Clinical Implications of Our Advancing Knowledge of Colorectal Cancer Genetics: Inherited Syndromes, Prognosis, Prevention, Screening and Therapeutics

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In the United States it is estimated that more than 145,000 individuals were diagnosed with colorectal cancer in 2005, and that more than 55,000 deaths were attributed to this disease [1]. It is likely that the genetics of no other cancer have been more intensely studied than those of colon and rectal cancer, and this is reflected by the approximately 1000 new scientific publications annotated annually in the National Center for Biotechnology and Information (NCBI) PubMed database (http://www.ncbi.nlm.nih.gov/entrez/) with both the medical subject headings (MeSH) of both “colorectal neoplasms” and “genetics.” It is therefore impossible to cover the vast field of colorectal cancer molecular genetics in any single article. This article highlights the aspects of colorectal cancer genetics that are either currently relevant to clinical management or are anticipated to be so in the near future.

Cancer is fundamentally a genetic disease in which a number of genetic alterations present in a cancer cell allow for its uncontrolled growth, evasion of cell death, local invasiveness, and metastatic potential. Central to our understanding of colorectal carcinogenesis is the model proposed by Fearon and Vogelstein [2,3] that the pathological progression of adenoma to carcinoma is accompanied by distinct and reproducible genetic alterations such as APC gene inactivation, K-ras oncogene activation, and p53 mutation, and
that these genetic events are believed to initiate colorectal neoplasia and lead to its orderly pathological progression. Further studies have confirmed this model and implicated many more genes in colorectal carcinogenesis [4]. Furthermore, a more complex genetic understanding of colorectal carcinogenesis has led to the identification of multiple alternate genetic and epigenetic pathways (ie, chromosomal instability, microsatellite instability, and methylator pathways) that appear to give rise to colorectal cancer [5].

To date, perhaps the greatest clinical advances have been recognized secondary to understanding inherited, germline genetic alterations that lead to relatively rare colorectal cancer syndromes; however, a better understanding of the somatic genetic changes that occur within colorectal cancer cells has also led to recent advances in sporadic colorectal cancer clinical management, and is anticipated to have great impact in the near future. The first portion of this article highlights clinical genetic issues relevant to the inherited cancer disorders of familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNPCC), Peutz-Jeghers syndrome (PJS), juvenile polyposis syndrome (JPS), and Cowden disease. In the second portion of this article, clinical genetic advances in sporadic colorectal cancer management, including the genetic basis of prognosis, response to therapy, screening, chemoprevention and novel therapeutics, are discussed.

Inherited colorectal cancer syndromes

Although approximately 20% of patients who have colorectal cancer or adenomatous polyps have a first-degree relative who has a history of these neoplasms, causative inherited genetic alterations have been identified in fewer than 5% of patients who have colorectal cancer [6]. Inherited syndromes that predispose to colorectal cancer are generally categorized based on the presence of large numbers of adenomatous polyps, few (if any) adenomatous polyps, or the presence of hamartomatous polyps (Table 1).

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Familial adenomatous polyposis and the APC and MYH genes

FAP is a rare, autosomal dominant disease that is typically associated with the development of hundreds to thousands of colorectal polyps. FAP accounts for less than 1% of all colorectal cancer, and occurs with a prevalence of approximately 1/8,000 births [6,7]. Adenomatous polyps usually arise during childhood or adolescence, and if left untreated, colorectal cancer will develop in young adulthood. An attenuated form of FAP has also been recognized. In attenuated FAP, the number of adenomatous polyps is decreased (<100), onset may be later, the location of these polyps may be more proximal in the colon, and cancers may not develop until 50 or 60 years of age [6–9].

In addition to colorectal neoplasms, the occurrence rate of several extracolonic tumors is increased in FAP. The FAP variant of Gardner syndrome has been characterized by colonic polyposis, osteomas, and dermoid cysts, whereas Turcot syndrome is distinguished by the occurrence of colorectal and brain neoplasms [6,9]. Extracolonic manifestations of FAP are of particular clinical relevance, because the widespread use of colonic endoscopy and prophylactic proctocolectomy has effectively decreased the likelihood of developing an advanced staged colorectal cancer. As such, periampullary cancer and desmoid tumors have become leading causes of death in individuals who have FAP [10].

The clinical management of FAP (like that of the other colorectal cancer syndromes) is complex and involves counseling, genetic testing, clinical screening, and treatment of multiple organ systems in not only the affected individual, but their at-risk relatives as well [9,11]. Practice parameters for FAP management have recently been published by the American Society of Colon and Rectal Surgeons, and include referral of individuals who have FAP, or those whose personal or family history make them at-risk for FAP, to specialized cancer registries and genetic counselors who specialize in the coordinated multidisciplinary management of these individuals [11]. Although no consensus exists on the lower limits of adenomatous polyp numbers that would raise suspicion for attenuated FAP, the occurrence of twenty or more synchronous polyps has often been used as a guideline [12].

The underlying genetic cause of FAP is a germline mutation in the APC gene [13,14]. In addition to the inherited germline APC mutations that lead to FAP, somatic mutations of the APC tumor-suppressor gene are believed to initiate most sporadic adenomatous polyps and colorectal cancers [3,4,15,16]. For this reason, APC has been dubbed the “gatekeeper” of colorectal neoplasia (Fig. 1). In FAP, a person is born having one mutated copy of the APC gene, and somatic inactivation of the second copy of the gene in a colonic epithelial cell leads to adenoma initiation [3]. In contrast, in sporadic polyps, both copies of APC must be inactivated by somatic events. In approximately 80% of cases of FAP there is a family history of the disease [6]. In the remaining 20% of cases, FAP occurs because of
To perform genetic testing for FAP, germline genetic analysis begins with an affected individual [6]. Before genetic testing, informed consent must be obtained. According to practice parameters published by The American Society of Clinical Oncology, before consenting, patients must be informed of [17]:

1. Information on the specific test being performed
2. Implications of a positive and negative result
3. Possibility that the test may not be informative
4. Options for risk estimation without genetic testing
5. Risk of passing a mutation to children
6. Technical accuracy of the test
7. Fees involved in counseling and testing
8. Risks of psychological distress
9. Risks of insurer or employment discrimination
10. Confidentiality issues
11. Options and limitations of medical surveillance and screening following testing

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**Fig. 1. APC gatekeeper inactivation in colorectal neoplasia.** In sporadic cancer, both wild-type APC alleles (☐) must undergo somatic mutation to initiate adenomatous polyp formation. In FAP, one APC allele carries a germline mutation (●) and polyp initiation occurs after somatic mutation of the second allele. In APC I1307K carriers, the germline APC variant (□) undergoes mutation more readily than the wild-type sequence, but both APC alleles must be mutated somatically to lead to polyp initiation. In MYH-associated polyposis (MAP), both APC alleles must be somatically altered, but mutation rates of APC are increased due to the extrinsic forces of germline defects in base excision repair (MYH).
Despite the large size of the APC gene, several characteristics of the mutations observed in FAP have led to efficient detection strategies by which mutations are identified in 80% to 90% of classic cases of FAP [6]. Up to one third of germline APC mutations occur at “hotspot” codons 1061 and 1309 [16,18]. These can be assessed by a number of mutation specific methods that use polymerase chain reaction (PCR) amplification of these genomic DNA regions, such as direct sequencing, heteroduplex analysis, or single-strand conformational polymorphism analysis [6]. Approximately 95% of APC mutations lead to a predicted truncated protein (nonsense mutations) [18]. This has led to the development of an analysis technique known as the protein truncation test (PTT), in which RNA is used to synthesize protein in vitro [6,19]. If a nonsense mutation exists, a faster moving, smaller band is observed (as compared with the wild-type protein) when the PTT product is subject to gel electrophoresis.

