Special Article

Treatment and Prevention of Intraepithelial Neoplasia: An Important Target for Accelerated New Agent Development

Recommendations of the American Association for Cancer Research Task Force on the Treatment and Prevention of Intraepithelial Neoplasia[1]

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Abstract

Precancer or intraepithelial neoplasia (IEN) is a noninvasive lesion that has genetic abnormalities, loss of cellular control functions, and some phenotypic characteristics of invasive cancer and that predicts for a substantial likelihood of developing invasive cancer. The AACR Task Force on the Treatment and Prevention of IEN has delineated the relationship between IEN and cancer risk as well as the clinical benefit that can be derived from reducing IEN burden. Although several effective endoscopic and surgical treatments for IEN have become standard medical practice, these interventions can confer morbidity and do not treat the entire epithelial field at risk. The incidence of many epithelial cancers is continuing to rise, the number of individuals at risk is increasing with the aging population, and the rapid advancement of imaging and molecular diagnostics is bringing to light precancers that were heretofore clinically silent. There is therefore an urgent need to rapidly develop new treatment and prevention agents for IEN. The AACR IEN Task Force recommends focusing on established precancers as the target for new agent development because of the close association between dysplasia and invasive cancer and because a convincing reduction in IEN burden provides patient benefit by reducing cancer risk and/or by decreasing the need for invasive interventions. The IEN Task Force proposes several clinical trial designs that provide practical and feasible approaches to the rapid development of new agents to treat and prevent precancer.

Introduction

Carcinogenesis is a multiyear, multistep, multipath disease of progressive genetic and associated tissue damage (Fig. 1). Chemoprevention, the use of drugs or other agents to inhibit, delay, or reverse this process, is recognized as a very promising and important area in cancer research (1–5). Despite increasing research and development efforts (6), drug approvals for chemopreventive indications have been slow to emerge. The critical factor in this regard is defining and then demonstrating clinical benefit. Historically, reduced cancer incidence or mortality has been required to show chemopreventive efficacy. These endpoints make chemoprevention studies too long, large, and costly for most academic research centers and pharmaceutical manufacturers to undertake, thus limiting the number of drug candidates that can be developed. Continuing to rely on cancer incidence and mortality endpoints will lead to significant loss of opportunity to impact cancer. This Task Force, then, has focused on developing chemopreventive strategies that do not depend on the cancer endpoint *per se* but rather on treatment of the disease process of carcinogenesis as a measure of clinical benefit. Specifically, these strategies are prevention and regression of a significant precancerous lesion, IEN.[3]

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[3] The abbreviations used are: IEN, intraepithelial neoplasia; HPV, human papillomavirus; CIS, carcinoma *in situ*; DCIS, ductal CIS; LCIS, lobular CIS; PIN, prostatic intraepithelial neoplasia; HGPIN, high-grade

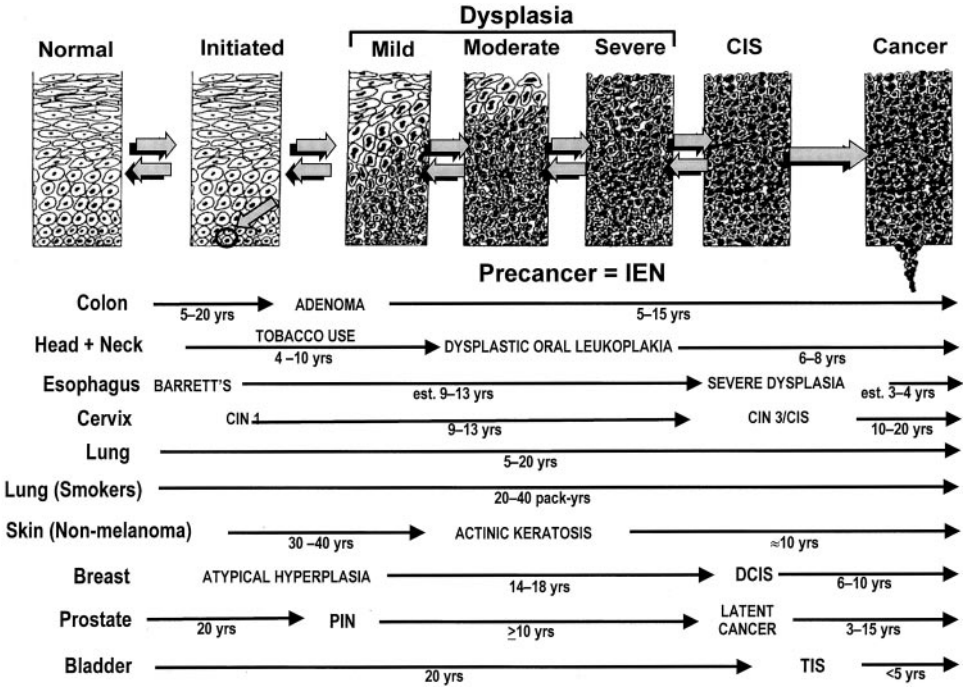

Fig. 1 Human carcinogenesis is a multiyear process. References for target organs are as follows: colon (10, 11), head and neck (90, 318–320), esophagus (28, 110, 321–323), cervix (324), lung (325–327), skin (200, 218, 234, 328, 329), breast (8, 9), prostate (14), and bladder (300).

Why IEN?

IEN Is a Near-Obligate Precursor to Cancer

IEN occurs in most epithelial tissue as moderate to severe dysplasia, is on the causal pathway leading from normal tissue to cancer, and is close in stage of progression to cancer (invasive neoplasia). Accumulating mutations (*i.e.*, genetic progression) and loss of cellular control functions are observed as the phenotype changes from normal histology to early dysplasia then to increasingly severe IEN, superficial cancers, and finally invasive disease (Table 1; Ref. 7). Although the progression of severe dysplasia to cancer may happen within months to a few years in situations where the process is relatively aggressive (*e.g.*, in the presence of a DNA repair-deficient genotype or viral transformant such as HPV), these changes generally appear to occur over a long time period. For example, in the breast it is estimated that progression from atypical hyperplasia through DCIS to adenocarcinoma requires 10–20 years or more (8, 9). Colorectal adenomas may form over a period as long as 5–20 years, and progression from adenoma to colorectal carcinoma usually requires another 5–15 years (10–13). PIN may develop over approximately 20 years. From PIN to early latent cancer may take 10 or more years, and clinically significant carcinoma may not occur until 3–15 years later (14).

Table 1 Why IEN?

- Near-obligate cancer precursor
- Risk marker for cancer
- Disease requiring surveillance and treatment interventions

Progression is mapped in target tissues by the appearance of specific molecular and more general genotypic damage associated with increasingly severe dysplastic histology. In many cases, early, critical steps include inactivation of tumor suppressor genes, such as *APC* in colon or *BRCA* in breast cancers, and activation of oncogenes, such as *ras* in colon, lung, and pancreas cancers. Carcinogenesis may take multiple paths and may be multifocal; not all cancers in a given tissue nor all cells in a given cancer will ultimately contain the same genetic lesions. Progression is influenced by factors specific to the host tissue's environment, such as the action of hormones and cytokines produced in stroma around the developing epithelial tumor and changes in tissue structure. Furthermore, carcinogenesis is not

PIN; CIN, cervical intraepithelial neoplasia; FAP, familial adenomatous polyposis coli; APC, adenomatous polyposis coli; FDA, Food and Drug Administration; CHD, coronary heart disease; CRC, colorectal cancer; ACF, aberrant crypt foci; NSAID, nonsteroidal anti-inflammatory drug; HNSCC, head and neck squamous cell carcinoma; HNPCC, hereditary nonpolyposis colorectal cancer; OPL, oral premalignant lesion; EGFR, epidermal growth factor receptor; RAR, retinoic acid receptor; 13-cRA, 13-*cis*-retinoic acid; CR, complete (histological) response; PR, partial (histological) response; PD, progressive disease; NR, no (histological) response to drug intervention; GERD, gastroesophageal reflux disease; PCNA, proliferating cell nuclear antigen; ODC, ornithine decarboxylase; LOH, loss of heterozygosity; SIL, squamous intraepithelial lesion; HGSIL, high-grade SIL; LGSIL, low-grade squamous intraepithelial lesion; HNPCC, hereditary nonpolyposis colorectal cancer; Pap, Papanicolaou; AAH, atypical adenomatous hyperplasia (lung); AK, actinic keratosis (or keratoses); BCC, basal cell carcinoma; SCC, squamous cell carcinoma; 5-FU, 5-fluorouracil; FNA, fine needle aspiration; PSA, prostate-specific antigen; GST, glutathione *S*-transferase; *GSTP1*, glutathione *S*-transferase B class gene; TGF, transforming growth factor; TCC, transitional cell carcinoma; TIS, transitional cell carcinoma *in situ*; BCG, *Bacille Calmette-Guérin*; *FHIT*, fragile histidine triad gene (tumor suppressor gene); LIFE, lung-imaging fluorescence endoscope; UVR, ultraviolet radiation.

solely driven by the order in which genetic changes appear; the resultant cellular and tissue disorganization may also be important. This disorganization is an obvious manifestation of carcinogenesis and reflects the evolution from normal tissue to invasive cancer. IEN provides a suitable target for treatment intervention because of its phenotypic and genotypic similarities and evolutionary proximity to invasive cancer.

IEN as Precancer Is a Risk Marker for Cancer

Subjects with IEN, particularly severe IEN, are at significantly higher risk than unaffected populations for developing invasive cancer in the same tissues. This risk in fact exceeds other measurable risk factors with the exception of germ-line mutations that occur in genetic syndromes. The invasive cancer risk associated with IEN can be illustrated by two notable examples, colon (adenomas) and prostate (PINs).

The very strong evidence characterizing the adenoma-carcinoma sequence and associating the presence of colorectal adenomas with subsequent invasive cancer development is described fully in another section of this report. As recounted therein, the correlation between extent of IEN progression and risk of cancer is established by multiple factors; the potential of colorectal adenomas to progress to cancer varies with histological growth pattern, size, and severity of dysplasia. For example, 2–5% of tubular, ~20% of tubulovillous, and 20–55% of villous adenomas progress to invasive disease. Risk of malignancy is negligible for adenomas <1 cm in diameter and increases with larger diameters. Of the 70% of adenomatous polyps that are mildly dysplastic, no more than 5% progress to cancers, whereas a much higher fraction (up to 55%) of the 10% of adenomas that are severely dysplastic become cancerous. In one study, one-third of severely dysplastic adenomas contained invasive carcinoma, and severe dysplasia is found most commonly in larger adenomas with villous histology (12, 13, 15).

PIN is a risk marker for prostate cancer, and the characteristics of PIN progression, have been described by Bostwick and Brawer (14, 16–19) and by Sakr *et al.* (20) and Foster *et al.* (21). This evidence, also cited in another section of this report, includes similar cellular morphology and atypia of HGPIN and prostatic adenocarcinoma (cellular atypia observed in HGPIN is virtually indistinguishable from invasive cancer, except that in HGPIN, no invasion has occurred). It also includes the spatial and temporal association of HGPIN to prostate cancer, with both being found primarily in the peripheral zone, and much more infrequently in the transition zone. As PIN progresses, the likelihood of damage to the basal cell layer and basement membrane increases. PIN and prostate cancer share other phenotypic characteristics. For example, certain cytoskeletal proteins, secreted proteins, and degree of glycosylation are shared by PIN and cancer but not by benign prostatic hyperplasia or normal prostate epithelium. Also, PIN is associated spatially and temporally with cancer. The most compelling data come from studies described by Bostwick and by Sakr (20), where patients with HGPIN and no detectable cancer progressed to a 40% incidence of cancer in 3 years and to ~80% incidence in 10 years.

IEN Is Precancer and in Its Own Right Is a Disease: Treatment Provides Clinical Benefit

Because IEN is a near-obligate precursor to invasive cancer, it is standard clinical practice to use invasive surgical interventions to reduce the burden of IEN, *e.g.*, oral leukoplakia, CIN 2/3, breast DCIS, and others. Reducing IEN burden, therefore, is an important and suitable goal for medical (noninvasive) intervention to reduce invasive cancer risk and to reduce surgical morbidity. Achieving the prevention and regression of IEN confers and constitutes benefit to subjects and, in the opinion of this Task Force, demonstrates effectiveness of a new treatment agent.

Established IEN places individuals at high risk for developing life-threatening invasive cancer. New agents are needed to effectively treat not only the clinically apparent IEN but also the entire epithelial sheet at risk, as well as to reduce the need for surgical extirpation of IEN and its associated morbidity. Several groups of high-risk individuals can be targeted for clinical trials to demonstrate the effectiveness of a new agent to treat IEN.

Prevention or Treatment of IEN in Subjects at High Risk Associated with Genetic Predisposition (*e.g.*, prevention of colorectal adenomas in patients with FAP). FAP is characterized by germ-line mutations in the *APC* tumor suppressor gene. Starting usually when they are teenagers, patients with FAP develop hundreds of colorectal adenomatous polyps. If untreated, FAP patients almost certainly develop colorectal cancer by age 50. Additionally, they are also at risk for developing other neoplastic lesions, such as duodenal polyps and cancers. Once colorectal adenomas appear, patients are monitored by periodic colonoscopy (at ~6-month intervals), removal of existing polyps, and cancer screening. When the polyp burden becomes unmanageable, most patients have partial or total colectomies, usually by age 21 (22). Thereafter, they continue to be monitored for duodenal disease and for polyp formation in the retained rectal segment or the ileal pouch. Agents that prevent or slow the progression of the adenomas could benefit these patients by delaying the time to primary colectomy, by allowing for longer intervals between surveillance colonoscopies and cancer screenings, and by decreasing the need for additional surgical procedures. Other benefits potentially include decreasing or delaying the occurrence of other neoplastic complications, such as duodenal cancer and desmoids. In this regard, celecoxib has already been approved by the FDA (accelerated approval under Subpart H) for treatment of colorectal polyps in FAP patients as an adjunct to standard-of-care (23).

Prevention or Treatment of IEN for Which Organ Removal or Other Major Surgery with High Morbidity Is Standard-of-Care (*e.g.*, Barrett's Esophagus, Superficial Bladder Cancers, and Head and Neck Cancers). Current treatment for dysplastic Barrett's esophagus, a precursor of esophageal cancer, may involve partial or total esophagectomy (24). Because of the high rate of recurrence and potential for progression, treatment for superficial bladder cancers includes periodic surveillance (every 3 months) and removal of new lesions and may include cystectomy (25). Patients with previous head and neck cancers are at high risk (20–40%) for developing new primary cancers throughout the upper aerodigestive tract (26). Treatment of either cancer or IEN (oral leukoplakia) may

involve multiple surgeries resulting in severe morbidity and decreased function, depending on the location of the cancers. For example, laryngeal and pharyngeal cancers may require total or partial laryngectomy, leaving the patient with difficulty in speaking and swallowing. In all of these diseases, treatment has profound detrimental effects on quality of life. They are examples of situations in which agents to prevent and/or treat IEN provide clinical benefit by reducing the need for these surgeries.

