Cancer chemoprevention rose and fell on precipitous waves before molecular-targeted research in colorectal neoplasia produced some of the most demoralizing and promising indications for the future of this field. In mid-1990, 2 large-scale, randomized, controlled trials (RCTs) showed that β-carotene actually harmed heavy smokers at risk of lung cancer. We learned in 1998 that tamoxifen reduced breast cancer risk by 50% and in 2003 that finasteride reduced prostate cancer risk by 25%. In both cases, adverse effects ended up overshadowing the positive effects, and neither agent was accepted for standard prevention. Getting back to colorectal research, 1999 results showed that a cyclooxygenase-2 (COX-2)-selective inhibitor (coxib), was significantly active in familial adenomatous polyposis (FAP). This was a seminal finding and promised a bright future for coxibs, which were thought to be safer than aspirin. Then in 2005, large, sporadic, adenoma-prevention RCTs found that celecoxib and rofecoxib had alarming cardiovascular toxicity. This result deepened concerns over the risk–benefit ratio of all chemopreventive agents, darkening the future of cancer chemoprevention despite beneficial preventive effects also evident in the coxib trials.

Events since 2005 have helped to lift the coxib-related pall of cardiovascular toxicity. Serious cardiovascular events subsided in the longer term after stopping celecoxib and apparently were not associated with a low baseline cardiovascular risk. Mixed data on aspirin were clarified by recent data confirming aspirin reductions in colorectal adenoma and cancer risks. A signal advance in combination chemoprevention came with findings that a low-dose, non-coxib combination reduced colorectal adenomas by 70% (>90% for advanced adenomas) with no significantly increased adverse events. New information on tamoxifen and finasteride has lessened concerns about the adverse effects of these active preventive agents. Helicobacter pylori antibiotics dramatically reduced stomach cancer risk. Last, raloxifene and human papilloma virus (HPV) vaccine reduced cancer risk, had acceptable side effects, and approved by the US Food and Drug Administration (FDA), with a good chance of widespread community acceptance for prevention.

The future of cancer chemoprevention depends on the continued development of molecular-targeted approaches. This commentary discusses highlights of the biology of preventive molecular targeting, cancer risk modeling, and trials of molecular-targeted agents, all moving toward the development of personalized preventive medicine.

Biology of Molecular-Targeted Cancer Prevention

Chemoprevention began with the fundamental premise that it is possible to intervene within multistep carcinogenesis to prevent the step of invasion. Early prevention biology gave potentially preventive agents unfocused rationales for activity within early, middle, or late stages of multistep premalignancy. Evolving molecular biology has advanced the development of agents capable of targeting specific, rate-limiting events within multistep carcinogenesis. Much of the impetus for molecular-targeted chemoprevention came from pioneering biological studies of COX-2, and COX-2 study in FAP is a paradigm for translating targeted research in rare germline disorders to the sporadic setting. FAP is characterized by germline APC mutations, which are associated with increased COX-2 levels. Most sporadic adenomas have acquired APC mutations, which also are associated with increased COX-2 levels; therefore, COX-2 targeting in FAP is relevant to sporadic adenoma prevention (celecoxib and rofecoxib are active in both settings).

This translation of germline to sporadic biology in the colon–rectum is being extended to other settings such as germline and sporadic vhl mutations in renal cancer development. Cardiovascular toxicity of COX-2 inhibitors has stimulated increased interest in targeting events downstream of COX-2 or related pathways, including E-prostanoid (EP) receptors, prostacyclin, 15-LOX-1, peroxisome proliferator-activated receptors, 15-hydroxyprostaglandin dehydrogenase, and the prostaglandin transporter in the colon-rectum, esophagus, lung, and other sites.

Other areas of biology vital to molecular-targeted chemoprevention include epidermal growth factor receptor (EGFR) signaling, the PI3K/Akt/mammalian target of rapamycin pathway, cMET, bronchioalveolar stem cells (comprising abnormalities in KRAS, Pten, PI3K and cyclin-dependent kinase pathways), and...
mokines and the microenvironment\textsuperscript{16,19} epigenetics,\textsuperscript{20} and promising combinations of molecular-targeted agents. Combined targeting of COX and ornithine decarboxylase (ODC) is highly active in preventing mouse and human colorectal neoplasia, and combined targeting of COX and EGFR is very promising in preventing mouse colorectal neoplasia.\textsuperscript{21,22}

\textbf{Cancer Risk Models}

Prevention RCTs with the slow-developing, definitive end point of cancer can have extremely large sample sizes, durations, and costs. A major dilemma has been a lack of surrogate end points (including premalignant lesions and molecular markers that reliably predict cancer) or suitable, high-risk populations, which could substantially reduce RCT logistics.\textsuperscript{23,24} Many potential surrogate end points have been studied, but none established.\textsuperscript{25} The state of the risk-modeling art has yet to reliably identify truly high-risk populations; most known higher risk populations have a somewhat elevated but still relatively low cancer risk. Fortunately, novel risk modeling with molecular risk factors integrated with clinical and classical epidemiologic factors is moving forward at a rapid pace, and promises to break through the barrier to high-risk identification and lead to substantial breakthroughs (including smaller and shorter definitive trials) in clinical prevention as well (Figure 1).

