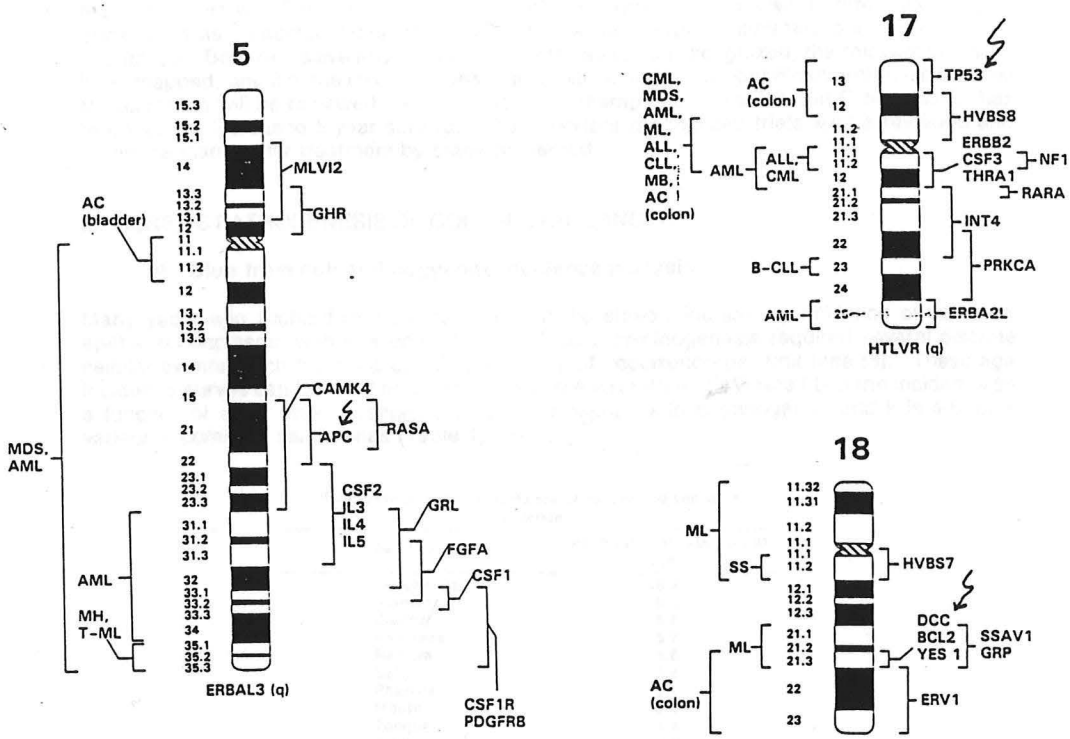
COLORECTAL CARCINOMA
PATHOGENESIS AND THERAPY



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I. INTRODUCTION

Carcinoma of the large bowel and rectum is a formidable public health problem in Western countries. Over 150,000 Americans will develop colorectal cancer this year, and about half of these patients will ultimately die of this disease. Mortality is remarkably stage-dependent; 5-year survivals for Dukes' stages A, B and C disease were 82%, 73% and 40% in a large series of U.S. patients (1). Thus, screening for early lesions is critical to lowering mortality.

Since John Dietschy last reviewed this topic at Grand Rounds (2), significant progress has been made in 2 areas. First, the pathogenesis of the these malignancies is now more clearly understood as an accumulation of many genetic lesions leading ultimately to a fully malignant phenotype. Both recessive and dominant effects have been recognized, the relevant loci have been mapped, and 3 of the involved genes have been cloned. The significance of these findings for screening will be reviewed. Second, adjuvant therapy for Dukes' B and C neoplasms has reproducibly increased 5-year survival. The important randomized trials will be reviewed and recommendations for treatment by stage presented.

II. GENETIC PATHOGENESIS OF COLORECTAL CANCER

A. Clue from epidemiology- age incidence analysis.

Many years ago Richard Doll pointed out that the steeply increasing incidence of common epithelial neoplasms with age could be explained if carcinogenesis required several discrete cellular events, each having a certain probability of occurrence per unit time (3). These age incidence curves can be fitted to an equation of the form $I(t) = at^k$, where I(t) is the incidence as a function of age t (more precisely, duration of exposure to carcinogens), and k is 4-6 for a variety of common carcinomas (Table 1).

Relationship between incidence of cancer and age in 11 populations

Site of cancer	Mean value of exponent of age[a]
Esophagus	6.2
Stomach	6.0
Bladder	5.8
Pancreas	5.7
Rectum	5.6
Colon	5.4
Pharynx	5.1
Mouth	5.0
Tongue	4.4
Kidney	4.4
Skin	4.3
Lip	4.0

[a] Exponent (k) in equation $I_t \propto t^k$, where I_t is incidence at age t.

Examples of these steeply rising curves for colon cancer are shown in Dr. Dietschy's Grand Rounds (2). To account for these curves, Doll suggested a model in which the number of cellular changes required to produce a malignant neoplasm was one more than the exponent (k) of the duration of exposure. This idea fits nicely with a host of experimental and other clinical data in many systems which supports the concept of multistage carcinogenesis (4). An equally impressive body of information points to the genetic nature of these cellular changes (5). In summary, the epidemiologic evidence suggests that colorectal carcinogenesis requires 6-7 discrete cellular events.

B. Clue from the clinic- adenomas are premalignant lesions.

Much clinical and histopathological observation clearly documents the premalignant nature of colorectal adenomas (6). In particular, patients with adenomas (tubular or villous) are at increased risk of development of carcinomas either within or outside of the adenomas. Large (> 2cm) adenomas often develop histologic dysplasia and contain areas of invasive carcinoma. Thus, some of the earlier cellular steps in the development of colorectal carcinoma are likely to contribute to the development of adenomas.

C. Clue from clinical genetics- familial colorectal cancer.

1. Introduction

About 5% of all colorectal neoplams affect patients whose family history suggests inherited predisposition. The syndromes can be divided into familial adenomatous polyposis (FAP) and hereditary nonpolyposis colon cancer (HNPCC). Affected kindreds provide the opportunity to map the involved genes by the techniques of linkage analysis.

2. Familial adenomatous polyposis (FAP), also called adenomatous polyposis coli

These familial syndromes, carefully reviewed in Dr. Dietschy's Grand Rounds, illustrate an autosomal dominant genetic predisposition to innumerable intestinal polyps followed by colorectal cancer. FAP is a generic term that includes the syndromes familial polyposis coli (FPC) and Gardner's syndrome. Bodmer's (7) and Leppert's (8) groups studied a total of 9 informative FAP kindreds. Each group reported consistent linkage of the FAP locus to markers on chromosome 5 band q21-22. The locus for FPC and Gardner's syndrome appears to be identical; in other words, these conditions derive from different alleles at the same locus. Further confirmation of the significance of this region for FAP syndromes came from cytogenetic observations of a patient with Gardner's syndrome, whose blood cells contained an interstitial deletion of 5q (9). At the present time, therefore, both FPC and Gardner's syndrome are thought to originate in allelic mutations mapping to 5q21-22.

3. Hereditary nonpolyposis colon cancer (HNPCC)

These syndromes are defined by the occurrence of colorectal cancer in family members in a pattern compatible with monogenic, generally autosomal dominant inheritance, without any evidence of diffuse polyposis (10,11). These kindreds outnumber those afflicted with familial polyposis (12). Clinical features of these patients are summarized in Table 2.