Treatment or Prevention of Recurrence of IEN in Patients at High Risk for Invasive Cancer. Eradication of IEN in high-risk cohorts, *e.g.*, women with breast atypical hyperplasia and a family history of breast cancer, men with HGPIN, or patients with bronchial dysplasia, decreases such individuals' risk of invasive cancer. IEN treatment agents that induce complete pathological eradication of high-grade IEN in a proportion of high-risk subjects provide clinical benefit to these subjects by reducing the risk that the high-grade IEN will progress to invasive cancer. In instances where it is known that the total burden of IEN is related to a person's risk of invasive cancer, *e.g.*, number and size of colorectal polyps (12, 13, 15), number of dysplastic nevi (27), and length of the Barrett's column (28), partial reductions in IEN burden with a new treatment agent may also provide clinical benefit by proportionally reducing invasive cancer risk.

Prevention or Treatment of Subclinical IEN in Patients at Risk for Recurrence. New adenomas occur within 1–3 years after resection in ~30% of patients with sporadic colorectal adenomas or cancers (13). These patients are screened routinely at 1–5-year intervals, undergoing colonoscopies with removal of clinically apparent new lesions. Prevention or regression of subclinical adenomas and hyperplastic colorectal mucosa will reduce the number of clinically apparent adenomas and will supplement the clinical benefit obtained by screening (which routinely results in missed polyps). Successful treatment of this "at-risk" epithelium could potentially provide benefit by increasing the screening interval, thereby decreasing associated morbidity and lowering health care costs.

Challenges of Using IEN in Establishing Cancer Risk Reduction

Carcinogenesis Is Multifocal and Multiclonal. Field cancerization as described by Slaughter *et al.* (29) is the consequence of exposing an epithelial field to carcinogens with the resultant development of genetic damage in normal-appearing mucosa. The entire field is then susceptible to the multifocal development of IEN and cancer. It is the whole field that is the target of treatments to reduce cancer risk. With appropriate sampling, IEN represents the total field of abnormal epithelium and provides identifiable lesions that can be targeted for evaluating the efficacy of new treatment interventions.

Few IEN Progress to Cancer. A limitation in using treatment of IEN to establish reduced cancer risk is that relatively small percentages of IEN progress to cancer. Thus, in patients with low-risk IEN, which constitute a significant part of the population, it will be important in future drug development efforts to reduce cancer risk to determine that the successfully treated lesions had potential to progress and, thus, that the patient benefited from treatment of IEN. Histological and clinical regression of low-grade IEN may not be sufficient to demonstrate clinical benefit unless fewer surgical or surveillance interventions are possible as a result. The evaluation of IEN should ideally consider the extent of genetic and molecular progression. For example, demonstrating clinical benefit from a partial reduction in IEN burden may be more persuasive if the IEN remaining after treatment has the same or less genetic progression than that of placebo-treated subjects. Advances that have been made in laser microdissection, genome sequencing, and functional genomics and proteomics provide the opportunity to evaluate gene patterns involved in progression. Two types of genotypic analyses, after extensive standardization of sampling and statistical analysis, could be used as endpoints for IEN treatment studies. The first is monitoring prespecified sets of genetic lesions that are strongly associated with neoplastic progression. Many academic researchers and commercial sources are now designing and producing gene chips that can be used to measure specific cancer-related genotypic changes. The second method is a more generalized comparison of gene expression in posttreatment and baseline lesions using gene microarray analyses.

Not All IEN Can Be Easily Detected and Measured. The development of standardized, appropriate, and quantitative techniques are required for sampling IEN and the field at risk to assure that the statistical power is sufficient to detect a meaningful treatment effect. Thus far, most progress has been made in tissues that can be directly visualized—oral cavity, colon, larynx, bladder, esophagus, cervix, bronchus, and skin. In these tissues, the focal lesion can be identified and stained, and the area of cancerization can be defined and imaged (*e.g.*, cervix). However, in tissues not readily visualized—prostate, ovary, breast, liver, and pancreas—detection of the focal lesion is less certain, and it is difficult to map and image the cancerization field. Even in cases where adequate sampling is difficult, the use of randomized, controlled trials along with standardized sampling procedures increases the opportunities for detecting meaningful effects. Advances in genomics, proteomics, and imaging provide the tools for further refining IEN detection and measurements. Improved diagnostic methodologies such as gene-chip analyses, the confocal microscope, breast ductal lavage and ductoscopy, the LIFE scope for visualizing bronchial tissue, and the magnifying endoscope for colorectal monitoring will be critical in assuring adequate visualization and monitoring of IEN. Table 2 recaps the strategies discussed in the target organ summaries for evaluating efficacy of treating IEN. In addition, Table 2 includes a list of molecular and cellular biomarkers associated with IEN and carcinogenesis in each of the target organs. As described above and throughout the target organ summaries, many studies with IEN endpoints evaluate effects on these earlier biomarkers, as secondary or tertiary endpoints. These biomarkers may be measured in the IEN directly or, less invasively, in cells sloughed from the target tissue (*e.g.*, stool, urine, and sputum). It is anticipated that results of these evaluations may lead to the validation of some of these biomarkers as surrogates for IEN in determining efficacy of IEN treatment and for identification of cohorts at risk for cancer.

Table 2 Strategies for treatment and prevention of IEN in major cancer target organs

Target organ	Cohorts for clinical studies	Primary endpoints	Early associated biomarkers
Colon/rectum	Previous adenomas	Prevention of adenomas	ACF, proliferation antigens (PCNA, Ki-67), apoptosis, differentiation antigens (Lewis^x, Lewis^y, T, Tn, and sialyl-Tn antigens and apomucins), K-*ras*, DNA methylation
	FAP	Regression and prevention of adenomas	
Head and neck	Adolescent FAP (prephenotype expression)	Prevention of adenomas	
	OPLs (atypical hyperplasia, hyperkeratosis, mild to severe dysplasia)	Regression of existing OPL and/or prevention of new OPL	LOH (3p, 9p), *p53*, cyclin D1, growth factors (*e.g.*, EGFR), retinoid receptors (RARβ), DNA content, proliferation antigens (PCNA, Ki-67), apoptosis
Esophagus (adenocarcinoma)	Barrett's esophagus (mild to severe dysplasia)	Regression and/or slowed progression of Barrett's dysplasia	DNA contents, proliferation antigens (PCNA, Ki-67), ODC, growth factors (*e.g.*, EGFR), cyclin D1, p16, *p53*, Cdx1 and Cdx2
Cervix	HGSIL (CIN 2/3)	Regression and prevention of CIN	LOH (3p, 4p, 4q, 11q), proliferation antigens (PCNA), growth factors (*e.g.*, EGFR), K-*ras*, differentiation antigens
Lung	Bronchial dysplasia (current or former smokers)	Regression and prevention of bronchial dysplasia	Proliferation antigens (Ki-67), LOH (3p, 8p, 9p), K-*ras*, retinoid receptors, *p53*, FHIT, DNA methylation, apoptosis
Skin (nonmelanoma)	Transplant patients (with previous nonmelanoma skin cancer)	Prevention of skin SCC (and, as a secondary endpoint, of AK)	Proliferation antigens (PCNA), ODC, growth factors (*e.g.*, EGFR, TGFβ), *p53*
	High-risk AK (>10 AK within previous year)	Prevention of AK (and, as a secondary endpoint, of skin SCC and, as a tertiary endpoint, regression of AK)	
Breast	Hyperplasia without atypia	Prevention of atypical hyperplasia	DNA methylation, LOH, growth factors (*e.g.*, EGFR, *erb*B-2, IGF), proliferation antigens (Ki-67, PCNA), *p53*, apoptosis
	Atypical hyperplasia	Regression of atypical hyperplasia	
Prostate	HGPIN without cancer	Regression of HGPIN	DNA methylation, GST, pc-1 chromosomal loss or gain (8p, 9p, 16q), apoptosis, proliferation antigens (Ki-67), growth factors (IGF, TGF-α, TGF-β)
		Prevention of prostatic carcinoma	
Bladder	T_a, T_1 superficial bladder cancer (papillary TCC)	Prevention of new superficial cancers and of progression (incidence or time to event)	LOH (3p, 9p, 11p, 17p, 18q), *p53*, proliferation antigens (Ki-67, PCNA, M344), differentiation antigens (γ-actin), growth factors (*e.g.*, EGFR), autocrine motility factor receptor
	T_a, T_1 ± TIS superficial bladder cancer (after BCG treatment)		

Precedents in Development of Drugs for Disease Prevention: Lipid Lowering Drugs in Prevention of Cardiovascular Disease

Because of the long time required for clinical disease to develop, the multiple paths by which the disease progresses, and the chronic administration of preventive drugs, the course of cardiovascular disease closely parallels carcinogenesis (30, 31). In the cardiovascular setting, the cholesterol level, which predicts for risk of CHD, is an established endpoint for drug development trials (32). Modulation of cholesterol levels has been used to gain marketing approval for gemfibrozil and hydroxymethylglutaryl coenzyme A reductase inhibitors such as lovastatin, simvastatin, and pravastatin (33–37). Gould *et al.* (38) have carried out a meta-analysis of 35 randomized clinical trials that summarize the evidence supporting cholesterol lowering as a predictor of reduced CHD risk. The results show that cholesterol lowering is correlated to CHD, non-CHD, and overall mortality. Specifically, it was found that for every 10% lowering of cholesterol, CHD mortality was reduced by 13% ($P < 0.002$) and total mortality by 10% ($P < 0.03$), whereas no effect was found on non-CHD mortality.

The research on cholesterol lowering and modulation of other risk markers for cardiovascular disease provides a model for studies needed to validate the association between successful treatment of IEN and reduced cancer risk, both in the relationship of the risk marker to the ultimate disease and in its ability

Table 3 Validation of drug intervention endpoints: Cholesterol lowering and CHD

Association of cholesterol level and CHD from Framingham study, data for total population	
Serum cholesterol	Relative risk (Observed/ Expected × 100)
1st quartile (<193 mg/100 ml)	57
2nd quartile (194–219 mg/100 ml)	85
3rd quartile (220–249 mg/100 ml)	102
4th quartile (>250 mg/100 ml)	152

Effect of drug intervention on cholesterol level, leading to approval by FDA for prevention of CHD

- Simvistatin decreased serum cholesterol by an average of 26% compared with placebo controls

Effect of cholesterol lowering intervention on CHD, meta-analysis of trials >2 yrs in duration

- For every 10% of cholesterol lowering, CHD mortality was reduced by 13% ($P < 0.002$) and total mortality was reduced by 10% ($P < 0.03$)

to predict activity of a given drug on that disease (Table 3). Lowering the cholesterol level has been validated as an endpoint for CHD risk reduction; analogous data might be applied to validating IEN for cancer risk reduction.

Despite these remarkable results on cholesterol and CHD, several caveats apply here as to all studies with risk markers. The relationship between lower CHD risk and lower cholesterol was established by averaging individual responses without full consideration of other (possibly, covariant) risks. There are many individuals in whom the correlation is not seen. For example, an analysis of CHD risk *versus* total serum cholesterol levels by age (30–62 years) using subjects in the Framingham study showed that CHD incidence was virtually unaffected by cholesterol levels in subjects 50–62 years of age and, even in those subjects 30–49 years of age, most CHD was not associated with hypercholesterolemia (39). In other words, when the parameter evaluated is one of several in a multifactorial disease process, other variables along with secondary or indirect processes contributing to the disease (in the case of heart disease, examples are age, smoking history, homocysteine levels, and diabetes mellitus) or protecting the subject may confound interpretation. The proportion of disease attributable to a specific effect may vary according to this diversity of contributing factors characteristic of a particular population, and the more distant temporally the risk marker is from the clinical disease, the lower the percentage of disease that will be attributable. Using this same line of reasoning, effects on early risk markers such as the cholesterol level may not be equally good predictors of disease effect for all classes of drugs. Many drugs have multiple mechanisms of activity that may confound extrapolation of their effects on a single early risk marker to an effect on disease incidence or mortality. For example, fibrates, like statins, lower cholesterol levels but do not protect from CHD mortality.

However, because IEN is directly on the causal pathway and is close temporally and in progression to invasive cancer, evidence for IEN as a risk marker for cancer is likely to be more compelling than that of cholesterol for CHD. For example, it has been well established that the presence of colorectal adenomas increases the risk for colorectal cancer. It is noteworthy that the risk of colon cancer conferred by even the early to moderately advanced colon adenomas far exceeds the risk of CHD from high serum cholesterol levels (10–13, 15). These facts combined with the known reduction of colorectal cancer risk with polyp removal can be used to demonstrate that successful treatment of this IEN provides clinical benefit. Also, because IEN is so close genotypically and phenotypically to cancer, these lesions reflect the end results of multiple carcinogenic processes and so would be expected to predict the activity of multiple drug classes (*i.e.,* multiple mechanisms of drug action).

Table 4 Risks of IEN drug treatment

- Drug toxicities resulting from extended treatment
- Drug effects that mask progression
- Possible reduced rigor of surveillance
- Reduced use of definitive treatment, if available

Establishing Clinical Benefit in Treating IEN with New Agents

A wealth of preclinical and clinical data, much of which are summarized in this report, support the validity of IEN as precursor lesions for invasive cancer and establish the link between reduction in IEN burden and reduction in cancer risk. However, efficacy is only one aspect of successful treatment agents. The mission of drug developers is to ensure that the risks of a drug do not outweigh the benefits of its use. Table 4 outlines the risks that must be calculated. Clearly, IEN treatment studies must monitor patient safety and efficacy long enough to ensure that risks associated with the agent do not exceed its benefit. Alternatively, the agent's long-term safety must have been evaluated in other patient populations.

