Supplementary on-line only

Appendix 1: Example of Regulatory Approvals for Direct Targeted Risk Reduction Drugs (A2-A8)

Appendix 2: Example of Cancer Risk Reduction by Immunologic Agents (A9-A14)

Appendix 3: A*n Updated* Clinical Developmental Pathway to Regulatory Approval, Some Practical Guidelines (A15-A19)

Appendix 4: *Biomarker Issues in Cancer Risk Reduction Development and Therapeutic Index (A20-A22)*

Appendix 1: Example of Regulatory Approvals for Direct Targeted Risk Reduction Drugs

The Example of Celecoxib for Colon Cancer (from results of a single phase 2b trial)

Celecoxib has a known molecular target (COX-2). Non-COX targets may also contribute to the effect. In 1999, FDA granted accelerated marketing approval for Celecoxib "to reduce the number of adenomatous colorectal polyps in familial adenomatous polyposis (FAP) as an adjunct to usual care" (FDA, December 23, 1999). The rationale for testing a specific COX-2 inhibitor in FAP is based on mechanistic data and preclinical efficacy studies, as well as epidemiologic and clinical intervention investigations (for reviews, see (1 - 3). This supporting evidence includes the observation that COX isoenzymes are overexpressed in colorectal adenomas and tumors (4 -12). Targeted deletion of the COX-2 gene prevents colorectal cancer in animals, and celecoxib as well as other selective COX-2 inhibitors (*e.g.*, JTE-522, NS-398, MF tricyclic, nimesulide, and rofecoxib) are also effective in preclinical models (13-20). In addition, substantial epidemiologic evidence supports a 40–50% protective effect of NSAIDs (primarily aspirin) against colorectal carcinogenesis (see [21-26] and more than 30 additional studies reviewed in [25, 27, 28]). A number of studies have also shown that the NSAID sulindac regresses adenomas in FAP patients (reviewed in [29]). In a study of 21 FAP patients, rofecoxib (25 mg qd for nine months) modestly reduced rectal polyp number (9.9% decrease) and size (21.7% decrease) (30).

The Subpart H approval was based on a randomized, double-blind, placebo-controlled study of 83 FAP patients in which 400 mg bid celecoxib for six months significantly reduced adenoma number by 28% (P=0.003 compared to the 4.5% reduction with placebo) (29). In addition, this celecoxib dose significantly reduced adenoma burden (the sum of adenoma diameters) by 30.7% (P=0.001 compared with the 4.9% reduction with placebo). In patients receiving 100 mg bid celecoxib, reductions in adenoma number and burden were 11.9% and 14.6% (P=0.33 and P=0.09 for the comparison with placebo, respectively). A blinded physicians' assessment indicated a qualitative improvement in the colon and rectum, and to a lesser extent in the duodenum, of treated subjects (13).

Post-marketing studies to show the clinical benefit of celecoxib in FAP are ongoing. These include a two-part phase 2 study to evaluate safety and FAP phenotype

suppression in genotype-positive children, and a phase 2 study to determine whether greater efficacy against adenomas and other manifestations of FAP can be achieved by combination of celecoxib with the antiproliferative agent 2-difluoromethylornithine. A separate phase 2 study is evaluating the biomarker-based efficacy of celecoxib in hereditary non-polyposis colorectal cancer patients, a cohort in whom no data are yet available regarding the effect (adverse or beneficial) of any potential colorectal cancer chemopreventive agent.

The prevalence of the disease FAP clearly falls under the required maximum prevalence of 200,000 to qualify for orphan disease status and remains of high interest for those developing drugs and seeking Orphan Drug status. However, the developers of celecoxib did not apply for Orphan status for celecoxib in FAP patients, probably because celecoxib was already approved for the labeled indication for FAP patients and there was every expectation that it would be approved for preventing sporadic adenomas. It should be clear that Orphan Drug status only provides exclusivity for the developer (for a defined period), and does not provide an approval to market a drug for an approved indication. This approval process still requires the developer to establish acceptable safety and efficacy in a defined population for the drug to be marketed.

This positive result in patients with FAP supported the hypothesis that celecoxib would be effective in preventing sporadic adenomas and provided important information for selecting potentially effective doses (400 and 800 mg/day). New adenomas occur within one to three years post-resection in approximately 30% of patients with sporadic colorectal adenomas (or cancers) . These patients are screened routinely at one- to five-year intervals, undergoing colonoscopies with removal of clinically apparent new lesions. Prevention and/or regression of subclinical adenomas and hyperplastic colorectal mucosa reduce the development of clinically apparent adenomas and supplements clinical benefit obtained by screening (which routinely results in missed polyps). In 2002, the FDA Gastrointestinal Drugs Advisory Committee estimated that clinical effectiveness could be shown by a statistically meaningful 35% reduction in adenoma incidence, or a 15–20% increase in patients without adenomas, compared with placebo three years after initial polypectomy. Successful treatment of this at-risk epithelium could also potentially provide benefit by increasing the screening interval, thereby decreasing associated morbidity and lowering health care costs. For example, adenoma reduction would include

a 25–50% reduction in colonoscopy complications (which are primarily associated with polypectomy). Risks of perforation on colonoscopy and sigmoidoscopy are estimated at 1.96 and 0.88 per 1,000 procedures, respectively (31). Perforation is, in turn, associated with an approximately nine-fold increased risk of death from either procedure.