Interestingly, mutational analyses in FAP have revealed significant genotype-phenotype correlations [16]:

1. Severe polyposis (> 5000 polyps) is associated with mutations between codons 1250 and 1464.
2. Attenuated polyposis (<100 polyps) occurs when mutations are at extreme 5’ and 3’ ends of APC gene.
3. Congenital hypertrophy of the retinal epithelium (CHRPE) is associated with mutations between codons 457 and 1444.
4. Desmoid tumors are associated with mutations between codons 1403 and 1578.

Only after an APC mutation is found in an affected individual can unaffected, at-risk members of the same family be appropriately tested. At-risk analysis is site-specific—that is, the specific familial APC mutation is sought, not APC mutations in general [6]. If an at-risk individual does not carry the APC mutation observed in his FAP-affected relative, the at-risk relative is “negative,” and can be counseled to receive “normal” population colorectal cancer screening [6,20]. If an APC mutation is not found in testing the initial affected individual, the test is “uninformative.” All the first-degree relatives of a genetically uninformative individual who has FAP have a 50% chance of developing polyposis, and should therefore receive counseling and clinical screening. In the case of uninformative testing, linkage analysis may be useful if sufficient affected individuals are available for testing [6]. In FAP linkage analysis, a number of genetic markers near the APC gene are evaluated. Depending on the pattern of these markers in an at-risk individual as compared with multiple affected individuals in the same family, the likelihood for having inherited the disease-causing gene can be estimated. For clinical practicality, only likelihoods of greater than 95% or less than 5% are relevant.

An analysis of commercial APC tests ordered by US physicians in 1995 [20] revealed that fewer than 20% of patients received pretest genetic counseling, that written informed consent was not obtained in nearly 85%
of cases, and that the referring physician could not appropriately interpret test results more than 30% of the time. In the same study, testing was not indicated in 17% of cases, and a further 30% of physicians employed an incorrect testing strategy. These results underscore the potential complexity of FAP management, and the need to refer those affected or at-risk to centers specializing in the management of inherited colorectal cancer syndromes.

Individuals at-risk for FAP, as assessed by personal or family history or positive APC mutation analysis, are advised to begin clinical screening around puberty every 6 to 12 months by flexible sigmoidoscopy [9,11]. When polyps are detected, prophylactic surgery should be undertaken. The timing and extent of surgery depends on the severity of polyposis and whether or not there is rectal sparing. Surgical options include total proctocolectomy with pelvic pouch-anal anastomosis, abdominal colectomy with ileal-rectal anastomosis, or total proctocolectomy with ileostomy. For most cases of classic FAP, a pelvic pouch reconstruction is now the standard of care. Technical issues, including whether or not a mucosectomy is performed and whether or not a hand-sewn versus stapled anastomosis is created, are relatively patient-specific and remain the subjects of some debate. Postoperatively, lifetime endoscopic surveillance of the pelvic pouch, rectum, or ileostomy is required.

In addition to clinical colorectal screening, those who have or are at risk of FAP are recommended to undergo regular screening esophagogastroduodenoscopy starting at approximately 20 years of age [9,11]. The majority of FAP patients develop gastric or duodenal polyps. In contrast, approximately 5% develop duodenal or periampullary cancers. Duodenectomy or pancreaticoduodenectomy is advised in the case of persistent or recurrent severe dysplasia [9,11].

Treatment of desmoid tumors complicating FAP can be difficult [9,11]. Small, well-defined abdominal wall desmoids may be removed surgically. Intra-abdominal desmoids, particularly those involving the small bowel mesentery, should be treated according to their rate of growth and symptoms. Slow-growing, mildly symptomatic tumors may be treated with sulindac, tamoxifen, or vinblastine and methotrexate. Aggressive desmoid tumors may require high-dose tamoxifen, antisarcoma combination chemotherapy such as doxorubicin and dacarbazine, and possibly radiation.

In contrast to the truncating APC mutations observed in FAP, APC I1307K is a single nucleotide substitution (a nontruncating, missense mutation) that leads to a single amino acid difference in the approximate 3000 amino acids that constitute the APC protein [7,21,22]. The APC I1307K variant is carried by an estimated 6% of the Ashkenazi Jewish population, and approximately doubles the risk of developing colorectal polyps and cancers in heterozygous carriers [23]. This type of significant but relatively modest increased cancer risk is explained by incomplete penetrance—that is, those who have the genotype have a modestly increased risk of developing the phenotype. Given the previous successes in identifying the genetic cause of most highly penetrant colorectal cancer syndromes (such as FAP), it is
likely that future advances in this field will be in identifying common, lower penetrant alleles, such as APC I1307K.

The APC I1307K variant creates a tract of eight consecutive adenine nucleotides [(A)\textsubscript{8}] in the DNA sequence that encodes the APC gene, and is not believed to significantly alter the function of the APC protein [21,22]. Instead, the (A)\textsubscript{8} offers a nucleotide sequence that is more prone to somatic mutation than the wild-type sequence. Mechanistically, APC I1307K thus behaves like a mutation “hot spot” or a “premutation” (see Fig. 1). Importantly, unlike the highly penetrant, truncating APC mutations observed in FAP that almost universally lead to the development of polyps, the APC I1307K confers an approximate 10% to 15% lifetime risk of polyp or cancer development [22]. Moreover, APC I1307K carriers do not appear to develop colorectal cancer at a clinically significant younger age compared with those who have sporadic cancers [23]. Although the American College of Medical Genetics and American Society of Human Genetics do have guidelines for clinical APC I1307K genetic testing [24], existing literature suggests that neither a positive nor a negative result of this testing is predicted to change recommendations regarding clinical colorectal screening based on family history alone [25,26]. Specifically, a positive genetic test result confirms (but not alters) a recommendation for colonoscopic screening that may be modified based on family history and age of colorectal cancer onset alone. Similarly, a negative APC I1307K genetic result is insufficient to rule out the need for clinical screening should a significant family history exist.

In addition to APC mutations associated with FAP, a second genetic predisposition to colorectal polyposis and cancer has been identified with inherited mutations of the MYH gene [7,27–29]. In general, the polyposis observed in MYH carriers is less severe and is classified as attenuated. MYH participates in a DNA proofreading system known as “base-excision repair,” and mutations of the MYH gene are thought to lead to somatic mutations of APC. In particular, specific G:C to T:A transversion mutations of the APC gene occur, which then give rise to colorectal neoplasia. For this reason the MYH gene is thought to be a “caretaker” gene, and increases mutation rate, as compared with the APC gene, which is a “gatekeeper” and initiates neoplasia directly (see Fig. 1).