Development Strategy for Demonstrating Clinical Benefit

An attractive strategy for developing agents for cancer risk reduction is to obtain proof of clinical activity in high-risk populations in whom routine surveillance is the standard of care (including long-term follow-up). Two examples are prevention or regression of colorectal adenomas in individuals with FAP and prevention of newly detected sporadic colorectal adenomas in subjects with a prior history of such adenomas. As described above, based on a study fitting this model, celecoxib has already been approved by the FDA (accelerated approval under Subpart H) for treatment of colorectal polyps in FAP patients as an adjunct to standard-of-care (23). In both cohorts, scheduled standard-of-care surveillance colonoscopy is required. Other examples are studies of PIN, where an annual prostate biopsy schedule is maintained, and superficial bladder cancer, where 3-month follow-up cytoscopies are done. IEN treatment studies in these populations are carried out with the intent of proving efficacy in the high-risk setting while gathering safety data. Benefits from observed reductions in IEN burdens would include potential organ preservation, less need for surgical interventions, and an expected reduced risk of developing invasive cancer. New drug approvals for treatment of IEN in high-risk populations will provide rationale for incrementally extending

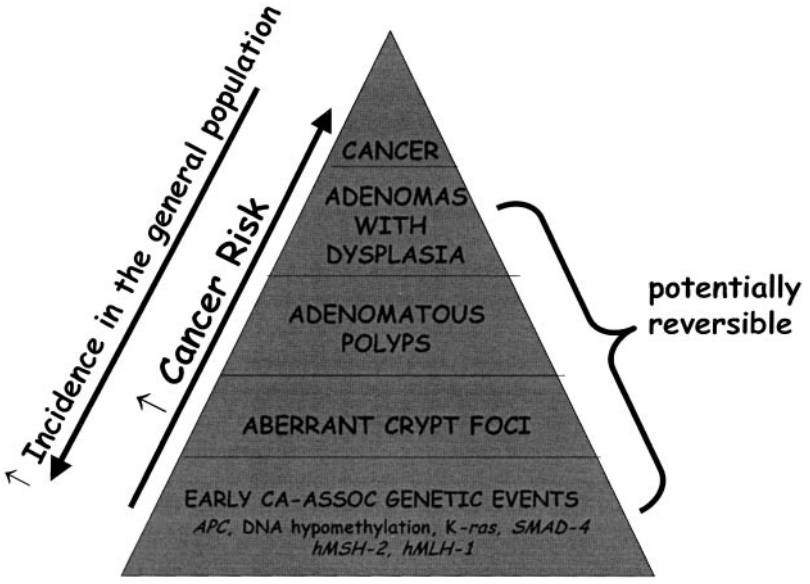

Fig. 2 Association of precancerous genetic and histological lesions with colorectal adenocarcinoma.

studies to lower-risk populations to gain drug approvals that will have broader public health impact.

Target Organ-specific Approach to Prevention and Treatment of IEN

The remainder of this report surveys individual IEN organ sites, summarizes the current knowledge linking IEN to invasive cancer, suggests promising agents for IEN treatment in these organ sites, and describes practical examples of clinical trials that can be used to demonstrate a significant reduction in IEN burden in populations where such reduction is expected to substantially reduce invasive cancer risk. These target organ summaries represent the consensus of the AACR IEN Task Force Subcommittees dedicated to each organ site and are offered as expert perspectives on this complex and rapidly evolving discipline. The IEN Task Force recognizes that the concept of linking reduced IEN burden with cancer risk reduction depends on the force of evidence documenting the cancer risk associated with the particular IEN. Some examples provided in this report are stronger than others, but all represent best current thinking and are provided to stimulate further investigation and opportunities for refinement. Results from ongoing IEN treatment trials will prove or disprove the utility of the clinical trial designs described herein. This first report from the AACR IEN Task Force represents a work in progress.

Treatment and Prevention of Colorectal Premalignant Lesions

CRC is the third most common cause of cancer-related death in the United States, after lung and prostate cancer in men, and lung and breast cancer in women (40, 41). Approximately 57,000 people in the United States are expected to die of colon or rectal cancer in 2001, accounting for ~10% of all cancer deaths. In addition, 135,400 new cases of CRC are expected in 2001, and overall these patients can expect 1-, 5-, and 10-year survival rates of 82, 61, and 55%, respectively (40, 41). The peak incidence of CRC is in the seventh decade of life, and the disease is fairly evenly distributed between men and women. The incidence of CRC has been declining since 1985, at a rate of 1.6% per year through 1997. This may be attributable to an increase in CRC screening and adenoma removal, practices that eliminate premalignant lesions and reduce the incidence of advanced dysplasia and carcinoma (42–44).

Colorectal carcinogenesis is a multistage process, occurring over 10–20 years, in which the successive accumulation of cancer-associated gene mutations results in the progression of an initiated enterocyte to an invasive cancer (Ref. 45; Fig. 2). Changes in oncogenes and tumor suppressor genes (*e.g.*, mutations in *APC*, K-*ras*, or changes in DNA methylation state) are the earliest evidence of IEN (46), and they precede the development of architectural irregularities in the mucosa of the colon or rectum. The first clinically detectable evidence of IEN consists of subtle alterations in the regular pattern of the intestinal crypts known as ACF. ACF may show loss of wild-type APC protein (47), as well as mutations of K-*ras* (48), suggesting that they represent a premalignant genetic event rather than simply a nonneoplastic hyperproliferative state (47). The number, size, and dysplastic features of ACF have been shown to correlate to the number of colorectal adenomas (49), which, as precursor lesions for CRC, are considered one of the most established examples of IEN as a cancer risk marker (50). The rate at which adenomatous polyps progress to cancer is estimated at ~2.5 polyps per 1000/year (51). Approximately 50% of men and 30% of women develop adenomatous polyps of the colorectum by age 50 (52, 53). The risk of cancer development in an adenoma relates to its architectural type, with tubular histology associated with the lowest risk (5% overall), villous lesions the highest (up to 50%), and tubulovillous lesions of intermediate risk (15–20%; Ref. 54). Adenoma size is also an important predictor of malignant potential. The risk of carcinoma development is approximately 1% for lesions <0.5 cm in diameter, 5% for intermediate-sized adenomas (6–9 mm), and 20% in adenomas >1

cm in diameter (55). The risk of cancer development also increases with the number of adenomas. Although direct evidence that CRC arises from adenomatous polyps is difficult to obtain, indirect evidence from multiple case-control studies and controlled clinical trials demonstrates that removal of adenomas decreases the incidence of CRC (42, 56–60).

Additional indirect evidence for the adenoma-carcinoma sequence are the observations that adenomas and CRCs have the same anatomical distribution, that cancer and polyp remnants are found together, and that most patients with CRC also have had adenomatous polyps (61). CRC screening studies have found that the average age at CRC diagnosis is approximately 10–15 years later than the average age of adenoma diagnosis (62, 63). Some CRCs contain a noninvasive adenomatous component, strongly suggesting that the cancer arose in a preexisting adenoma (64). Many observational studies linking agents such as NSAIDs to a decrease in CRC incidence or mortality also show a parallel decrease in adenoma incidence (65–71). Finally, the 6% lifetime incidence of CRC in the United States suggests that ~10% of patients with adenomatous polyps will develop invasive disease. For patients with CRC, 98% have identified cancer-associated somatic mutations in their cancers, 100% have ACF, and 20–30% have synchronous adenomatous polyps (72, 73). The strength of this indirect evidence ethically precludes study of the natural history of unresected adenomatous polyps in humans.

It is clear that the risk of developing invasive cancer increases with progression of IEN, although not all initiated cells, ACF, or adenomas progress to CRC. Studies of IEN progression show that there is a rough correlation between tumor-associated mutations and histological phenotype. For example, an APC mutation is a very early event, likely occurring before microscopically visible adenomas develop, whereas a *p53* mutation is a later event, marking the transition from adenoma to dysplasia (45, 50). Several studies in populations at high risk for developing CRC suggest that alterations in the growth of intestinal epithelial cells precede architectural changes associated with increased enterocyte proliferation (74), and that defects in apoptosis precede architectural changes (75). These preinvasive stages of CRC appear to be reversible under certain conditions, and spontaneous regression (76, 77), as well as regression in response to colectomy (78), diet (79), or NSAID therapy (23, 80), have been described.

The biology of CRC presents a strong case for the existence of significant environmental-genetic interactions. Environmental factors including dietary constituents, such as a diet rich in saturated fat and red meat and deficient in fruit and vegetables, as well as lifestyle factors such as physical activity, differ greatly between industrialized and developing nations (81). Multiple inherited and acquired genetic factors have been linked to the etiology of CRC (82). Patients at highest risk for CRC are those with FAP, a hereditary CRC syndrome caused by the autosomal dominant transmission of a mutation in the *APC* gene. The lifetime risk of CRC in patients with FAP is nearly 100%. Although FAP accounts for <1% of all cases of CRC, an understanding of its biology is essential for prevention and treatment of sporadic CRC, because as many as 95% of sporadic CRCs contain a mutation in either *APC* or an associated oncogene, β-catenin (82–84). Celecoxib has been demonstrated to reduce the number of adenomatous polyps in patients by 28% compared with placebo. This reduction in polyp burden led to FDA approval of celecoxib for treating patients with FAP (23). HNPCC, a cancer family syndrome caused by germ-line mutation in one of several genes governing DNA mismatch repair (85), has an associated lifetime risk of CRC of up to 90% (86). HNPCC accounts for roughly 5% or less of all CRC cases. Although FAP and HNPCC families make up a small fraction of CRC cases, familial clustering of CRC is common. Other high-risk populations include patients with a history of CRC and patients with chronic ulcerative colitis.

Clinical Trial Designs

Potential CRC risk reduction strategies (pharmacological agents or procedure-based) should be evaluated in prospective, randomized trials in patients who have undergone colonoscopy with polypectomy. The new intervention is compared with placebo, and the recurrence of adenomatous polyps is the primary endpoint. This endpoint can be further analyzed by severity, depending upon the number, size, and histological type of the recurrent adenoma(s). Study participants can be selected to include those at high risk for CRC, and trials should carefully control for familial risk and for dietary and life-style factors that may affect outcome, particularly concomitant aspirin or NSAID use. Available data concerning the natural history of adenoma development suggest that adenoma recurrence endpoints should be assessed a minimum of 1 year after index colonoscopy and polypectomy and preferably at 3 years after polypectomy (56, 60, 87). Such trials, if large, are also optimal settings to investigate the relationship between other forms of IEN and colorectal adenoma recurrence. For example, nested cohort studies can examine the reliability of measuring ACF or other mucosal growth characteristics and can correlate the results of these studies with the outcome of the larger trial. Molecular studies can compare the differences between IEN that recur despite the new treatment and IEN that are obtained pretreatment or in the polypectomy control arm. The results of nested cohort studies addressing these issues can help validate other forms or characteristics of IEN as risk markers of adenoma recurrence and by inference, CRC incidence. Because optimal colorectal IEN treatment trials require large numbers of subjects and are expensive and time consuming, it is important to develop and validate IEN endpoints of shorter duration. Such endpoints can then be used to efficiently screen potential CRC risk reduction strategies.

An important issue that must be addressed in CRC risk reduction trials is the durability of response to an IEN treatment intervention. Important unanswered questions include: (*a*) Which patients will have significant recurrent IEN after endoscopic polypectomy and thus require short follow-up intervals? (*b*) Can patients with no evidence of IEN by a given age safely discontinue CRC screening? (*c*) Will adenoma treatment interventions such as calcium, vitamin D, and/or NSAIDs require lifetime use? The answers to these questions are not likely to come from nested cohort studies and will require either randomized trials or long-term follow-up of treated cohorts.

For CRC risk reduction clinical trials, the primary measure of clinical benefit is reduction in the incidence of adenomatous polyps. The benchmark to be improved upon is the baseline

colonoscopy with polypectomy, followed by the standard 3-year repeat colonoscopy to assess recurrent disease. The expectation is that 25–35% of adenoma patients treated with placebo alone will have additional disease discovered at follow-up colonoscopy (42, 56), a figure that includes both newly formed adenomas and adenomas missed at the time of the original intervention. A 30% reduction in the number of adenomatous polyps found in patients treated with an intervention agent compared with placebo 3 years after initial polypectomy would be considered evidence of clinical effectiveness. In a Phase III study, approximately 500–700 evaluable patients treated with either placebo or an intervention agent over 3 years would be needed to have a 90% probability of detecting a 30% decrease in recurrent polyps.

Assessment of net clinical benefit must take into account the morbidity/mortality, patient acceptability and utilization, and the economic impact of the method for treating or preventing adenomatous polyps. New interventions that provide adenoma incidence reduction with increased safety, reduced inconvenience to the patient, and decreased cost will add to overall clinical benefit. One of the tragedies of CRC is that colonoscopy with polypectomy, which has the potential to reduce CRC incidence by 75%, is used by only 10–20% of the population. Effective new interventions will help increase the use of CRC screening, will reduce colorectal IEN burden, and may eventually permit less frequent surveillance colonoscopies.

Treatment and Prevention of Oral Premalignant Lesions

In 2001, oral cavity, oral pharynx, and laryngeal cancers (which comprise HNSCC) will represent 3% of all new cancer cases, with an estimated 40,100 new cancer cases and 11,800 cancer deaths in the United States (40, 41, 88). The primary risk factors include smoking tobacco and alcohol consumption. Despite availability of refined diagnostic tools and treatment modalities, overall survival rates for these cancers (~55%) have not significantly improved over the last three decades (89). The main reasons for treatment failure are the development of second primary tumors in subjects with early-stage disease (Stages I and II) and the development of local recurrence and metastases for subjects with locally advanced HNSCC (89). HNSCC results from a multistep carcinogenesis process, in which increasing degrees of mucosal atypia and dysplasia occur over large areas of the carcinogen-exposed upper aerodigestive tract epithelium, according to the "field cancerization" hypothesis initially proposed by Slaughter *et al.* (29). Therefore, potential approaches to reduce the incidence of head and neck cancer are either to decrease an individual's exposure to carcinogens or to reduce risk in exposed individuals through the use of selected natural and synthetic substances to halt or reverse carcinogenesis (2). Although numerous changes contribute to epithelial carcinogenesis, histologically defined intraepithelial premalignant lesions are still considered to be a better predictor of cancer risk than any individual genetic lesion.

OPLs are white and/or red mucosal patches in the oral cavity or oropharynx that occur in 1–10% of the adult population in the Western world. The histological patterns of OPLs vary from hyperkeratosis with hyperplasia to severe dysplasia or CIS. Prospective studies of subjects with OPLs revealed a 17.5% rate of malignant transformation at 8 years and a rate of 36.4% for those with dysplasia on the initial biopsy (90). In the larynx, similar premalignant lesions are observed, and approximately 30–50% of these lesions will progress to cancer within a 5-year period (91). Although surgical excision is the standard therapy for OPLs, this treatment is not feasible if the area is extensively involved and is associated with considerable morbidity. In addition, surgery treats only the end result of carcinogenesis and does not prevent recurrence or development of *de novo* lesions in the field at risk. OPLs are an excellent human system for clinical testing of new treatment agents for several reasons. These IEN lesions are: (*a*) clearly related to tobacco exposure; (*b*) associated with a well-defined risk for subsequent cancer development; and (*c*) easy to monitor and biopsy. Moreover, OPLs are linked by their etiology and biology to carcinogenesis in other sites of the upper aerodigestive tract.

OPLs are also an excellent model system for study of molecular markers of cancer risk and response to therapy. Chromosomal aneuploidy and LOH at critical chromosomal loci, such as 3p and 9p, the locus of the tumor suppressor gene *p16*, are frequently found in head and neck cancers (92, 93). Allelic losses at 3p and 9p in OPLs are associated with subsequent development of head and neck cancer (94). *p53* alterations are frequent in both head and neck cancers and premalignant lesions, and P53 protein overexpression in OPLs is associated with resistance to retinoid treatment (95). Cyclin D1 overexpression is a poor prognostic factor in head and neck cancer, is frequently identified in premalignant lesions, and is associated with eventual cancer development (96). A stepwise increase in EGFR expression has been described in the process of head and neck tumorigenesis (97, 98). Loss of RARβ also occurs frequently, and restoration of expression correlates with response to retinoid intervention in OPLs (99). Study of these genetic markers within OPL treatment studies will help elucidate the correlation between OPLs with various genotypes and responsiveness to treatment.