Risk models based on clinical/classical epidemiologic factors have been developed for several sites and can include established precursor lesions defined by clinical/histologic criteria.\textsuperscript{26–28} Such models are being improved by the addition of specific molecular alterations that drive carcinogenesis. For example, in the precursor Barrett esophagus (a well-established but modest predictor of absolute cancer risk), a striking model incorporating a chromosome instability panel of loss of heterozygosity (LOH) and DNA content profiles distinguished between individuals at a high (79\% in 6 years) and low (0\% in >6 years) cancer risk.\textsuperscript{29} LOH profiles (eg, at specific loci in chromosomes 3p and/or 9p)\textsuperscript{30,31} substantially increase the relatively low oral cancer risk of oral leukoplakia,\textsuperscript{32–34} especially in patients with a previously treated oral cancer.\textsuperscript{35} The expression of biomarkers indicating an abrogated response to cellular stress predicts a worse outcome for breast ductal carcinoma in situ (DCIS) patients.\textsuperscript{36} A panel of methylation markers in sputum marked a high lung cancer risk in chronic smokers.\textsuperscript{37} Germline genetic variations (eg, in the lung and prostate)\textsuperscript{38,39} also are promising risk factors for multidimensional models. Recently published lung cancer risk models integrating genomic (somatic gene expression arrays and host DNA repair capacity) and clinical features were more accurate than were clinical models alone.\textsuperscript{40,41} Cyclin D1 genotype and expression are associated with a high cancer risk in patients with dysplastic head and neck premalignant lesions.\textsuperscript{42} Important molecular risk models are also being designed for the adjuvant setting.\textsuperscript{43}

Many molecular risk factors not only improve the accuracy of traditional risk models, but also are steps of carcinogenesis potentially targeted by chemoprevention. Cyclin D1 is a case in point; although no direct cyclin D1 inhibitors are yet in clinical use, several agents inhibit targets (eg, EGFR and mammalian target of rapamycin) upstream of, and thus ultimately could down-regulate, cyclin D1. One such agent, erlotinib, is being tested in a Phase III oral cancer prevention trial in oral leukoplakia patients selected for a high risk owing to the LOH profile mentioned.\textsuperscript{44} A Phase II trial of erlotinib is ongoing in intraductal papillary mucinous neoplasms, which are driven by EGFR signaling (Figure 1).\textsuperscript{45,46}

\textbf{Colorectal Neoplasia and Prevention}

Several COX inhibitors have been studied in RCTs to prevent colorectal adenomas.\textsuperscript{3} Sulindac and celecoxib are effective treatments for adenomas in FAP patients.\textsuperscript{6,47} The COX inhibitor aspirin significantly reduced sporadic adenoma risk in 3 relatively short-term RCTs published in 2003.\textsuperscript{48–50} Although generally positive, these RCTs were complex, difficult to interpret, and did not lead to the acceptance of aspirin for reducing the risk of sporadic adenomas. Further complicating the interpretation of aspirin, the Physician’s Health and Women’s Health studies found no protective effect of aspirin on colorectal cancer risk. New 2008 data from the United Kingdom Colorectal Adenoma Prevention study\textsuperscript{51} have helped to clarify the mixed results of the previous RCTs by demonstrating that aspirin significantly reduced adenoma risk by 21\%. Furthermore, recent pooled analyses of the British Doctors Aspirin Trial and the United Kingdom Transient Ischaemic Attack Aspirin Trial found that aspirin was associated with a significant 26\% overall reduction in colorectal cancer risk; the reduction was greatest in people treated with ≥300 mg/d for ≥5 years and did not appear before 10 years.\textsuperscript{52} These pooled results are consistent with those of a recent large cohort study from the Health Professionals Followup Study.\textsuperscript{53}