Table 2. Clinical Features of HNPCC

1. Absence of diffuse polyposis
2. Cancer diagnosis at younger age (< 50 yr)
3. Multiple synchronous or metachronous cancers
4. More right-sided cancers than in sporadic type
5. Other cancers (endometrium > ovary, breast, stomach) (Lynch syndrome II or cancer family syndrome)

These features help distinguish chance associations from monogenic heritable disease (13). In taking family histories it is worth remembering that the older individuals are the most informative for expression of the trait, even though the average age of diagnosis of colorectal cancer in affected family members is 20 years under that of patients with sporadic disease (45 vs 65 yr).

Analysis of 7 kindreds with the cancer family syndrome (Lynch syndrome II) strongly supported linkage of cancer predisposition with the Kidd blood group gene on the long arm of chromosome 18 (18q) (14,15).

D. Clues from cytogenetic analysis of colorectal neoplasms.

Cytogeneic analysis of colorectal carcinomas has revealed 2 major patterns of abnormality (16-19). The first is nonrandom loss of certain chromosomes or their fragments, followed by duplication of many of the remaining chromosomes. The structures lost most commonly are 18 and 17p. Other frequently lost chromosomes or fragments are 1p, 4, 14 and 5q. The second type of abnormality is nonrandom gain of chromosomes (trisomy), including 7, 12, X, 5 and 8 in decreasing order of frequency. None of these changes is found in every tumor. The data are consistent with a role in pathogenesis for decreased function of some genes and increased function of other genes.

Frequent loss of 18 and 17p suggest the presence of tumor suppressor genes on these chromosomes.

E. Allele loss in colorectal neoplasms

1. Introduction

Search for loss of potential suppressor genes is now done by Southern hybridization technology, which resolves much smaller deletions than does cytogenetic analysis. The methodology relies on the presence of numerous restriction fragment length polymorphisms (RFLPs) in the human genome (Figure 1).

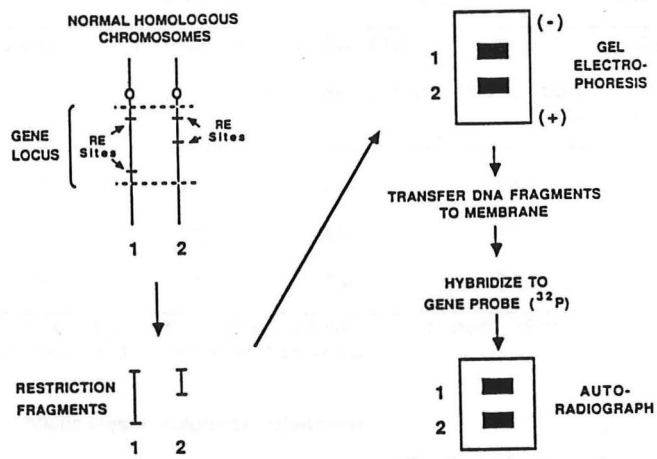

Figure 1 DNA restriction fragments as markers of homologous chromosomes (see text for full explanation). *Upper left*, A pair of homologous chromosomes "1" and "2" in normal tissue, containing a gene locus within which each homolog has two cutting sites for a specific restriction enzyme ("RE sites" denoted by *small arrows*). *Lower left*, When normal tissue DNA is cut by this restriction enzyme, unique restriction fragments "1" and "2" (released from homologs "1" and "2") are present among thousands of others from elsewhere in the genome. *Upper right*, All DNA restriction fragments present are size-separated using agarose gel electrophoresis. *Right center*, All restriction fragments are transferred to a nylon membrane and subsequently hybridized with a ^{32}P-labeled cDNA probe specific for the gene region denoted (*upper left*). *Lower right*, Fragments "1" and "2," which mark the pair of homologs of interest ("1" and "2"), are located by exposing x-ray film to the rinsed membrane and viewing the resulting autoradiograph.

For any given tumor to be informative, the patient must be constitutionally heterozygous for a RFLP within a surrogate marker sequence closely linked to the putative tumor suppressor locus. An especially useful form of RFLP is the variable number tandem repeat (VNTR) (20). As more RFLPs are recognized in the human genome, an ever-increasing fraction of tumors yield valuable data from which a map of the suppressor locus can be constructed. Such maps provide important landmarks in the process of cloning the actual suppressor gene.

The reported frequencies of allelic loss are subject to 2 sources of error. First, since the technique searches for sequences in normal tissue which have been lost in tumors, the tumor tissue must be rigorously microdissected free of normal tissue prior to DNA extraction. Substantial contamination with normal cells will result in an underestimate of the fraction of tumors showing allelic loss. Cryostat sectioning methods for enrichment of tumor cells have been described. Second, if the lesion common to a group of tumors is deletion of a specific suppressor gene, the results will depend on how close the surrogate marker probe is to this gene. Some deletions will be large, even visible cytogenetically, so that surrogate probes within a few million bases of the critical suppressor gene will pick up the loss. Other deletions, however, may only involve portions of the suppressor gene itself and will not be detected until probes for this gene are available.

Allelic loss from chromosomes 5q, 17p and 18q in colorectal neoplasia is summarized in Table 3.

Table 3. Allelic loss in colorectal neoplasia

	Adenoma			Carcinoma	
	Class I	Class II	Class III	FAP*	Sporadic
	(% of tumors with deletion)				
5q	0	29	29	24	36
18q	13	11	47	40	73
17p	6	6	24	31	75

From (21) and (22). * FAP carcinomas were not microdissected; thus figures could be underestimates.

2. Allelic loss in colorectal adenomas

Since adenomas are precursors of carcinomas, characterization of allelic loss in adenomas is crucial to an understanding of the sequence and significance of these genetic lesions in colorectal carcinogenesis. Vogelstein studied 3 classes of adenomas (21). Class I adenomas were derived from patients with FAP. These lesions were small with low grade dysplasia. Class II adenomas came from patients without FAP. The size and degree of dysplasia in these lesions was greater than in Class I adenomas, but no areas of carcinoma were seen. Class III adenomas were from patients without FAP and contained areas of invasive carcinoma. In the analysis of Class III lesions, areas of carcinoma were microdissected away from the adenomatous tissue prior to DNA extraction.

a. FAP (Class I) adenomas

Unlike their sporadic counterparts, none of 34 low-grade FAP adenomas deleted 5q alleles (21). Recall that the FAP trait maps to 5q21-22. Therefore, the scale of most inherited genetic lesions in FAP must be small. The frequency of 17p and 18q deletion in low-grade FAP (Class I) adenomas was low, about equal to that in Class II sporadic adenomas (Table 3).

b. Sporadic (Class II) adenomas

When adequately purified from normal surrounding tissue, 29% of sporadic Class II adenomas were found to have lost 5q alleles. A smaller fraction of sporadic adenomas deleted 17p or 18q alleles (6 and 11% of polyps, respectively) (21) (Table 3).

c. Sporadic (Class III) adenomas

There was no difference between Class II and III adenomas in frequency of 5q deletion, but many more of the higher-grade Class III lesions deleted 17p and especially 18q alleles (Table 3).