The clinical genetics of MYH-associate polyposis are not as well-studied, and are more complex than those of APC-associated FAP [28,29]. Mutations of MYH appear to confer a codominant risk. Germline mutations of both MYH alleles (biallelic) are associated with the greatest risk of polyposis and cancer (similar to an autosomal recessive disease). In contrast, carriers of a single mutated copy of the MYH gene are at a moderately increased risk of developing polyps and cancers as compared with noncarriers (similar to an autosomal dominant disease with incomplete penetrance), but less so than biallelic MYH mutation carriers. Thus, MYH mutation carriers may present with attenuated polyposis, or cancer in the absence of synchronous adenomatous polyps. In addition to colorectal
polyposis and cancer, adenomatous polyps of the duodenum and pilomatrixomas (benign cutaneous hair follicle neoplasms) have been reported in MYH mutation carriers [30,31]. In FAP cases that are uninformative for APC mutation, germline MYH mutational analysis should be undertaken, because up to one third of these individuals have been observed to harbor biallelic MYH mutations [28].

**Hereditary nonpolyposis colorectal cancer, microsatellite instability, and DNA mismatch repair**

HNPCC (also known as Lynch syndrome) is an autosomal dominant disorder characterized by colorectal cancer in the absence of marked polyposis. HNPCC appears to account for approximately 2% to 4% of all colorectal cancer [6,7,32]. Although probands (the incident case) from HNPCC families are diagnosed with colorectal cancer at approximately 45 years, the actual median age of colorectal cancer diagnosis in HNPCC now appears to be approximately 60 years [33].

In addition to colorectal cancer, numerous other cancers appear to occur at increased frequency in HNPCC kindreds (see Amsterdam II criteria, below) [34]. Most notably, the lifetime risks for endometrial or ovarian cancer in a female who has HNPCC are approximately 55% and 15% respectively [6,35]. Historically, Turcot syndrome (colorectal and brain cancers) can be a variant of HNPCC with glioblastoma multiforme, or FAP, where medulloblastoma is usually observed [36]. The HNPCC variant Muir-Torre syndrome is characterized by sebaceous gland adenomas or keratoacanthomas and visceral cancers [37].

In HNPCC diagnosis is less straightforward than in FAP. In HNPCC, a more diverse range of cancers are observed, there is a lack of profound polyposis, and penetrance is lower than that observed in FAP. Individuals affected with HNPCC have an approximate 50% to 60% lifetime risk of developing a colorectal cancer (compared with a near 100% chance of colorectal polyposis or cancer in FAP), and women who have HNPCC have an approximate 55% risk of developing endometrial cancer [6,35].

Clinically, HNPCC has been defined by the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (ICG-HNPCC) in terms of the following Amsterdam II criteria [32]:

1. Three or more relatives who have HNPCC-associated cancer (colorectal, endometrial, stomach, ovary, ureter or renal pelvis, brain, small bowel, hepatobiliary tract cancers or sebaceous tumors)
2. One affected individual should be a first-degree relative of the other two.
3. Two or more successive generations should be affected.
4. One or more of these cancers should be diagnosed before the age of 50 years.
5. FAP should be excluded.
6. Tumors should be verified by pathological examination.
Studies of large numbers of cancers have shown that certain characteristics appear more commonly in HNPCC compared with sporadic colorectal cancers. Colorectal cancers in HNPCC tend to arise proximal to the splenic flexure, and are associated with a variety of histological features, including tumor infiltrating lymphocytes, Crohn’s-like lymphocytic reaction, mucinous, or signet ring differentiation, and a medullary growth pattern [32,34,38,39].

In addition to frequent differences in their clinical appearance, HNPCC tumors often display a molecular phenotype known as high-frequency microsatellite instability (MSI or MSI-H), also known as replication error positive, (RER+)) [16,40]. This molecular hallmark arises because the underlying genetic cause of HNPCC is a germline mutation in any one of several genes that participate in a DNA replication proofreading system known as mismatch repair [7,16,32,41]. As a “caretaker” system, a deficiency in mismatch repair leads to an increased mutation rate and secondary mutations in the genes, which then give rise to the various cancers observed in HNPCC [6,16]. Additionally, mismatch repair-deficiency causes “by-stander” mutations in short, repetitive DNA repeats known as microsatellites (ie, cytosine-adenine dinucleotide repeats [(CA)n], or adenine mononucleotide repeats [(A)n]) (Fig. 2). It is estimated that the human

![Fig. 2. Microsatellite instability. For a particular microsatellite locus, two consistent sets of (CA)n bands (allele 1 and 2) are amplified from DNA extracted from normal tissue (N). In contrast, allele 1 has undergone microsatellite instability expansion at this locus in DNA extracted from this individual’s colorectal cancer (Ca).](image)
genome contains hundreds of thousands of microsatellite repeat DNA regions, largely in noncoding (intronic) regions [42,43]. Microsatellite regions are highly polymorphic, and as such, microsatellite repeat numbers often differ among individuals, but are the same in all cells of any single individual. Instability of a microsatellite is apparent when the copy number of that particular microsatellite DNA region is different in a cancer when compared with normal tissue from that same individual; for example, (CA)$_3$ versus (CA)$_4$. MSI-H is defined as instability in two or more of the five National Cancer Institute-recommended panels of microsatellite loci [44]. Mutations of microsatellite DNA generally have no direct functional consequence on the cell, unless the microsatellite is located in the coding region of a gene [42,44].

To date, germline mutations in four mismatch repair genes—MLH1, MSH2, MSH6 and PMS2—appear to give rise to HNPCC [6,7,41]. The majority of HNPCC occurs because of MLH1 or MSH2 mutations. As with FAP, individuals who have HNPCC are born with one inactivated mismatch repair gene, and the second copy of this gene is then lost as a somatic event. In very rare instances, biallelic germline mismatch repair gene mutations have been identified in individuals who have severe cancer syndromes, leading to colorectal, hematological, and other cancers at very young ages [45].

Immunohistochemical analysis of paraffin-embedded specimens is now available for MLH1, MSH2, MSH6 and PMS2 [46]. In cases of MLH1-deficiency both MLH1 and PMS2 are immunohistochemically absent, because the PMS2 protein is rapidly degraded in the absence of MLH1. Similarly, in MSH2-deficiency both MSH2 and MSH6 protein expression are absent. In contrast, in the case of either PMS2 or MSH6-deficiency, only the gene of interest is not expressed. Although it is sensitive, immunohistochemistry testing may miss a proportion of mismatch repair protein deficiencies that arise because of functionally relevant substitution (missense) mutations that have been observed in 10% to 37% cases of HNPCC [41].

Based on the previously described clinical and genetic knowledge, the ICG-HNPCC now recommends that individuals fulfilling any of the following Revised Bethesda criteria be genetically assessed for HNPCC [32]:

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age
2. Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumors (as outlined in the Amsterdam II criteria), regardless of age
3. Colorectal cancer with the MSI-H histology (as described above) diagnosed in a patient who is less than 60 years of age
4. Colorectal cancer diagnosed in one or more first-degree relatives who have an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives who have HNPCC-related tumors, regardless of age
If the Revised Bethesda Criteria are met, the ICG-HNPCC recommends the following approach to genetic testing [32]:

1. The optimal approach to evaluation is microsatellite instability or immunohistochemical analysis of tumors, followed by germline MSH2/MLH1 testing in patients who have MSI-H tumors or tumors with a loss of expression of one of the mismatch repair genes.
2. After the mutation is identified, at-risk relatives should be referred for genetic counseling and tested if they wish.
3. An alternative approach, if tissue testing is not feasible, is to proceed directly to germline analysis of the MSH2/MLH1 genes.
4. If no mismatch repair gene mutation is found in a proband with an MSI-H tumor or a clinical history of HNPCC, the genetic test result is noninformative. The patients and the at-risk individuals (ie, relatives) should be counseled as if HNPCC were confirmed, and high-risk surveillance should be undertaken.
5. There is a need to assure patients of confidentiality to allay fears related to discrimination based on genetic status.

