adenoma	• High relevance in population • Easy to find eligible patients for inclusion in trials	• Very large trials needed • Cancer or mortality not typical end points • Trials often very expensive

CAPP, Cancer Prevention Programme; EMA, European Medicine Agency; FAP, familial adenomatous polyposis.

Study design

Owing to the increased risk in hereditary CRC syndromes, the hope was that smaller trials would enable a clinical response to be seen. Indeed, the sample sizes for trials in FAP (usually <100 patients) have been consistently much smaller than in average-risk populations, for which sample sizes >1,000 have been required to show a reduction in incident adenomas. Regression of prevalent adenomas and/or prevention of new polyp formation in patients with FAP over very short times (as short as 6 months) have been used as end points in most FAP chemoprevention trials (FIG. 1; see Supplementary information S1 (table))[47]. Changes seen in such a short time might not reflect a disease response or a robust effect, particularly in light of the data from aspirin trials suggesting the benefit in cancer prevention should be measured in multiples of years, not months (TABLE 2; see Supplementary information S1 (table)).

One important issue is related to the willingness of participants to take part in a placebo-controlled trial. To overcome this problem and boost recruitment, some trials (for example, the CAPP-3 for Lynch syndrome and sulindac plus DFMO for FAP) have been designed without a placebo arm but with effective agents that can be used as comparators. However, we believe that placebo-controlled trials are essential in situations in which no clinically approved chemopreventive agent exists and approval is needed from regulatory authorities. Furthermore, all patients involved in placebo-controlled trials should receive standard of care for that specific disease.

Another issue is placebo contamination of chemoprevention trials using aspirin or nutritional supplements because their use is common in the USA and Europe. To accommodate this reality, low-dose aspirin was allowed in the calcium and vitamin D trial for sporadic adenomas, and the outcomes were analysed among aspirin users and nonusers[78]. Furthermore, the CAPP-3 trial allows patients on low-dose aspirin to be included without a placebo arm. Similar accommodations were made for women taking calcium in the calcium and vitamin D polyp prevention trial[78]. Trials need to be designed to accommodate the use of these agents with an appropriate *a priori* calculation that might substantially affect the number of patients needed to be recruited for substratification.

Finally, multicentre international trials are needed to test chemopreventive agents in hereditary cancer syndromes because they are uncommon. Developing these trials has been quite challenging as follow-up protocols differ across countries, making it more difficult for some centres to participate. For example, US centres in the CAPP-2 trial had difficulties in participating because of the limited budget that was available; an NIH application to support CAPP-2 in the USA was submitted, but it was not funded (P.M. Lynch, personal communication). There were also concerns about aspirin, particularly the relatively high dose and possible toxicity. Thus, protocols need to take these restrictions into account and harmonize strategies for eligibility and follow-up to allow adequate trial recruitment in multiple countries. These strategies, although costly, would provide more reliable data that could be useful for regulatory approvals.

The need for relevant end points

FAP. One of the biggest challenges to chemoprevention trial design is deciding on relevant and convincing end points that are indicative of a true clinical benefit for the patient population (BOX 2). Initial chemoprevention studies in FAP relied on real-time counts of polyps by endoscopists, sometimes limited to the rectum in patients after subtotal colectomy[47], whereas others used comparisons of photos taken before and after treatment from dense polyp areas that were tattooed at baseline[36,43] (see Supplementary information S1 (table)). The latter method is far from optimal since the precise assessment of changes in polyp size is challenging and it is arguable that a single tattoed area might not be representative of whole organ disease activity. Only in the past 15 years has routine video surveillance been possible, enabled with convenient archiving for later audit and more

Box 2 | **Suggested end points for clinical trials in hereditary CRC**

Familial adenomatous polyposis

- Number of polypectomies
- Number of patients undergoing surgery
- Cumulative number of polyps ≥10 mm removed by polypectomy
- Duodenal cancer
- Effects on desmoids
- Decrease in the number of surveillance colonoscopies
- Proportion of patients requiring polypectomy for polyp(s) ≥10 mm

Lynch syndrome

- Prevention of first cancer
- Prevention of metachronous cancers
- Prevention of cancer-related mortality
- Decrease in the number of surveillance colonoscopies
- Proportion of patients requiring polypectomy for polyp(s) ≥10 mm

CRC, colorectal cancer.

precise measurements with digital tools. Newly developed web-based tools might also be extremely helpful for polyp burden and disease assessment[79].