3. Allelic loss in colorectal carcinomas.

a. Colorectal carcinomas in FAP patients

These cancers have been difficult to obtain because of prophylactic screening and colectomy programs. However, recently Sasaki and collaborators reported data from a large series of FAP adenomas and carcinomas in Japan (22). The results confirmed Vogelstein's observation (21) that 5q allelic loss is rare in FAP adenomas. However, 5q deletions were found in about the same fraction of FAP carcinomas as in sporadic carcinomas (Table 3). Moreover, in one informative FAP carcinoma, the allele lost at this stage was from the normal parent (22,cited in (23)). This sequence is predicted by the Knudson 2-hit theory of carcinogenesis (24).

b. Sporadic colorectal carcinomas.

Several laboratories have confirmed frequent loss of alleles on chromosome regions 5q15-22, 17p13 and 18q21-qter in sporadic colorectal carcinomas (Table 3) (18,19,21,25). However, the totality of allelic loss in these tumors appears to be much greater.

Vogelstein's group has conducted an exhaustive analysis of allelic loss from all 39 nonacrocentric autosomal arms in 56 primary colorectal carcinomas (26). Vogelstein calls these studies "allelotypes", in analogy to karyotypes. The results are striking for the extent and complexity of the deletions observed (Figure 2). Losses of alleles on 17p and 18q were the most common changes, noted in > 75%of tumors. Deletions of loci on 9 other nonacrocentric arms (1q, 4p, 5q, 6p, 6q, 8p, 9q, 18p and 22q) were seen in 25-50% of the tumors. From 7 to 25% of tumors had deleted alleles on the remaining 28 arms. In 65% of informative cases, only one of 2 arms was involved, indicating that the majority of losses were subchromosomal.

In individual tumors, the median fractional allelic loss (number of arms with loss/number of informative arms = FAL) was 0.2. In 12 tumors, more than one third of evaluable arms suffered deletions. The degree of genetic deletion was prognostically significant. The risk of tumor recurrence and death with colorectal cancer was over twice as high in the group of patients with high FAL tumors as in those with low FAL tumors (Table 4).

Although multiple allelic losses are extremely common in colorectal cancer, loci on chromosomes 17p and 18q are deleted much more commonly than others. These regions are candidates for further mapping and cloning in the search for suppressor genes.

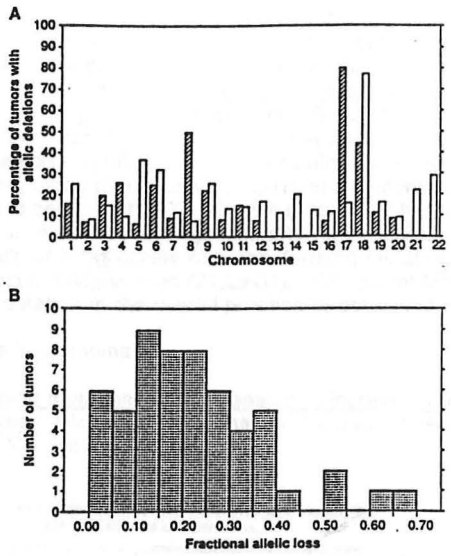

Fig. 2 (A) Frequency of allelic deletions in individual chromosomal arms. Allelic deletions were evaluated with RFLP analyses, examples of which are in Fig. 2. DNA from paired normal colonic mucosa and tumor tissues was cleaved with one of three enzymes (Taq I, Msp I, or Hind III), and evaluated with probes from each nonacrocentric autosomal arm. The probes used are listed (*23*), together with references describing their derivation and polymorphism patterns. Only informative tumors, that is, those in which DNA from the normal tissue exhibited a heterozygous pattern for one or more allelic markers from the indicated chromosomal arm, were used to determine allelic loss frequencies. The number of tumors informative for each chromosomal arm is listed in (*23*). An allelic loss was scored if an RFLP fragment present in normal DNA was lost in at least 80% of the neoplastic cells, as assessed by comparison of the autoradiographs with histologic evaluation of the cryostat sections from which the tumor DNA was purified. Open bars, q arm; hatched bars, p arm. (B) Frequency of allelic deletions in individual tumors. The FAL in each tumor was defined as the number of chromosomal arms on which allelic loss was observed divided by the number of chromosomal arms for which allelic markers were informative. The chromosomal arms on which the allelic deletions occurred in each tumor are listed in Table 1.

Table 4 Relation of FAL to clinical and histologic features. In comparing the means for group I with those of group II, age, follow-up period, tumor size, and Dukes' classification were nonsignificant on the basis of the *t* test. For the comparisons of percentages, *RAS* was nonsignificant and tumor recurrence and death were significant on the basis of the Fisher exact test ($P < 0.01$).

Group*	FAL (mean)	Number of patients†	Age (years)	Follow-up period‡ (months)	Tumor size (cm)	Dukes' class.§	*RAS* mutation‖ (%)	Tumor recurrence¶ (%)	Death# (%)
I	0.11	27	67	38	5.3	2.3	52	30	26
II	0.32	25	67	38	5.6	2.4	52	68	64

*Group I patients had tumors with an FAL less than the median value (0.2) of the 56 tumors listed in Table 1; group II patients had tumors with an FAL greater than 0.2. †All patients from Table 1 with a single carcinoma were included. ‡Mean follow-up period in patients who survive is listed. The mean follow-up period in all patients combined (that is, those who are still alive plus those who died) was 31 and 17.5 months for group I and II patients, respectively. §Dukes' classification scored as 1.0 for Dukes' A tumors (confined to muscularis propria), 2.0 for Dukes' B tumors (extension through muscularis propria), and 3.0 for Dukes' C tumors (metastatic to regional lymph nodes). ‖*RAS* gene mutations in this group of tumors were reported in (*8*) and (*13*). ¶Distant metastases developed in all except one patient who developed tumor recurrence. #Death with or from carcinoma. An additional 6 and 12% of group I and II patients, respectively, died without definite evidence of recurrent carcinoma.

F. Ras gene mutations

1. Introduction

The genetic lesions described above involve deletion of normal loci, some of which may encode tumor suppressor genes. Another type of abnormality noted in a large fraction of colorectal neoplasms is mutation in a gene called k-ras. In this case, the mutation seems to activate a growth-promoting function rather than delete a growth-restraining function as do the allelic losses already discussed. The K-ras gene was first identified in an acutely transforming retrovirus called Kirsten murine sarcoma virus. The normal gene product is a protein which is located at the inner face of the cytoplasmic membrane, binds and hydrolyzes GTP, and is involved in transduction of various external signals affecting cell growth (27). Certain mutants of K-ras which have lost GTP hydrolase activity can transform cells to a neoplastic phenotype (28). Therefore, much interest has centered on the finding of K-ras alleles with transforming mutations in a significant fraction of colorectal and pancreatic malignancies (21,29-31). Of special interest for today's presentation is the role of K-ras mutation in the overall process of colorectal carcinogenesis.

2. K-ras mutations in adenomas

The likelihood of finding transforming mutations in K-ras seems to increase with the size of and degree of dysplasia in the adenoma. Nine % of adenomas < 1 cm and 58% of larger lesions contained such mutations ($P < 0.0001$) (21) (Table 5).

Table 5 Relation between *Ras*-Gene Mutation and Histopathological Features of Adenomas.

