



Published in final edited form as:

Clin Cancer Res. 2014 December 1; 20(23): 5937–5945. doi:10.1158/1078-0432.CCR-14-1269.

Pilot Clinical Trial of Hedgehog Pathway Inhibitor GDC-0449 (Vismodegib) in Combination with Gemcitabine in Patients with Metastatic Pancreatic Adenocarcinoma

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Abstract

Background—The hedgehog (HH) signaling pathway is a key regulator in tumorigenesis of pancreatic adenocarcinoma (PDA) and is up-regulated in PDA cancer stem cells (CSCs).

GDC-0449 is an oral small-molecule inhibitor of HH pathway. This study assessed the effect of GDC-0449-mediated HH inhibition in paired biopsies, followed by combined treatment with gemcitabine, in patients with metastatic PDA.

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Declaration of potential conflicts of interest:

None

Author contributions: Edward J. Kim contributed to the tissue acquisition, correlative studies, data interpretation, patient accrual and manuscript development. Vaibhav Sahai contributed to the statistical analysis, manuscript development and interpretation of the data. Ethan V. Abel contributed to the correlative studies and provided substantive changes to the manuscript. Kent Griffith conceived the statistical section of this trial and contributed to the statistical analysis. Joel K. Greenson performed the microscopic and immunohistochemical evaluation of the tissue specimens. Gazala N. Khan contributed patients to the study. John L. Blau, Ronald Craig and Ulysses G. Balis performed the trichrome staining and evaluation of the tissue specimens. Naoko Takebe assisted with study evaluation, study design and manuscript preparation. Mark M. Zalupski assisted with clinical trial design, patient accrual, manuscript preparation and interpretation of data. Diane M. Simeone conceived and planned the work, assisted with clinical trial design, interpreted correlative studies and assisted with manuscript preparation. All authors approved the final submitted version of the manuscript.

Methods—Twenty-five patients were enrolled of which 23 underwent core biopsies at baseline and following 3 weeks of GDC-0449. On day 29, 23 patients started weekly gemcitabine while continuing GDC-0449. We evaluated GLI1 and PTCH1 inhibition, change in CSCs, Ki-67, fibrosis, and assessed tumor response, survival and toxicity.

Results—On pre-treatment biopsy, 75% of patients had elevated sonic hedgehog (SHH) expression. On post-treatment biopsy, *GLI1* and *PTCH1* decreased in 95.6% and 82.6% of 23 patients, fibrosis decreased in 45.4% of 22 and Ki-67 in 52.9% of 17 evaluable patients. No significant changes were detected in CSCs pre- and post-biopsy. The median progression-free and overall survival for all treated patients was 2.8 and 5.3 months. The response and disease control rate was 21.7% and 65.2%. No significant correlation was noted between CSCs, fibrosis, SHH, Ki-67, GLI1, PTCH1 (baseline values, or relative change on post-treatment biopsy) and survival. Grade >3 adverse events were noted in 56% of patients.

Conclusion—We show that GDC-0449 for 3 weeks leads to down-modulation of GLI1 and PTCH1, without significant changes in CSCs compared to baseline. GDC-0449 and gemcitabine was not superior to gemcitabine alone in the treatment of metastatic pancreatic cancer.

Keywords

GDC-0449; stem cells; hedgehog; SMO; GLI1; PTCH1; fibrosis

Introduction

Pancreatic ductal adenocarcinoma (PDA) remains a disease with an exceptionally poor prognosis (1). Gemcitabine has been the cornerstone chemotherapeutic agent for PDA based on clinical benefit compared to 5-fluorouracil (2). Attempts to enhance response with combinations of cytotoxic agents have not yielded clinically meaningful results until recently (3-9). In 2010, the FOLFIRINOX regimen demonstrated an improvement in median overall survival (OS) in metastatic PDA to 11.1 months as compared to 6.8 months with gemcitabine [10]. Toxicity and morbidity of this combination, however, limit broad applicability. More recently, the addition of nab-paclitaxel to gemcitabine demonstrated improved median overall survival (8.5 months vs. 6.7 months) compared with gemcitabine alone (10). Despite these recent advances, however, the overall outcome remains dismal for this patient population.