In addition to these recommendations, recent publications suggest that MSH6 and PMS2 immunohistochemistry should be performed if MSH2 and MLH1 expression are intact [46]. Furthermore, despite theoretical concerns, studies showing high sensitivity of mismatch repair protein immunohistochemistry as an initial screening tool for HNPCC detection raise the possibility that more laborious microsatellite testing may not be necessary in future clinical screening algorithms [46,47].

Mutational analysis in HNPCC is more complicated than in FAP because: (1) there are more genes to potentially screen; (2) mutations observed in the mismatch repair genes are less likely to occur at recurrent “hotspots”; (3) these mutations are more commonly nontruncating, missense mutations; and (4) these mutations are more commonly large genomic rearrangements, as compared with germline APC mutations [6,18,41]. If tumor tissue is available for analysis, the question of which mismatch repair gene to best assess initially can be screened using immunohistochemistry [47]. In certain populations, such as that of Finland, recurrent founder mutations account for a large percentage of HNPCC germline mutations, and thus genetic analysis begins with sequence-specific analysis for the specific founder mutation [48]. In most populations, founder mutations are not common, and genetic analysis incorporates methods to detect large genomic rearrangements and smaller genetic mutations [41,49]. Large genomic rearrangements account for 10% to 20% of MSH2 mutations and a lesser percentage of MLH1 mutations [6,41]. These mutations are effectively screened for using a recently developed assay known as multiplex ligation-dependent probe amplification (MLPA) [6,49]. In MLPA specific probes are hybridized to genomic DNA and then the probes (as opposed to the DNA) are amplified and quantified. Although germline mutations predisposing to HNPCC often lead to
a truncated mismatch repair protein, 10% to 37% of mutations reported in MSH2, MLH1, and MSH6 are thought to be nontruncating, missense mutations [41,44]. Furthermore, germline mutations in HNPCC appear to be roughly equally distributed throughout all exons of the mismatch repair genes. Thus screening of these genes is optimally performed using full sequencing or other methods that may detect either missense or nonsense mutations. When detected, truncating, nonsense mutations are considered to be pathological; however, determining pathogenicity of sequence changes that lead to amino acid substitutions, splice-site changes, or in-frame nucleotide deletions/additions is less straightforward [50]. Predicting whether these alterations are variants of normal or disease-causing relies on a number of factors. Favoring disease causation would be: (1) a nonconservative amino acid substitution (versus conservative or semiconservative), (2) a change in an amino acid evolutionarily conserved between diverse species, (3) the absence of the genetic variant in normal populations, (4) cosegregation of the genetic alteration with disease, and (5) the association of the alteration with tumor MSI-H or lack of specific mismatch repair protein expression [44].

In addition to the high incidence of proximal colon cancer in HNPCC, it is believed that the timeframe of adenoma to carcinoma progression may be markedly accelerated as compared with sporadic colorectal cancer [51]. Thus, a polyp may progress to an invasive cancer in 2 to 3 years, rather than the 8 to 10 years this process is estimated to require in sporadic colorectal carcinogenesis. Mechanistically, this is believed to occur because of the rapid accumulation of somatic mutations associated with neoplastic initiation and progression secondary to mismatch repair deficiency [16]. Practically, this has led to the recommendation that those at risk of HNPCC undergo full colonoscopy, as opposed to flexible sigmoidoscopy, every 1 to 2 years beginning between ages 20 and 25 years [25]. Furthermore, because of the high incidence of endometrial or ovarian cancers in HNPCC, some authors recommend transvaginal ultrasonography, endometrial aspiration for pathological assessment, and plasma CA-125 (an ovarian cancer genetic marker) determination annually beginning at age 30 years in women at-risk for HNPCC [52].

Surgical recommendations in HNPCC remain controversial [11]. This primarily stems from a relative lack of high-level evidence to support or refute the theoretical advantages of prophylactic surgery or extended resection beyond what would normally be oncologically necessary. Given these relative uncertainties, proper counseling is critical to all decision-making and informed consent. The American Society of Colon and Rectal Surgeons recommendations for HNPCC include that individuals who fulfill the Amsterdam criteria and who are diagnosed with more than one advanced adenoma or a colon cancer be offered subtotal colectomy with ileorectal anastomosis or segmental colectomy, whereas those who have rectal polyps or a cancer may be offered total proctocolectomy with ileal-pouch anal anastomosis or anterior resection, assuming the sphincters can be saved
Prophylactic hysterectomy should be considered in women who have HNPCC undergoing other abdominal surgery or once their family is complete. Significant reduction in endometrial cancer, and to a lesser extent ovarian cancer, has been documented with prophylactic hysterectomy and bilateral salpingo-oopherectomy for women who have germline HNPCC mutations [53]; however, in this recent retrospective study, there was no standardized clinical screening in the group of women who did not undergo prophylactic surgery.

Interestingly, individuals whose family history satisfies the Amsterdam criteria for HNPCC but whose colon cancers do not display MSI-H appear to have a syndrome that is distinct from HNPCC, and has recently been referred to as “Familial Colorectal Cancer Type X” [54]. In a population-based study, Familial Colorectal Cancer Type X was nearly as common as HNPCC. However, as compared with HNPCC, the risk of colorectal cancer in Familial Colorectal Cancer Type X was observed to be more moderate and age of onset later. Furthermore, in Familial Colorectal Cancer Type X, the risk of extracolonic cancers such as endometrial cancer was not appreciably elevated [54]. These results have significant clinical screening and treatment implications, because currently individuals who fulfill the Amsterdam criteria are usually considered to have HNPCC and counseled as such.

**Hamartomatous polyposis syndromes**

Intestinal hamartomas are frequent in PJS, JPS, and Cowden disease (including Bannayan-Ruvalcaba-Riley syndrome). All these syndromes are very rare, with incidences below 1 per 100,000 [6,7].

PJS is an autosomal dominant disease characterized by perioral pigmentation, pathologically distinct Peutz-Jeghers–type hamartomatous polyps throughout the gastrointestinal tract, and an approximate 30% lifetime risk of colon cancer and 50% risk for breast cancer [6,7]. In PJS, patients are at risk for other extracolonic cancers, including pancreatic, gastric, small bowel, ovarian, uterine, and lung. Approximately 50% of PJS cases are believed to occur because of germline mutations of the STK11 gene [55,56].

Although solitary colonic juvenile polyps are believed to be one of the most common sources of lower gastrointestinal bleeding in children, multiple juvenile polyps are rarely observed [6,7,57]. JPS should be considered when more than three to five juvenile polyps are identified in the colon. The lifetime colon cancer risk in JPS approaches 60%, and patients are additionally at risk of developing stomach, small bowel, and pancreatic cancers. In approximately 50% of JPS cases, germline mutations of either the SMAD4 or BMPR1A genes, both involved in TGFβ signaling, are believed to confer an autosomal dominant risk [58,59]. In addition to genetic testing, colonoscopy, gastroscopy, and small bowel examination are recommended in PJS and JPS [6,57]. Endoscopic or surgical excision of large or symptomatic polyps is recommended.
Cowden disease is an autosomal dominant disease characterized by facial trichilemmomas, oral papillomas, multinodular goiter, fibrocystic breast disease, esophageal glycogenic acanthosis, and intestinal hamartomas [6,7]. Breast and thyroid cancer risk are most pronounced in Cowden disease, with colon cancer developing in up to 10% of patients. Germline mutations of the PTEN gene have been identified in the majority of patients who have Cowden disease, and also predispose to Bannayan-Ruvalcaba-Riley syndrome, which shares characteristics with Cowden disease and additionally includes slowed psychomotor development and pigmented spotting of the penis [60,61].