Feature and Subtype	Class I	Class II	Class III	Total
	percent of tumors with mutation (no. analyzed)			
Morphologic category				
Tubular	0 (22)	0 (3)	100 (1)	4 (26)
Tubulovillous	29 (17)	55 (11)	53 (15)	44 (43)
Villous	0 (1)	40 (5)	60 (5)	45 (11)
Dysplasia				
Low grade only	0 (24)	25 (4)	100 (1)	7 (29)
Focal high grade	30 (10)	17 (6)	75 (4)	35 (20)
Extensive high grade	33 (6)	67 (9)	50 (16)	52 (31)
Size				
<1 cm	8 (36)	12 (8)	0 (0)	9 (44)
1–2 cm	33 (3)	20 (5)	0 (1)	22 (9)
>2 cm	100 (1)	100 (6)	60 (20)	70 (27)
Total	12 (40)	42 (19)	57 (21)	

Mutations were infrequently found in small FAP adenomas showing low-grade dysplasia. When several adenomas from one patient were studied, each adenoma contained a singular composite ras genotype, implying a separate clonal origin for each lesion.

3. K-ras mutations in carcinomas

The likelihood of finding transforming mutations in K-ras in colorectal carcinomas is the same as in large and/or dysplastic adenomas, namely about 40-60% (21,32) (Table 5). Burmer and Loeb separated diploid and aneuploid cells from histologically purified carcinoma tissue (32). The frequency of K-ras mutations in diploid (9/13 = 69%) and aneuploid (17/27 = 63%) cell populations was not significantly different, again suggesting that ras mutations can be rather early events in carcinogenesis. Interestingly, Burmer and Loeb found K-ras mutations in histologically normal mucosa surrounding 2 carcinomas. The frequency of this finding around a large series of cancers needs to be determined.

G. Summary of the genetic pathogenesis of colorectal cancer.

Doll's inference that multiple cellular steps underlie the production of malignancy has been amply confirmed in colorectal cancer. The fundamental changes are genetic in nature and are probably largely mediated by carcinogenic substances in the diet. The number of changes is approximately 8. Genetic predisposition is decisive in pathogenesis of about 5% of all of these malignancies (12). In these families, the "first event" is inherited; subsequent events represent somatically acquired gene alterations. The inherited change may increase the probability of developing subsequent abnormalities.

Studies of linkage, cytogenetics, allele loss and K-ras mutation in normal, adenomatous and carcinomatous tissues have defined 4 loci of importance in pathogenesis. The frequency of these abnormalities as a function of tumor progression has been determined (21) (Figure 3).

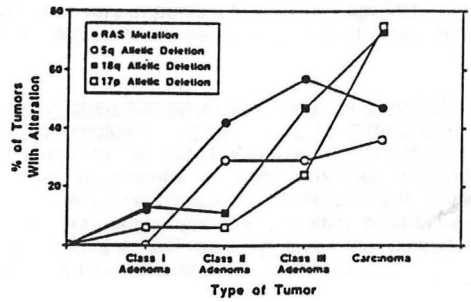

Figure 3 Genetic Alterations during Colorectal-Tumor Progression.
The percentage of tumors with the indicated genetic alteration is plotted for each type of tumor.

These findings allow us to state whether a given change is important early or late in the carcinogenic sequence. As in all aspects of tumor biology, heterogeneity is the rule; and no one change or sequence of events is found in all cancers. The true significance of any particular genetic abnormality for colorectal carcinogenesis cannot be known until the relevant gene has been cloned.

In the vast majority of FAP and Gardner's syndrome patients, an inherited subcytogenetic defect at 5q appears responsible for the syndrome. Since the gene has not been cloned, we do not know the nature of the defect. In keeping with other inherited defects, point mutations, deletions and perhaps insertions will probably be found. Loss of the second 5q allele, commonly observed in FAP carcinomas, is likely to be significant in tumor progression because it is always the normal allele that is lost. This fact, plus the dominant inheritance of polyposis, suggests that one abnormal allele is sufficient to produce polyposis. This sequence fits with Knudsen's 2-hit hypothesis of carcinogenesis (24), with the twist that a single (inherited) hit is sufficient to yield a premalignant marker lesion. Clearly, acquired loss of alleles on 5q can lead to sporadic adenomas. Thus, fap locus damage of both germ-line and somatic nature may function early and late in the pathogenesis of cancer.

In sporadic colorectal cancer, loss of alleles on chromosomes 17p and 18q correlates with larger polyps, advancing dysplasia and foci of carcinoma during the later stages of progression. Therefore, these deletions may contribute to such features as dedifferentiation, invasiveness and metastasis. Chromosome 17p alleles are probably lost after 18q deletion. One form of hereditary colorectal cancer, the cancer family syndrome or Lynch syndrome II, has been linked

to the Kidd marker on 18q. Perhaps inherited 18q lesions (point mutations?) may predispose to colorectal as well as to endometrial, breast, ovarian and gastric adenocarcinomas. As with fap loci, deletion of the normal allele could then contribute to malignant progression.

The awesome degree of genetic deletion revealed in Vogelstein's carcinoma allelotypes prompts caution in interpreting studies of allelic loss. Can all of these deletions be contributing significantly to the malignant phenotype of the tumor? It seems more likely that some of the alleleic losses result incidentally from the genetic instability of the tumor rather than from a process of selection for increased malignancy. Therefore, we need criteria for judging the significance of these large-scale deletions. The frequency of the loss is one obvious standard. Another test is whether a large group of apparently similar deletions defines a locus small enough to suggest that a single gene is involved. As more and more RFLPs become available, this test will be easier to apply. Finally, study of kindreds segregating alleles for hereditary nonpolyposis colon cancer could provide valuable clues to the significance of loci deleted in sporadic colorectal cancers. Judging the relevance of particular alleles which are lost to tumor progression is important in setting priorities for the difficult task of cloning the key gene. Once the gene is cloned, another very important test can be applied. If a gene functions as a tumor suppressor, both of its alleles should be damaged or deleted in a significant fraction of late-stage tumors.

Activating K-ras mutations often appear at the adenoma stage of tumor progression, and may even be found in morphologically normal mucosa immediately adjacent to a carcinoma. Therefore these mutations may facilitate the clonal dominance of subsets of initiated mucosal cells in a field where such dominance is normally not allowed (33). Since many aggressive carcinomas do not contain these mutations, other changes must be able to substitute for whatever function these K-ras alleles provide. Another possibility is that these mutations are functionally insignificant. This seems unlikely, given the powerful transforming capability of mutated ras genes in experimental systems.

Based on epidemiologic surveys, Cannon-Albright and co-workers suggested a model wherein the majority of individuals with apparent sporadic colorectal cancer have inherited a low-penetrance dominant gene for susceptibility whose frequency in the population is 19% (34). It seems more likely that multiple susceptibility genes may be involved. If this is true, genetic screening followed by primary dietary prevention may become an effective strategy for dramatically reducing the incidence of colorectal cancer. However, first the relevant genes must be cloned.

H. Progress in identification of colorectal cancer genes

1. Closing in on the fap gene

To date, this gene has eluded the cloners. However, Tops and collaborators have succeeded in mapping the gene between 2 closely linked polymorphic DNA markers on 5q (35). This finding refines the map published by White's laboratory (36) and has important implications for genetic screening (Section III). With luck, this gene should be cloned in the near future.