Several agents have been shown to have promise as treatment for OPLs (100). 13-cRA (1–2 mg/kg/day) treatment for 3 months was shown to be significantly more effective than placebo in reversing oral leukoplakia in a randomized, placebo-controlled, double-blind trial (101). However, this agent was associated with substantial toxicity (skin effects, conjunctivitis, and hypertriglyceridemia), and rapid relapses occurred upon treatment cessation. Low-dose maintenance therapy with 13-cRA reduced the rate of progression of oral leukoplakia over a 9-month period in a randomized, double-blind trial of low-dose 13-cRA *versus* β-carotene after induction therapy with high-dose 13-cRA (102). The recently updated long-term follow-up of this study revealed similar malignant transformation rates for the two maintenance groups. A trend toward an extended latency period for cancer development was observed in the maintenance 13-cRA group, suggesting a potential need for longer intervention (103). Although retinoids have proven activity in treating early premalignant lesions, single-agent retinoid has not been effective in reversing moderate and severe dysplasia of the oral cavity. A treatment study using a combination of 13-cRA, α-tocopherol, and IFN-α was designed to address advanced premalignant lesions of the upper aerodigestive tract that are

resistant to single-agent retinoid. Although laryngeal lesions responded (47% complete response rate at 6 months and 50% at 12 months), other oral lesions did not (9% at 6 months and none at 12 months; Ref. 104). Thus, even aggressive treatment combinations are not effective in reversing advanced premalignant lesions of the oral cavity and oropharynx, suggesting an urgent need for innovative approaches.

Clinical Trial Designs

In a clinical treatment trial testing efficacy of new agents, subjects are randomized to either placebo or active treatment and are stratified for the presence of either early (atypical hyperplasia, hyperkeratosis, mild dysplasia) or advanced (moderate or severe dysplasia) oral premalignant lesions. Subjects are treated with the new agent or placebo daily for 3–6 months. Clinical assessment and biopsies are obtained at baseline, after 3 and 6 months of active intervention, and 3 months after completing the intervention. Biomarkers including measurements of proliferation, apoptosis, angiogenesis, and genotypic markers of the carcinogenic process are analyzed and correlated with response to intervention.

The primary objective of this prototype study is to evaluate the efficacy of a new agent in reversing premalignant lesions (as defined by histological and clinical response) and in preventing the formation of any new OPLs. The secondary objective is to evaluate the effects of the treatment in modulating the expression of genomic and proliferative markers for early or advanced index OPLs. On the basis of results that have been obtained with other OPL treatment agents, an effective new treatment will result in clinical responses (defined by standard measurement criteria) in >40% of the patients with early premalignant lesions and in histological responses (defined as reversal of dysplasia or improvement in the grade of dysplasia) in >20% of patients with advanced premalignant lesions. Such a level of effectiveness in the absence of significant toxicity would constitute clinical benefit in patients at risk for HNSCC, provided the possible need for chronic treatment was addressed.

The notion that reversal of OPLs might not be of significant clinical benefit by itself and that a reduction in HNSCC incidence is needed to demonstrate clinical benefit of a new treatment has been challenged. Maintenance therapy with 13-cRA increased the latency period for cancer development (22), suggesting that treating early premalignant lesions provides clinical benefit by slowing the rate of progression to cancer.

Treatment of Barrett's Esophagus

Esophageal cancer has been estimated to account for 1% (13,200) of all cancer cases and for 2.2% (12,500) of cancer-related deaths in the United States in 2001 (40, 41). It is associated with the third lowest 5-year survival rate among all cancers (behind pancreas and liver); in the early to mid-1990s, this rate was approximately 11–12% (88, 105). Also, most esophageal cancers (50–60%) are not curable by resection at the time of diagnosis (106), and for those which are, esophagectomy is a highly morbid procedure that significantly lowers quality of life. Adenocarcinoma is the predominant form of esophageal cancer in the United States and other Western countries, accounting for ~60% of such cancers among white males (105, 107, 108).

Barrett's esophagus, a condition in which normal esophageal squamous epithelium is replaced by a metaplastic columnar lining resembling intestinal epithelium, is the primary IEN precursor to esophageal adenocarcinoma. Barrett's esophagus is seen in up to 3.9% of all upper aerodigestive tract endoscopies and is also strongly associated with chronic GERD (24, 107, 109, 110). The prevalence of Barrett's esophagus secondary to GERD is difficult to determine, but in patients undergoing endoscopy for GERD symptoms, rates average from 8 to 12%, increasing to ~40% in patients with peptic strictures (111–113). It is most commonly a lesion of middle-aged white males with a male:female incidence ratio of 3.5:1 (111–113).

On the basis of retrospective studies, the best estimate of the overall prevalence of carcinoma in patients with Barrett's esophagus is 8–10% (111–113). Incidence studies suggest lower risks of malignancy in Barrett's patients, ranging from one case per 52 years to one case per 441 years of follow-up, representing an approximate 30–40-fold increased risk compared with the general population, and corresponding to an annual incidence up to 2.1% (114). The incidence of esophageal adenocarcinoma has increased markedly since the 1970s with an annual rate of increase between 4 and 10% (106, 108, 109). Approximately 64–85% of esophageal adenocarcinomas arise within the metaplastic columnar epithelium (106, 108, 109), evidence further supporting Barrett's esophagus as a precursor of esophageal adenocarcinoma. The increased risk of cancer in patients with Barrett's esophagus is related to the duration of time that a person has Barrett's esophagus and, in all likelihood, to the length of the Barrett's segment. Patients who develop esophageal cancer tend to be about 8–10 years older than Barrett's esophagus patients. Apart from GERD (110), other risk factors for Barrett's esophagus are minor and do not result in high relative risks.

Barrett's esophagus is detected endoscopically as characteristic mucosal lesions projecting from the gastroesophageal junction and by histological confirmation. Barrett's esophagus can progress from metaplasia through various stages of dysplasia to adenocarcinoma (114, 115). Dysplasia demonstrates a combination of architectural and cytological abnormalities that usually increase in a parallel fashion as the lesions progress from low- to high-grade dysplasia. Intramucosal adenocarcinoma is identified by the presence of sheets of neoplastic cells infiltrating the lamina propria. The natural history regarding time to progression of the dysplasia is variable; nevertheless, the finding of high-grade dysplasia on biopsy is typically associated with concurrent adenocarcinoma in about one-third of cases. This figure is appreciably higher in patients with high-grade dysplasia in association with a mass lesion. Low-grade dysplasia without a mass lesion may progress to carcinoma over a highly variable period of time ranging from 1 to 10 years.

Evidence of genetic progression has been described in Barrett's esophagus (116–123). Abnormal DNA content or aneuploidy has been associated with both metaplasia and dysplasia (118), suggesting that clonal expansion of single progenitor cells occurs. Evaluation of mucosal biopsies by flow cytometry has shown that diploid cell populations are converted to single aneuploid cell populations and eventually to multiple aneuploid cell populations. This clonal evolution has been documented by cytogenetic analysis and chromosomal genomic hybridization.

This genetic progression is manifested in dysplastic Barrett's esophagus by overexpression of proliferation markers including PCNA and Ki-67 (119), elevated ODC, and changes in oncogenes (overexpression of EGFR and its ligands and cyclin D1) and tumor suppressor genes [*p53* mutations and LOH (120–122); *p16* inactivation by mutation, deletion, or promoter hypermethylation]. Microsatellite instability has been noted in both dysplasia and adenocarcinoma, reflecting alterations in DNA mismatch repair genes. Screening methods that include analysis of these genetic markers will be useful in improving early detection methods and in monitoring response to therapy [for example, see Galipeau *et al.* (123)].

In endoscopic screening of Barrett's esophagus, meticulous attention is required to comprehensively evaluate the Barrett's epithelium as well as the gastric cardia and the nearby normal esophageal squamous mucosa. It is reasonable to offer periodic endoscopic screening to middle-aged patients in whom acid reflux symptoms are frequent and persistent and/or those who do not respond to acid-suppressive measures. Once Barrett's esophagus is identified, the frequency of subsequent surveillance endoscopies is dictated by the presence or absence of histological evidence of dysplasia (124). It is important to emphasize that the pathologist's experience in evaluating dysplasia is critical in determining the course of surveillance and treatment for a given patient. Metaplasia without dysplasia calls for endoscopic surveillance approximately every 3 years, and the presence of low-grade dysplasia warrants endoscopic surveillance at least annually. High-grade dysplasia requires more frequent endoscopy, at least every 6 months, and usually surgical therapy (esophagectomy). Photodynamic therapy is another option for high-grade dysplasia (125) but may not represent definitive treatment because case reports of recurrent Barrett's esophagus with associated cancer following this therapy exist. Because of the potential morbidity and the uncertain effectiveness of current treatments, new agents or interventions that effectively treat dysplastic Barrett's esophagus are needed.

Clinical Trial Designs

The target population for treatment intervention trials is patients with Barrett's esophagus and dysplasia. In these trials, an agent is administered orally for at least 6–12 months. Careful endoscopy is performed at time points 0, 6, and 12 months with rigorous biopsy methods for histological evaluation. Follow-up endoscopy at scheduled intervals thereafter assesses the persistence of the treatment effects. From a histopathological viewpoint, evidence of either reversal of high-grade dysplasia to low-grade dysplasia or metaplasia, or arresting progression of the low-grade dysplasia would provide a definitive endpoint in trials of adequate size and duration. Molecular markers evaluated in the biopsy specimens would be useful investigative correlates and could include those discussed above as well as early progression markers such as homeodomain transcription factors (Cdx1 and Cdx2) that are linked to the transdifferentiation from squamous mucosa to intestinal metaplasia.

Drug-induced reversal or arrested progression of dysplasia while patients continue to undergo rigorous standard-of-care surveillance endoscopy would constitute clinical benefit. In the absence of significant drug toxicity, patients would derive net clinical benefit from delaying or avoiding the morbidity associated with esophagectomy (by delaying the need for this surgery) and from enhancing the efficacy or avoiding the incomplete efficacy of photodynamic therapy in patients with dysplastic Barrett's esophagus.

Treatment and Prevention of Cervical Intraepithelial Neoplasia

Cervical cancer is the third most common cancer in women worldwide and is an important cause of morbidity and mortality in the developing world, where 80% of cases arise.

Parkin *et al.* (126) estimated that ~371,200 women had been diagnosed with cervical cancer worldwide in 1990, accounting for 10% of all cancers diagnosed in women. In 2001, an estimated 12,900 cases of invasive cervical cancer are expected in United States women, and ~4,400 women will die of this neoplasm (40, 41).

The precursor lesion to invasive cervical cancer, CIN, is also known as SIL of the cervix. It arises at the junction between the primary columnar epithelium of the endocervix and the squamous epithelium of the ectocervix, a site of continuous metaplastic change. SILs are classified as low-grade (LGSIL) and high-grade (HGSIL). LGSILs include CIN 1 lesions; HGSILs include CIN 2 and 3 and CIS, according to the Bethesda System (127, 128). The prevalence of SIL ranges from 1% in women attending family planning clinics to 13.7% in women attending sexually transmitted disease clinics (129). The National Breast and Cervical Cancer Early Detection Program reported >26,000 cases of SILs among 851,818 Pap smears from underserved women, a prevalence of 3% (130). Kurman estimates that of the 50 million Pap smears performed in the United States annually, 2.5 million show LGSIL and cervical atypias (127). Using the prevalence rates of 1 and 13.7% and the estimate of 50 million screening Pap smears annually, the overall prevalence of SILs can be estimated to be 525,000–6,850,000 women.

The prevalence of HGSILs in the United States can be estimated from two large cohort studies. In a cohort of 8,026 patients screened in a military setting, 0.3% of the Pap smears obtained revealed HGSILs (131). In a study by the National Breast and Cervical Cancer Detection Program, 1.1% of 100,500 Pap smears revealed HGSILs (132). Using the prevalence rates of 0.3 and 1.1% and Kurman's estimate of 50 million Pap smears annually, the overall prevalence of HGSILs can be estimated to be 150,000–550,000 women. It is now believed that HPV is involved in the pathogenesis of CIN and all cervical cancers (133–136). Koutsky (137) estimates that millions of Americans are infected with HPV. How many of those infected with HPV will go on to develop CIN is unknown.

Although still a matter of debate, some experts consider it a reasonable standard-of-care to follow LGSILs (CIN 1 and HPV infection) without active treatment, because approximately 50–60% of these lesions will regress, and only ~11% will progress to CIS (138). Treatment of CIN 2 and 3 involves superficial ablation or surgical removal of transformed tissue and some portion of the endocervical canal. Superficial ablative therapies involve either cryosurgery or

laser therapy. Surgical excision generally involves loop diathermy excision of the entire transformation zone and distal canal. Superficial ablative therapies achieve 2-year cure rates of 80%. It is often possible to treat the cervix multiple times. Ablative therapy repeated a second time achieves 2-year cure rates approaching 95%. In comparison, loop excision removes more tissue and achieves cure rates approaching 98%. The principal morbidities of both ablative therapy and loop excision are bleeding, infection, and cervical stenosis, which occur in 2–5% of cases (139). Lifelong surveillance of patients with CIN is required because recurrence rates are up to 20%.

HPV infects the vulva, vagina, and cervix. Nearly 40% of patients with CIN/SILs have multifocal lesions involving two to three sites on the female genital tract. Treating the vagina and vulva with surgical ablation is more challenging than treating the cervix and results in considerably more disfiguration and in higher rates of recurrence (139). Therefore, methods of treating IEN of the female genital tract that do not involve surgical ablation are needed.

Cervical cancer develops along a well-established pathway of progression from benign to malignant disease (138). In the 1960s, Richart (140) first described the progression of normal cervical epithelial cells to dysplasia to CIS to invasive cancer. This model has been well established by obtaining repeated cytological specimens (*i.e.,* Pap smears) from annual screening exams. Further validation that CIN is precancer that can progress to CIS and invasive cervical cancers comes from studies of genetic alterations that accumulate during cervical carcinogenesis. There is extensive evidence that chromosomes 3p, 4p, 4q, and 11q harbor tumor suppressor genes and LOH in these regions are commonly associated with invasive cervical cancers. Larson *et al.* (141) have shown no LOH in these four chromosomal regions in CIN 1, whereas 25% of CIN 2 and 88% of CIN 3 contained LOH for one or more of these chromosomal regions. Forty-one % of CIN 3 lesions exhibited LOH for three or more of these regions. In addition, when LOH was scored for the same locus of a particular chromosomal region in multiple CIN lesions from a single patient, the same allele was lost at each locus, without exception (141). This same-locus LOH analysis strongly suggests that topologically diverse CIN lesions are related and likely arise from a common precursor cell. These molecular analyses demonstrate the genetic relationship between CIN 2 and 3 and invasive cervical cancers with persistent specific allele loss during cervical carcinogenesis.