Interim cardiovascular event rates were unexpectedly increased in 2 of 3 RCTs, the Adenomatous Polypl Prevention on Vioxx (APPROVe) and Adenoma Prevention with Celecoxib (APC) trials but not a third, the Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP) trial, of selective COX-2 inhibitors (vs placebo) in preventing sporadic adenomas.\textsuperscript{54–56} All 3 RCTs were stopped early, and rofecoxib was withdrawn from the world market by the manufacturer because of this serious safety issue, despite significant coxib reductions in adenomas in the trials. These developments cast a dark shadow over cancer prevention. A recent extension anal-
Mini-Reviews and Perspectives continued

A

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Months 0 36 Up to 144

B

Real-time molecular risk assessment

Before dissection

After dissection

Lesion dissection DNA extraction

PCR amplification (microsatellites)

DNA fragment separation and quantification

Patient eligibility determined within 7 business days

7 Days
ysis of APC patients suggested that the serious cardiovascular event rate wore off 2 years after stopping celecoxib and that the suppression of adenomas persisted (albeit at a reduced level), particularly for a dose of 200 mg BID in advanced adenomas.\textsuperscript{37} A recent pooled analysis of the major RCTs of celecoxib in nonarthritis diseases suggested that there was no increase in serious cardiovascular events at any dose (up to 400 mg BID) in people with a low baseline cardiovascular risk.\textsuperscript{38} In PreSAP, celecoxib at 1 daily dose of 400 mg did not significantly increase cardiovascular toxicity.

An RCT of combined difluoromethylornithine (DFMO, which targets ODC) and sulindac significantly reduced colorectal adenoma risk by 70\% and >90\% for advanced or multiple adenomas.\textsuperscript{21} Low doses of each agent were developed through preclinical testing and, in case of DFMO, in novel clinical de-escalation trials. Serious adverse event (grade ≥3) rates and hearing changes (associated with DFMO) were not significantly different between the combination and placebo. This trial heralds the bright future of combination chemoprevention for enhancing the activity while reducing the toxicity of the active constituent agents.\textsuperscript{59}

A colorectal prevention study of aberrant crypt foci (ACF)\textsuperscript{60} bears closely on the dilemma of surrogate end points, which (as mentioned earlier in Cancer Risk Models) are not yet established, but potentially could reduce the size, duration, and cost of definitive prevention RCTs. Evidence that dysplastic ACF are the earliest clonal precursor of colorectal cancer supported ACF as a potential surrogate end point for drug development in mice and humans. Unfortunately, a randomized, placebo-controlled, prespecified substudy of the APC trial found that dysplastic ACF were extremely rare in sporadic adenoma patients and that nondysplastic ACF were not a useful marker of synchronous or advanced adenomas or of celecoxib activity. The only placebo-controlled analysis of ACF reported to date, this study substantially dampened the hope that ACF would be validated as a surrogate end point for sporadic colorectal adenomas and thus cancer in clinical trials.\textsuperscript{61}

\textbf{Risk/Benefit Lessons From Prevention in the Breast and Prostate}

The selective estrogen-receptor modulator tamoxifen and 5-α-reductase inhibitor finasteride confront the same risk–benefit dilemma faced by celecoxib in colorectal adenoma prevention. Each agent produced a highly significant reduction in breast or prostate cancer risk,\textsuperscript{62,63} but did not change the standard of care, largely because of serious actual or perceived adverse drug effects. Recent analyses of tamoxifen and finasteride have cast a new perspective on their value in prevention.

The opposing tamoxifen effects of reducing breast cancer risk and, primarily, increasing endometrial cancer risk have made tamoxifen’s use a complex, highly individualized decision at best,\textsuperscript{64} and more often removed it from consideration in women at a higher risk but without a history of invasive or in situ breast cancer. Long-term follow-up studies of the RCTs in these women suggest that the beneficial tamoxifen effects on breast cancer risk persist for ≥10 years, but most side effects resolved after the 5-year treatment period, including all serious adverse events such as thrombotic events and endometrial cancer.\textsuperscript{65,66} Tamoxifen reduced the 5-year incidence of breast cancer by 40\% (vs placebo) after resection and radiation of DCIS.\textsuperscript{67} In this setting, tamoxifen is an accepted standard of care, notwithstanding known side effects, because in general women want to reduce their risk of DCIS recurrence and the attendant implications for invasive breast cancer. Adjuvant tamoxifen also reduces the risk of contralateral breast cancer, including in sporadic\textsuperscript{64} and germline (BRCA-1 and BRCA-2 mutation carrying)\textsuperscript{68} cancer patients.