2. The DCC gene- a member of the integrin family

In a dazzling technical feat, Vogelstein's group has succeeded in cloning most of the DCC gene on 18q21-q22 that is deleted in colon cancer (37). Difficulties were encountered in isolating the gene because of its large size (2-3 megabase), complex exon structure and extremely low level of expression in most tissues. In fact, expression in most tissues could be demonstrated only by in vitro amplification of transcripts by the polymerase chain reaction (PCR). Northern blots of human brain mRNA revealed a transcript of 10-12 kb. Evidence that the gene is a human colorectal carcinoma suppressor gene is summarized in Table 6.

Table 6. DCC is probably a colorectal cancer suppressor gene

1. One allele was deleted in 29/41 (71%) of colorectal cancers tested.
2. The gene was expressed (at low levels) in almost all normal tissues, including normal colorectal mucosa.
3. Expression was very low or absent in 15/17 (88%) colorectal cancer cell lines examined.
4. Somatic mutations were detectable by Southern analysis in 12/94 (13%) of colorectal cancers.
5. A homozygous deletion was found in one tumor, one allele being destroyed by deletion of its 5' half.
6. Point mutation creating an abnormal 3' splice acceptor site was found in another tumor.
7. In 10 tumors, insertions of 120-300 bp within a hotspot for recombination were found.

Although the 3' end of the coding region of the cDNA has not been fully characterized, the available sequence predicts a protein whose recognizable features provide clues to the function of the DCC gene. Four regions in tandem, each about 100 amino acids long, share extensive homology to each other and to the C2 class of domains found in members of the immunoglobulin super-family. Immediately downstream of these immunoglobuling-like domains is a 195-amino acid region with significant homology to fibronectin type III-related domains. Both of these domain homologies are characteristic of a family of cell surface receptors known as the integrins. The homology of DCC to an integrin known as the neural cell adhesion molecule (NCAM) is 42% in the immunoglobulin-like C2 domains and 31% in the fibronectin type III-related domains. In addition, a number of conservative amino acid substitutions within these domains are evident in comparisons of DCC and NCAM.

The relationship of DCC to the integrins is particularly intriguing because these cell membrane receptors interact with extracellular matrix proteins and with other cell surface molecules called counter receptors (38). Integrins bind to specific sequences on these molecules such as the RGD sequence in fibronectin. The integrins participate both in extracellular matrix assembly and cell-cell adhesion. Interaction with ligand can result in changes in cytoskeletal organization, cell motility and even state of differentiation. The integrins are thought to regulate cell trafficking. With regard to tumors, many experiments have revealed altered patterns of integrin expression on transformed cells. Specific inhibition of integrin-matrix binding has been shown to block invasive and metastatic behaviors of mouse melanoma cells (39,40). A current model relates these malignant properties to abnormalities in cell trafficking mediated in part by abnormal expression and regulation of specific integrins. In this context, the relationship of DCC to other integrin family members, its probable tumor suppressor function and its deletion late in the pathogenesis of colorectal cancer suggest a role in restraining epithelial cell invasiveness. Testing this and other hypotheses will follow complete cloning and expression of the gene. For example, the cloned gene can be transfected into colorectal cancer cells whose behavior in xenograft models can then be tested.

3. The p53 gene- a tumor suppressor in multiple cell types.

Deletion of alleles on 17p, found in >75%of colon carcinomas, is also commonly observed in other prevalent human malignancies. Therefore, identification of the gene or genes involved has become a priority goal. To address this problem, Vogelstein and co-workers used a panel of 20 DNA markers covering known regions from 17p11.2 to 17p13.3 to map a common region of allele loss in 8 informative colorectal tumors (41) (Figure 4).

The common region was large, extending over approximately 3 cytogenetically visible bands. However, an extremely interesting gene, p53, was known from previous work to map to this region. The product of this gene was discovered 10 years earlier as a protein which bound to the SV40 tumor virus large T antigen. Large T antigen is the viral protein responsible for transforming infected cells to a neoplastic phenotype. Because it was expressed at elevated levels in a variety of tumor cells, p53 was initially thought to be a dominantly acting oncogene. However, recent in vitro observations showed that only mutant forms of p53 function in a dominant fashion. Suspicion began to mount that wild-type p53 might function as a tumor suppressor after the discovery that another tumor suppressor, the Rb protein, also bound SV40 large T antigen.

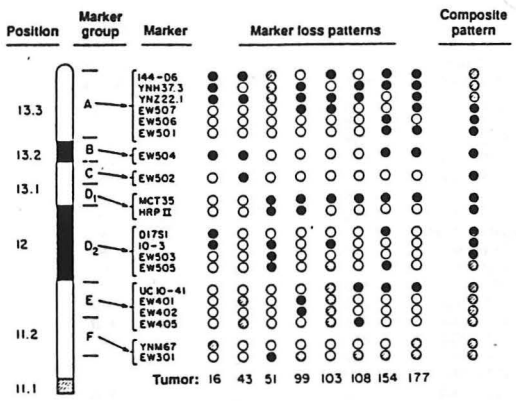

Fig. 4 Map of the common region of 17p deletion in colorectal tumors. Chromosomal positions of 20 markers from chromosome 17p are indicated. The markers were previously localized (5) to seven subchromosomal regions (A to F). Hybridization results for eight tumors are shown on the right, with patient identification numbers indicated at the bottom. For each of the 20 markers, a filled circle indicates that one parental allele was lost in the tumor; a cross-hatched circle indicates that both parental alleles were retained in the tumor; an open circle indicates that the marker was not informative (the patient's normal tissue was not heterozygous for the marker). The composite pattern (far right) assumes that there was only one target gene on chromosome 17p, so that markers for which heterozygosity was retained in any of the eight tumors would be outside the target gene locus. The region between probes YNZ22.1 and EW505 was deleted in every tumor in which markers in this region were informative.

Reasoning that if p53 were a tumor suppressor gene both alleles should be defective in advanced colorectal tumors, Vogelstein and collaborators systematically analyzed the remaining gene in a large number of cancers which had deleted one p53 locus. None of 82 tumors showed any rearrangements of p53 by Southern hybridization analysis, and none of 22 showed any mRNA abnormalities by Northern blot analysis. However, 11 of 12 revealed point mutations in one p53 gene (41,42). Ten of 12 of these tumors had deleted one p53 allele. Most importantly, these mutations clustered in highly conserved, presumably functionally vital regions of the gene. Moreover, some of these mutations were in regions involved in mutant mouse p53 alleles which are oncogenic. Since point mutations are thought to be rare events on a per nucleotide basis even in tumors, the finding of mutations in one allele and deletion of the other allele strongly support the hypothesis that p53 is a tumor suppressor gene in colorectal and a variety of other types of cells (42) (Table 7).

Clearly, deletion of a p53 gene is a late phenomenon in colorectal carcinogenesis, probably usually happening after deletion of a DCC allele (Figure 3). Current data do not indicate when the non-deleted allele is mutated. Theoretically, such mutation could be found in the germline of some patients in cancer-prone families, or could occur at any time during the life of the patient with sporadic colorectal carcinoma. If segregating as a germline mutation, a phenotype of multiple tumor types within families would be expected.

The interplay between wild-type and mutant p53 alleles is thought to account for "dominant-negative" oncogenic effects of point mutation in experimental systems. Thus, an abnormal molecule may dimerize with a normal molecule and reduce its function (43,44). As with the FAP and DCC genes, mutation of one p53 allele could contribute to an early stage of colorectal tumorigenesis, while deletion promotes a later effect.

