SUPPLEMENTARY MATERIALS AND METHODS

Analysis of circulating biomarkers

Levels of free IGF-1 (fIGF-1) were measured in plasma using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s recommendations (Beckman-Coulter Diagnostic System Laboratories [Webster, TX]). The lower limit of quantitation (LLOQ) for fIGF-1 was 0.118 ng/mL.

Levels of IGFBP-3 were measured in diluted serum by radioimmunoassay at Esoterix (Calabasas, CA). This assay was a competitive binding radioimmunoassay that utilized high-affinity rabbit antisera specific for IGFBP-3 as the primary antibody and radioiodinated purified IGFBP-3 as the tracer. The amount of tracer bound to the immunocomplexes formed in the calibrator sample was used to establish a standard curve from which the IGFBP-3 concentrations of control and test samples were read by scintillation counting in a gamma counter. The LLOQ for IGFBP-3 was 0.3 mg/L.

Levels of IGF-2, IGFBP-1, IGFBP-2, and IGFBP-4 were measured in serum using ELISA kits according to manufacturers’ recommendations. The assay kits for IGF-2, IGFBP-1, and IGFBP-4 were from Beckman-Coulter Diagnostic System Laboratories (Webster, TX). The IGFBP-2 DuoSet® ELISA kit was purchased from R&D Systems (Minneapolis, MN) The LLOQ were 300 ng/mL for IGF-2, 1.60 ng/mL for IGFBP-1, 0.06 ng/mL for IGFBP-2, and 4 ng/mL for IGFBP-4.
Analysis of somatic mutations in tumor tissues

DNA was extracted from archival formalin-fixed, paraffin-embedded (FFPE) tumor samples, and sequencing libraries were generated for analysis on the 454 GS FLX platform (454 Life Sciences, Branford, CT). The exon targets of the primers used for polymerase chain reaction (PCR) were as follows: HRAS, exons 2 and 3; KRAS, exons 2 and 3; NRAS, exons 2 and 3; BRAF, exon 15; PIK3CA, exons 2, 3, 10, 11, and 21; PTEN, all exons; and TP53, all exons. Amplified sequencing libraries were evaluated by capillary electrophoresis using the Agilent 2100 Bioanalyzer and DNA 1000 Kit (Agilent Technologies, Inc., Santa Clara, CA) prior to sequencing. Libraries with little or no amplification of target amplicons and/or a molar excess of nonspecific PCR artifact relative to target amplicons were not used for sequencing. Sequence data were base-called and filtered using the default 454 GS FLX software settings. Variant detection against standard reference sequences (human genome assembly build 37, hg19) for each exon was performed using the 454 GS Amplicon Variant Analysis software, version 2.0 (454 Life Sciences). Only nonsynonymous variants lacking a corresponding entry in dbSNP v131 were reported as mutations. In summarizing the data for each patient, mutant status was assigned to gene-subject combinations where a mutation was observed. Wild-type status was assigned to gene-subject combinations with no observed mutations in which the median number of sequence reads per amplicon was >50. If no mutation was observed but the median number of sequence reads per amplicon was not >50, the status of that gene-subject intersection was reported as not determined.
The number and percentage of patients in the ganitumab arm were summarized by KRAS mutational status. Associations between KRAS mutational status and OS were evaluated in the ganitumab arm using a Cox proportional hazards model.

**Analysis of PTEN expression in tumor tissues**

Archival FFPE tumor samples were analyzed for nuclear and cytoplasmic expression of PTEN by immunohistochemistry at HistoGenex (Antwerpen, Belgium). Tumor tissues were sectioned and analyzed by hematoxylin and eosin staining to identify regions of high tumor cell content. Immunohistochemistry staining was performed by first incubating sections with an affinity-purified polyclonal rabbit anti-human/mouse/rat PTEN antibody (R&D Systems, Inc., Minneapolis, MN). The primary antibody was detected using the DakoCytomation EnVision+ System-HRP (Dako North America, Inc., Carpinteria, CA), which includes a peroxidase-labeled polymer conjugated to goat anti-rabbit immunoglobulin and diaminobenzidene for chromogen visualization. Nuclear and cytoplasmic staining were scored according to intensity of staining on a relative scale of 0 to 3 (0=no stain; 3=maximal stain), and the percentage of stained tumor cells at each grade was recorded. Tumor cells with relative staining intensity ≥1 in the nuclear or cytoplasmic compartments were considered PTEN-positive. Endothelial cells and/or inflammatory cells within each tumor sample, as well as control specimens, were used as quality controls.

PTEN expression was evaluated using the following two parameters: an H-score, defined as the sum of product of staining score (ie, 0, 1, 2, 3) and the percentage of cells at each staining score, and the percentage of cells that are PTEN positive, where
PTEN positive was defined as a staining score >0. Summary statistics for PTEN expression were provided for the ganitumab arm. Associations between PTEN protein expression and OS were evaluated in the ganitumab arm using the two parameters.