The cellular and biochemical factors that underlie intrinsic resistance of PDA to therapy remain poorly understood. Notably, both the primary tumor and distant metastasis are associated with an intense desmoplastic reaction which has been suggested to limit the delivery of chemotherapy and initiate cross-talk between stromal cells and the cancer cells, promoting chemoresistance at the microenvironment level (11, 12). The hedgehog (HH) signaling pathway is a developmental pathway that is dormant in the adult pancreas but is reactivated early in PDA development (13). Paracrine HH signaling from PDA cells to stromal cells promotes stromal desmoplasia. HH pathway inhibition has been shown to deplete this desmoplastic stroma in a genetically engineered mouse model (GEMM) of pancreas cancer (14). The HH ligand binds to its receptor patched 1 (PTCH1). In the unbound state, PTCH1 inhibits smoothened (SMO), a G-protein coupled phosphoprotein

receptor, presumably by preventing its localization to the cell surface. Signaling by SMO results in the activation of *GLI* transcription factors and consequent induction of HH target genes, including *GLI* and *PTCH1*. Pre-clinical data suggests that inhibition of the HH pathway via small molecule inhibitors of SMO can lead to decrease in growth and tumorigenesis of human PDA cell lines (15), as well as prevent distant metastasis from orthotopic xenograft cancers in mice (16). In addition to targeting this upstream component of the hedgehog (HH) signaling pathway, efforts are also being made to target the final step of this pathway, by directly inhibiting the *GLI* family of transcription factors (17).

In addition to desmoplasia, cancer stem cells (CSCs), capable of unlimited self-renewal in both primary tumor and metastases, have been proposed as a mechanism for cancer progression and chemotherapy resistance (18). We have previously reported that SHH is upregulated in PDA CSCs, with a distinct population of CD44⁺/CD24⁺/ESA⁺ cancer stem cells shown to have SHH expression 46-fold higher than the CD44⁻/CD24⁻/ESA⁻ cells (19). Also, overexpression of *GLI1* is observed at the mRNA level in a subset of SSC-low/aldehyde dehydrogenase (ALDH) -“bright” cells with increased clonogenic potential (20). Upregulation of the HH pathway may, therefore, play a significant role in CSC-driven carcinogenesis. These observations raise the possibility that inhibition of the HH signaling pathway will enhance tumor control by targeting underlying tumor initiating CSCs.

GDC-0449 (vismodegib) is a small-molecule SMO antagonist which inhibits the HH signaling pathway. In a phase I study, 68 patients with solid malignancies refractory to standard therapies were treated with GDC-0449. Tumor responses were observed in 20 (29.4%) patients (19 with basal cell carcinoma) and GDC-0449 was noted to have an acceptable safety profile (21). In this pilot study, we intended to evaluate the effect of GDC-0449 inhibition of the HH signaling pathway, initially used alone to evaluate the effect of GDC-0449 on paired biopsies, and then in combination with gemcitabine, in patients with previously untreated, metastatic PDA. This trial uniquely provided a prospective evaluation of HH pathway inhibition directly in PDA by incorporating paired core biopsies of tumor before and after treatment with GDC-0449. The primary end point was to evaluate the effect of HH signaling on PDA CSCs. Additional objectives were to evaluate inhibition of *GLI1* and *PTCH1*, change in Ki-67 and the stromal component of the tumors. Key secondary end points included progression free survival at 3 months, overall response and disease control rate, overall survival and evaluation of toxicity of GDC-0449 alone, and in combination with, gemcitabine. This report summarizes the efficacy, safety and biomarker results for 25 patients enrolled on this study.

Patients and Methods

Eligibility

Patients with pathologic confirmation of, and previously untreated metastatic pancreatic carcinoma, including patients receiving adjuvant therapy at least 6 months prior to development of metastatic disease, were eligible for this study. Patients were required to have measurable disease and tumor accessible for serial core needle biopsies. Further eligibility criteria included life expectancy greater than 12 weeks, Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1, and adequate hematologic,

renal, and hepatic function. Exclusion criteria included prior systemic chemotherapy for metastatic disease. The study was approved by the Institutional Review Board at the University of Michigan and performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. Written, informed consent was obtained from all patients prior to study entry. This trial is registered on the clinical trials site of the United States National Cancer Institute Web site (<http://clinicaltrials.gov/ct2/show/NCT01195415>).