TABLE 7 *p53* gene mutations in human tumours

Tumour	Tumour name	Tumour type*	Tumour cells tested†	Number of 17p alleles‡	Codon	Mutation Nucleotide	Amino acid
1	D263	Brain	B, X	1	175	CGC → CAC	Arg → His
2	D274	Brain	X	1	273	CGT → TGT	Arg → Cys
3	D303	Brain	B, X	1	216	GTG → ATG	Val → Met
4	D317	Brain	B, X	1	272	GTG → ATG	Val → Met
5	D247	Brain	C	1		None detected	
6	MDA 468	Breast	C	1	273	CGT → CAT	Arg → His
7	T47D	Breast	C	1	194	CTT → TTT	Leu → Phe
8	BT123	Breast	B	1		None detected	
9	1012	Lung	B	1	293	Deleted a G	Frameshift
10	5855	Lung	B	1		None detected	
11	H231	Lung	C	2	134	TTT → TTA	Phe → Leu
12	88-3/14	NFS	B, C	1	179	CAT → TAT	His → Tyr
13	C×4A	Colon	B, X	1	239	AAC → AGC	Asn → Ser
14	C×5A	Colon	X	1	248	CGG → TGG	Arg → Trp
15	C×6A	Colon	X	1	132	AAG → AAC	Lys → Asn
					133	ATG → TTG	Met → Leu
16	C×7A	Colon	B, X	2	281	GAC → GGC	Asp → Gly
17	C×19A	Colon	X	2		None detected	
18	C×20A	Colon	B, X	1	175	CGC → CAC	Arg → His
19	C×22A	Colon	X	1	175	CGC → CAC	Arg → His
20	C×26A	Colon	X	1	141	TGC → TAC	Cys → Tyr
21	SW480	Colon	C	1	273	CGT → CAT	Arg → His
					309	CCC → TCC	Pro → Ser
22	SW837	Colon	C	1	248	CGG → TGG	Arg → Trp

* The brain tumours were glioblastoma multiforme; the colon and breast tumours were adenocarcinomas, the NFS tumour was a neurofibrosarcoma developing in a patient with type-I neurofibromatosis; H231 was a small cell carcinoma of the lung, and the other two lung tumours were non-small-cell carcinomas.

† B, Tumour biopsy; C, cell line passaged *in vitro*; X, xenograft derived from biopsy, passaged in athymic nude mice. Whenever two sources of tumour cells are listed, both contained the indicated mutation.

‡ The number of alleles was determined by RFLP analysis, as described in the text.

III. IMPLICATIONS OF THE GENETIC FINDINGS FOR SCREENING

A. Introduction

Screening is designed to increase survival by detecting neoplasia at an early stage (Table 8). Dr. Dietschy reviewed current screening recommendations for patients in various categories of risk for colorectal neoplasms (2). In a Grand Rounds to be given next month, Dr. Kathy Zeller will review more general aspects of screening for colorectal cancer. I would like to speculate on the implications of the recent genetic discoveries for screening certain populations at risk for colorectal carcinoma.

Table 8. Survival in colon cancer by stage

Stage	5-Year Survival (%)	Reference
T1N0M0	97	(45)
T2N0M0	90	
T3N0M0	78	
T4N0M0	63	
T2N1M0	74	
T3N1M0	48	
T4N1M0	38	
Dukes' A	82	(1)
Dukes' B	73	
Dukes' C	40	
1-4 nodes	56	
>4 nodes	26	
Dukes' D	5	

B. Members of FAP kindreds

At the present time, all members of FAP kindreds need flexible sigmoidoscopy every 6 months beginning in the early teens and continuing until age 50. However, only half of these patients will develop polyposis/cancer. Genetic screening by RFLP analysis now should identify which patients carry the fap allele with > 95%confidence. This is probably sufficient insurance to avoid endoscopic screening in subjects who test negative. Genetic screening is now available only in research centers such as the University of Utah.

Genetic detection of FAP at an early age will become an essential tool in selecting patients for primary prevention trials (46,47).

C. Hereditary nonpolyposis colorectal cancer

First-degree relatives of patients with HNPCC features (Table 2) are at high risk for colorectal cancer. These family members require annual fecal occult blood testing and triennial colonoscopy beginning at age 20.

The genes involved in hereditary nonpolyposis colorectal cancer have not been identified. Information on the possible role of fap, DCC and p53 in these kindreds should be available within the next few years. Other candidate HNPCC genes could reside on chromosomes 1q, 4p, 6q, 8p, 9q, 18p or 22q which are commonly deleted in sporadic cancers (Figure 2). Linkage analysis with RFLPs specific for these loci could be done now on a research basis to aid in mapping the involved genes. As in FAP, genetic screening could then help focus surveillance and prevention efforts on affected patients.

D. Adenomas or carcinomas in first-degree relatives

Cannon-Albright et al studied the risk of adenomas in individuals having a first-degree relative with colorectal adenoma or having a cluster of relatives with colorectal carcinoma (34). Spouses of the individuals served as controls for environmental influences. FAP and HNPCC kindreds were excluded from the study. The incidence of adenoma was significantly higher in the population of relatives (19%) than in the spouse controls (12%, $P < 0.02$). The data fit a model of dominant inheritance of colorectal tumors with a gene frequency of 19%. Although this type

of study can never provide definitive evidence of inheritance, kindreds with apparent sporadic adenomas and carcinomas may be segregating low-virulence alleles predisposing to colorectal neoplasms. In one such kindred, White's group found linkage of neoplasia to chromosome 5q markers near the fap locus (cited in (23)).

Deciding which individuals of this type to screen with expensive genetic techniques will be difficult. Cannon-Albright and collaborators found that clusters of colorectal neoplasia in most kindreds were not associated with the clues described above, namely tendency to early age of onset or right-sided lesions (48). These authors concluded that presentation with ordinary distal colorectal cancer which develops during late middle age should not exclude the possibility of inherited predisposition if family history is significant.

IV. ADJUVANT THERAPY OF LOCOREGIONAL COLORECTAL CANCER

A. Introduction

If efforts at early detection of colorectal cancer fail and cancer is discovered at an advanced stage, what can be done to increase survival? For the past 35 years, efforts have focused on post-surgical adjuvant chemotherapy in patients with completely resected disease. Radiotherapy of patients with resected rectal cancer has also been tested. Carefully designed randomized trials, conducted in large cooperative groups, were begun in the late 1970's. These trials yielded a mixed harvest of negative and positive results (49). Six of these trials, 3 in colon and 3 in rectal cancer patients, demonstrated a modest but statistically significant benefit in favor of adjuvant treatment. Concomitantly, other trials in patients with metastatic disease have established a modest but significant enhancement in rates of response to newer "modulated" 5-fluorouracil (5-FU) therapy, when compared to 5-FU alone (50). Based on these results, third-generation adjuvant trials of national scope have been initiated to address which of several regimens will provide the best survival with the least toxicity in patients with completely resected Dukes' B2 and C cancer. Based on recent results, it is now assumed that inclusion of a control arm of no adjuvant treatment is unnecessary and perhaps unethical. What are the results upon which this optimism is based?

In reviewing the results of the studies, it is important to remember that survival is the only hard endpoint in adjuvant trials and that subset analysis can be misleading.

B. Studies in Dukes' B2 and C colon cancer

Results of the important recent trials are summarized in Table 9.