In comparison to the “gatekeeper” function of the APC gene and the “caretaker” roles of the mismatch repair and MYH genes, the genes predisposing to hamartomatous polyposis have been dubbed “landscaper” genes [62]. In sporadic circumstances, non-neoplastic hamartomatous polyps are not believed to confer a significant cancer risk. In comparison, germline mutations and somatic inactivation of the STK11, SMAD4, BMPR1A, and PTEN genes in hamartomatous polyposis syndromes are believed to create an epithelial milieu (or landscape) at risk for neoplastic development.

**Molecular genetic advances in sporadic colorectal cancer management**

Numerous studies to date have focused on the clinical relevance of various genetic alterations in sporadic colorectal cancer management [63,64]. Genetic analyses of blood, stool, and tumors have been proposed as methods for primary polyp and cancer screening, monitoring for cancer response to therapy or recurrence, estimating prognosis and predicting response to adjuvant chemotherapy [64–66]. Furthermore, exploitation of cancer-specific genetic alterations has been proposed as a strategy for chemoprevention and colorectal cancer therapeutics [67,68]. Although some biologic-based chemotherapeutic agents have recently been adopted into colorectal cancer care, molecular genetic testing has not yet gained acceptance in the standard clinical management of sporadic colorectal cancer. Nonetheless, it does appear that genetic-based decision making will play a significant role in colorectal cancer management in the near future.

**Genetic markers of prognosis and response to therapy**

To date, the prognostic and predictive potential of dozens of genetic alterations in colorectal cancer have been explored [65]. Recently, a number of meta-analyses or systematic reviews of the scientific literature have been published evaluating the most promising of these molecular genetic markers.

**Microsatellite instability**

Tumor MSI-H is not only observed in the 2% to 4% of colorectal cancers that arise in the context of HNPCC, but is also observed in approximately
15% of sporadic colorectal cancers [2,42,43]. Sporadic MSI-H colorectal cancers appear to arise due to an epigenetic (nonmutational) phenomenon causing mismatch repair deficiency, as compared with the genetic (mutational) cause of mismatch repair deficiency and MSI-H observed in HNPCC [69]. In the majority of sporadic MSI-H colorectal cancers, the MLH1 gene has been silenced by promoter hypermethylation. Interestingly, hypermethylation and silencing of other genes such as p16 appear to be important in colorectal carcinogenesis and in a general sense, a cancer-specific methylation pathway (CpG island methylator phenotype, CIMP) has been proposed [70]. Although the vast majority of the 15% of sporadic colorectal cancers that display MSI-H are also CIMP, this epigenetic phenomenon is also observed in approximately 5% of the 80% to 85% of sporadic colorectal cancers that are microsatellite stable (Fig. 3) [71].

Arguably, MSI status has emerged the most consistent independent molecular genetic predictor of survival [38,72,73]. In a recent systematic review of the literature involving 32 studies and more than 7600 patients who had colorectal cancer [74], 17% were MSI-H, and the vast majority of the remaining 83% of cancers were microsatellite stable (MSS). A small number of cancers were observed to have an intermediate genotype referred to as low-frequency microsatellite instability (MSI-L). MSI-H was associated with a hazard ratio of 0.65 (95% CI, 0.59–0.71) of dying when compared with MSS cancers. That is, the risk of a patient who had MSI-H cancer dying was 65% that of a patient who had MSS colorectal cancer, even after controlling for pathological stage and other clinical predictors of survival.

Although cancer MSI-H status is associated with independent improved survival in patients who have colorectal cancer, it appears likely that patients who have MSI-H cancers do not derive the same benefit from

Fig. 3. Genetic and epigenetic pathways of colorectal carcinogenesis. Colorectal cancer evolves through either the chromosomal instability (CIN) pathway, which is additionally characterized by microsatellite stability (MSS) or the microsatellite instability (MSI-H) pathway. The CpG island methylator pathway (CIMP) appears to overlap both MSI-H and CIN/MSS colorectal cancers. Percentages indicate estimated prevalence as a proportion of all colorectal cancer.

<table>
<thead>
<tr>
<th>Cancer Characteristics</th>
<th>Sporadic Colorectal Cancer</th>
<th>Inherited Colorectal Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN/MSS</td>
<td>80%</td>
<td>FAP &lt;1%</td>
</tr>
<tr>
<td>CIMP/MSS</td>
<td>5%</td>
<td>not reported</td>
</tr>
<tr>
<td>CIMP/MSI-H</td>
<td>10-15%</td>
<td>rare‡</td>
</tr>
<tr>
<td>MSI-H</td>
<td>rare‡</td>
<td>HNPCC 2-4%</td>
</tr>
</tbody>
</table>

‡There have been two case reports of an HNPCC variant secondary to apparent germline CIMP [145]. †Some sporadic MSI-H colorectal cancers may arise in the absence of CIMP [146].
fluorouracil (5-FU)-based adjuvant chemotherapy as patients who have MSS colorectal cancers. In two studies of patients who had Stage II or III colorectal cancer enrolled in randomized control trials of surgery alone compared with surgery plus adjuvant 5-FU-based chemotherapy [75,76], patients who had MSS were observed to benefit from 5-FU adjuvant chemotherapy, whereas those who had MSI-H cancer did not benefit, and may in fact have been harmed by the addition of 5-FU. A similar observation was noted in two recently published case series [77,78], but not in another [79], perhaps because of treatment biases not present in the randomized control trials. Clinically, this suggests that 5-FU adjuvant chemotherapy should be withheld in the 15% to 20% of individuals who have MSI-H colorectal cancer; however, the current observations are based on retrospectively reanalysis of older clinical trials in the context of MSI-status. Given the widespread acceptance of adjuvant 5-FU in Stage III colorectal cancer and Stage II cancers with poor prognostic features, it appears that new prospective randomized trials of MSI-status and 5-FU will only be possible in patients who have Stage II cancers without poor prognostic features [80,81]. The ethical dilemma of withholding established 5-FU treatment may be circumvented, however, if subgroup analyses of newer chemotherapeutic agents such as irinotecan or oxaliplatin show specific benefit to patients who have MSI-H colorectal cancer [65,82].

Chromosomal instability, p53, and DCC

Whereas 15% to 20% of colorectal cancers display MSI-H, the majority of colorectal cancers are MSS [42,43]. In comparison with MSI-H colorectal cancers, which display frequent micro-genetic (intragenic) mutations, most MSS colorectal cancers display widespread alterations at the chromosomal level. These macro-genetic alterations may be measured by techniques such as flow cytometry, G banding cytogenetics, comparative genomic hybridizations (CGH), DNA allelotyping, or DNA fingerprinting [83]. Chromosomal instability (CIN) measured by these methods may be characterized by abnormal chromosome number (aneuploidy) or gross changes within a chromosome such as allelic imbalance or loss of heterozygosity (LOH) [44,83]. MSI-H cancers rarely display CIN/LOH and are thus chromosomal stable, and conversely, most MSS cancers do display CIN/LOH. In contrast to microsatellite status, no widely accepted consensus definition of CIN exists. Furthermore, unlike MSI-H, which is known to be caused by an underlying defect in DNA mismatch repair, no generalized mechanistic cause for CIN has been discovered to date [84].