Study Design and Treatment

This was a single arm pilot study in 25 patients to evaluate the effect of GDC-0449 inhibition of the HH signaling pathway, initially alone, and then in combination with gemcitabine. GDC-0449 was administered orally at a dose of 150 mg daily as mono-therapy for the first 4-week cycle. While continuing on daily GDC-0449, intravenous gemcitabine at a dosage of 1000 mg/m² was infused over 30 minutes on days 1, 8 and 15 of each subsequent 4-week cycle beginning with cycle 2. Two sets of biopsies from the same lesion (3 cores) were required for study participation; one set prior to start of therapy and a second set 3 weeks after initiation of single agent GDC-0449. GDC-0449 was generously provided by Genentech Inc. and the National Cancer Institute, NIH.

GDC-0449 was held for up to 4 weeks for grade 3 or 4 toxicity attributable to GDC-0449 but no dose reductions were permitted. Dose modifications for gemcitabine were based on toxicity experienced during prior therapy and platelet count and absolute neutrophil count (ANC) measured on each day of treatment. For an absolute ANC > 1,000/mm³ and platelets > 75,000/mm³, no adjustments were made and full dose of drugs delivered. For an ANC >500/mm³ and <1000/mm³ and/or platelets >50,000/mm³ and <75,000/mm³, gemcitabine was given with a 50% reduction. Additionally, gemcitabine was also held for any non-hematologic toxicities > grade 3 (except alopecia, nausea and vomiting not optimally treated with anti-emetics, or grade 3 liver function test abnormalities not attributable to treatment) with treatment resumption upon improvement to < grade 1 with 25% dose reduction. Patients were continued on study regardless of disease status following the initial 4 weeks on study with GDC-0449 mono-therapy provided their performance status permitted continuing treatment.

Assessment

Tumor assessments were performed at baseline (BL1), after the first cycle of GDC-0449 mono-therapy (BL2), and then subsequently following every 2 cycles of combination therapy. Response to treatment was assessed using revised Response Evaluation Criteria in Solid Tumors (RECISTv1.1) and confirmed at least 4 weeks after first noted. CA 19-9 serum levels were drawn at baseline and repeated at the start of each cycle. Safety assessments included monitoring adverse events (AEs), performing laboratory tests (hematology, serum chemistry and urinalysis) and physical examinations. Severity of AEs was assessed by using the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4.01. Serious AEs (SAEs) were defined in accordance with the International Conference on Harmonization Guidelines for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, Topic E2A.

Statistical Analysis

Clinical outcome parameters, including progression free survival (PFS) at 3 months, median PFS, overall response rate (ORR), disease control rate (DCR) and OS were measured. OS was defined as the time from date of first protocol therapy until death (considered an event) or last patient contact (considered censored). PFS was calculated from date of first dose of protocol therapy to date of documented disease progression or death from any cause, whichever came first. Experiments for biomarker evaluation were done in triplicate.

The relationship between variables was tested by χ^2 and Fisher's exact test. Comparison of changes in biomarkers between serial biopsy specimens was conducted using paired t-test after appropriate normalizing transformation. Specifically for the proportion of CSCs, the arcsin of the square root of the proportion was used. Efficacy analysis for PFS and OS was done using the Kaplan-Meier method and the log-rank test for inference testing. The Cox proportional hazards model was used to estimate hazard ratios (HRs). The P-values of <0.05 were considered statistically significant. The survival analysis was completed using the SAS v9.3 (SAS Institute, Cary, NC). The secondary analyses of PFS and OS were event driven and all evaluable patients were included. Safety analysis included patients who received at least one dose of the study treatment.

Serial Biopsies

The biopsy samples were evaluated for presence of tumor, and immediately processed in the biopsy suite and partitioned as follows for correlative experiments. Cores of tumor tissue were divided into ~5 mm pieces. One piece was placed in RNAlater and transferred on ice to minus 20 degree for storage until further processing. A second piece was placed on ice prior to freezing in OCT media. Small segments from each core were placed immediately into 10% formalin. The remaining tissue was placed in Media 199 on ice and processed into single cell suspension for flow cytometry analysis.