For FAP, important questions have been raised as to whether the reduction of polyp number and size in a limited area represents a more global clinical change or not. Although this finding might be the case in patients who have undergone ileorectal anastomosis, it is probably more difficult to accept for those with intact colons. After the withdrawal of celecoxib, the European Committee for Medicinal Products for Human Use (CHMP) noted "the uncertain clinical benefit" of decreases in polyp size and number alone[80] and, in the USA, the FDA stated that changes in adenoma number and size are insufficient for regulatory approval, with evidence of clinical benefit required. Suitable examples of clinical benefit cited include decreases in CRC or the need for surgery.

To design better future trials, we should consider looking at disease changes that translate into real clinical benefit (BOX 2; FIG. 2). One approach has been the development of a staging system in which each disease stage is paired with a treatment choice made by the physician, resembling the Spigelman score for duodenal polyposis[81]. In this system, stage shift would be used as a clinically meaningful outcome, incorporating several relevant end points such as delaying endoscopies, polypectomies and surgery, and preventing procedure-associated complications (perforation or bleeding). Lower stages are associated with less frequent endoscopic surveillance and higher stages are closer to surgical options. Such a system could be uniquely valuable in standardizing the approach to therapy and for monitoring clinical benefit in chemopreventive trials. A clinical trial using this system is currently underway (sulindac plus DFMO in FAP[59]).

Desmoid tumours represent an additional notable burden, both in terms of morbidity and mortality in patients with FAP. Several case series have looked into the possible effects of different compounds including sulindac, indomethacin and tamoxifen on desmoid size[82,83], but there is a need for controlled trials targeting desmoids as primary end points. Another critical point is the target population. For FAP, in addition to those with ileorectal anastomosis, future clinical trials should include teenagers and young adults with intact colons, as very few trials have targeted these vulnerable populations. In our opinion, postponing surgery in young teenagers is a very important clinical end point.

Lynch syndrome. Again, for Lynch syndrome the situation is more difficult, mostly because of the difficulty of finding reliable and assessable end points in a suitable time frame. Polyp development or prevention alone is not a sufficient end point because of the unpredictability of polyp growth and progression in this disease[68]. This aspect, together with the small number of identified MMR mutation carriers, makes the selection of agents critical. Eventually, improvements with universal microsatellite instability and immunohistochemistry testing should increase the number of detected Lynch syndrome mutation carriers[84]. Although cancer prevention is difficult to assess in short-term trials, multicentre studies that are designed to assess long-term effects on cancer risk as primary end points, such as that started in the CAPP-3 trial, should be supported. Given that aspirin is commonly taken in the setting of Lynch syndrome, data on its effect could be obtained from observational studies of outcomes in mutation carriers who elect to take aspirin and those who do not. This nonrandomized approach, although not ideal, could add information on possible effects of aspirin in the long term.

Another major requirement in this area is a pipeline of promising chemopreventive agents to test, perhaps using the improved trial designs suggested above. One initial approach could be the running of short-term multicentre pilot studies (with very few patients in each centre) that would provide extensive safety profiles, as well as effects on molecular targets and surrogate end points. This method would then set the stage for large multicentre or cooperation-driven randomized-controlled studies run for appropriate time lengths (>6 months) (FIG. 2).

Conclusions

Chemoprevention should ultimately be aimed at delaying colectomy, reducing endoscopies and polypectomies, and preventing cancer development. However, major barriers exist to the future of chemoprevention studies in hereditary syndromes, which makes them valuable but difficult to undertake. Future clinical trials must be carefully designed and conducted to be successful, with an effort to avoid wasting resources and time. We have to remind ourselves that we are dealing with a very sensitive population of patients who are determined to try potential protective molecules, and will thus have high expectations from proposed clinical trials. Experience of the criticisms from previous trials might help design the next generation of chemoprevention trials in hereditary syndromes.

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L.R. researched the data for the article and drafted the manuscript. D.J.A. and P.M.L. edited and reviewed the manuscript and provided a critical contribution to discussions of the content.

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L.R. has received an unrestricted research grant from SLA Pharma UK. D.J.A. serves on the Scientific Advisory Boards for EXACT Sciences and Cancer Prevention Pharmaceuticals. P.M.L. declares no competing interests.

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Competing interests statement

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