Consistent with the survival benefit observed for MSI-H, colorectal cancers displaying CIN as measured by either aneuploidy [85–87] or widespread LOH [88] have been observed to be associated with poor survival; however, this association has not been as consistently reported [63,64,83]. Furthermore, no meta-analysis or systematic review of the literature has been published summarizing colorectal CIN and survival. Thus, neither American
Society of Clinical Oncology, nor College of American Pathologists recommend the routine use of DNA content determination in colorectal cancer management [63,64].

In general, LOH is believed to eliminate either one or both copies of a tumor suppressor gene. Chromosomes 5q, 17p, and 18q all display considerable levels of LOH in colorectal cancer [89]. In the cases of 5q and 17p, the somatic genetic targets are believed to be the APC and p53 tumor suppressor genes, respectively [3,16]. In the case of 18q, debate continues as to whether the target is DCC, SMAD2, SMAD4, some combination of these putative tumor suppressor genes, or an as-yet-unidentified gene target [90].

Both 17p/p53 and 18q/DCC have been the focus of considerable research in colorectal cancer prognosis [91,92]. In the case of chromosome 17p, an intragenic mutation of the p53 gene commonly accompanies LOH, leading to inactivation of both copies of the p53 tumor suppressor gene [92,93]. Mutations of p53 (paradoxically) lead to stabilization of the mutant p53 protein, and can be detected by positive p53 expression by immunohistochemistry [94]. By comparison, the wild-type p53 protein is unstable and not visualized by immunohistochemistry. Approximately 45% to 55% of colorectal cancers harbor chromosome 17p LOH, p53 expression by immunohistochemistry, or p53 mutations by direct analysis, and there is strong correlation between these detection methods [92,94].

A recent systematic review of p53 abnormalities and colorectal cancer outcome from 241 publications involving nearly 19,000 patients [92] found that either p53 mutation or positive expression of p53 by immunohistochemistry was associated with a survival hazard ratio of approximately 1.24 to 1.43. Thus, the presence of a p53 mutation appears to confer an approximate 30% worse survival when compared with patients whose colorectal cancers do not have a p53 mutation. In neither this systematic review nor a recent reanalysis of three previous randomized trials of surgery alone versus surgery plus 5-FU-based adjuvant chemotherapy [95] was p53 status predictive of response to 5-FU adjuvant chemotherapy.

Chromosome 18q LOH and loss of DCC expression are observed in approximately 55% of colorectal cancers [91,96,97]. A recent meta-analysis of 17 studies of nearly 2200 patients [91] concluded that 18q LOH or loss of DCC expression were associated with a survival hazard ratio of 2.00 (95% CI 1.49–2.69) when compared with patients whose tumors did not show 18q LOH or had intact DCC expression. Thus, patients whose cancers display 18q LOH or DCC loss appear to have twice the risk of dying compared with those who do not show these molecular genetic features. This meta-analysis did not address the predictive impact of 18q LOH or DCC expression on response to 5-FU.

MSI status, p53 mutation, and DCC expression are nonrandomly associated with one another. MSI-H colorectal cancers infrequently harbor chromosome 17p or 18q LOH, p53 mutations, and usually express DCC [5,98,99]. Conversely, MSS/CIN cancers frequently display 17p or 18q
LOH, p53 mutations, and are often DCC-deficient. Thus future studies must address whether these survival associations previously identified are pathway-specific (ie, MSI-H versus MSS/CIN) or somatic target-specific (ie, DCC or p53 wild-type versus mutant).

**Thymidylate synthase**

Thymidylate synthase (TS) is an enzyme that catalyzes the methylation of deoxyuridine-5'-monophosphate (dUMP) deoxythymidine-5'-monophosphate (dTMP), providing the sole intracellular de novo source of thymidine [100]. The main mechanism of 5-FU is thought to be inhibition of TS. A recent meta-analysis of 20 studies with approximately 3500 patients [101] found that increased cancer TS expression was observed in approximately 50% of cancers and was associated with poorer patient survival. The combined hazard ratio for high TS expression was 1.74 (95% CI, 1.34–2.26), implying that patients whose tumors expressed high levels of TS had a 74% greater chance of dying compared with patients who had low TS expressing tumors. The association between TS expression and 5-FU responsiveness remains controversial. In the two largest trials to date to analyze TS expression in colorectal cancer patients randomized to surgery alone or surgery plus adjuvant 5-FU, one study of 862 patients [102] concluded that high TS expression compared with low was associated with 5-FU responsiveness (ie, improved survival of patients who received surgery plus 5-FU), whereas a second study of 706 patients [95] failed to demonstrate this association.

In addition to MSI-status, p53, DCC, and TS, numerous other genetic markers have been explored less extensively for their role in prognosis or as predictors of response to adjuvant or palliative chemotherapy [65,83,103–105]. Included in this list are chromosomal loci, tumor suppressor genes, oncogenes, and genes regulated by methylation believed to be involved directly in colorectal carcinogenesis, as well as genes whose product may be important specifically in pathways affected by 5-FU or newer colorectal cancer chemotherapeutic agents such as oxaliplatin, irinotecan, bevacizumab, or cetuximab. Although these studies have not yet led to specific recommendations for clinical management, it is likely that some of these genetic markers will make their way into the clinic in the near future [63,64]. Furthermore, new genomic and proteomic technologies offer the potential for interrogating whole genome [106,107] and proteome [108] associations with survival and response to therapy, and the future possibility of individualized molecular medicine.

**Molecular genetic colorectal cancer screening**

Given the common occurrence of adenomatous polyps and colorectal cancer in Western society, and the relative invasiveness of current screening procedures such as colonoscopy, the potential to identify cancer- or
polyp-specific genetic markers from bodily fluids including blood or stool offers a potentially attractive avenue for sporadic cancer screening [66,109,110]. In general, both blood- and stool-based assays attempt to detect the same genetic alterations that are observed in colorectal cancers, via cells shed from the tumor into the bloodstream or gastrointestinal tract lumen. Currently, the most promising approach to genetic-based colorectal cancer screening appears to be stool-based DNA detection technologies [66,110]. Based on current research results, genetic-based screening for sporadic colorectal cancer should still be viewed as investigational. Despite this, commercial clinical DNA stool testing is currently available through licensed health care provider requisition in the United States (http://www.exact sciences.com/medical/pregen.html).

Detection of several different cancer genetic alterations from stool has been proposed as a possible clinical colorectal neoplasia screening strategy. Included within the genetic targets are APC, p53, and K-Ras mutations, as well as markers of MSI-H, LOH, and CIMP [66,110]. Another interesting strategy involves detection of intact long DNA within stool samples. The rationale for this lies in the fact that normal colonic epithelium exfoliates after undergoing apoptosis (programmed cell death), whereby the cell and its DNA are fragmented, and thus the presence of intact long DNA in stool is most likely to have originated from sloughed cancer or adenoma cells.