Immunohistochemistry

Tissue samples were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Formalin-fixed, paraffin-embedded sections were cut 4 μm thick, mounted on poly-L-lysine-coated slides (Sigma, St. Louis, MO), and dried overnight at 37°C. Sections were then dewaxed in xylene, rehydrated according to standard histopathologic procedures, and stained with hematoxylin and eosin. A blinded pathologist reviewed the pathology slides and evaluated for presence of tumor cells, degree of fibrosis by trichrome staining and Ki-67 (Abcam, Cambridge, MA; clone SP6; 1:200 dilution) and SHH (Millipore, clone EP1190Y) according to standard IHC procedures described previously (22). Stained samples were graded by two variables: percentage of stained cancer cells (0-100%) and the intensity of staining (0-3) on IHC. A baseline SHH level (H-score) was calculated by multiplying the two variables for a range of scores from 0 to 300.

Quantitative Real Time-PCR analysis

Patient biopsies were dissociated in QIAzol lysis reagent (Qiagen) with 0.143M β -mercaptoethanol added to further denature RNases. Total RNA was isolated following the

RNeasy Plus Universal kit and protocol (Qiagen) and converted to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). qRT-PCR was performed using a Rotor-Gene Q thermocycler (Qiagen) and Taqman® Universal Master Mix II (with UNG) (Applied Biosystems, Foster City, CA). Cycling conditions were as follows: 50°C for 2 minutes, 95°C for 10 minutes, and 70 repeats of 95°C for 15 seconds and 60°C for 1 minute. Fold change in *GLI1* and *PTCH1* mRNA was calculated using the

Ct method normalized to GAPDH. For calculation purposes, any samples that did not amplify were given a CT value of 70. Taqman® probes for GAPDH (Hs99999905_m1), *GLI1* (Hs01110766_m1), and *PTCH1* (Hs00181117_m1) were purchased from Applied Biosystems (Foster City, CA).

Flow cytometry analysis for CSCs

The primary objective was to evaluate the effect of GDC-0449 on the percentage of pancreatic CSCs on serial biopsy. Single cell suspensions of tumor cells was prepared as described previously (19) with modifications as noted below. Primary human PDA tissue was minced completely and then suspended in 200 unit/mL ultrapure collagenase IV (Worthington Biochemicals, Freehold, NJ) in Media 199 (Invitrogen, Carlsbad, CA). After enzyme digestion at 37°C for 45 to 60 minutes and mechanical dissociation by pipetting every 15 minutes with a 10 mL pipette, the digested and dissociated cells were filtered through a 40 µm nylon mesh cell restrictor (B.D. Franklin Lakes, NJ) and washed with HBSS (Invitrogen, Carlsbad, CA) twice. The cells were then re-suspended in 2% FBS in HBSS for experiments.

Antibodies PerCP-Cy5.5-conjugated mouse anti-human CD44 (B.D., Franklin Lakes, NJ), FITC-conjugated mouse anti-human CD24 (B.D., Franklin Lakes, NJ), APC conjugated mouse anti-human ESA (Miltenyi Biotec, Bergisch Gladbach, Germany), PE-conjugated anti-human CD30 and anti-CD45 (B.D., Franklin Lakes, NJ), were added at a 1:50 dilution, and the sample was incubated for 45 minutes on ice and then washed twice with HBSS/2%FBS. Cells were re-suspended in HBSS containing 3 µM 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen, Carlsbad, CA). Flow cytometry was done using a XDP cell sorter (Beckman Coulter, Brea, CA). Side scatter and forward scatter profiles were used to eliminate cell doublets. CD30 and CD45 positive profiles were used to eliminate hematopoietic and endothelial lineage cells. ESA was used for positive selection of epithelium-derived tumor cells to distinguish from stromal cells. The number of CSC was determined as a percentage of live cancer cells (DAPI⁻/CD30⁻/CD45⁻) that were profiled as ESA⁺/CD44⁺/CD24⁺ by flow cytometry.