Large cohorts have been followed in countries with established screening programs, and the results have shown that the morbidity and mortality of cervical cancer are decreased by screening for and treating precancer lesions. Ostor (138) summarized a series of natural history studies that delineated the rates of progression, regression, and stable disease for CIN 1, 2, and 3 (Table 5). Richart and Barron (140) reported mean times to development of CIS of 58, 38, and 12 months for patients with CIN 1, 2, 3, respectively, and predicted that 66% of all dysplasias would progress to CIS within 10 years.

A Phase III trial of topical *trans*-retinoic acid showed a significantly superior rate of regression of CIN 2 with treatment compared with placebo (26, 142). However, no improvement in severe dysplasia (CIN 3) was observed. In a Phase I treatment study of CIN, 2-difluoromethylornithine was found to be associated with a promising response rate of 50% (143). Phase II and III intervention studies have shown that folic acid and β-carotene are not effective in treating established CIN (144–147).

Clinical Trial Designs

Patients most often present clinically with two or more distinct CIN lesions of differing histological severity identified by colposcopy. At present, regression of intraepithelial lesions in the cervix is best evaluated by pathology. Because LGSILs (CIN 1 and HPV infection) are generally followed clinically and are not actively treated, regression of CIN 2 or 3 to normal histology or of CIN 3 to a LGSIL is a clinically beneficial effect and is considered a pathological response (142, 148).

The assessment of new oral and vaccine treatments for CIN is feasible because delay in the definitive surgical treatment of CIN lesions is practical, safe, and acceptable to patients within a clinical trial. In addition, colposcopic as well as pathological assessment of CIN are measurable clinical and pathological endpoints for treatment clinical trials. New treatment agents for cervical precancer should first be tested in small Phase I trials to define the toxicities and tolerability of the preparations. Randomized, placebo-controlled Phase II trials should then be conducted to control for the expected spontaneous regression of high-grade CIN, which is in the range of 20–30% (149).

The following clinical trial design is offered as one scientifically reasonable model in which to demonstrate clinical benefit from treating HGSILs with a new agent. Patients with biopsy-proven unresected and measurable CIN 2/3 undergo careful delineation of measurable lesions by colposcopy and optical mapping (fluorescence and reflectance spectroscopy with confocal imaging) to precisely define their location and to rule out spontaneous regression of the lesion after biopsy.

Three hundred thirty-five patients with measurable CIN 2/3 are randomized to 6 months of treatment with the new agent or to placebo and then undergo repeat colposcopic-guided biopsy of all indicator lesions using the optical map. Repeat colposcopy with optical mapping and biopsy of any lesions are also done at 6 and 12 months after therapy ends to study the duration of any regression of the CIN lesions and to assess whether new lesions have appeared. Patients with persistent CIN 2/3 lesions undergo surgical removal or superficial ablation therapy to eradicate the lesions. Because ablative therapies are associated with objective response rates (CR and PR) in ~80% of patients, obtaining a 50% objective regression rate with a new treatment agent is considered clinically meaningful. A CR is defined as a pathological regression of the CIN 2/3 lesion to histologically normal epithelium, and a PR is improvement of a CIN 3 lesion to CIN 1. A 335-patient, randomized, placebo-controlled trial has 80% power to detect a 50% objective regression rate (CR plus PR) of the CIN 2/3 lesions with a type 1 error rate of 5%, taking into account the expected 20–30% spontaneous regression rate. An improvement in CIN 2/3 to either pathologically normal cervix or of CIN 3 to CIN 1, with no new

Table 5 Progression of CIN[a]

Study	Behavior of lesion				
	% lesions regressed	% lesions persisted	% lesions progressed to higher grade CIN	% lesions progressed to CIS	% lesions progressed to ICC[b]
A. Studies clustered by study design					
All grades CIN followed by Pap smear only	34.0	41.0	25.0	10.0	1.0
All grades CIN followed by Pap smear and biopsy	45.0	31.0	23.0	14.0	1.4
CIS followed by biopsy	—[c]	—	36.0	—	36.0
B. Studies clustered by CIN grade					
CIN 1	57.0	32.0	—	11.0	—
CIN 2	43.0	35.0	—	22.0	—
CIN 3	32.0	56.0	—	12.0	—
Overall all grades of CIN	—	—	—	—	1.7

[a] Based on Ostor and Mitchell's literature reviews (138, 143).
[b] ICC, invasive cervical cancer.
[c] —, not available.

CIN 2/3 lesions appearing in at least 50% of the treated patients, is evidence of clinical benefit of the new agent.

Treatment and Prevention of Bronchial Intraepithelial Neoplasia

In the United States, lung cancer is the most common cause of cancer death in both men and women. Lung cancer represents 14% of all new cancer cases in men and 13% of all new cancer cases in women (40, 41, 88). In the year 2001, ~31% of cancer deaths in men and 25% of cancer deaths in women will be attributable to lung cancer with an estimated total of 162,500 deaths. There are more patients who die from lung cancer than from breast, colon, and prostate cancers combined. The overall 5-year survival rate of lung cancer evaluated for the 1989–1996 was 14% (40). The survival rate has not improved substantially over the last two decades.

Approximately 85% of all lung cancers are related to tobacco smoking. Other risk factors include exposure to occupational carcinogens such as asbestos, indoor and outdoor pollutants, and the presence of airflow obstruction on lung function testing. The risk of lung cancer varies with the number of cigarettes smoked/day, the duration of smoking, and the age at which the individual began smoking. In long-term heavy smokers, the risk of lung cancer persists despite smoking cessation (150). At the present time, there are approximately 45 million current smokers and 45 million former smokers at risk of lung cancer in the United States.

Lung cancers, similar to other epithelial malignancies, are preceded by a series of precursor lesions. This sequence has been defined for SCC but is less well understood for other lung cancers (151–160). Serial sputum cytology examinations in uranium miners and in smokers showed that invasive lung cancer develops through a series of stages from mild, moderate, and severe atypia, CIS, and then invasive cancer over an average of 10 years (152, 157). The prevalence of bronchial IEN (dysplasia and CIS) has been studied using sputum cytology examination (161) and fluorescence bronchoscopy (162, 163). In smokers with chronic obstructive pulmonary disease and a smoking history of ≥40 pack-years, the prevalence of mild, moderate, or severe atypia and CIS on sputum cytology examination was found to be 48, 25, 0.8, and 0.9%, respectively (161). In current and former smokers >40 years of age with a smoking history of ≥30 pack-years, the prevalence of mild, moderate, or severe dysplasia and CIS on fluorescence bronchoscopy and biopsy was found to be 44, 14, 4.3, and 1.2%, respectively (162, 163).

Little is known about the development of other tumor types, although AAH is considered to be the preinvasive lesion of adenocarcinoma (164–166). AAH are usually <7 mm in diameter and are detectable on CT scan as small, "ground-glass" densities (164, 167). In resected lungs, the incidence of AAH was estimated to be 9–21% in patients with primary lung cancer and 4–10% in patients without lung cancer (165). Laboratory investigations demonstrate that AAH cells have ultrastructural features of Clara cells or type II pneumocytes (168) and that many of the molecular changes present in lung adenocarcinomas are present in these lesions. AAH cells exhibit active proliferation, aneuploidy, 3p and 9p deletions, K-*ras* codon 12 mutations, and disruption of cell cycle control, but *p53* gene aberrations are rare and telomerase activation is absent (169). These findings support the concept that AAH lesions are precursor lesions of peripheral adenocarcinomas.

For small cell carcinoma, no characteristic morphological precursor lesions have been described. Recent studies demonstrate extensive molecular damage in normal or hyperplastic epithelium of small cell carcinoma lung cancer patients, suggesting that these tumors may arise directly from such epithelium without undergoing extensive preneoplastic changes (170).

Smoking damages the entire upper aerodigestive tract. Heavy smokers have multiple foci of abnormal fluorescence and

dysplasia. These patches are small, usually <1.5 mm in size, which is smaller than the mean diameter of a bronchial biopsy.[4] In addition, the study of carefully microdissected lobectomy specimens indicates that multiple small clonal or subclonal patches containing molecular abnormalities are present in normal or slightly abnormal bronchial epithelium of patients with lung cancer (171). As much as one-third of the bronchial epithelium may demonstrate molecular changes (171).

Molecular changes have been studied in smokers with and without lung cancer (170, 172–177). These changes begin in histologically normal epithelium, increase in frequency with progressive degrees of histological change, and are rare or absent in the epithelium of never smokers. The changes are not random but follow a definite sequence, with losses of chromosomes 3p, 9p, and 8p being relatively early events and losses of *p53* and *FHIT* genes being later events (174–176).

Although the prevalence of mild dysplasia may decline on smoking cessation, the prevalence of moderate to severe bronchial dysplastic lesions and of CIS are similar in current and former smokers (162). Of interest, no differences have been found between the patterns of molecular changes in current and former smokers (173). These findings suggest that smoking-induced severe histopathological and molecular changes do not regress spontaneously on smoking cessation. Although the damage already sustained persists, the rate of development of new IEN or invasive lesions either decreases or ceases. Thus, the risk of subsequent lung cancer development is reduced with smoking cessation compared with continued smoking but never returns to baseline.

The proportion of individuals with mild, moderate, or severe sputum cell atypia who will develop invasive lung cancer within 10 years has been found to be 4, 10, and 40%, respectively (156, 157). Serial bronchoscopy and biopsy in patients with bronchial dysplasia have shown that ~25% of patients with the dysplastic lesions developed invasive cancer over a mean period of 36 months. The dysplastic lesions were found to persist in another 42% of patients (178, 179). More than 50% of patients with CIS were found to progress to invasive cancer within 30 months (180, 181). These figures are probably low because small lesions might have been removed by the biopsy procedure.

Bronchial intraepithelial neoplasia does not usually result in symptoms. A variety of treatments have been used for carcinoma *in situ*, depending on the size and location of the tumor and the operability of the patient. These treatments include surgery (182, 183), photodynamic therapy (184), YAG laser therapy (185), electrocautery (186), cryotherapy (187), or brachytherapy (188). Currently, there is no standard treatment for bronchial dysplasia.

Clinical Trial Designs

Two proof-of-principle clinical trials suggest that treatment of bronchial IEN might be an effective strategy for reducing lung cancer risk. A study by Hong *et al.* (189) showed that 13-cRA given daily for 12 months was more effective than placebo in preventing second primary cancers in the upper aerodigestive tract and lung in patients with cured head and neck cancer. Another study by Pastorino *et al.* (190) showed that daily retinol palmitate for 12 months was more effective than placebo in preventing second primary lung cancers. However, subsequent Phase III clinical trials using β-carotene or retinol failed to show a reduction in lung cancer incidence compared with placebo in high-risk individuals, such as heavy smokers with or without exposure to asbestos. In fact, the use of β-carotene in those who continued to smoke while on study was found to increase the risk of lung cancer.

Successful treatment of established bronchial dysplasia in current or former smokers is required to reduce lung cancer risk. The advantage of treating patients with bronchial dysplasia is that the severity of the dysplasia can be quantified using image cytometry (191–193).

An example of a prototype treatment clinical trial for bronchial IEN is the ongoing National Cancer Institute-sponsored Phase IIb trial examining the potential efficacy of inhaled budesonide *versus* placebo in smokers with bronchial dysplasia. The trial was designed based on promising preclinical studies in animal models and budesonide's safety and ease of administration (194, 195). Delivery of dry budesonide powder via a turbuhaler facilitates optimal distribution of the compound in the respiratory tract with minimal systemic absorption. The schema of the study is shown in Fig. 3. Patients with bronchial dysplasia on fluorescence-guided bronchoscopy biopsies are randomized to 6 months of treatment with budesonide *versus* placebo and then undergo repeat bronchoscopy and biopsies. Patients with CR at 6 months come off study; those with PR continue treatment, and those in the placebo group with no response or PD cross-over to receive budesonide for 6 months.

The primary endpoints of this study are the number of sites of bronchial dysplasia after treatment and their pathological grade. On a lesion site-by-site analysis, CR is defined as pathological regression of the dysplastic lesion to hyperplasia/normal epithelium. PD is defined as the appearance of new lesions that are mild dysplasia or worse, irrespective of whether the site was biopsied at baseline, or worsening of the dysplastic lesions present at baseline by two grades or more (*i.e.*, mild dysplasia to severe dysplasia or worse, moderate/severe dysplasia to CIS/invasive cancer). NR refers to sites that are not a CR or PD.

On a participant-by-participant basis, response is defined as a CR or PR. CR refers to regression of all dysplastic lesions found at baseline to severity no higher than hyperplasia, as defined on the site-by-site analysis at 6 months, and no appearance of new dysplastic lesions that are mild or higher grade dysplasia. PR is defined as regression of some but not all of the dysplastic lesions and no new lesions that are mild or higher grade dysplasia. PD is defined as progression of one or more sites to a higher grade as defined on the site-by-site analysis or appearance of new dysplastic lesions that are mild or higher grade dysplasia at 6 months. NR refers to subjects who do not have CR, PR, or PD.

On the participant-by-participant analysis, a 40% superiority in the objective response rate (CR plus PR) associated with the new agent compared with placebo constitutes effectiveness of the agent. A lesion by lesion histological and molecular

[4] Dr. Stephen Lam, British Columbia Cancer Agency, unpublished data.

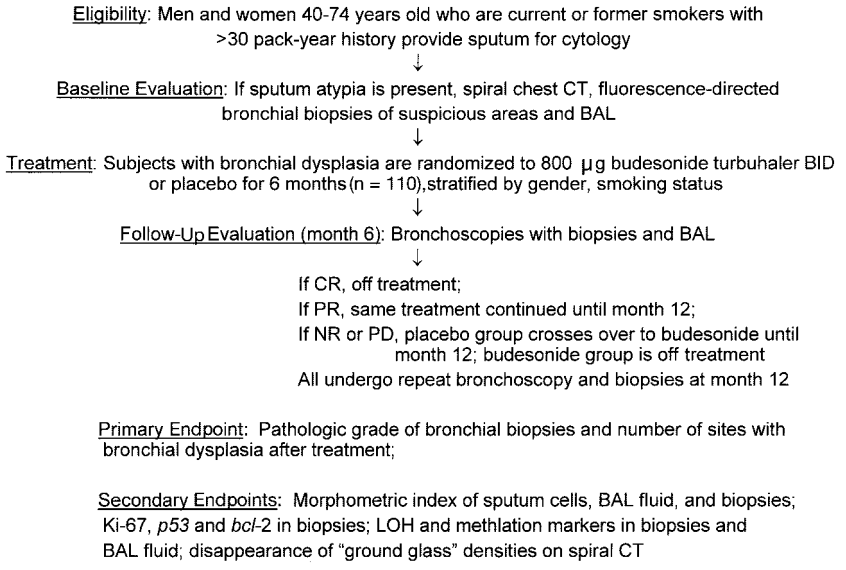

Fig. 3 Schematic of prototype clinical study for treatment of bronchial IEN. *CT,* computed tomography; *BAL,* bronchoalveolar lavage; *BID,* twice per day.

analysis may provide insight into why a given agent may or may not be effective in causing regression of the dysplasia.