Results of two celecoxib studies, both showing significant efficacy, were published recently. In the Adenoma Prevention with Celecoxib (APC) study (23), adenoma incidence at three years was reduced to 43.2% in patients receiving 400 mg/day celecoxib and 35.7% in patients treated with 800 mg/day, compared with 60.7% in the placebo group. The similar Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP) study (24) found adenoma incidence at three years was reduced to 33.6% in the celecoxib group compared with 49.3% in the placebo group. As with selective estrogen receptor modulators (SERMs), chronic safety concerns are challenging the potential utility of selective COX-2 inhibitors (32, 33). Both studies showed > 2-fold increased risk of cardiovascular events (including myocardial infarction, stroke, and heart failure), and a trend to dose-related increases in these events and to increased blood pressure (32). Further analyses are on-going to identify subpopulations at reduced cardiovascular risk and to assess the efficacy of dose-reducing regimens.

The Example of Raloxifene in Breast Cancer (from serial phase 3 trial results)

The FDA approved the SERM tamoxifen for reducing breast cancer risk in 1999 based on results of the Breast Cancer Prevention Trial, a randomized, placebo-controlled clinical trial in 13,388 women 35–70 years old, with risk greater than or equal to that of a 60-year old without breast cancer (35, 36). This trial was well designed, with a strong scientific hypothesis based on the role of estrogen signaling in breast cancer, and the already approved use of the drug as a treatment for late stage breast cancer and as an adjuvant treatment for preventing second breast cancers or cancer recurrence. Despite significantly reduced risks of breast cancer and ductal carcinoma *in situ* (DCIS), physicians and women in the target population have not subsequently embraced tamoxifen. For example, among 350 primary care physicians surveyed in the US, only 96 (27.4%) prescribed tamoxifen for breast cancer prevention at least once within a year of the survey (36). For many women, potential side effects such as uterine cancer, thromboembolic events and cataracts, as well as quality of life issues such as hot flashes

and weight gain, outweigh what appears to be a small reduction in cancer risk. In one study, only two (4.7%) of 43 eligible women elected to take tamoxifen for prevention (38). In a follow-on study in > 19,000 postmenopausal women with an increased risk of developing breast cancer within five-years, another SERM, raloxifene, was equivalent to Tamoxifen in reducing breast cancer incidence, but showed fewer side effects (39). However, raloxifene did not reduce the incidence of DCIS for reasons that are not yet clear.

The sponsor filed for and obtained Orphan Drug status for raloxifene before its NDA for the labeled indication was approved, as is the usual sequence for a sponsor seeking Orphan Drug approval. The Orphan status was approved presumably because the sponsor was able to demonstrate reasonable expectation that the cost of developing and making raloxifene available for women at high risk of developing breast cancer would not be recovered by sales in the U.S.

References

1. Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer 2001;1:11–21.
2. Howe LR, Dannenberg AJ. A role for cyclooxygenase-2 inhibitors in the prevention and treatment of cancer. Semin Oncol 2002;29:111–9.
3. Hawk ET, Viner JL, Umar A, Anderson WF, Sigman C, Guyton KZ. Cancer and the cyclooxygenase enzyme - Implications for prevention and treatment. Am J Cancer 2003;2:27–55.
4. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994;107:1183–8.
5. Kargman SL, O'Neill GP, Vickers PJ, Evans JF, Mancini JA, Jothy S. Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer. Cancer Res 1995;55:2556–9.
6. Kutchera W, Jones DA, Matsunami N, Groden J, McIntyre TM, Zimmerman GA, White RL, Prescott SM. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect. Proc Natl Acad Sci U S A 1996;93:4816–20.
7. Gustafson-Svard C, Lilja I, Hallbook O, Sjodahl R. Cyclooxygenase-1 and cyclooxygenase-2 gene expression in human colorectal adenocarcinomas and in azoxymethane induced colonic tumours in rats. Gut 1996;38:79–84.
8. Fujita T, Matsui M, Takaku K, Uetake H, Ichikawa W, Taketo MM, Sugihara K. Size- and invasion-dependent increase in cyclooxygenase 2 levels in human colorectal carcinomas. Cancer Res1998;58:4823–6.
9. Maekawa M, Sugano K, Sano H, Miyazaki S, Ushiama M, Fujita S, Gotoda T, Yokota T, Ohkura H, Kakizoe T, Sekiya T. Increased expression of cyclooxygenase-2 to -1 in human colorectal cancers and adenomas, but not in hyperplastic polyps. Jpn J Clin Oncol 1998;28:421–6.
10. Sheehan KM, Sheahan K, O'Donoghue DP, MacSweeney F, Conroy RM, Fitzgerald DJ, Murray FE. The relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA 1999;282:1254–7.
11. Dimberg J, Samuelsson A, Hugander A, Soderkvist P. Differential expression of cyclooxygenase 2 in human colorectal cancer. Gut 1999;45:730–2.
12. Tomozawa S, Tsuno NH, Sunami E, Hatano K, Kitayama J, Osada T, Saito S, Tsuruo T, Shibata Y, Nagawa H. Cyclooxygenase-2 overexpression correlates with tumour recurrence, especially haematogenous metastasis, of colorectal cancer. Br J Cancer 2000;83:324–8.
13. Reddy BS, Rao CV, Seibert K. Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res 1996;56:4566–9.
14. Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res 1998;58:409–12.