1. Negative studies

The Southwest Oncology Group (SWOG) (51), Veterans Administration Surgical Oncology Group (VASOG) (52), the Gastrointestinal Tumor Study Group (GITSG) (53) and the Cross Cancer Center (54) each studied the efficacy of post-surgical 5-FU and methyl-CCNU (semustine) combination in patients undergoing curative resection. Three of these studies also tested the effect of nonspecific immunotherapy. Each of these 4 studies contained a control (observation only) arm. None of these studies found a statistically significant increase in survival in the chemotherapy groups. With regard to methyl-CCNU, another study conducted by the Eastern Cooperative Oncology Group (ECOG) failed to demonstrate any advantage of the combination of 5-FU and methyl-CCNU over 5-FU alone (55). No control arm of observation alone was included in the ECOG study.

Table 9. Controlled adjuvant trials in Dukes' B2 and C colon cancer

Trial (Ref.)	Patients Evaluable	Treatment	Survival Advantage
SWOG (51)	279	Observation	-
		5-FU/methyl-CCNU	No
		5-FU/methyl-CCNU/BCG	No
VASOG (52)	645	Observation	-
		5-FU/methyl-CCNU	No
GITSG (53)	572	Observation	-
		MER	No
		5-FU/methyl-CCNU	No
		5-FU/methyl-CCNU/MER	No
CCC (54)	253	Observation	-
		BCG	No
		5-FU/methyl-CCNU/BCG	No
ECOG (55)	701	5-FU	-
		5-FU/methyl-CCNU	No
NSABP C-01 (56)	1116	Observation	-
		Methyl-CCNU/Oncovin/5-FU	Yes
		BCG	Yes
NSABP C-03	Closed	MOF	Too early
		5-FU/high-dose folinic acid	
NSABP C-04	Active	5-FU/high-dose folinic acid	Accruing patients
		5-FU/levamisole	
		5-FU/folinic acid/levamisole	
NCCTG (57)/ Intergroup 1 (58)	401/ 1247	Observation	-
		Levamisole	No
		Levamisole/5-FU	Yes (Stage C)
Intergroup 0089	Active	5-FU/low-dose folinic acid	Accruing patients
		5-FU/high-dose folinic acid	
		5-FU/levamisole	
		5-FU/low-dose folinic acid/levamisole	

Survival advantage means statistically significant benefit over indicated control group.

2. NCCTG and Intergroup levamisole ± 5-FU trials

In the North Central Cancer Treatment Group (NCCTG) trial, 401 patients were randomized to observation alone, levamisole or 5-FU plus levamisole (57). After a median followup of 7 years 9 months, analysis revealed a significant decrease in recurrence rate for the combination of 5-FU and levamisole compared to observation alone. This benefit translated into a significant overall survival benefit for Dukes' C patients only (53% for treated vs 45% for control, $P = 0.03$). Levamisole alone was associated with a marginally significant improvement in disease-free survival in Dukes' C patients ($P = 0.06$). The toxicity of these regimens was minimal. The mechanism of action of levamisole, an antihelminthic and immunostimulant, is unclear since it is nontoxic for colon carcinoma cells in vitro.

These results were confirmed in a larger Intergroup trial with 1247 evaluable patients, involving the NCCTG, ECOG and SWOG (58). This trial was modified after a median followup of 3 years, when the disease-free survival in the 5-FU/levamisole group significantly exceeded that of the observation control. At this point (September 1989), the monitoring committee discontinued the observation alone control arm and a new Intergroup 0089 study was designed (see below).

3. NSABP C-01 Trial

The National Surgical Adjuvant Breast and Bowel Project (NSABP) C-01 trial randomized patients to observation, BCG immunotherapy or chemotherapy with methyl-CCNU, Oncovin (vincristine) and 5-FU (MOF regimen) (56). A total of 1,116 patients were randomized. The differences between overall survival curves, although small, were statistically significant in favor of both BCG and chemotherapy over observation alone. The 5-year survival for patients in the chemotherapy, BCG and control groups was 67%, 67% and 59%, respectively.

This results of this trial have been viewed with skepticism because several similar trials showed no significant benefit for 5-FU/methyl-CCNU chemotherapy (Table 9). Moreover, chemotherapy and immunotherapy were equally superior to observation alone, and the value of nonspecific immunotherapy alone has been negligible (59). The benefits in any case were small and are likely to diminish further over time as the leukemogenic potential of methyl-CCNU is realized.

4. NSABP C-03 trial

Based on the results of the C-01 trial, NSABP investigators organized a successor adjuvant trial without a control arm of observation alone. This trial compares MOF to 5-FU/folinic acid chemotherapy. The rationale for this so-called "enhanced" or "modulated" 5-FU therapy came from a variety of studies in metastatic colorectal cancer, all of which showed improved antitumor activity when compared to 5-FU alone (50). Folinic acid is thought to increase the intracellular pool of reduced folate, which in turn enhances the binding of 5-FU to its target enzyme, thymidylate synthetase (60).

This trial was closed to further entry about a year ago. The analysis is eagerly awaited since efficacy of 5-FU/folinic acid which is equal or superior to that of MOF therapy would terminate study of the leukemogenic MOF regimen.

5. Currently active "high-priority" trials

a. NCI Intergroup Trial 0089

At the time that the surgery-alone control arm of the original Intergroup study was eliminated, 4 treatment arms were proposed: 5-FU/levamisole, 5-FU/low-dose folinic acid, 5-FU/high-dose folinic acid and 5-FU/levamisole/low-dose folinic acid. The trial asks which of these 4 programs, each thought to be more active than 5-FU alone against colorectal cancer, is best in the adjuvant setting. Our institution participates in this trial through membership in the SWOG "high priority" consortium.

b. NSABP C-04 trial

Interestingly, the NSABP investigators have dropped the methyl-CCNU containing MOF regimen and now randomize between 5-FU/high-dose folinic acid, 5-FU/levamisole and 5-FU/high-dose folinic acid/levamisole. This trial was designed without benefit of final results of the NSABP C-03 trial, as the statisticians must await accumulation of sufficient tumor recurrences for analysis.

C. Interpretation and current recommendations: Dukes' B2 and C colon cancer.

Small but reproducible benefits of post-surgical adjuvant therapy for Dukes' B and especially C colon cancer have been demonstrated in recent, well-designed large trials. However, a standard of therapy has not been established. Potentially leukemogenic regimens containing methyl-CCNU should be avoided. If possible, patients should be entered onto one of the current "high-priority" trials which, when complete, may establish a standard of practice for the next several years. For patients who cannot be entered onto these trials, 5-FU plus levamisole is a safe alternative (57,58). Levamisole can be obtained from the Cancer Therapy Evaluation Branch,

NCI. Another possible alternative is 5-FU plus low-dose (synonym: low-cost) folinic acid, which is reproducibly more active than 5-FU alone in metastatic disease (50). However, this regimen is only now being formally evaluated in the randomized adjuvant trials. If 5-FU/folinic acid is chosen, the risk of life-threatening diarrhea (50) must be recognized so that therapy can be stopped promptly and fluids replaced. With this precaution, the 5-FU/folinic acid regimens have been safe.

