Appendix

Colorectal cancer

Approximately 608,000 deaths resulting from colorectal cancer are estimated worldwide, accounting for 8% of all cancer deaths, making it the fourth most common cause of death resulting from cancer (http://globocan.iarc.fr/factsheets/cancers/colorectal.asp). Most patients die with metastatic disease (http://www.cancerresearchuk.org/cancer-info/cancerstats/survival). The use of epidermal growth factor receptor (EGFR) –targeted therapy has led to the discovery of the importance of *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* (exon 9) mutations in prediction of lack of response to EGFR-targeted therapy.[36] Oncologists now face major challenges in identifying effective treatments for patients with colorectal cancer after the overall disappointing results seen with bevacizumab and cetuximab/panitumumab, the modest benefits seen with aflibercept and regorafenib, and the failure of multiple other targeted agents in recent commercial trials. Many leading scientific authors have reviewed this issue (Gutierrez ME et al: Nat Rev Clin Oncol 6:259-265, 2009; Hait WN: Nat Rev Drug Discov 9:253-254, 2010; Kelley R et al: Curr Oncol Rep 11:175-185, 2009; Lord CJ et al: BMC Biol 8:38, 2010), including a systematic review of EGFR-targeted therapies in colorectal cancer (Vale CL et al: Cancer Treat Rev 38:618-625, 2012). A more efficient, structured, and systematic approach to both treatment and biomarker evaluation is urgently required. The convergence of molecular understanding of the disease and the clinical development of a wide range of targeted therapies demands the evaluation of new therapies, at least initially within subsets of the population whose tumors are more likely to benefit.

Biomarkers and treatments

Appendix Table A1 provides a summary of the proposed interventions and biomarkers for FOCUS4.

BRAF *mutation.* In the Medical Research Council COIN (Continuous or Intermittent) trial,[38] 8% of patients had *BRAF* V600E mutations, and their median survival was only 8.8 months. This poor prognostic effect of *BRAF* mutation in advanced disease has been confirmed in other series. However, the impressive though short-lived benefits of vemurafenib in melanoma (Chapman PB et al: N Engl J Med 364:2507-2516, 2011) did not translate to colorectal cancer (Kopetz S et al: J Clin Oncol 28:269s, 2010 [suppl; abstr 3534]), apparently because of signaling through the EGFR (Prahallad A et al: Nature 483:100-103, 2012). Combination therapies of BRAF inhibition and EGFR inhibition with or without MEK inhibition are being evaluated for safety, and one such combination will be tested in this group.

AKT pathway activation. The AKT pathway plays a key role in multiple cellular processes, such as glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration. *PIK3CA* mutations have been identified as one of the most common mutations in cancer and were identified in 12.8% of patients in the COIN trial. In addition, approximately 10% to 20% of patients have a complete loss of PTEN function, the mechanisms of which include mutation, methylation silencing of the promoter, and mRNA inhibition. Data from the Cancer Genome Atlas Report suggest that alteration of *IGF2* or *IRS2* also drives this pathway (Cancer Genome Atlas Network: Nature 487:330-337, 2012). These molecular alterations lead to activation of the AKT signaling network, which seems to be activated in more than half of colorectal cancers. In addition, approximately half of the patients also have *KRAS* mutations in their tumor. In FOCUS4, we will enrich this population by identification of *PIK3CA* mutations or profound loss of PTEN function, which together would account for up to an estimated 30% of the colorectal cancer population. The agent to be tested will be a combined PIK3CA-mTOR inhibitor, which will block the AKT pathway at two critical points.

KRAS *or* NRAS *mutation.* The COIN trial[38] showed that the prognosis of patients with stage IV disease with either *KRAS*- or *NRAS*-mutated tumors is similar (median survival of approximately 14 months). However, expression profile analysis shows a variation in gene expression patterns with *KRAS* mutation, with signaling down the canonic RAS-RAF-MEK-ERK pathway dominating in approximately one quarter, signaling through the PI3K-AKT-mTOR pathway in others, and diverse signaling in other tumors. An estimated 33% of patients present with these mutations (without *PIK3CA*-activating mutations or profound loss of PTEN function). In this group, we will be testing the combination of an AKT inhibitor and MEK inhibitor to effectively inhibit both major signaling transduction pathways.