Overall detection from stool samples of any single genetic marker of neoplasia is only predicted to afford a sensitivity of approximately 40% [66,110]. In part this is because of the fact that no one alteration is present in all colorectal cancers and adenomas. Additionally, although concordance between stool sample and tumor testing is usually in the order of 80%, detection of a cancer DNA from stool is not perfect. To overcome these limitations, more recent efforts have involved panels of genetic markers. Using panels of tumor DNA markers for stool screening, sensitivities of 63% to 100% have been reported for colorectal cancer detection; however, virtually all studies of genetic-based screening in stool samples reported to date have involved a relatively small numbers of patients who had known cancer or adenomas.

Only one fully reported study to date has included a substantial average risk population [111]. A DNA fecal panel of 21 alterations from five different genetic markers was investigated in 2507 asymptomatic individuals. Thirty-one of these subjects had colorectal cancer, whereas 1051 had adenomas. Genetic stool screening sensitivity was only 51.6% for cancer and 10.5% for adenomas. The specificity of the test panel was 94.4%. Although fecal DNA testing outperformed fecal occult blood screening in this study, low sensitivities for both fecal DNA and occult blood testing mean that the majority of all neoplasms were not detected by either, and were discovered only by colonoscopic screening. Furthermore, given a relatively low prevalence of colorectal cancer in the general population, even a specificity of 94% is predicted to mean that just 2% of adults 50 to 59 years with a positive fecal DNA screen have colorectal cancer [112].
present, fecal DNA screening is estimated to cost $400 to $800, and is predicted to cost at least three times as much per year of life gained compared with fecal occult blood testing. Thus, although promising, stool-based cancer DNA screening performance must improve and the cost must decrease if this technology is going to rival current colorectal cancer screening strategies.

Chemoprevention

A number of interventions with pharmaceuticals, vitamins, and minerals have been undertaken in high-, intermediate-, and average-risk populations to prevent formation of adenomatous polyps and colorectal cancer [67,113]. Although the absolute effect of all agents tested to date is modest, good scientific data do exist to support significant chemopreventative roles in colorectal neoplasia for nonsteroidal anti-inflammatories (NSAIDs), calcium carbonate, selenium and hormone replacement therapy (HRT).

Nonsteroidal anti-inflammatories

At present, NSAIDs are the most well-established agents in colorectal neoplasia chemoprevention, both in terms of the mechanism of effect and the risk reduction they afford [67,113]. The molecular basis of NSAID chemoprevention is primarily attributed to inhibition of cyclooxygenase (COX) enzymes in the conversion of arachidonic acid to prostaglandins [67,114,115]; however, in addition to inhibition of COX enzymes, NSAIDs may inhibit colorectal neoplasia through non-COX–mediated pathways [67,116]. For instance, aspirin has been shown to cause nuclear translocation of the NF-κB transcription factor and cancer cell apoptosis [116].

The COX enzyme comes in two isoforms: COX-1 and COX-2. COX-1 is a housekeeping protein that is constitutively expressed in many tissues, and is thought to lead to production of cytoprotective prostaglandins in the gastrointestinal tract [114,115]. In comparison, COX-2 expression is induced in response to growth factors, mitogens, and cytokines. It is through the inhibition of COX-2 by NSAIDs or specific COX-2 inhibitors that anti-inflammatory, analgesic and antipyretic effects are thought to occur [114,115].

Compared with normal colonic epithelium, COX-2 is overexpressed in approximately 50% of colorectal adenomas and in 85% of cancers [117]. It is hypothesized that this overexpression occurs as a result of transcription induction by oncogenic pathways. In support of this, wild-type p53 suppresses COX-2 expression, whereas mutant p53 leads to COX-2 overexpression [118]. Abundant experimental data also support the hypothesis that inhibition of COX-2 causes suppression of colorectal neoplasia. Preclinical in vivo evidence includes dose-dependent attenuation of the polyp phenotype in mouse models of FAP when these mice were crossed with heterozygote and homozygote COX-2 deficient mice [119] or were fed increasing doses of the COX-2 inhibitor celecoxib [120].
COX-2 overexpression is thought to drive tumorigenesis through multiple mechanisms [67,114,115]. COX-2 overexpression is associated with: (1) vascular endothelial growth factor (VEGF) secretion and angiogenesis; (2) increased expression of the BCL2 protein and resistance to apoptosis; (3) increased expression of metalloproteinases (MMPs) and CD44, and increased cancer cell invasiveness and metastasis; and (4) impairment of tumor infiltrating lymphocytes (TILs), circulating T-cells, and macrophages and host immunosuppression.

The most compelling evidence for NSAID chemoprevention of colorectal neoplasia comes from two double-blind, randomized-controlled trials. In one trial [121], 635 patients who had previously resected early colon cancer were randomized to aspirin 325 mg per day versus placebo for 3 years. Aspirin use was associated with a significant decrease of adenoma relative risk of 0.65 (95% CI, 0.46–0.91) on follow-up colonoscopy at a median of 12.8 months. Thirty percent of those on aspirin and 49% of those on placebo developed adenomas, and the time to detection of first adenoma was significantly longer in the aspirin group. In the second study [122], 1121 patients who had previous adenomas were randomized to aspirin 325 mg or 81 mg per day or placebo. In this trial, low-dose aspirin use was associated with a significantly reduced adenoma relative risk of 0.81 (95% CI, 0.69–0.96) and a relative risk of 0.59 (95% CI, 0.38–0.92) for advanced adenomas (≥1 cm in diameter, tubulovillous, villous, severe dysplasia, or invasive cancer) compared with placebo. Thirty-eight percent of individuals on aspirin 81 mg developed adenomas compared with 47% of those on placebo. No significant risk reduction was observed for those on aspirin 325 mg, 45% of whom developed adenomas.

Although modest but significant Level I evidence for colorectal neoplasia risk reduction has been demonstrated for nonspecific COX inhibition by aspirin, two recent chemoprevention trials with specific COX-2 inhibitors have been associated with significant increases in cardiovascular death and complications [123,123a]. In both trials, patients with previous colorectal adenomas were randomized to either placebo or one of the specific COX-2 inhibitors, celebrex or rofexoxib. In one trial [123], a total of 2035 patients who had previous adenomas were randomized to celebrex 200 mg twice daily, or 400 mg twice daily, or placebo. At approximately three years, a significant dose-response association was observed between celebrex use and death from myocardial infarction, stroke or heart failure with 3.4%, 2.3% and 1% cardiovascular mortality observed for those on celebrex 400 mg twice daily, 200 mg twice daily, and placebo, respectively. In the second chemoprevention trial [123a], a total of 2586 patients who had previous adenomas were randomized to rofexoxib 25 mg daily or placebo. Adverse thrombotic cardiovascular events, primarily myocardial infarctions and ischemic cerebrovascular events, were observed more often in those on rofexoxib (1.50 events per 100 patient-years) compared with those on placebo (0.78 events per 100 patient-years). A significantly increased relative risk of 1.92 (95% CI,
1.19–3.11) was observed after 18 months of rofecoxib use. Additionally, the rates of congestive heart failure, pulmonary edema, or cardiac failure may have been increased in those taking rofecoxib. Although cardiovascular events differed significantly in this trial, mortality from cardiovascular events was similar in those on rofecoxib compared to those taking placebo.

Chemoprevention in FAP has been an area of great interest [67]. The nonspecific COX inhibitor sulindac [124] and the COX-2 inhibitor celecoxib [125] have both been shown in several randomized, double-blind, placebo-controlled clinical trials to cause significant regression of existing polyps and to decrease new polyp formation in FAP patients [67,113,114]; however, there have also been case reports of FAP patients who had ileorectal anastomoses developing rectal cancers while on sulindac [126,127]. Furthermore, a 4-year randomized controlled trial failed to show an effect of sulindac on primarily prevention of polyps in at-risk patients who had APC mutations, but without polyposis at study enrollment [128]. Additionally, trials have not typically found that sulindac effectively treats duodenal adenomas [11]. For these reasons, NSAIDS and COX-2 inhibition should not be used as an alternative to surgery in FAP.