15. Jacoby RF, Seibert K, Cole CE, Kelloff G, Lubet RA. The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. Cancer Res 2000;60:5040–4.
16. Sasai H, Masaki M, Wakitani K. Suppression of polypogenesis in a new mouse strain with a truncated Apc(Delta474) by a novel COX-2 inhibitor, JTE-522. Carcinogenesis 2000;21:953–8.
17. Yoshimi N, Shimizu M, Matsunaga K, Yamada Y, Fujii K, Hara A, Mori H. Chemopreventive effect of N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide (NS-398), a selective cyclooxygenase-2 inhibitor, in rat colon carcinogenesis induced by azoxymethane. Jpn J Cancer Res 1999;90: 406–12.
18. Fukutake M, Nakatsugi S, Isoi T, Takahashi M, Ohta T, Mamiya S, Taniguchi Y, Sato H, Fukuda K, Sugimura T, Wakabayashi K. Suppressive effects of nimesulide, a selective inhibitor of cyclooxygenase-2, on azoxymethane-induced colon carcinogenesis in mice. Carcinogenesis 1998;19:1939–42.
19. Oshima M, Murai N, Kargman S, Arguello M, Luk P, Kwong E, Taketo MM, Evans JF. Chemoprevention of intestinal polyposis in the Apcdelta716 mouse by rofecoxib, a specific cyclooxygenase-2 inhibitor. Cancer Res 2001;61:1733–40.
20. Lal G, Ash C, Hay K, Redston M, Kwong E, Hancock B, Mak T, Kargman S, Evans JF, Gallinger S. Suppression of intestinal polyps in Msh2-deficient and non-Msh2-deficient multiple intestinal neoplasia mice by a specific cyclooxygenase-2 inhibitor and by a dual cyclooxygenase-1/2 inhibitor. Cancer Res 2001;61:6131–6.
21. Kune GA, Kune S, Watson LF. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Res 1988;48:4399–404.
22. Thun MJ, Namboodiri MM, Heath CW, Jr. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 1991;325:1593–6.
23. Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW, Jr. Aspirin use and risk of fatal cancer. Cancer Res 1993;53:1322–7.
24. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann Intern Med 1994;121:241–6.
25. Giovannucci E, Egan KM, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC, Speizer FE. Aspirin and the risk of colorectal cancer in women. N Engl J Med 1995;333:609–14.
26. Garcia-Rodriguez LA, Huerta-Alvarez C. Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. Epidemiology 2001;12:88–93.
27. Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 2002;94:252–66.
28. Hawk ET, Viner JL, Umar A. Non-steroidal anti-inflammatory and cyclooxygenase-2-selective inhibitors in clinical cancer prevention trials. Prog Exp Tumor Res 2003;37:210–42.

29. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK, Levin B. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000;342:1946–52.
30. Higuchi T, Iwama T, Yoshinaga K, Toyooka M, Taketo MM, Sugihara K. A randomized, double-blind, placebo-controlled trial of the effects of rofecoxib, a selective cyclooxygenase-2 inhibitor, on rectal polyps in familial adenomatous polyposis patients. Clin Cancer Res 2003;9:4756–60.
31. Gatto NM, Frucht H, Sundararajan V, Jacobson JS, Grann VR, Neugut AI. Risk of perforation after colonoscopy and sigmoidoscopy: a population-based study. J Natl Cancer Inst 2003;95:230–6.
32. Solomon SD, Pfeffer MA, McMurray JJ, Fowler R, Finn P, Levin B, Eagle C, Hawk E, Lechuga M, Zauber AG, Bertagnolli MM, Arber N, Wittes J. Effect of celecoxib on cardiovascular events and blood pressure in two trials for the prevention of colorectal adenomas. Circulation 2006;114:1028–35.
33. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, Konstam MA, Baron JA. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med 2005;352:1092–102.
34. Jensen EV, Jordan VC. The estrogen receptor: a model for molecular medicine. Clin Cancer Res 2003;9:1980–9.
35. Armstrong K, Quistberg DA, Micco E, Domchek S, Guerra C. Prescription of tamoxifen for breast cancer prevention by primary care physicians. Arch Intern Med 2006;166:2260–5.
36. Port ER, Montgomery LL, Heerdt AS, Borgen PI. Patient reluctance toward tamoxifen use for breast cancer primary prevention. Ann Surg Oncol 2001;8:580–5.
37. Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, Bevers TB, Fehrenbacher L, Pajon ER, Jr., Wade JL, 3rd, Robidoux A, Margolese RG, James J, Lippman SM, Runowicz CD, Ganz PA, Reis SE, McCaskill-Stevens W, Ford LG, Jordan VC, Wolmark N. Effects of tamoxifen vs raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. Jama 2006;295:2727–41.

Appendix 2 Example of Cancer Risk Reduction by Immunologic Agents

Human Papilloma Virus Vaccines as an Example of Cancer Risk Reduction by Immunologic Agents

The identification of chronic infection by one of ~15 sexually transmitted human papilloma viruses as etiologic for the transformation of the squamo-columnar cervix epithelium provided an opportunity to prevent cervical cancer (1). Two vaccines, one produced by GSK (Cervarix) and the second produced by Merck (Gardasil), have notable differences. The GSK vaccine is bivalent, containing virus-like particles from HPV16 and HPV18, the two types found in 70% of cervix cancers world-wide. The vaccine uses a proprietary adjuvant, AS04, consisting of aluminum salts monophosphoryl lipid A. Merck's vaccine is quadravalent, containing virus-like proteins from HPV types 6, 11, 16, and 18, and uses a simple aluminum salt adjuvant. HPVs 6 and 11 cause 90% of cutaneous genital warts. For this reason, the quadrivalent vaccine targets two distinct hyperproliferative diseases (2-4).