All wild type for aforementioned mutations. Patients with all wild type (ie, wild-type *KRAS*, *NRAS*, *BRAF*, and *PIK3CA*) with preserved function of PTEN include those shown to be most sensitive to EGFR inhibition by cetuximab or panitumumab.[36] In addition, up to 10% have alterations in human epidermal growth factor receptor (HER) 2 or HER3 function (Cancer Genome Atlas Network: Nature 487:330-337, 2012). Therefore, we plan to evaluate an inhibitor of HER1, HER2, and HER3 in this patient cohort to block the primary signaling through these receptors and compensatory resistance mechanisms through HER3 that may occur after EGFR inhibition. It is estimated that 27% of patients would fall into this cohort.

Nonstratified. There will inevitably be a small number of patients whose biomarker assessment fails, estimated to be approximately 2% of the population. These patients may benefit from conventional maintenance therapy or potentially from an antiangiogenic agent (for which no validated biomarker has been identified to date). In light of the uncertainty regarding fluoropyrimidine maintenance therapy, we plan to randomly assign such patients to either capecitabine or no treatment. This will also provide a default random assignment for those patients for whom a biomarker-specific trial is not open at the suitable time and for any patients unwilling or unable to travel to an experimental cancer medicine center for one of the novel combinations early in its evaluation in FOCUS4.

Challenges of FOCUS4 biomarker selection

Quality assurance of the biomarker assessment is a critical process both before and during the study. Our approach is to have two well-established laboratories working collaboratively to deliver biomarker assessments for the trial. These laboratories already participate in external quality assurance schemes for assays such as *KRAS* mutation. For more-novel assays, they have set up a mutual quality assurance process to assure each other's analyses and assess both interpatient variation and variation over time.

Recent data have underlined the previously suspected extent of tumor heterogeneity that exists within different parts of a cancer (Gerlinger M et al: N Engl J Med 366:883-892, 2012). So does a single formalin-fixed, paraffin-embedded sample analyzed for a small set of mutations provide an acceptable or sufficient assessment of the biology of the disease? The answer clearly is no, at least in some respects. However, mutations that occur early in the disease course, such as *KRAS*-activating mutations, have confirmed utility as predictive biomarkers. Both our understanding of the biology of the disease and our technical ability to define it will undoubtedly develop over time with the use of combinations of wider panels of gene mutations, gene expression signatures, and other parameters. These issues are being actively researched within FOCUS4 with sequential biopsies before and after therapy and with more extensive characterization of the so-called tumor omics. These analyses, along with preclinical and other clinical analyses ongoing in multiple institutions, will feed into and refine our understanding of the detailed allocation of individual patients to biomarker-defined comparisons in our adaptive clinical trial.

Metastatic disease comprises many clones of competing tumor cells, including low levels of numerous mutations, within which the mechanisms of resistance are either already present or easily inducible in response to selection pressure of chemotherapy and targeted therapies. Sequential biopsies at randomization and on progression will enable us to look for and identify such mechanisms and will inform future trial design. Use of comparable strategies much earlier in the disease, such as in the perioperative setting, may enable more robust selection of patients for specific adjuvant therapy and thereby have an impact on curative outcome.

Table A1. Biomarker-Defined Cohorts and Novel Agent Interventions for FOCUS4

Cohort (trial)	Agent
BRAF-mutant tumors (FOCUS4-A)	
Intervention	Specific *BRAF*-mutated kinase inhibitor in combination with panitumab (EGFR-targeted monoclonal antibody) and/or MEK inhibitor
Control	Dual placebo
PIK3CA-mutant tumors and/or loss of PTEN IHC (FOCUS4-B)	
Intervention	Dual PI3K/mTOR inhibitor monotherapy
Control	Dual placebo
KRAS- or *NRAS*-mutant tumors (FOCUS4-C)	
Intervention	Dual-pathway inhibition using AKT and MEK inhibitor
Control	Dual placebo
BRAF, *PIK3CA*, *KRAS*, and *NRAS* wild-type tumors (FOCUS4-D)	
Intervention	HER1, HER2, and HER3 inhibitors
Control	Placebo
Nonstratified unclassified biomarker patients or those unable or unwilling to enter trial cohorts (FOCUS4-N)	
Intervention	Capecitabine
Control	No therapy (treatment break)

Abbreviations: EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; IHC, immunohistochemistry; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide-3 kinase.