\textbf{Figure.} Personalized cancer chemoprevention. (A) The personalized trial (top) highlights the role of molecular targeting in risk assessment, intervention, and risk–benefit results. Striking contrasts between this trial and the nonpersonalized one depicted below it are its sample size of 150 patients (vs up to 35,000), duration of 36 months (vs up to 144), and relative risk (RR) results of 0.1 for cancer (vs 0.75 or 0.5) and 1.0 for serious adverse event(s) (SAE; vs 1.25 or 2.5). These comparative data are extrapolated from or based on actual studies and trials. Examples of each step (shown below the line within the boxes) are now being implemented or developed for implementation in clinical trials (and are described in the text). Steps 1, 2, and 3 select patients with the highest cancer risk, low adverse event risk, and high drug sensitivity. Step 4 reflects an ongoing personalized trial, the Erlotinib Prevention of Oral Cancer (EPOC) trial, which is randomizing 150 oral IEN patients at a high risk of oral cancer to receive either erlotinib, which targets the EGFR, or placebo.\textsuperscript{44} Monitoring in step 5 could lead to offering early treatment stoppage to patients with biomarker indications predicting that they likely will benefit and/or likely will experience ≥1 SAEs. Although step 6 results may seem dramatic, they do not differ radically from results of a recently completed colorectal adenoma prevention trial of combined sulindac and DFMO, which is discussed in the text. The trial duration (36 months) noted below step 6 is based on the EPOC design. The nonpersonalized trial (bottom) reflects data from ongoing or completed trials. The step 2 figure of 35,000 men reflects accrual to the ongoing Selenium and Vitamin E [prostate] Cancer Prevention Trial (SELECT),\textsuperscript{46} which also provided the timeline noted below step 3 (up to 144 months). The RRs in step 3 reflect actual data from the completed PCPT (0.75 RR of prostate cancer overall and 1.25 RR of the SAE high-grade prostate cancer) and Breast Cancer Prevention Trial (BCPT; 0.5 RR of breast cancer and 2.53 RR of the SAE endometrial cancer).\textsuperscript{62,63} (B) The sequence shown in this panel represents the real-time molecular risk profiling being used to select high-risk oral IEN patients for the ongoing EPOC trial. \textit{Abbreviations:} CV, cardiovascular; LOH, loss of heterozygosity; ODC, ornithine decarboxylase; SNP, single-nucleotide polymorphism.
The Study of Tamoxifen and Raloxifene found that raloxifene was similar to tamoxifen in reducing the risk of invasive breast cancer and produced fewer cases of uterine cancer, thromboembolic events, and cataracts. Effects on noninvasive breast cancer, however, favored tamoxifen. Raloxifene was recently approved by the FDA for invasive breast cancer risk reduction in postmenopausal women at a high risk or with osteoporosis, and represents a major advance in improving risk–benefit ratios of chemopreventive agents. With promise shown in adjuvant trials, the aromatase inhibitor anastrozole may have a more favorable risk–benefit ratio than raloxifene or tamoxifen and is in RCTs in the settings of DCIS and higher risk women. Tamoxifen, raloxifene, and aromatase inhibitors are only active against estrogen receptor–positive breast cancer, and a major thrust of breast cancer prevention is the development of agents such as RXR-selective retinoids and inhibitors of COX-2, EGFR, and HER-2 for preventing estrogen receptor–negative breast cancer.

In the large-scale Prostate Cancer Prevention Trial (PCPT), the 5-α-reductase inhibitor finasteride reduced the risks of prostate cancer overall, high-grade prostatic intraepithelial neoplasia and benign prostatic hypertrophy, but also increased the risk of sexual functioning side effects and, apparently, high-grade prostate cancer (vs placebo). A recent analysis of the sexual functioning side effects showed them to be minimal. The adverse finding of increased high-grade prostate cancer has sharply limited public interest in finasteride for prostate cancer prevention and, coupled with the suggestion that finasteride reduced mainly clinically insignificant cancer, led to several assessments of whether these unfavorable effects actually occurred. The concern about insignificant cancer arose from rigorous screening (prostate-specific antigen [PSA]/digital rectal examination) and the detection of a substantial number of cancers associated with PSA <4.0 ng/mL in the PCPT.

The primary end point of the PCPT was biopsy-detected prostate cancer. Finasteride biases toward improved prostate cancer detection and accuracy in prostate cancer grading at biopsy. A multifaceted statistical modeling analysis accounting for these and other biopsy biases and incorporating prostatectomy data found no increased actual risk of high-grade disease and also showed a trend of reduced high-grade cancer risk with finasteride across a plausible range of biopsy-sensitivity values (greater detection in finasteride vs placebo). This statistical modeling report was consistent with an earlier, rigorous pathology analysis based on “gold standard” grading of radical prostatectomy samples from PCPT men; this analysis suggested that finasteride effects on prostate volume and selective inhibition of low-grade cancer contributed to the initially reported increase in high-grade cancers with finasteride and that high-grade cancer was detected earlier and was less extensive on finasteride (vs placebo) in the PCPT.