Results

Patient Characteristics

Thirty patients were consented and registered on the study between July 2010 and August 2012. Five patients were screen failures due to hyponatremia (1), absence of cancer on biopsy (1), death secondary to progression prior to treatment (1), and hyperbilirubinemia (2). Twenty-five patients were treated on this trial and received a mean (range) of 5.2 (1 to 15) cycles of therapy. Two patients did not undergo repeat biopsy on day 22 due to rapid

progression within first few weeks and were not considered evaluable for biomarker assessment. There were 15 men and 10 women with a median age of 65 (range, 46 to 80) years (Table 1). Twenty-four had adenocarcinoma while one patient had acinar cell carcinoma. A majority (64%) had ECOG PS 0 while the remainder (36%) had PS 1. All patients have discontinued therapy due to progressive disease (80%), patient withdrawal (8%), or AEs (12%).

Efficacy Results

The 3-month PFS in all treated patients was 40%, median PFS 2.8 months (95% CI, 1.4 to 4.7 months) (Figure 1A), median OS 5.3 months (95% CI, 3.6 to 8.4 months) (Figure 1B), and the one-year survival 20% (Table 2). Twenty-three patients with serial biopsies and evaluable for the primary study endpoint had imaging performed after 4 weeks (BL2) on single agent GDC-0449, and subsequently 15 patients (65%) had at least one additional scan after 2 months of combined treatment with gemcitabine and GDC-0449. All 23 patients had evidence of increase in tumor measurements from BL1 to BL2 (range 4 to 84%; progressive disease in 9 (39.1%) and stable disease in 14 (60.9%)) and were continued on combination therapy with gemcitabine. Using BL1, and as per RECISTv1.1 criteria, 5 patients had confirmed partial response for an ORR of 21.7% with mean OS of 16.2 months. An additional 10 patients had stable disease on imaging for a DCR of 65.2%, however, 3 of those patients had clinical progression for an overall clinical DCR of 52.2% (Table 2).

In the 23 patient evaluable for response to combination treatment, CA19-9 was elevated at baseline in 19 (82.6%) patients. Three (13.0%) had normal values throughout the course of therapy, 1 (4.3%) had missing data, 6 (26.1%) had >50% decrease at best CA19-9 response, and 13 (56.5%) had CA 19-9 similar to or increasing from baseline throughout treatment (Table 3). CA 19-9 was correlated with OS in these 23 patients and those 6 patients with >50% decrease in CA 19-9 had significantly increased median OS compared to those with <50% decrease or continued increase (13.8 versus 4.9 months; HR 0.06, 95% CI 0.01 to 0.49; $p=0.008$) (Supplementary Figure 1).

Safety results

No minor or major complications occurred as a result of the first or second for research biopsies. Out of 25 treated patients, 21 (84%) patients had at least one AE attributable to therapy and more than half the treated patients (56%) had AE > grade 3. The most common treatment-related AEs of any grade were fatigue (60%), dysgeusia (56%), hyponatremia (52%), nausea/vomiting (50%), anorexia (48%), elevation in liver enzymes (36%), anemia (32%), and alopecia (32%) (Supplementary Table 1). The most common grade 3 treatment-related AEs were anemia (12%) and elevation in liver enzymes (12%). One patient on GDC-0449 and gemcitabine had grade 4 thrombocytopenia and another developed colonic perforation at splenic flexure at the site of contact with splenic mass and required transverse colostomy. One patient died within the first 30 days on the study due to rapid disease progression.

Correlative studies

CSCs—We have previously demonstrated an increased level of SHH expression in pancreatic CSCs (19). The median (range) percentage of CD44+/CD24+/ESA+ CSCs in 22 patient samples was 4.79% (0.43 to 37.2%). Following treatment with GDC-0449, the median (range) percentage decreased to 3.09% (0.74 to 45.9%) with a relative and absolute decrease by 35.4% and 1.7%, which was not statistically significant ($p = 0.21$, t test after normalizing the distribution). There was no significant correlation between change in percentage of CSCs and OS (Table 3).

Sonic hedgehog (SHH) level—The median (range) baseline H-score was 270 (10 to 300) (Figure 2A). Nine of the 20 patients had a maximal H-score of 300 and 15 of 20 had an H-score > 200 demonstrating high expression of SHH level at baseline which suggests an activated SHH pathway in PDA.