D. Studies in Dukes' B2 and C rectal cancer

1. Introduction

In contrast to colon cancer, a major issue in rectal cancer is local (pelvic) recurrence. Twenty-five % of stage B2 and 50% of C rectal cancers will recur locally, resulting in major morbidity. The reason for this problem is the difficulty in obtaining adequate radial margins in the pelvic soft tissues surrounding the infraperitoneal rectum. Therefore, adjuvant radiotherapy has been widely practiced in high-risk (Dukes B2 and C) rectal cancer. Several randomized trials attest to the ability of preoperative or postoperative radiotherapy to reduce local recurrence (61-66). However, the magnitude of the effect is small and the dose required rather high (≥4500 cGy). Consequently, toxicity can be substantial. Preoperative radiotherapy has also been effective in reducing pelvic recurrences. The best approach to improving local control of Dukes' B2 and C rectal cancer remains controversial.

What is clear is that deaths in rectal cancer are primarily related to metastatic disease and that adjuvant radiation therapy has no impact on overall survival (61,62,64-66). Therefore, in an attempt to improve both the quality and quantity of life, the complex issues of combined radiotherapy/chemotherapy (combined modality therapy) have been addressed in a number of randomized adjuvant trials. The results of these trials, summarized in Table 10, suggest beneficial effects but do not establish a preferred approach.

2. GITSG 7175 trial

This small trial (about 50 subjects per arm) randomized patients between observation alone, radiation therapy, 5-FU/methyl-CCNU and radiation plus 5-FU/methyl-CCNU (64,67). Disease-free and overall survival were sigificantly greater than control only in the group of patients treated with combined modalities. Fifty-nine% of patients who received combined modality therapy survived 5 years compared to 43% of the control group.

3. NCCTG 79-47-51 trial

About 90 subjects each were randomized to either radiation therapy alone or 5-FU/methyl-CCNU plus radiation therapy (68). Five-year survival in the combined modality group was 55% compared to 49% in the radiotherapy alone group, a significant difference. Notice that this trial did not address the role of radiation therapy in the combined modality approach; conceivably, chemotherapy alone would have had an equally beneficial effect.

4. NSABP R-01 trial

About 180 patients each were randomized to observation alone, radiation therapy alone or MOF chemotherapy alone (69). Disease-free and overall survival were significantly higher in the MOF than in the other control arms, but only in males under age 65. This peculiar outcome has cast doubt on the biologic significance of the results.

Table 10. Controlled adjuvant trials in Dukes' B2 and C rectal cancer

Trial (Ref.)	Patients Evaluable	Treatment	Survival Advantage
GITSG 7175 (64,67)	202	Observation	-
		Radiotherapy (RT)	No
		5-FU/methyl-CCNU	No
		RT/5-FU/methyl-CCNU	Yes
NCCTG 79-47-51 (68)	186	RT	-
		RT/5-FU/Methyl-CCNU	Yes
NSABP R-01 (69)	555	Observation	-
		RT	No
		Methyl-CCNU/Oncovin/5-FU	Yes *
Intergroup 86-47-51	455 (Active)#	5-FU/RT-bolus 5FU	Too early
		5-FU-meCCNU/RT-bolus 5-FU	
		5-FU/RT-infusion 5FU	
		5-FU-meCCNU/RT-infusion 5-FU	
NSABP R-02	Active	5-FU/high-dose folinic acid	Accruing patients
		RT/5-FU/high-dose folinic acid	
		MOF$	
		RT/MOF$	

Survival advantage means statistically significant benefit over indicated control group. * Benefit only in males <65 yr. # Study continuing without meCCNU-containing arms. $ MOF therapy only for males.

5. Currently active "high-priority" trials

Based on these results, two ongoing "high-priority" trials were initiated which reflect different interpretations of the role of radiotherapy in management.

a. Intergroup 86-47-51 trial

This trial assumes that adjuvant combined radiation and chemotherapy is standard management of high-risk rectal cancer and asks which of 4 combined modality regimens is superior. Based on the results of the GITSG 7175 trial, which suggested a role for 5-FU as a radiation sensitizer, the Intergroup trial randomizes patients between radiotherapy/ bolus 5-FU and radiotherapy/continuous infusion 5-FU. Radiotherapy is sandwiched between 2 courses of chemotherapy, either 5-FU alone or 5-FU/methyl-CCNU. Thus, patients are randomized to one of 4 treatment arms. The radiotherapy is technically demanding, and the continuous IV infusion 5-FU arm (lasting 5 weeks) is expensive and inconvenient. Moreover, half of the patients are to receive methyl-CCNU, a leukemogenic agent. For these reasons, we have not entered patients on this trial.

As of February 28 of this year, the target accrual of 455 patients was achieved. The study is still open, pending finalization of a successor trial. The 2 5-FU/methyl-CCNU arms have been dropped. Therefore, the trial now randomizes between 2 modes of administration of 5-FU as a radiation sensitizer. We plan to participate actively in this modified trial.

b. NSABP R-02 trial

Based on the results of the NSABP trial R-01, R-02 does not assume a standard role for radiotherapy in the management of high-risk rectal cancer; rather the effect of radiotherapy becomes a study question. Another question is whether MOF or 5-FU/folinic acid is superior adjuvant chemotherapy. As in the Intergroup study, patients are randomized to one of 4 treatment arms. Again, half of the patients will receive methyl-CCNU as a component of the MOF regimen.

E. Interpretation and current recommendations: Dukes' B2 and C rectal cancer.

There is no standard approach for post-surgical adjuvant therapy for Dukes' B2 and C rectal cancer. Although 3 controlled trials have shown an advantage for chemotherapy with or without radiotherapy, the benefits are small and the costs high. In spite of problematic aspects in their design, currently active high-priority trials should provide much-needed answers over the next few years. For example, the effect of methyl-CCNU will have to be very substantial, which is doubtful, to justify its use in standard therapy. Also, the role of radiotherapy in adjuvant programs is being re-evaluated in the NSABP R-02 trial. If chemotherapy alone provides equal or greater benefit, then radiotherapy will best be used to treat, rather than prevent, disease recurrence. This approach would save many dollars and spare much toxicity.

For now, should physicians choose not to enter patients onto one of the high-priority trials, treatment with high-dose pre- or postoperative radiotherapy, together with postoperative 5-FU/folinic acid chemotherapy, would be a reasonable approach for a patient with locoregionally advanced rectal cancer. Obviously, individual decisions will depend on the preferences of well-informed patients and factors such as age, co-morbid disease and finances.

V. SUMMARY OF TREATMENT RECOMMENDATIONS BY STAGE

A. Colon cancer

Dukes' A, B1	Resection, followup according to standard guidelines (70)
Dukes' B2, C	Resection, adjuvant chemotherapy*, followup
Dukes' D	Chemotherapy†

* High-priority trial or 5-FU/levamisole or 5-FU/low-dose folinic acid.
† Modulated 5-FU trial, such as methotrexate + 5-FU/folinic acid + interferon (E. P. Balaban in our Division has organized a trial of this type).

B. Rectal cancer

Dukes' A, B1	Resection, followup
Dukes' B2, C	Resection, adjuvant radiotherapy/chemotherapy* for selected patients, followup
Dukes' D	Chemotherapy†

* High-priority Intergroup 86-47-51 trial; or ~4500 cGy external beam radiotherapy plus 500-900 cGy boost to tumor bed, followed by 5-FU/low-dose folinic acid chemotherapy.
† Same as for colon cancer.

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