

SUPPLEMENTARY METHODS

Amendments to CALGB (Alliance)/SWOG 80405 protocol during enrollment

Subjects were participants of an NCI-sponsored phase III trial for metastatic or advanced colorectal adenocarcinoma (mCRC) of irinotecan, 5-fluorouracil, and leucovorin (FOLFIRI) or oxaliplatin, 5-fluorouracil, and leucovorin (mFOLFOX6) combined with either cetuximab, bevacizumab or cetuximab plus bevacizumab (Cancer and Leukemia Group B [CALGB, now part of the Alliance for Clinical Trials in Oncology]/SWOG 80405; ClinicalTrials.gov identifier NCT00265850). Participants did not receive any systemic therapy for mCRC prior to trial enrollment (1). The clinical trial design of CALGB/SWOG 80405 underwent major changes during enrollment due to evolving science in the field of mCRC. The trial was initially activated in September 2005 but put on hold in June 2008 when *KRAS* was determined to be predictive of response to cetuximab (2,3). The trial was amended and reopened in October 2008, limiting to *KRAS* exon 2 wild-type patients (1). After 2 trials raised concern regarding combination of epidermal growth factor receptor inhibitors with bevacizumab (4,5), the trial was amended and reopened in September 2009 to the final trial design of physician/patient choice of FOLFIRI or mFOLFOX6 and randomization to cetuximab or bevacizumab.

Molecular Analyses

Molecular analyses were performed for cases with patient consent and tumor specimen ascertainment from study sites. Tumor DNA was obtained from 852 formalin-fixed, paraffin embedded (FFPE) tumor blocks (91% primary, 5% metastatic, 4% unknown). DNA was extracted by QIAamp DNA FFPE tissue kits (Qiagen, Hilden, Germany). Allele-specific polymerase chain reaction (PCR) was used to genotype mutation hotspots in *APC*, *PIK3CA*,

BRAF, and *TP53*, as previously described (6,7). Analyses were conducted at Genentech (South San Francisco, CA). *KRAS* status used for covariate adjustment was derived from 3 variables: *KRAS* used to determine trial eligibility (1), an expanded *RAS* assay (1), and *KRAS* status from a Genentech panel (6,7). Microsatellite instability (MSI) status was determined using both the MSI Analysis System (Promega, Madison, WI), as previously described (7), and next-generation sequencing using the FoundationOne platform conducted at Foundation Medicine (Cambridge, MA) following standard procedures. Specimens were categorized as MSI if either method indicated MSI.

References

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