Background: The Wnt/β-catenin pathway signals through the Frizzled (Fzd) receptor family and is implicated in many human cancers. Vantictumab is a monoclonal antibody that blocks canonical WNT/β-catenin signaling through binding of five Fzd receptors (1, 2, 5, 7, 8) at a conserved epitope within the extracellular domain. Mouse xenograft studies using minimally-passaged, patient-derived xenografts show that vantictumab inhibits tumor growth, promotes differentiation, and reduces cancer stem cell (CSC) frequency in multiple tumor types and synergizes with many chemotherapeutic agents. In nonclinical models, vantictumab modulates gene expression in tumor cells associated with stem cell and differentiation pathways and down-regulates Wnt pathway genes in the tumor and stroma. As such, vantictumab is a novel anti-cancer agent that inhibits tumor growth through direct actions on tumor cells, including CSCs, and effects on the stroma. We sought to determine the pharmacodynamic (PD) effects of various doses of vantictumab on Wnt signaling and stem cell pathways by examining surrogate tissues and serial tumor tissues from Phase 1b patients.

Methods: PD biomarker analysis of surrogate tissues and tumors was performed in the vantictumab Phase 1a dose escalation study in patients with solid tumors. Hair follicle samples collected at Day 0 and Day 28 or Day 35 from 9 patients enrolled in 4 dose-escalation cohorts with vantictumab administered 0.5 mg/kg weekly (QW), 0.5 mg/kg every other week (QoW), 1 mg/kg weekly (QW), 1 mg/kg every three weeks (Q3W), and 1 mg/kg every three weeks (Q3W). Three biopsy cohorts were collected at Day 0 and Day 28 after vantictumab administration. Additionally, serum samples from 24 patients in 7 dose-escalation cohorts with vantictumab administered 0.5 mg/kg weekly (QW), 0.5 mg/kg every other week (QoW), and 1 mg/kg every three weeks (Q3W) were analyzed. Three biopsy cohorts were collected at Day 0, Day 7, and Day 35 from vantictumab administration. Tumor tissues were collected at time points post-dosing as indicated and described above from the vantictumab clinical phase 1a dose escalation study in patients with solid tumors.

Microarray Analysis: RNA was isolated from the hair follicles and biopsy samples and global gene expression profiles were assessed using Affymetrix HG-U133 plus2. GCRMA was used to normalize the arrays and summarize the signals. Empirical Bayes analysis (LIMMA) was used to identify the genes differentially expressed in the samples between pre-dose and post-dose time points, or between different dosing groups. Pairwise sample bootstrapping was used to access the significance of fold changes. The 95% CI (Bias-Corrected adjusted, BCA) was calculated according to standard methods, applying a non-parametric bootstrap procedure.

Results: Wnt pathway genes (e.g., AXIN2, and stem cell and differentiation genes (e.g., BMI1, GFI1) were found to be regulated in hair follicles by vantictumab treatment. In tumors, vantictumab inhibited Wnt target (e.g., AXIN2, TCF4), stem cell and EMT genes (HMXA2, ZEB2) and increased the expression of differentiation genes, including MUC4, MUC5B, and MUC20. These PD effects were observed 1 to 2 weeks after dosing and were evident at the lowest vantictumab Phase 1a dose (0.5 mg/kg QoW). There was dose- and schedule-dependent modulation of bone turnover markers.

Conclusions: The PD effects of vantictumab on Wnt target, stem cell and differentiation pathway genes in surrogate tissues and tumor tissues from serial biopsies, as well as on bone turnover markers, were clearly established in this first-in-human study.

STUDY DESIGN

Patient Samples: Hair follicles, serum, and 3 biopsied tumors were collected at time points post-dosing as indicated and described above from the vantictumab clinical phase 1a dose escalation study in patients with solid tumors.

Microarray Analysis: RNA was isolated from the hair follicles and biopsy samples and global gene expression profiles were assessed using Affymetrix HG-U133 plus2. GCRMA was used to normalize the arrays and summarize the signals. Empirical Bayes analysis (LIMMA) was used to identify the genes differentially expressed in the samples between pre-dose and post-dose time points, or between different dosing groups. Pairwise sample bootstrapping was used to access the significance of fold changes. The 95% CI (Bias-Corrected adjusted, BCA) was calculated according to standard methods, applying a non-parametric bootstrap procedure. The fold-change represents the gene expression ratio comparing post-treatment to pre-treatment (Day 0) samples. Gene Set Enrichment Analysis (GSEA) was performed to obtain the biological processes effected by vantictumab in the biopsied tumors.

Bone markers: Bone resorption (iCTX) and bone formation (BSAP, total osteocalcin, and P1NP) markers in serum were measured at designated time points. Enzyme immunoassay (EIA) was used to measure BSAP activity. iCTX was assayed by electrochemiluminescence and the concentrations of total osteocalcin and P1NP were measured using a chemiluminescence immunoassay. Pairwise sample bootstrapping was used to measure the significance of the 4 bone marker changes. The effects of vantictumab at different dose groups were assessed by mixed-effects model analysis.

References
6. Smyth and Gurney are employees of the University of Michigan which has an equity interest in and has licensed intellectual property to OncoMed Pharmaceuticals and may receive royalties.

SUMMARY OF FINDINGS

• Pharmacodynamic effects of vantictumab established in tumor biopsies, hair follicles, and blood
• PD effects are consistent with Wnt biology.
• Vantictumab inhibits Wnt pathway and cancer stem cell genes in hair follicles and tumors.
• Vantictumab up-regulates differentiation genes in hair follicles and tumors.
• Vantictumab increases bone degradation marker iCTX & decreases bone formation markers P1NP & Osteocalcin.
• PD effects of vantictumab were observed at lowest dose levels & extended beyond serum exposure.
  • Starting at dose of 0.5 mg/kg (with half-life of 1.5 days), PD effects of vantictumab persist in tumors 1 to 2 weeks after dosing.
  • Increases of iCTX were also observed at lowest dose levels & decreases of bone formation markers Osteocalcin and P1NP only occurred at higher dose levels.