Calcium carbonate, selenium, and hormone replacement therapy

In addition to NSAIDs, Level I evidence exists for colorectal neoplasia chemoprevention with calcium carbonate, selenium, and HRT [67,113], although not all large placebo-controlled trials of these agents have demonstrated colorectal neoplasia risk reduction. Furthermore, in the case of HRT use, significant adverse health risks have been observed [129].

Unlike the COX-2 mediated effects of NSAIDs, the chemopreventative mechanisms of calcium carbonate, HRT, and selenium are poorly understood [67]. Calcium is believed to exert its antineoplastic effects through both luminal binding of mutagenic bile acids in addition to direct effects [130,131]. Extracellular calcium can activate a number of signaling pathways and intracellular calcium influences several nuclear proteins [130]. HRT is believed to directly and indirectly reduce bile acid production and inhibits insulin-like Growth Factor I [67,132]. Selenium is antioxidant [133]; however, at the present time, molecular genetic mechanisms linking calcium carbonate, HRT, and selenium and colorectal neoplasia chemoprevention remain largely speculative.

Genetic-based colorectal cancer treatment

One hope accompanying the complete sequencing of the human genome was the rapid development of genetic-based therapeutics for a myriad of diseases. In no disease were post-genomic clinical advances more anticipated than in cancer. To date two drugs, bevacizumab and cetuximab, have received United States Food and Drug Agency approval for use in colorectal cancer management [134].
**Bevacizumab**

Bevacizumab is a monoclonal antibody that targets and binds vascular endothelial growth factor-A (VEGF-A) [135]. This reduces the amount of VEGF ligand available to bind to its receptor, and prevents receptor activation. The VEGF pathway is believed to be critical in cancer angiogenesis. Cancer cells secrete substances such as VEGF to recruit host vasculature to supply cancer growth.

Bevacizumab has been evaluated in first-line combination therapy for metastatic colorectal cancer. The first Phase III trial of bevacizumab [136] involved 813 patients who had metastatic colorectal cancer randomized to irinotecan, 5-FU, or leucovorin (IFL) plus placebo or bevacizumab. In this study, patients receiving IFL plus bevacizumab had significantly longer median survival compared with IFL alone, 20.3 versus 15.6 months. Additionally, patients receiving bevacizumab had longer progression-free survival, higher response rate, and longer median duration of response compared with those who did not. This benefit was accompanied by greater toxicity; grade 3 or 4 adverse events were observed significantly more often in patients receiving bevacizumab compared with those who did not, 85% versus 74%.

Two further Phase II and III trials have recently been reported using combination treatment with bevacizumab in metastatic colorectal cancer [137,138]. In the Phase III trial [137], 210 patients who had metastatic colorectal cancer were randomized to treatment with IFL or 5-FU, leucovorin (5-FU/LV), and bevacizumab, and a nonsignificant median survival advantage was observed for the 5-FU/LV and bevacizumab treatment group compared with IFL (18.3 versus 15.1 months). The toxicity was roughly equivalent in the two groups—Grade III/IV adverse events: 5-FU/LV and bevacizumab, 77.1%; IFL, 81.6%. In the other study [138], 237 patients who received 5-FU/LV or IFL were compared with 244 patients receiving 5-FU/LV and bevacizumab. Median survival for those receiving 5-FU/LV plus bevacizumab was 17.9 months, compared with 14.6 months in the patients who received 5-FU/LV or IFL. Grade III or IV adverse events were observed in 81% of those who received 5-FU/LV and bevacizumab, compared with 73% of patients who received 5-FU/LV or IFL. In summary, these clinical trials show that IFL plus bevacizumab offers the best survival in metastatic colorectal cancer [136,137], and 5-FU/LV and bevacizumab offer comparable survival to IFL with similar or less toxicity [138].

**Cetuximab**

Cetuximab is a monoclonal antibody that targets the epidermal growth factor receptor (EGFR) [135,139]. This competitively inhibits ligand binding of the receptor and EGFR activation. EGFR signaling is complex and has been shown to be involved in: (1) cancer cell proliferation; (2) degradation of extracellular matrix, tumor migration and invasion; and (3) endothelial proliferation and angiogenesis [139]. Inhibition of EGFR has proven
a successful genetic target for cancer treatment, and in addition to cetuximab, two additional EGFR inhibitors, gefitinib and erlotinib, are in clinical use in non-small–cell lung and pancreatic cancer treatment [135].

One Phase III clinical trial using cetuximab in metastatic colorectal cancer has been reported to date [140]. This study aimed to demonstrate that the addition of cetuximab in second-line treatment of metatstatic disease could resensitize patients to a palliative chemotherapeutic agent that they had previously failed. As such, 329 patients who had progressed on irinotecan-based therapy were randomized to cetuximab alone or cetuximab with irinotecan. Significantly greater response rates (22.9% versus 10.8%) and median time to progression (4.1 versus 1.5 months) were observed in patients who received irinotecan and cetuximab compared with those who received cetuximab monotherapy. No significant difference in median survival (irinotecan and cetuximab, 8.6 months; cetuximab 6.9 months) was observed, however. Thus, although somewhat inconclusive in terms of efficacy, current results of cetuximab in colorectal cancer offer hope that the addition of cetuximab to first-line treatment will improve survival.

The cetuximab clinical trial discussed above did not observe a correlation between colorectal cancer EGFR expression and clinical response [140]. Furthermore, although EGFR overexpression has been observed in 25% to 82% of colorectal cancer specimens [139], oncogenic EGFR mutations (as opposed to protein overexpression) are rarely observed in colorectal cancers [141,142]. In contrast, EGFR gain of function mutations has been observed to predict response to the EGFR inhibitor, gefitinib, in non-small–cell lung cancer [143,144].

The introduction of biologic agents such as bevacizumab and cetuximab dramatically highlights the cost of such advances [134]. Eight weeks of 5-FU/LV are estimated to cost $63 to $304, and the addition of irinotecan or oxilaplatin brings this price to $9381 to $11889. The further addition of bevacizumab or cetuximab bring the drug cost of 8 weeks of therapy to a staggering $21,033 to $30,790. Obviously such costs for significant but relatively modest clinical gains will pose both financial and ethical challenges for the future.

Summary

Recent genetic advances in our knowledge of colorectal cancer genetics are beginning to pay translational dividends in the management of this common clinical problem. We are now able to accurately screen and counsel individuals at risk of rare inherited cancer syndromes. We have recently introduced two of what are sure to be numerous biologic-based therapies, and have shown that colorectal neoplasia risk can be modestly reduced by various chemopreventative agents. Finally, our advancing knowledge has led to significant inroads into understanding what genetic alterations define prognosis and predict response to specific chemotherapeutic agents, and we
are beginning to explore the utility of this knowledge in mass genetic-based clinical screening efforts. Enthusiasm must be tempered, however, by the extraordinary cost that often accompanies relatively modest gains. Finally, although genetic-based therapy often receives the greatest attention, molecular genetics, will likely have the greatest cost-effective impact in primary prevention and early diagnosis.

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