Although a decreased incidence of invasive lung cancer may be the ultimate "gold standard" for risk reduction intervention trials, such studies require lengthy observation of numerous subjects. Thus, regression of established bronchial dysplasia is a suitable treatment endpoint because of the high risk of invasive lung cancer associated with this lesion. However, the small size of the dysplastic lesions (usually smaller than a bronchial biopsy) presents problems for sequential monitoring. Development of *in vivo* optical imaging methods such as confocal microscopy or optical coherence tomography may allow more accurate outcome assessment without removing the IEN lesions by the biopsy procedure. Currently, we do not have the means to perform risk reduction intervention trials in patients at risk for developing peripheral adenocarcinoma. Coupling low-dose spiral computed tomography with optical imaging methods via fiberoptics to detect and to characterize small lung nodules may allow us to target AAH in treatment studies aimed at reducing adenocarcinoma risk.

Treatment and Prevention of Actinic Keratosis

Nonmelanoma skin cancer is by far the most common type of malignancy and has a tremendous impact on public health and healthcare expenditures. The incidence of skin cancer is continuing to grow at an alarming rate. Approximately 1.3 million new cases of BCCs and SCCs are expected to be diagnosed in 2001 (41), compared with 900,000 new cases in 1997 (196). These numbers are probably underestimates, because many skin cancers are treated or removed in clinics without being reported to cancer registries. Despite the tremendous number of nonmelanoma skin cancer cases, mortality attributable to this disease is relatively low, with death rates $<$1.5/100,000 (197). However, morbidity can be dramatic because of excision of lesions in cosmetically sensitive areas (198, 199).

Keratinocytic nonmelanoma skin cancers originate in the epidermis and consist of basal cell and squamous cell neoplasms. Approximately 80% of nonmelanoma skin cancers are BCCs (200). These neoplasms, originally described by Jacob (201) in 1827, appear to originate from basal cells of the epidermis and occasionally those of the infundibular and outer root sheath of the hair follicles (198). These are slow-growing tumors that are locally invasive but rarely metastasize. Death is rare and usually attributable to neglect if it occurs. Morbidity can be high, because tumors are often disfiguring and located in facial areas. SCC originates in the keratinizing cells of the epidermis. These tumors are generally more aggressive than BCCs and have a much higher potential for metastasis. Mortality from nonmelanoma skin cancer is mainly attributable to SCC, with 1,200–1,500 deaths reported each year in the United States (199, 202). The number of deaths attributable to nonmelanoma skin cancers is approximately equivalent to the number of deaths from Hodgkin's disease (88).

Risk factors are well defined for nonmelanoma skin cancer. Exposure to UVR and fair skin type susceptible to sunburns are the predominant risk factors (203). Increasing frequency of exposure (204), age (205), and male gender (200) also contribute to increased risk. UVR is well known as a complete carcinogen (206). The incidence of skin cancers, especially SCCs, is also increased among organ transplant recipients (207–210). One study showed that 2,561 kidney and heart transplant recipients had a 66-fold increased risk of SCC compared with the general population (211, 212). A comprehensive study of 5,356 transplant recipients in Sweden showed that they had a 100-fold increased relative risk of developing nonmelanoma skin cancer, almost exclusively in sun-exposed areas (213). The increased frequency of SCC in these patients, especially in individuals with chronic actinic damage, is presumably attributable to long-term immunosuppressive therapy (214), although nonimmune mechanisms may play a role (215).

AK (also known as solar or senile keratosis) is by far the

most common premalignant dermatosis and is attributable to UVR (216). AK appears as a proliferation of transformed, neoplastic keratinocytes confined to the epidermis. A clinical precursor to SCC (217), AK is characterized by thickened, cornified, scaly lesions that develop on the surface of the skin because of improper maturation of keratinocytes. These lesions are characterized histologically by irregular arrangement of cells and atypical hyperchromatic nuclei. Many experts believe that AK represents early SCC *in situ* (218), because it is indistinguishable from SCC on a cytological basis alone (219). Because AK has the potential to invade and metastasize, several authorities have proposed revising the nomenclature and grading of AK to more closely reflect IEN as described in other tissue types (220).

AK is an extremely common lesion, especially in older Caucasian populations. In the United States, an estimated 3.7 million physician office visits/year for AK were made in 1993 and 1994 (221). On the basis of limited available data, the best estimate for current prevalence of AK ranges from 5 to 14% of the adult population in the United States (222). In high-risk groups, prevalence may be as high as 26% (223, 224). In Australia, where skin cancer incidence is highest, prevalence of AK ranges from 40 to 60% in the adult population (225).

Significant morbidity can result from AK, as well as from its treatment. The appearance of AK in sun-exposed areas, including the face and arms, is cosmetically objectionable to many patients. High-risk subjects may have numerous lesions. Common treatments include cryotherapy, curettetage with or without electrosurgery, and topical 5-FU (226). High-risk lesions should be biopsied, because it is impossible to distinguish AK from SCC without histological confirmation (217). Topical 5-FU is used when multiple lesions exist in a specified area to ensure treatment of lesions that are not clinically evident. Side effects of 5-FU include severe erythema, inflammation, pain, pruritis, and burning. Compliance is problematic, because it may require several weeks of treatment until erosion, necrosis, and ulceration are evident (226). Diclofenac gel (Solaraze®) has been approved recently by the FDA as a topical treatment for AK. In a total of 427 patients, 90 days of treatment with diclofenac completely cleared the AK in ~40% of the specified 5 × 5-cm skin regions when assessed 30 days after treatment, compared with 18% among those treated with vehicle alone (227). This clinical development strategy provides an important precedent for future IEN treatment trials.

Models for both UVR-induced and chemically induced skin carcinogenesis and progression are well established (228, 229). IEN lesions in skin can progress to invasive disease, but most do not. In fact, estimates of AK progression range from 0.1 to 14%. Lowest rates of conversion (<1%) were seen in the seminal studies by Marks *et al.* (230, 231), which have been criticized because they describe only the 1-year conversion rate. Higher rates seen in later studies were obtained over longer follow-up periods, taking into account multiple lesions, and are likely more accurate (200). Approximately 60% of SCCs arise from preexisting AK and/or contiguous skin surfaces (231–233). On the basis of data from a series of longitudinal studies conducted in Australia among moderate-risk AK patients (230, 232, 234), the estimated 10-year risk of malignant transformation for an average patient with AK ranges from 6.1 to 10.2% (235). An even higher rate of malignant transformation has been observed in southeastern Arizona; among 1,140 moderate-risk AK patients, the cumulative probability of developing a first new SCC was 14.1% over 5 years (236). On the basis of these data, the presence of AK should not be taken lightly, as it indicates a significant risk for skin cancer and serves as a valuable risk marker for individuals at high risk for SCC (200).

Clinical Trial Designs

Clinical trials designed to demonstrate the effectiveness of a new agent in treating or preventing skin IEN and/or primary SCCs are outlined in Table 6. High-risk populations include patients with multiple AK and organ transplant recipients. Thus, one trial design with considerable merit involves randomizing organ transplant recipients, who have had primary skin cancers removed and who are at high risk for developing additional skin cancers, to active treatment or placebo. Outcome measures include interval to development of new AK and/or SCCs.

A second trial design involves identifying patients who have multiple AK over a measurable body surface area, *e.g.*, a 5 × 5-cm defined area, at the time of study enrollment. Such patients generally continue to develop AK, and treatment is often associated with significant cosmetic morbidity. These patients are randomized to treatment with a new agent or to placebo, with the primary endpoint being the regression of existing AK and prevention of new AK in the defined 5 × 5-cm area. The secondary endpoint is development of new SCCs. An example of this clinical trial design is the one that led to the recent approval of diclofenac gel for treatment of AK described above. An example of a clinical trial design that had a cancer endpoint is that by Moon *et al.* (237) in which 2,297 participants with a history of 10 or more AK (and fewer than two prior SCCs or BCCs) were randomized to placebo or retinol for at least 3 years. The primary endpoint was the rate of development of SCC. This study showed a hazard ratio of 0.74 (95% confidence interval, 0.56–0.99; $P = 0.04$) for the development of a new SCC in retinol-treated patients compared with those receiving placebo.

If successful, these trials will demonstrate that agents that are effective in treating skin IEN significantly decrease the burden of AK in the high-risk populations that have significant morbidity from these lesions and, in the organ transplant population, the incidence of SCC and/or AKs. If toxicity is low, a net clinical benefit is realized because of reduction in morbidity in treating both AK and SCCs in these high-risk patients, as well as from potential reduced mortality from SCC.

Treatment of Breast Intraepithelial Neoplasia

Breast cancer is the most common cancer in women excluding BCCs and SCCs of skin and the second most common cause of cancer deaths in women. An estimated 192,200 cases of invasive breast cancer will be diagnosed in 2001 (40, 41). Although <30% of women diagnosed today will ultimately die of their disease (126), the morbidity from treatment is substantial among survivors.

Breast IEN, which spans the continuum from simple hyperplasia without atypia to CIS, is a recognized risk factor for invasive cancer (238, 239). The relative risk for invasive breast

Table 6 Clinical trials: Designs for treatment of skin IEN

	Organ transplant patients	Patients with multiple AK
Study design	Randomized, placebo-controlled, double-blind Phase III trial of a new treatment agent given for 3 years	Randomized, placebo-controlled, double-blind Phase III trial of a new treatment agent
Endpoints	Primary, new, pathologically confirmed SCC Secondary, new AKs	Primary, regression of existing AK to clinically normal-appearing skin and extent of new AK in 5 × 5-cm surface area Secondary, new pathologically confirmed SCCs
Monitoring schedule	12-week intervals (217)	Monthly intervals (240)
Biopsy schedule	Baseline Suspicious lesions as they occur	Baseline Suspicious lesions as they occur
Sample size with assumptions	• n = 103 per group (206 total) • α = 0.05, power = 80% • 50% of controls will have progressive AK to biopsy-proven SCC • Ability to detect 40% reduction in AK progression to SCC	• n = 328/group (656 total) • α = 0.05, power = 80% • 14% of controls will develop SCCs, and 50% of controls will develop new AK • Ability to detect complete clearance of AK in the 5 × 5-cm area of skin in at least 33% more treated patients compared with placebo. • Ability to detect 40% reduction in SCC incidence and/or a 40% reduction in the incidence of new AK

cancer after a breast biopsy for a palpable mass or mammographic abnormality is increased by 2-fold for simple hyperplasia, 4–5-fold for atypical hyperplasia without a family history, 10-fold for atypical hyperplasia with a family history or LCIS, and 20-fold for DCIS (238–242). The 5-year risk for invasive breast cancer development/detection after a histopathological diagnosis of IEN is ~1% for hyperplasia without atypia, 2.5% for hyperplasia with atypia, 5% for hyperplasia with atypia plus a family history of breast cancer or LCIS, and 10% for DCIS (238, 239, 242, 243). Approximately a half million cases of breast IEN are diagnosed yearly in the United States including 360,000 with simple hyperplasia, 60,000 with atypical hyperplasia, 46,000 with DCIS, and 5,600 cases of LCIS (40, 41, 242, 244–246). Occult IEN appears prevalent in the adult female population. In an autopsy study by Nielsen *et al.* (247), 68% of Caucasian women of 20–54 years of age dying from causes other than breast cancer had evidence of breast IEN (18% *in situ* cancer, 7% atypical hyperplasia, and 43% simple epithelial hyperplasia). An additional 2% had invasive breast cancer. Hyperplasia and atypical hyperplasia were more frequently observed in women with concomitant *in situ* and/or invasive cancer. Ninety-five % of women with *in situ* or invasive cancer had concordant atypical hyperplasia *versus* only 9% of women without *in situ* or invasive cancer (247).

Diminished expression of a number of tumor suppressor genes is observed in the early stages of IEN and may result in part from hypermethylation of gene promoter regions (248–252). Decreased expression of estrogen receptor can also result from methylation abnormalities (251, 253), although the predominant phenotype in breast IEN is a progressive increase in the proportion of cells expressing ER (254). An increase in growth factors, growth factor expression, receptor tyrosine kinase activity, and proliferation as well as diminished apoptosis is also observed in early breast IEN (255–261). Oxidative stress and DNA damage may result in increased expression of wild-type *p53* in early IEN, although *p53* mutation may be uncommon prior to development of DCIS and invasive cancer (262–267). Chromosomal aneuploidy and LOH have been observed in early-stage IEN and appear to be progressive through late-stage IEN and invasive cancer (268–271). Twenty to 40% of simple and atypical hyperplasias and 80% of DCIS are found to have LOH regions that are also present in synchronous invasive breast cancers (269).

Most histomorphological features associated with breast IEN can be appreciated in cytological preparations, although questions have been raised about whether histopathological terminology should be applied to cytological preparation (272). Intra- and interobserver variance has been a major issue for both cytological and histological interpretation of breast IEN (273–276). Standardized criteria have been developed for histomorphological (277–279) and cytomorphological classifications of breast IEN that should improve diagnostic reproducibility. Semiquantitative index scoring (280) and image morphometry (281) are also being explored as means of decreasing intra- and interobserver variance associated with morphological designations.

Up to 70% of women at increased risk on the basis of family history or a prior precancerous breast biopsy will have occult IEN detected by nipple aspiration or random FNA (267, 282, 283). Risk of subsequent breast cancer development is substantial after detection of occult hyperplasia with atypia by random FNA in high-risk women and is ~3%/year in the first 4 years after detection of atypia in the series by Fabian *et al.* (267). In ~600 high-risk women with proliferative hyperplasia without atypia on random bilateral breast, periareolar FNA, one-fourth showed progression (or detection) of hyperplasia with atypia with a median interval between the FNAs of 11 months.[5]

[5] Dr. Carol Fabian, University of Kansas, unpublished data.

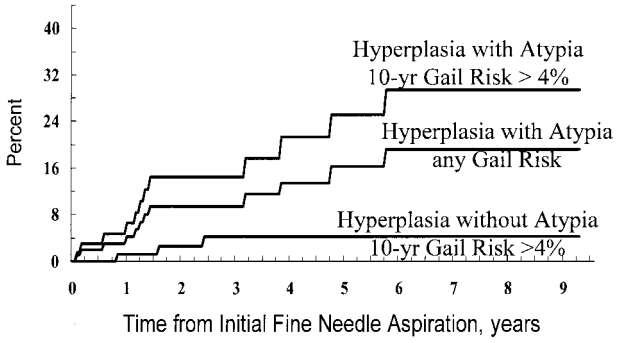

Fig. 4 Hazard function for subsequent detection/development of DCIS or invasive breast cancer as a function of time after initial FNA of high-risk women. Groups are defined as having a 10-year Gail risk that is >4% plus FNA evidence of epithelial hyperplasia without atypia (group 1); FNA evidence of epithelial hyperplasia with atypia (group 2); and FNA evidence of epithelial hyperplasia with atypia plus a 10-year Gail risk >4% (group 3). Note that the groups are not exclusive. Modified from Fabian *et al.* (267).