Both vaccines are remarkably effective in preventing persistent HPV infections and low- (Grade 1) and high- (Grade 2 and 3) grade CIN (5,6). Two phase 3 multi-center international trials of the tetravalent (Merck) vaccine involved 15,057 women aged 15 to 26 years, with endpoints of preventing HPV16- or HPV18-related CIN 2/3 and adenocarcinoma *in situ* (FM). In women with no evidence of relevant vaccine HPV type (by HPV PCR and serology) who received all three doses of the vaccine, the tetravalent vaccine has high efficacy (96.9%; 95% CI 81.3%–99.9%) against incident infection HPV-16 and HPV-18 endpoints, sustained for up to 4.5 years (7). The vaccine also has efficacy against cervical intraepithelial neoplasia lesions (100%; 95% CI 42.4%-100%) associated with vaccine type. The vaccine does not protect against persistent infection, CIN, or genital warts in females who were infected at the time of vaccination. The vaccine partially cross-protects for incident infections with HPV-45 and HPV-31, the third and fourth most common HPV types associated with cervical cancer (2,8).

To date, few safety issues have been documented, although public concern has risen over several deaths and adverse events occurring post-vaccine (8). In 83% of Merck vaccine administrations and 73% of placebo injections, recipients had injection site erythema, pain, and swelling with severe intensity more often reported in the vaccine

recipients. Fever, headache, and nausea were reported in a similar proportion of vaccine and placebo recipients. One case of bronchospasm, one case of gastroenteritis, one case of headache with hypertension, and one case of vaginal hemorrhage occurred. The Merck vaccine affects pregnancy outcomes compared to the placebo arm (2,4).

The vaccine was approved using HPV viral infection as a surrogate biomarker endpoint for risk of neoplastic transformation of the cervix. Such a biomarker is valid based upon the extensive data demonstrating the causal role of HPV in squamous cell cervical carcinogenesis. This endpoint reduced the time and expense of providing sufficient safety and efficacy data to the FDA for regulatory review. The safety data were sufficient to merit approval of a cancer risk reduction as well. Thus, the immuno-prevention experience can inform the regulatory process for other cancer risk reductions.

This experience provides strong rationale for continued development of immuno-preventive approaches targeting known viruses or other immunogenic pathogens thought to be causal in carcinogenesis. The risk-benefit ratio favors use of a vaccine. A clearly defined surrogate biomarker, HPV viral infection, rather than a neoplastic event provided a rapid endpoint that can be readily assayed with current technologies.

Immunologic Prevention

Major infective carcinogens include the human hepatitis viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV) for hepatocellular carcinoma (9), *Helicobacter pylori* for gastric adenocarcinoma (10), human papilloma viruses (HPV) in cervix, anus, vulva, penis, and oral cavity and pharynx (2), herpes virus-8 for Kaposi's sarcoma (11), and schistosomes for bladder carcinoma (12). A mass vaccination program against HBV launched in Taiwan in 1984, when 15–29% of the population were estimated to be HBV carriers and incidence of hepatocellular carcinoma was very high, especially in children. This is an outstanding example, after 25 years, of an effective cancer immuno-prevention strategy (13). The HBV carrier rate among children born after 1987 is now close to zero (14) and in parallel, the rate of hepatocellular carcinoma has declined to insignificant levels (15). The effectiveness of the HBV vaccine correlates highly with successful elicitation of immunity against the virus. Recent development of effective HPV vaccines represents another major advance in cancer immunoprevention (15). Induction of immunity to HPV proteins contained in the vaccine has correlated with an effective

prevention of infection and clearance of cervical epithelial neoplasia (CIN). The success of HPV and HBV vaccines in reducing the incidence of epithelial neoplasia of the cervix and liver cancer, respectively, demonstrates the potential of immuno-cancer risk reduction for epithelial targets for which an infectious etiologic agent can be identified. For the majority of human cancers, infectious etiology has not been determined. However, numerous cancer-associated antigens, as candidates for vaccines, have been identified (16). In preclinical animal model studies, induction of immune responses to many of these antigens prevents cancer growth without causing toxicity (17,18). In patients diagnosed with cancer, evidence has been obtained that preexisting immunity to some of these antigens correlates with better disease outcome (19) or reduced cancer risk (20), as does induction of immune responses to these antigens through vaccines (21-25).

Passive immunotherapy, *i.e.*, infusion of antibodies that recognize cancer-associated antigens, is already an effective FDA-approved cancer therapy (26-28). Cancer vaccines based on some of the same antigens or on several other antigens with a superior safety profile can induce cancer-specific antibodies and T cells expected to last a lifetime. As with HBV and HPV vaccines, the potential of cancer vaccines to lower lifetime risk of cancer in high-risk individuals and overall cancer incidence in the general population will have to be determined over a period of 10–20 years. However, their ability to generate an immune response can be measured efficiently in phase 2a trials, and as with risk reduction drugs, their effects on IEN can be assessed in phase 2b trials analogous to those designed for drugs.