The concern that finasteride prevented a substantial proportion of biologically inconsequential tumors was examined in biopsy specimens stratified by level of PSA for men in the placebo group who developed cancer. Tumor pathology characteristics showed that 75% of all cancers and 62% of Gleason score ≤6 cancers in the PCPT were clinically significant, with risks of insignificant disease decreasing from 51.7% (PSA, 0–1.0 ng/mL) to 17.8% (PSA, 2.6–4.0 ng/mL) and concomitantly increasing risks of high-grade disease. These findings indicate that finasteride prevented clinically significant prostate cancer, including Gleason score ≤6 cancer. The statistical modeling and pathology analyses support the conclusion of these and other investigators that finasteride is an effective prevention option that should be offered to men at risk for prostate cancer.

Vaccines and Infection-Related Cancers

Vaccines against the infections hepatitis B (to prevent hepatocellular carcinoma) and HPV (to prevent cancers of the cervix, vulva, and vagina) are striking successes of targeted prevention. The hepatitis B and HPV vaccines generate immune responses against the specific protein hepatitis B surface antigen or L1 HPV viral capsid protein, respectively. Molecular targeting through immunization against infections related to neoplasia prevents early steps of host cell damage that otherwise can lead to cancer. As reported >10 years ago, immunizing children against hepatitis B dramatically reduced the incidence and mortality of liver cancer in Taiwan. HPV infection is a major risk factor for cervical cancer and other gynecologic diseases. The results of several recent large-scale RCTs of HPV vaccines in girls and young women include a 100% reduction in the coprimary end points of genital warts, vulvar or vaginal intraepithelial neoplasia (IEN) or cancer, and cervical IEN, adenocarcinoma in situ, or cancer. These RCTs led to the FDA approval of an HPV vaccine for reducing the risk of cervical cancer, adenocarcinoma in situ, and high-grade IEN and vulvar and vaginal IEN in girls and women from 9 to 26 years old. HPV vaccines do not accelerate HPV clearance and so are unlikely to prevent cancer in already infected patients. HPV also is an important risk factor for oropharyngeal cancer, which thus may be prevented by the vaccine. Preclinical and observational data suggest that vaccines against tumor antigens may be an effective preventive strategy.

*H pylori* is the major worldwide cause of stomach cancer, and a recent RCT demonstrated that *H pylori* eradication decreased the incidence of metachronous gastric carcinoma after endoscopic resection of early gastric

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cancer. Efforts in this setting, however, illustrate the complexity of controlling infection-related cancers. Eradicating \textit{H pylori} not only decreases stomach cancer, but may increase esophageal adenocarcinoma.

### Conclusions and Future Directions

Although the ups and downs of chemoprevention certainly will continue, as in cancer therapy, the recent string of solid gains involving raloxifene, HPV vaccine, \textit{H pylori} treatment, and recent studies of celecoxib, aspirin, finasteride, and tamoxifen has had a steadying effect on clinical cancer chemoprevention and secured its future. Our understanding of cancer risk and preinvasive neoplasm is advancing with translational studies of cyclin D1 genotype and expression, LOH, and many other molecular factors and processes. Extension studies of the tamoxifen and celecoxib prevention trials are better defining risk–benefit profiles and optimal durations of treatment with these drugs. Personalized approaches to identifying patients most likely to benefit and least likely to be harmed by aspirin and celecoxib are evolving from continued study of these agents in colorectal neoplasia showing, for example, that an \textit{ODC} single-nucleotide polymorphism may mark patients most likely to benefit from aspirin and a low baseline risk of cardiovascular events marks patients least likely to be harmed by celecoxib (Figure).

Future RCTs should be designed to personalize patient selection (Figure) for greatest potential benefit and least potential harm and to detect efficacy early (as did the APC trial in finding celecoxib efficacy at 1 year [similar to that at 3 years]). The strong colorectal adenoma results of the DFMO-sulindac trial discussed earlier show that combinations to increase the ratio of benefit (activity) to risk (toxicity) for effective single agents may be at the threshold of standard clinical reality and strongly support moving other active combinations into clinical trials. With reductions of 70% overall and >90% in advanced or multiple adenomas, this trial supports designing future RCTs for a \(\geq 50\%\) preventive effect. Perhaps with molecular-targeted combinations leading the way, the future of cancer chemoprevention may hold advances in molecular biology, cancer risk modeling, and clinical medicine that will substantially lessen the burden of cancer.

### References


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