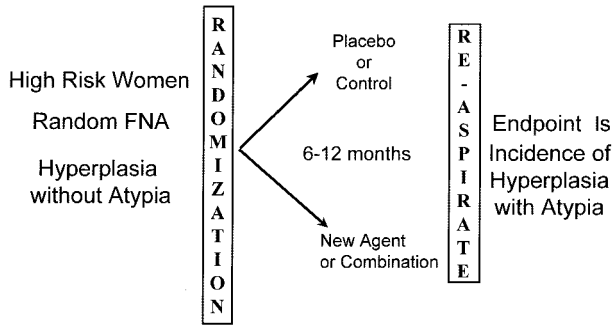

Fig. 5 Schematic of the FNA failure-to-progress model. High-risk women with random FNA evidence of epithelial hyperplasia without atypia are randomized between placebo and investigational agent for 6–12 months and then undergo repeat FNA. The study endpoint is the incidence of hyperplasia with atypia detected in the repeat aspiration.

Women with newly diagnosed advanced IEN (atypical hyperplasia, LCIS, DCIS) are often offered treatment with 5 years of tamoxifen to reduce the risk of invasive breast cancer. Risk of invasive breast cancer in women with a prior diagnosis of DCIS, LCIS, or atypical hyperplasia can be reduced by 40, 56, and 86%, respectively, with 5 years of tamoxifen treatment (243, 284). Tamoxifen has been approved by the FDA for reduction of the risk of invasive breast cancer in high-risk women and for treatment of DCIS in patients who have undergone lumpectomy and radiation therapy.

Clinical Trial Designs

Because of the close biological proximity of DCIS to invasive cancer, partial or complete eradication of DCIS would be accepted as definitive evidence of reduction of breast cancer risk. However, because the standard of care for DCIS is surgical removal via lumpectomy or mastectomy, Phase III new agent treatment trials with eradication of DCIS as a therapeutic endpoint are not currently feasible in the United States.

A recent study has demonstrated the feasibility of repeatedly obtaining breast tissue by bilateral periareolar FNA in women at elevated risk for breast cancer (267). Using this technique, >90% of women had sufficient cells for cytomorphological interpretation as well as multiple other biomarkers (267). Both 10-year Gail risk and evidence of hyperplasia with atypia in the random periareolar FNA were significantly and independently predictive of subsequent breast cancer development in a group of 480 women with a median follow-up of ~4 years (Fig. 4).

On the basis of the above, two clinical trial models are suggested for demonstrating effectiveness of a new agent for the treatment or prevention of advanced breast IEN: (*a*) a prevention of progression to hyperplasia with atypia model; and (*b*) a reversal of hyperplasia with atypia model that requires feasibility and validation testing.

Random periareolar FNA is proposed as the tissue sampling technique because of the reliability and validation of this method in predicting risk, in repeatedly obtaining breast epithelial cells, and because of demonstrated patient acceptance. Other tissue sampling methods, such as ductal lavage, may be useful in the future, depending on evidence obtained from ongoing studies (285, 286).

In the prevention of progression to atypia model (Fig. 5), high-risk women with proliferative cytology without atypia in their baseline FNA are randomized to between 6 and 12 months of the investigational treatment agent or to placebo. The primary endpoint is the difference in the incidence of progression to atypia between the two groups. Lack of progression to atypia would be interpreted as an effective intervention because cytological atypia has already been shown in a prospective study to be associated with a high short-term risk of developing breast cancer (267). Assuming a 75% failure to progress rate in the placebo arm, this trial design would allow detection of a 50% reduction in the number of women who demonstrated progression to atypia in the treatment arm relative to placebo with entry of approximately 335 subjects with a 5% type 1 error rate and 80% power. This trial could be performed by a multi-institutional consortium with 2–3 years of accrual. A variation of the prevention of progression to atypia model would be to require high-risk women to have baseline cytology designated as benign (nonproliferative or proliferative without atypia) as per the new standard criteria adopted by the Diagnostic Terminology Subcommittee at the National Cancer Institute/NIH Workshop for FNA Cytopathology (279). The primary endpoint would still be the difference in the incidence of progression to atypia (Workshop-designated categories of intermediate/atypia or suspicious).

In the reversal of hyperplasia with atypia model, women with a baseline random FNA showing proliferative cytology with atypia are randomized to 6 months or more of the investigational agent *versus* placebo in a double-blind fashion and then undergo repeat FNA. The primary endpoint is eradication of cytological atypia in the treatment *versus* placebo groups (Fig. 6). Even with as much as a 50% decrease in the incidence of FNA-detected atypia in the placebo arm because of sampling or interpretive variation, a 50 or 33% decrease in the incidence of hyperplasia with atypia in the active treatment group relative

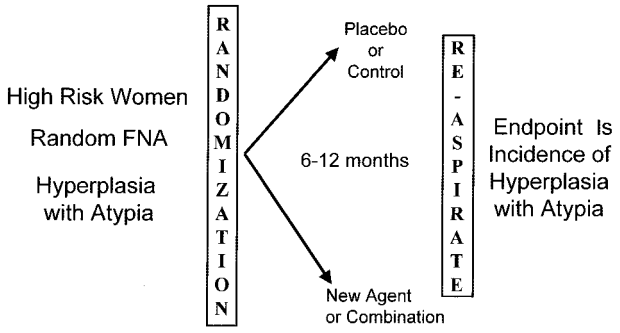

Fig. 6 Schematic of the FNA reversal-of-atypia model. High-risk women with random FNA evidence of epithelial hyperplasia with atypia are randomized between placebo and investigational agent for 6–12 months and then undergo repeat FNA. The study endpoint is the reversal of cytological atypia in the repeat FNA.

to placebo can be detected with 130 and 280 subjects, respectively, with a 5% type 1 error rate and 80% power, presuming good population homogeneity and compliance. At the present time, it has yet to be demonstrated that atypia can be eradicated with 6–12 months of an antiproliferative and/or pro-apoptotic drug treatment. Hyperplasia with atypia may be more focal in the breast than proliferative changes without atypia and thus subject to greater variance by random tissue sampling. Therefore, reversal of atypical cytology should not be considered a definitive efficacy endpoint unless the improved cytology correlates to a reduced risk of developing breast cancer or until tamoxifen, which has proven benefit in reducing breast cancer risk, is found to reduce the incidence of FNA-detected atypia relative to placebo in high-risk women.

Treatment of Prostatic Intraepithelial Neoplasia

Prostate cancer is the most common cancer in United States males, accounting for 180,400 (29%) of all new cancers and 31,900 (11%) of cancer deaths in males (40, 41). Risk factors for prostate cancer include age >50 years, family history, high serum testosterone, high fat diet, and prostatitis with geographic background (prevalence being highest in the United States, Canada, and Northwest Europe; Ref. 287). In the United States, ~8% of males are diagnosed with prostate cancer during their lifetimes, with about 2–3% of all male deaths attributable to prostate cancer. Besides clinically evident disease, microscopic foci of adenocarcinoma have been found at autopsy in the prostates of men who died from other causes (288). The frequency of such "latent" tumors has been shown to increase with each decade of life from the 50s (5.3–14% incidence) to the 90s (40–80%; Ref. 289). With the dramatic increase in the use of PSA screening and resultant biopsies and stage shift, the death rate relative to incidence is trending downward.

Prostate cancer is slow-growing, often requiring decades to appear as a clinical cancer. Exogenous risk factors seem to contribute more to the disparity between latent and clinical prostate cancer than do hereditary factors. For example, the incidence of latent prostate adenocarcinoma does not vary widely among populations. In one study, the prevalence of microscopic lesions at autopsy was 20.6, 28.8, and 36.9 per 100,000 in Japanese, Germans, and African-Americans, respectively. On the other hand, rates of clinical cancer were 2.7, 21.1, and 67.1 per 100,000, respectively. Furthermore, within two generations after immigrating to the United States, the rate of clinical cancer in Japanese men approaches that of United States Caucasians (288). These data suggest that the baseline incidence of early microscopic prostatic neoplasia is accelerated to progressive PIN to varying degrees in specific populations and individuals, depending on exogenous environmental influences.

The evidence that PIN is a morphological and genetic precursor to prostate cancer is extensive and conclusive (16–18, 20, 289). When examined microscopically, PIN lesions are characterized by collections of proliferative prostatic epithelial cells confined within prostatic ducts that exhibit many morphological features of prostate cancer cells, including architectural disorganization, enlarged cell nuclei and nucleoli. PIN lesions are currently classified into two grades, low-grade PIN (formerly PIN 1) and high-grade PIN (HGPIN, formerly PIN 2 and PIN 3); current use of the term PIN generally refers only to HGPIN. In addition to the similarity of the cellular morphologies of HGPIN and invasive lesions, evidence that HGPIN is a precursor of prostatic adenocarcinoma includes the multifocality of both lesions and the presence of carcinoma in foci of PIN; among older men, foci of PIN are found in 82% of prostates with carcinoma but in only 43% of normal prostates. PIN is frequently located in the peripheral zone of the prostate, the site at which 70% of prostatic carcinomas occur. Additional similarities include enhanced proliferative activity of both PIN and carcinoma (3-fold that of benign tissue), cytokeratin immunoreactivity, lectin binding, and loss of blood group antigen with both PIN and carcinoma. Prevalence of PIN and its temporal association with invasive cancer are illustrated by the known 40–50% PIN incidence in men 40–60 years of age, evolving into the 40–50% incidence of prostate cancer in men 80 years of age (21). Autopsy data reveal that PIN lesions appear in the prostates of men in their 20s and 30s in the United States, preceding the appearance of prostate cancer lesions by as many as 10 years (21, 290). African-American men, who are at higher risk of prostate cancer mortality, appear to have a greater extent of PIN at any given age. PIN and prostate cancer lesions share a number of somatic genome abnormalities (291), including loss of DNA sequences at 8p and increased *GSTP1* CpG island DNA methylation (292, 293), among others. Finally, transgenic mouse strains prone to developing prostate cancers typically develop PIN lesions in advance of the appearance of invasive cancer (294, 295).

PIN lesions are always asymptomatic and cannot currently be diagnosed or detected by any reliable means other than examination of prostate tissue histologically. In autopsy studies, the incidence and extent of PIN increases with age, as does the incidence of prostate cancer. The incidence of PIN in men who are not known to have prostate cancer who undergo prostate biopsy has been reported to be as high as 25% (20).

All of the available data support the conclusion that the presence of PIN on prostate biopsy predicts for an increased risk for prostate cancer and that some PIN lesions give rise to prostate cancers. However, the limitations of prostate biopsy sampling techniques preclude repeated monitoring of any specific PIN lesion to assess its natural history (16, 20, 289). Thus,

PIN lesions detected on prostate biopsy are risk markers of prostate carcinogenesis and identify men at high risk for developing prostate cancer. When a diagnosis of HGPIN is combined with other risk factors such as serum PSA, age, race, and/or family history, cohorts of men at very high risk for developing prostate cancer are identified that have prostate cancer incidence rates of 40% over 3 years and 80% over 10 years (296).

Clinical Trial Designs

Most current or planned clinical trials of prostate cancer risk reduction for men who have PIN but not prostate cancer will determine whether candidate treatment agents reduce the prevalence or extent of PIN. In these trials, men undergo interval prostate biopsies to assess the presence of PIN and prostate cancer. As of yet, no clear data have been reported that ascertain whether reducing the extent of PIN (if this can be accurately estimated by patterned prostate biopsies) by treatment with a candidate treatment agent will result in lowered prostate cancer risk. Interestingly, it is known that the prevalence and extent of PIN lesions are reduced by treatment with androgen deprivation therapy, with external beam radiation therapy, and with prostate resection. All these treatments are known to be efficacious in treating prostate cancer. Phase II clinical trials of candidate prostate cancer risk-reduction agents in men with prostate cancer who will undergo radical prostatectomy hold promise for defining the usefulness of monitoring PIN prevalence and extent and PIN-associated cellular (*e.g.*, morphometry, DNA ploidy, apoptosis, and proliferation) and molecular (*e.g.*, GST, *bcl*-2, Ki-67, insulin-like growth factor, and TGF) markers as efficacy endpoints (296). With this clinical trial model, prostate cancer tissue is obtained by core biopsy at diagnosis and again at definitive surgery after several weeks of treatment with a new agent *versus* placebo. Because the majority of men with prostate cancer will have normal prostate epithelium, PIN, and prostate cancer in these tissue samples, treatment effects on each of these cell populations can be studied.

Prototype pivotal Phase III clinical trials for prostate cancer risk reduction have been developed, are feasible, and offer the promise of net clinical benefit in several patient populations (296, 297). At this time, because extent of PIN cannot be reliably measured by serial sampling procedures, a decrease in the extent of PIN with treatment is not a conclusive efficacy endpoint. Thus, clinical trials targeted at eliminating or reducing the extent of PIN are not likely to demonstrate net clinical benefit without additional data indicating that prostate cancer incidence (risk) has been reduced. Because prostate cancer incidence can be estimated in cohorts of patients with HGPIN, PSA abnormalities, and other risk factors, Phase III placebo-controlled trials that have prostate cancer incidence as the primary endpoint can be conducted with 300 patients/arm, with the control group having an expected 40% prostate cancer incidence over 3 years. This trial design will definitively evaluate candidate prostate cancer risk-reduction agents and will validate extent of HGPIN as a suitable efficacy endpoint for subsequent trials (296, 297). A 30% reduction in prostate cancer incidence in the HGPIN patients who are treated with the new agent compared with control patients constitutes clinical benefit, provided the intervention is safe.

Advances in imaging science will facilitate efforts to follow HGPIN lesions over time without relying on blind biopsies in evaluating a new treatment agent. Three-dimensional ultrasonographic image analysis methods developed for brachytherapy will also be valuable in directing the biopsy sampling that is required in definitive risk-reduction studies. New magnetic resonance imaging technologies and enhancing agents may not only improve resolution for staging and biopsy sampling but may point the way to noninvasive molecular spectroscopic evaluation of a specific lesion's risk of progression and/or its modulation by drug.

Treatment and Prevention of Superficial Bladder Tumors

Bladder cancer is the fourth most common cancer in United States males and in 2001 will account for 39,200 (6%) of new cancer cases and 8,300 (3%) of cancer-related deaths (40, 41). In United States females, the incidence is lower with 15,100 (2%) of new cases and 4,100 (<2%) of cancer deaths (40, 41). Of the 98% of these cancers that are confirmed histologically, 93% are TCCs (298, 299). Bladder cancers have been attributed to several factors including tobacco use and occupational exposure to aromatic amines; chronic inflammation and infection are also important etiological risk factors (299). In different regions of the world, carcinomas of the bladder exhibit varied histopathological features associated with diverse predisposing factors. For example, in contrast to the United States, SCC of the bladder, which arises in conjunction with endemic schistosomiasis, is the predominant pathological subtype in Egypt (298, 299). The association with environmental and lifestyle factors suggests that bladder cancer incidence may be modulated by chemopreventive agents. Perhaps as a result of the contribution of carcinogens to the pathogenesis of TCC, field cancerization appears to be quite common, with nearly one-half of all TCC cases having multiple tumor foci at initial presentation.