While many other cancer prevention agents require development of new biomarkers of their activity, cellular and humoral immune responses generated by a vaccine are established, time-tested functional biomarkers of effectiveness of all vaccines (29-31). However, the effectiveness of cancer vaccines in preventing cancer will require long term follow up of immunized persons.

References

1. Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB, Scott DR, Sherman ME, Kurman RJ, Wacholder S, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 1993;85: 958–64.
2. Saslow D, Castle PE, Cox JT, Davey DD, Einstein MH, Ferris DG, Goldie SJ, Harper DM, Kinney W, Moscicki AB, Noller KL, Wheeler CM, Ades T, Andrews KS, Doroshenk MK, Kahn KG, Schmidt C, Shafey O, Smith RA, Partridge EE, Garcia F. American Cancer Society Guidelines for human papillomavirus (HPV) vaccine use to prevent cervical cancer and its precursors. CA Cancer J Clin 2007;57:7-28.
3. Schiller JT, Lowy DR. Prospects for cervical cancer prevention by human papillomavirus vaccination. Cancer Res 2006;66:10229–32.
4. Markowitz LE, Dunne EF, Saralya M, Lawson HW, Chesson H, Unger ER. Quadrivalent Human Papillomavirus Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Rcomm Rep 2007;56:1-24.
5. Harper DM, Franco EL, Wheeler CM, Moscicki AB, Romanowski B, Roteli-Martins CM, Jenkins D, Schuind A, Costa Clemens SA, Dubin G. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomized control trial. Lancet 2006;367:1247–55.
6. Villa LL, Costa RL, Petta CA, Andrade RP, Paavonen J, Iversen OE, Olsson SE, Hoye J, Steinwall M, Riis-Johannessen G, Andersson-Ellstrom A, Elfgren K, Krogh G, Lehtinen M, Malm C, Tamms GM, Giacoletti K, Lupinacci L, Railkar R, Taddeo FJ, Bryan J, Esser MT, Sings HL, Saah AJ, Barr E. High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. Br J Cancer 2006;95:1459–66.
7. CDC. Reports of Health Concerns Following HPV Vaccination. Accessed at http://www.cdc.gov/vaccinesafety/vaers/gardasil.htm, ed.
8. Markowitz LE, Dunne EF, Saraiya M, Lawson HW, Chesson H, Unger ER. Quadrivalent Human Papillomavirus Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2007;56:1–24.
9. Seeff LB, Hoofnagle JH. Epidemiology of hepatocellular carcinoma in areas of low hepatitis B and hepatitis C endemicity. Oncogene 2006;25:3771–7.
10. Fox JG, Wang TC. Inflammation, atrophy, and gastric cancer. J Clin Invest 2007;117:60–9.
11. Mohanna S, Maco V, Bravo F, Gotuzzo E. Epidemiology and clinical characteristics of classic Kaposi's sarcoma, seroprevalence, and variants of human herpesvirus 8 in South America: a critical review of an old disease. Int J Infect Dis 2005;9:239–50.
12. Mostafa MH, Sheweita SA, O'Connor PJ. Relationship between schistosomiasis and bladder cancer. Clin Microbiol Rev 1999;12:97–111.
13. Huang K, Lin S. Nationwide vaccination: a success story in Taiwan. Vaccine 2000;18 Suppl 1:S35–8.

14. Su FH, Huang HY, Chang HJ, Jeng JJ, Liu YH, Chen CD. Forecasting the declining rate of chronic hepatitis-B carrier status at a Taiwanese university: twenty years after implementation of an universal HBV vaccination program in Taiwan. Chang Gung Med J 2007;30:521–8.
15. Schiller JT, Castellsagué X, Villa LL, Hildesheim A. An update of prophylactic human papillomavirus L1 virus-like particle vaccine clinical trial results. Vaccine 2008;26 Suppl 10:K53–61.
16. Graziano DF, Finn OJ. Tumor antigens and tumor antigen discovery. Cancer Treat Res 2005;123:89–111.
17. Ostrand-Rosenberg S. Animal models of tumor immunity, immunotherapy and cancer vaccines. Curr Opin Immunol 2004;16:143-50.
18. Cavallo F, Offringa R, van der Burg SH, Forni G, Melief CJ. Vaccination for treatment and prevention of cancer in animal models. Adv Immunol 2006;90:175–213.
19. von Mensdorff-Pouilly S, Vennegoor C, Hilgers J. Detection of humoral immune responses to mucins. Methods Mol Biol 2000;125:495–500.
20. Cramer DW, Titus-Ernstoff L, McKolanis JR, Welch WR, Vitonis AF, Berkowitz RS, Finn OJ. Conditions associated with antibodies against the tumor-associated antigen MUC1 and their relationship to risk for ovarian cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1125–31.
21. Butts C, Murray N, Maksymiuk A, Goss G, Marshall E, Soulieres D, Cormier Y, Ellis P, Price A, Sawhney R, Davis M, Mansi J, Smith C, Vergidis D, Ellis P, MacNeil M, Palmer M. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. J Clin Oncol 2005;23:6674–81.
22. Carbone DP, Ciernik IF, Kelley MJ, Smith MC, Nadaf S, Kavanaugh D, Maher VE, Stipanov M, Contois D, Johnson BE, Pendleton CD, Seifert B, Carter C, Read EJ, Greenblatt J, Top LE, Kelsey MI, Minna JD, Berzofsky JA. Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune response and clinical outcome. J Clin Oncol 2005;23:5099–107.
23. So-Rosillo R, Small EJ. Sipuleucel-T (APC8015) for prostate cancer. Expert Rev Anticancer Ther 2006;6:1163–7.
24. Fay JW, Palucka AK, Paczesny S, Dhodapkar M, Johnston DA, Burkeholder S, Ueno H, Banchereau J. Long-term outcomes in patients with metastatic melanoma vaccinated with melanoma peptide-pulsed CD34(+) progenitor-derived dendritic cells. Cancer Immunol Immunother 2006;55:1209–18.
25. Gould P. Sipuleucel-T shows partial advantage in prostate cancer. Lancet Oncol 2006;7:710.
26. Cheson BD. Monoclonal antibody therapy for B-cell malignancies. Semin Oncol 2006;33:S2–14.
27. Hortobagyi GN. Trastuzumab in the treatment of breast cancer. N Engl J Med 2005;353:1734–6.
28. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios CH, Steger G, Huang CS, Andersson M, Inbar M, Lichinitser M, Lang I, Nitz U, Iwata H, Thomssen C, Lohrisch C, Suter TM, Ruschoff J, Suto T, Greatorex V, Ward C, Straehle C, McFadden E, Dolci MS, Gelber RD.

Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005;353:1659–72.

29. June CH. Adoptive T cell therapy for cancer in the clinic. J Clin Invest 2007;117:1466–76.

30. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, Royal RE, Topalian SL, Kammula US, Restifo NP, Zheng Z, Nahvi A, de Vries CR, Rogers-Freezer LJ, Mavroukakis SA, Rosenberg SA. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 2006;314:126–9.

Appendix 3: A*n updated* clinical developmental pathway to regulatory approval – some practical guidelines (prepared by M. Kakarala and D. Brenner, U. Michigan)

Although the issues surrounding the early phase development for chemoprevention of cancer have been discussed in detail elsewhere, including information gleaned from experience with a drug in the intervention of patients with cancer, several specific adaptations are emphasized here within phases 1, 2,

Phase 1 Trial

Phase 1 cancer risk reduction trials define an optimal dose that might be considered for administration to a population over a long time period (years). An optimal cancer risk reduction dose is usually non or low-toxic, scheduled once daily, and modulates a tissue, cellular, or serum biomarker that is a direct mechanistic target of action of the intervention (for example, the dose of aspirin that inhibits prostaglandin production in a target tissue site). Definition of a maximal tolerable dose should not be essential, and indeed is an undesirable endpoint of phase 1 cancer risk reduction trials. Higher but non-toxic doses may reduce the efficacy of risk reduction. For example, beta-carotene at high doses in two large trials had pro-oxidant activity and enhanced the carcinogenesis process (1), while at low doses this compound may well be a potent antioxidant and differentiating agent (2).

Phase 1 designs employing a classical Fibonacci-type escalation with three subjects per dose level are inefficient, expensive, and do not identify optimal doses or maximum tolerated doses for individuals or populations. Newer Bayesian based designs, employing time-to-event and continual reassessment monitoring (3), may permit more efficient approaches to optimal dose identification. Such strategies depend on rapid throughput analytical methods that then feed back to toxicity and dose data. Alternatively, dose de-escalation strategies permit phase 1 trials to identify optimal doses on the basis of biomarker changes from a maximum dose known to have acceptable risk. These strategies (and perhaps others) may be useful rather than a one-size-fits-all design.

For regulatory purposes, an acceptable cancer risk reduction goal in phase 1 should be to identify a dose of the candidate drug that modulates a tissue or circulating biomarker, indicating that the intervention has reached a cellular target and that toxicity is acceptable using the NCI Common Toxicity Criteria; the number of acceptable events depend on the seriousness of the indication. Such biomarker-driven early phase trial

designs rely upon Good Laboratory Practice quality analytical methods in addition to standard Good Clinical Practice.

Phase 2 Cancer Risk Reduction

1.Short-term Phase 2a Risk Reduction Trials with Molecular or Biochemical Biomarker Endpoints

Phase 2 cancer risk reduction trials should begin to define risk reduction efficacy. These short- to medium-term (one month to three year) treatment periods gather evidence of risk reduction by assessing the effect of cancer risk reduction on tissue, cellular, or blood biomarkers reflective of the carcinogenesis biologic process. Phase 2a trials are short term (one to six months), usually non-randomized, biomarker modulation trials. In Phase 2a, modulation of tissue, cellular or blood biomarkers directly targeted by the risk reduction drug provides evidence of mechanistic efficacy.

Phase 2a trials may re-interrogate different doses of a cancer risk reduction, using a larger portfolio of biomarkers as endpoints. As such, novel designs such as Bayesian-driven time-to-event, continual reassessment monitoring designs, or dose de-escalation designs (3) may more efficiently yield higher quality biomarker modulation data than currently used escalation designs.

The results of phase 2a cancer risk reduction clinical trials alone are insufficient for regulatory approval. However, consistent results in phase 2a provide a basis for longer term trials that move a drug along the pathway.

The major purpose of phase 2a trials should be to demonstrate that the candidate risk reduction drug modulates a mechanistically relevant marker in the relevant tissue in the predicted manner. Without such data, proceeding to a larger and longer, randomized phase 2b trial is problematic.

2. Phase 2b With Endpoint of IEN and/or Biomarker Risk Reduction

Phase 2b randomized, placebo-controlled trials of a cancer risk reduction drug represent a major investment in a hundred (*e.g.*, oral leukoplakia) to a thousand (*e.g.*, colon polyps) or more subjects over a one- to three-year period, with an endpoint focused on a dysplastic epithelium (usually an IEN). These trials test the hypothesis that a drug will decrease recurrence of a pathologic endpoint. Such trials also may

incorporate molecular or biochemical biomarkers that would provide a preliminary basis for developing a surrogate endpoint for regulatory purposes in the future.

The use of such trials as the general basis for regulatory approval for cancer risk reduction remains controversial because the relationship of progression of an IEN or other biomarker endpoint to frank invasive malignancy varies greatly among IENs and is not highly predictable for an individual. If the majority of IENs do not progress to invasive neoplasm, then such lesions may not be acceptable as surrogate biomarkers for regulatory approval. Nevertheless, BCG was approved for carcinoma in-situ (CIS) of the bladder several decades ago (4), celecoxib was approved for reduction in the number of colorectal polyps, as an adjunct to standard care, in patients with FAP (5), and Photofrin for management of Barrett's esophagus more recently (6). Provisional regulatory concurrence, an approval with a sunset provision based on generation of further phase 3 data, may permit acceptance of favorable modulation of a particular IEN as a surrogate for cancer incidence. Such a regulatory approach will have the benefit of permitting commercial use of cancer risk reduction drugs at an earlier time than previously allowed (7). The approach will permit dissemination of potential cancer risk reduction drugs into broader clinical practice while encouraging investment in and completion of phase 3 cancer risk reduction trials, a strategy that has been highly successful in developing pharmacologic risk reduction for cardiovascular diseases.

However, the risk standard of cancer risk reduction in a phase 2b trial remains undefined, which is not unexpected, since the range of risk for disease is broad. Based on the paradigm in Figure 2, we might consider a graded set of risk endpoints for a given preventive indication. Such a standard might permit more frequent Grade 3 toxicity in individuals with high genetic risk while imposing stricter toxicity standards for individuals with a lower lifetime risk of cellular transformation. Phase 2b trials should also generate data that begin to link molecular endpoints with pathologic surrogate biomarker outcomes as well. Such data may eventually be used to validate biomarkers as surrogate endpoints for cancer incidence. If such validation data can be generated, then future regulatory decisions might employ molecular biomarkers as the primary approval criterion.

We suggest that regulatory approval should be expanded to include an accelerated approval mechanism that permits the labeling and use of cancer risk reduction drugs on the basis of large randomized phase 2b trials if predetermined

efficacy goals (as defined in collaboration with regulatory agency input, on a case-by-case basis) have been met and toxicity is acceptable for the cohort at risk.

Risk Benefit in Development of Phase 3 Cancer Risk Reduction

Phase 3 chemoprevention trials define risk reduction as a hard cancer endpoint such as cancer incidence or mortality. Such trials using large high-risk populations in a randomized, double blinded intervention are designed to identify a standard of preventive care for a given risk population. Trials of BCG for reducing bladder carcinoma in situ, tamoxifen for reducing breast cancer incidence, finasteride for reducing prostate cancer incidence, and beta-carotene for reducing lung cancer incidence have served as examples of well-conducted, definitive phase 3 chemoprevention clinical trials. Some investigators consider randomized, controlled clinical trials with a tissue-based IEN biomarker surrogate endpoint sufficient for regulatory review as phase 3. Using such a definition, a clinical trial with an endpoint of reduction in adenoma recurrence is considered a phase 3 trial. For example, a 28% reduction of polyp recurrence in patients with FAP led to regulatory approval of celecoxib in this high-risk cohort, as discussed above (6). For sporadic colorectal adenomas, a 35% decrease in polyp incidence or a 20% increase in polyp-free survival at three years in the active drug arm *versus* placebo was considered as acceptable efficacy for approval by an FDA Gastrointestinal Advisory Committee in 2002 (see Appendix 2) However, excessive cardiovascular toxicity precluded the approval of Celecoxib for the reduction of sporadic ademomas. In contrast, the favorable risk-benefit profile for Raloxifene has allowed its approval for the risk reduction of breast cancer.

References

1. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996;334:1150–5.
2. Pryor WA, Stahl W, Rock CL. Beta carotene: from biochemistry to clinical trials. Nutr Rev 2000;58:39–53.
3. Normolle D, Lawrence T. Designing dose-escalation trials with late-onset toxicities using the time-to-event continual reassessment method. J Clin Oncol 2006;24:4426-33.
4. FDA. Approval for BCG. Accessed at http://www.fda.gov/cber/sba/bcgorga082198S.pdf.
5. FDA. Approval Letter for Celecoxib. Accessed at http://www.fda.gov/cder/foi/appletter/1998/20998ltr.pdf.
6. FDA. Approval Letter for Photofrin. Accessed at http://www.fda.gov/cder/foi/appletter/1998/20451sap.pdf.
7. Kellof GJ, Sigman CC, Johnson KM, Boone CW, Greenwald P, Crowell JA, Hawk ET, Doody LA. Perspectives on surrogate endpoints in the development of drugs that reduce the risk of cancer. Cancer Epi Biom Prev 2000;9:127-37.