Invasive TCC are thought to arise from one of two precursor lesions, papillary TCC and TIS (298, 300, 301). Papillary transitional carcinomas, the most common bladder tumors, are characterized by papillae of varying sizes protruding into the bladder lumen, composed of several layers of urothelial cells. The urothelial cells exhibit a spectrum of cytological atypia and proliferative activity, with grade 1 lesions showing slight cytological atypia and few mitoses, grade 2 showing moderate cytological atypia and some mitoses, and grade 3 lesions showing marked nuclear atypia and frequent mitoses. Although experienced pathologists can distinguish papillary TCCs from most other papillary lesions in the bladder, grade 1 papillary TCCs can occasionally be difficult to differentiate from papillomas. Newer classification systems that categorize grade 1 lesions with little cytological atypia as papillary neoplasms of low malignant potential and those with somewhat more cytological atypia as low-grade papillary urothelial carcinoma are currently under consideration. TIS lesions that display many of the cytological features of grade 3 papillary TCCs appear as flat (rather than papillary) tumors. Lower grade flat lesions in the bladder, often termed mild dysplasia and moderate dysplasia, are also recognized. Both papillary TCC and TIS can be readily discriminated from invasive carcinoma, which is characterized by progressive penetration of cancer cells through the lamina

propria of the bladder urothelium into the underlying musculature. Superficial bladder cancers, *i.e.*, papillary TCC and TIS, account for ~75% of all initial presentations of bladder cancer, with invasive bladder cancers accounting for the remainder (298). Superficial and invasive bladder cancers often present with symptoms of hematuria or discomfort, prompting diagnosis, staging, and treatment (298). Superficial bladder cancers tend to be treated by transurethral resection, whereas invasive bladder cancers often require cystectomy (2).

Papillary TCC and TIS tend to recur after initial treatment with 50% recurring within 6–12 months of initial treatment, 60–75% within 2–5 years, and up to 85% over a lifetime (302–304). Recurrence rates increase with the severity of previous lesions, and recurrent lesions may have progressed in grade or type. After transurethral resection of papillary TCC, the risk of recurrence approaches 75%, but the risk of progression to muscle-invasive TCC is much lower. In contrast, as many as 50% of TIS ultimately progress to muscle-invasive cancers. An accumulation of somatic genome changes may underlie this clinical progression. Analyses of the genome alterations present in bladder tumors suggest that loss of chromosome 9 sequences, particularly at 9q21, the location of the *p14*, *p15*, and *p16* tumor suppressor genes, occurs in both superficial and muscle-invasive tumors, whereas loss of sequences at 3p, 11p, 17p, and 18q may occur as superficial bladder tumors and progress to malignant invasive cancers (305, 306). For superficial cancers, the stage, histological grade, tumor size, and multiplicity can all be used to predict risk of recurrence or progression (298). In addition, specific somatic genome alterations in superficial bladder tumors, such as loss of sequences at 17p accompanied by *p53* mutations, may serve as markers for an increased risk of progression (298, 305–307). A number of other candidate cellular and molecular risk markers have also been studied including Ki-67, PCNA, M344, γ-actin, EGFR, and autocrine motility factor receptor (305–310).

To reduce their risk of recurrence or progression after transurethral resection, many patients with superficial bladder cancers, particularly grade 3 papillary TCC and TIS, are treated with intravesical instillation of BCG, thiotepa, mitomycin C, Adriamycin, or other agents (298, 304). Intravesical BCG was approved by the FDA for treatment of TIS based on a study of 114 patients, which showed a significantly superior CR rate and median time to treatment failure with BCG compared with intravesical Adriamycin (311). These therapies have been shown to reduce recurrence rates in the near term by as much as 15–20%, but intravesical immunotherapy and chemotherapy are associated with significant cystitis in up to 30% of patients.

Clinical Trial Designs

Two patient populations with superficial bladder tumors have been targeted for evaluation of strategies for bladder cancer risk reduction. The first group is patients with lower grade papillary TCC that recur in up to 50% of the patients. These more indolent superficial tumors are generally not currently treated with intravesical immunotherapy or cytotoxic agents after transurethral resection. Nonetheless, because low-grade papillary TCC shares many epidemiological risk factors and somatic genome abnormalities with more aggressive muscle-invasive tumors, treatments that successfully reduce recurrence rates after resection may also reduce muscle-invasive bladder cancer incidence in populations at risk. The second cohort that may benefit from investigational bladder cancer risk-reduction strategies is patients with high-grade superficial bladder tumors, who have even higher risks of recurrence. For these more aggressive superficial tumors, treatment with new intervention agents are given after treatment with standard intravesical agents such as BCG; several such treatment trials are currently in progress (312–316). In both high- and low-risk patient cohorts, randomized, placebo-controlled clinical trials involving treatment with a new agent for 1–3 years with 200–300 patients/arm provide adequate power to evaluate the effectiveness of new interventions in keeping with standard of care. Primary efficacy endpoints include tumor recurrence, time-to-tumor recurrence, progression to muscle-invasive bladder cancer, and time-to-progression to muscle-invasive bladder cancer. New molecular markers may also be considered in these trials, such as PCR-detected DNA alterations in voided urine.

Papillary TCC and TIS are noninvasive precursors to muscle-invasive bladder cancer, are often symptomatic, and clearly merit treatment to reduce the risk of recurrence and/or progression. After transurethral resection, many patients with these lesions receive treatment with intravesical immunotherapy or chemotherapy as standard care to reduce these risks. Recurrence with progression may significantly diminish quality of life, because cystectomy may be required. Thus, safe and effective treatments that reduce the risk of recurrence of superficial bladder tumors will convey significant clinical benefit in these populations.

Discussion

The AACR Task Force on the Treatment and Prevention of Intraepithelial Neoplasia explored and delineated the relationship between IEN and cancer risk and the urgent need for more rapid development of effective interventions for the treatment and prevention of precancerous lesions. This first report is offered to stimulate debate about optimal high-risk populations, clinical trial designs, and efficacy endpoints that are practical and worth pursuing in the near term.

Why Target IEN for New Agent Development?

The Task Force's conclusions supporting the need for such interventions are based on several lines of evidence:

(*a*) Most importantly, development of cancer is a multistep process occurring over years to decades that offers ample opportunity for intervention, if precancer can be detected clinically.

(*b*) Conversion of normal tissue to invasive cancer is characterized by clinically, histologically, and genetically identifiable lesions, and such lesions are attractive targets for clinical intervention.

(*c*) Clinical experience demonstrates that treatment of precancerous lesions provides clinical benefit to the individual.

(*d*) Emerging technologies, both imaging and bioanalytical, will increase the number of such lesions identified, refine their definition, and increase the need for effective interventions.

(*e*) The process of carcinogenesis is a dynamic process that can be reversed or halted using pharmacological agents.

(*f*) The Task Force recognized that the complexity of conclusively demonstrating a reduction in the development of invasive cancer will, with few exceptions, require large and lengthy clinical trials that will delay the delivery of such agents to the public at large and that alternative, innovative trial designs endorsed by the medical and scientific community are required.

The purpose of this Task Force report is to provide a conceptual and practical basis for this discussion and to stimulate new ideas and refinement of these proposals in this complex and evolving field.

To dramatically reduce the incidence and mortality associated with invasive cancers, carcinogenesis must be viewed as a disease process that requires and is susceptible to treatment. The IEN Task Force defines precancer as a noninvasive lesion that has genetic abnormalities, loss of cellular control functions and some phenotypic characteristics of invasive cancer, and that predicts for a substantial likelihood of developing invasive cancer. To demonstrate effectiveness and patient benefit for new agents to treat IEN, the Task Force recommends targeting individuals with or at risk of developing precancerous lesions with moderate to severe dysplasia. Such lesions are close in stage of progression to invasive cancer and carry a substantial risk of developing invasive cancer.

Because high-grade IEN confers a substantially elevated risk for developing invasive cancer, it is standard medical practice to conduct routine surveillance examinations, including imaging and invasive interventions, to detect and surgically excise IEN. Examples of IEN that are established precancers and for which surgical extirpation is a standard-of-care include oral leukoplakia, colorectal adenomas, CIN 2/3, breast DCIS, bladder TIS, high-grade Barrett's dysplasia, and AK. To date, only five treatment agents have been approved by the FDA for the treatment of IEN: topical 5-FU and topical diclofenac for multiple AK; intravesical BCG for bladder CIS; tamoxifen for DCIS after lumpectomy and breast radiotherapy; and celecoxib for adenomatous polyps in patients with FAP. Although surgical excision of IEN is generally effective in eradicating clinically apparent lesions, these interventions can confer morbidity and do not treat the entire epithelial field at risk. A successful IEN treatment will reduce patients' risks of developing life-threatening invasive cancer, may reduce the need for possibly morbid and inconvenient endoscopies/surgeries, and will likely prevent the progression of subclinical IEN lesions in the epithelium at risk.

Demonstrating Clinical Effectiveness and Patient Benefit with a New IEN Treatment Agent

How can the effectiveness of a new IEN treatment agent be demonstrated convincingly? Foremost, in organ sites of IEN, the rate of progression to invasive cancer is too slow and too infrequent to support invasive cancer incidence as a practical efficacy endpoint. Furthermore, it is not possible to conduct the number of very large clinical trials that would be required to assess cancer incidence after treatment with the plethora of promising candidate treatment agents available currently. Clinical trials that measure eradication of IEN or prevention of recurrent IEN as the primary efficacy endpoint can be conducted with far fewer patient and time resources and can provide persuasive evidence of patient benefit. However, for such trials to provide convincing evidence of effectiveness, there must be a well-established link between the IEN and its associated invasive cancer, a reliable method of detecting and serially monitoring the IEN must exist, and a convincing reduction in IEN burden must be demonstrated.

The IEN Task Force identified several examples in which successful treatment of IEN would indicate clinical effectiveness and patient benefit, assuming an adequate safety profile of the new agent. Demonstration that an agent can convincingly reduce IEN burden and that this reduction is associated with the need for fewer surgical interventions provides strong evidence of effectiveness and patient benefit. Examples of IEN where fewer surgical interventions may be needed as a consequence of a substantial reduction in IEN burden include dysplastic Barrett's esophagus, oral and laryngeal premalignant lesions, CIN 2/3, breast DCIS, bladder TIS, and multiple AK.

In addition, eradication of clinically detectable high-risk IEN in a substantial proportion of patients, *e.g.,* complete responses in 20–30% of patients, with a new treatment agent would also provide evidence of effectiveness and patient benefit, regardless of whether the need for surgical intervention was decreased, because these patients' risk of developing invasive cancer would be substantially reduced. This example requires that a reliable method for serially monitoring the IEN be available to provide convincing evidence that the IEN was eradicated in 20–30% of patients. It also requires that the link between the IEN and invasive cancer is clear enough to conclude that eradication of clinically detectable IEN will result in reduced invasive cancer risk. Examples where the latter criterion are met include Barrett's esophagus, bladder TIS, CIN 2/3, bronchial dysplasia, oral or laryngeal dysplasia, colorectal adenomas, breast DCIS, cytological evidence of breast atypical hyperplasia in women with an elevated 5-year Gail risk or family history of breast cancer, and HGPIN.

At this time it is not clear whether a partial reduction in the burden of an IEN that is closely linked to invasive cancer such as Barrett's dysplasia, bronchial dysplasia, oral premalignant lesions, breast DCIS, or HGPIN will provide patient benefit by reducing the risk of invasive cancer. The IEN Task Force believes it is likely that partial reductions in high-grade IEN burden will result in a proportional reduction in invasive cancer risk because there are several examples where extent of precancer burden is believed to correlate with invasive cancer risk, *e.g.,* dysplastic nevi and melanoma (27), number of adenomatous polyps and ACF and colorectal cancer (49), length of the Barrett's column and esophageal cancer (317), and extent of oral premalignant lesions (104). However, a firm conclusion cannot be made presently that a partial reduction in precancer burden, without complete clinical eradication of the IEN in a proportion of patients, will result in reduced invasive cancer risk.

The IEN Task Force explored the relationship between the durability of the beneficial treatment effect of reduced IEN burden and patient benefit. For an agent with an excellent safety and tolerability profile, no minimal duration of benefit is required if the agent can be administered chronically to treat or prevent recurrence of IEN. Conversely, an agent that causes toxicity that limits its chronic administration, *e.g.,* topical 5-FU

and intravesical BCG, must be associated with a durable reduction in IEN burden to provide patient benefit.

Integrating a New IEN Treatment Agent into Standard Medical Care

In the final analysis, the overall clinical utility of a new IEN treatment option will be assessed in relationship to existing standard treatments on the basis of relative safety, efficacy, convenience and patient acceptance, and degree of utilization. For example, although colon polypectomy is highly effective in reducing invasive colon cancer risk, this life-saving intervention is used by <15% of the population that would benefit. A well-tolerated and accepted treatment agent that can prevent the recurrence of colon polyps in a proportion of patients may have substantial clinical utility, even if it is less effective than polypectomy in reducing IEN burden. It is clear that nonsurgical treatments for IEN must be developed, even if they are less effective than surgical extirpation, to provide nonsurgical treatment options to patients and to treat the entire epithelial sheet at risk.

A potential safety concern in treating and/or preventing IEN with a new agent is decreased utilization of established effective surveillance and surgical interventions such as colon polypectomy, cystoscopy, cervical loop excision, esophagectomy, or superficial ablative procedures. The IEN Task Force strongly emphasizes that new treatments for IEN should not alter standard IEN surveillance schedules without randomized trials of longer *versus* shorter surveillance and treatment intervals.

Remaining Challenges in Developing New IEN Treatment Agents

Several opportunities and challenges must be addressed to decrease the great unmet clinical need for safe and effective treatments for IEN: (*a*) IEN detection, quantitation, and serial monitoring methods that are consistent, reliable and broadly accessible are needed for the breast, prostate, and lung; (*b*) improved phenotypic and genotypic classifications of IEN are needed to identify individuals with high-risk IEN who require treatment and to select treatments; (*c*) it is critically important to educate the lay public, clinical investigators, the pharmaceutical industry, regulatory bodies, and legislators about the clinical implications of precancer, the need for surveillance and early detection of IEN, and the urgent need for support and participation in IEN treatment trials. Finally, an important practical issue to be considered is the composition and expertise of regulatory advisory committees that shepherd the development of new IEN treatment agents.

Conclusions

In summary, it is standard medical practice to conduct surveillance studies to detect and excise established precancer because reduction in IEN burden reduces invasive cancer risk. There is a great unmet clinical need for treatments for IEN that provide nonsurgical options to patients and that treat the entire epithelial field. A treatment agent that convincingly and substantially reduces IEN burden decreases invasive cancer risk and may decrease the need for surgical interventions. Individual patients as well as at-risk populations that underutilize existing surgical treatments for IEN will benefit from the development of convenient and safe treatments that reduce the burden of precancer. It is imperative that the pharmaceutical industry, lay advocates, clinical investigators, and regulatory committees aggressively develop new agents to treat IEN. It is time to enlarge the focus on treatment of invasive cancer to encompass the treatment and prevention of precancer.

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