Appendix 4 *Biomarker Issues in Cancer Risk Reduction Development and Therapeutic Index*

Table 1. Prototype "evidence map"—categorical description of different types of scientific evidence potentially relevant to biomarker qualification; subcategorical graded weight of evidence from least to most (from (1) with permission)

Evidence type	Grade D	Grade D+/C-	Grade C	Grade C+/B-	Grade B	Grade B+/A-	Grade A
Theory on biological plausibility	Observed association only	Theory, indirect evidence of relevance of the biomarker from animals	As for lower grade but evidence is direct	Theory, indirect evidence of relevance in humans	Theory, direct evidence in humans, non-causal pathway possible	As for lower grade, but biomarker on causal path	Human evidence based mathematical model of biology showing biomarker is on causal pathway
Interaction with pharmacologic target	Biomarker identifies target in *in vitro* binding			Biomarker identifies target in *in vivo* binding in animals	Biomarker identifies target in *in vivo* studies or from human tissue, no truth standard		Biomarker identifies target in *in vivo* studies or from tissues in humans, with accepted truth standard
Pharmacologic mechanistic response	*In vitro* evidence that the drug affects the biomarker	*In vitro* evidence that multiple members of this drug class affects the biomarker	*In vivo* evidence that this drug affects biomarker in animals	As for lower grade but effect shown across drug class	Human evidence that this drug affects the biomarker OR animal evidence of specificity	Human evidence across this mechanistic drug class	Human evidence that multiple members of this drug class affect the biomarker and the effect is specific to this class/mechanism
Linkage to clinical outcome of a disease or toxicity		Biomarker epidemiologically associated with outcome without any intervention	Biomarker associated with change in outcome from intervention in another drug class	As for lower grade but in this drug class	As for lower grade but multiple drug classes albeit inconsistent or a minority of disease effect		As for lower grade but consistent linkage and explains majority of disease effect
Mathematics replication, confirmation		An algorithm is required to interpret the biomarker and was developed from this dataset		Algorithm was developed from a different dataset and applied here prospectively			Algorithm developed from different dataset, replicated prospectively in other sets and applied prospectively here
Accuracy and precision (analytic validation)				Sources of technical variation are unknown but steps are taken to ensure consistent test application	Major sources or variation known and controlled to be less than biological signal; standardization methods applied		All major sources of technical imprecision are known, and controlled test/assay accuracy is defined against standards
Relative performance		Does not meet performance of benchmark		Similar performance to benchmark			Exceeds performance of benchmark or best alternative biomarker

Not all types of evidence required all seven grades to be completed.

Biomarker Issues in Cancer Risk Reduction Development and Therapeutic Index

The NCI's Definitions Working Group defined a biomarker to be a characteristic that is measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic interventions (2). A surrogate endpoint for cancer prevention assumes that a measured biological feature will predict the presence or future development of a cancer outcome (3). The primary motivation for development of such surrogate endpoints concerns the ability to diagnose cancer at an early stage, to identify individuals at high risk for cellular transformation, and to enable reduction in the size and duration of an intervention trial by replacing a rare or distal endpoint with a more frequent, proximate endpoint (4).

Although many advocate the use of IEN-based biomarkers as regulatory surrogate endpoints, others caution that IEN may not serve as sufficiently robust surrogate biomarkers for cancer incidence or mortality (5). Biomarkers may also be derived from biological products, such as a protein, gene, or quantitative cellular process used to predict cancer diagnosis or risk (6), for example, CA-125 for ovarian cancer and alpha-fetoprotein for hepatocellular cancer and testicular cancers.

In order to be useful as endpoints for risk reduction as a regulatory endpoint, any biomarker must have statistical accuracy, precision and effectiveness of results (7, 8) that demonstrates prediction of a "hard" disease endpoint—a cancer incidence or mortality endpoint. The validation dataset must address defined standards of validation, avoiding and accounting for overfitting and bias. The biomarker must be generalizable to the specific clinical or screening population. Future progress in linking the genetic changes in neoplastic progression with biologically important functional consequences will provide improved biomarkers, interventional targets, and strategies (8).

References

1. Altar CA, Amakye D, Bounos D, Bloom J, Clack G, Dean R, Devanarayan V, Fu D, Furlong S, Hinman L, Girman C, Lathia C, Lesko L, Madani S, Mayne J, Meyer J, Raunig D, Sager P, Williams SA, Wong P, Zerba K. A prototypical process for creating evidentiary standards for biomarkers and diagnostics. Clin Pharmacol Ther 2008;83:368–71.
2. De Gruttola VG, Clax P, DeMets DL, Downing GJ, Ellenberg SS, Friedman L, Gail MH, Prentice R, Wittes J, Zeger SL. Considerations in the evaluation of surrogate endpoints in clinical trials. summary of a National Institutes of Health workshop. Control Clin Trials 2001;22:485–502.
3. Schatzkin A, Freedman LS, Schiffman MH, Dawsey SM. Validation of intermediate end points in cancer research. J Natl Cancer Inst 1990;82:1746–52.
4. Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. Stat Med 1989;8:431–40.
5. Schatzkin A. Problems with using biomarkers as surrogate end points for cancer: a cautionary tale. Recent Results Cancer Res 2005;166:89–98.
6. Sidransky D. Emerging molecular markers of cancer. Nat Rev Cancer 2002;2:210–9,.
7. Feinstein A. (1996). "Multivariable Analysis: An Introduction." Yale University Press, New Haven, CT.
8. Ransohoff DF. Rules of evidence for cancer molecular-marker discovery and validation. Nat Rev Cancer 2004;4:309–14.