Hedgehog pathway—Single agent GDC-0449 decreased *GLII* and *PTCHI* mRNA levels in 22 (95.6%) and 19 (82.6%) patients on day 22 compared to baseline levels, with a median decrease of 93% and 38% respectively (Table 4). These results indicate that GDC-0449 inhibited HH pathway signaling after 3 weeks of daily administration. However, decreased levels of *GLII* or *PTCHI* did not correlate with OS (Table 3).

Fibrosis—The HH pathway has been implicated as a contributing factor to the development of desmoplastic stroma typical of PDA. GDC-0449 monotherapy decreased the degree of fibrosis in 10 (45.4%) of 22 evaluable patients (Figure 2B) with no median change over the 3 week period (Table 4).

Proliferative index (Ki-67)—The median (range) proliferative index was 40% (0.12 to 86%) at baseline in the 20 samples available for this marker (Figure 2C). Following monotherapy with GDC-0449, there was not a statistically significant change in median Ki-67 index and there was no correlation between change in Ki-67 and OS (Table 3).

Discussion

In this report, we evaluated the effect of GDC-0449, a small-molecule SMO antagonist which inhibits the HH signaling pathway, alone and then in combination with gemcitabine in patients with metastatic PDA. Serial biopsies were taken at baseline and after 3 weeks on GDC-0449. In the setting of target-based cancer drug development, it is critical to establish in early phase clinical trials that any observed clinical activity might be attributed to modulation of the target by evaluating sequential pre- and post-treatment biopsies. Out of 25 patients enrolled on this study, 23 (92%) had post-treatment biopsies, demonstrating that sequential tumor biopsies are feasible and safe during early phase clinical trials in patients with PDA. Significantly, we validate that GDC-0449 successfully inhibits HH pathway signaling by down-modulation of *GLII* and *PTCHI* in vivo after 3 weeks of daily administration of single agent GDC-0449.

Using a GEMM, Olive and colleagues (14) showed reduced stromal content and improved survival in mice with use of IPI-926, a SMO antagonist, in combination with gemcitabine

compared with gemcitabine alone which provided strong pre-clinical evidence to support its development in humans. However, the phase IB/randomized phase II study of GDC-0449 with or without gemcitabine in patients with metastatic PDA showed no significant difference in median PFS (4.0 and 2.5 months; HR, 0.81; 95% CI, 0.54 to 1.21; p, 0.03), or OS (6.9 and 6.1 months; HR, 1.04; 95% CI, 0.69 to 1.58; p, 0.84) (23). Similarly, the phase 2 IPI-926 plus gemcitabine or gemcitabine plus placebo study showed no improvement in efficacy on preliminary analysis following which the trial was closed (24). The median of 5.3 months on this trial is also somewhat lower than the historical data for gemcitabine alone (10, 23, 25). This could be either due to lack of single agent activity as evidenced by progression in all patients during first 4 weeks on single-agent GDC-0449, or secondary to possible deleterious effect of SHH inhibition on survival as shown recently in GEMMs by Rhim et al (26). In that study, genetic deletion of SHH in the neoplastic pancreatic epithelium of a pancreatic cancer GEMM led to development of aggressive tumors with reduced stromal content, undifferentiated histology, increased vascularity and heightened proliferation and consequent decreased OS compared to a matched pancreatic cancer GEMM with wild-type SHH (26). In the analysis of biopsy samples before and after GDC-0449 treatment in this study, we did not observe a relative decrease in stromal content after treatment. Possible explanations for the discrepancy in histologic findings in the Rhim study and our clinical trial in patients include: shorter exposure to HH pathway inhibition prior to repeat biopsy, tumor heterogeneity on repeat biopsy, differences in levels of change in HH signaling in the two model systems, or lack of correlation in mouse and human tumor responses.

Interestingly, the observed ORR of 21.7% for GDC-0449 in combination with gemcitabine is higher than reported for gemcitabine alone (5 - 9%) in phase III clinical trials (2, 10). This is probably due to variability from a small sample size in this trial, and less likely from the efficacy of the GDC-0449 and gemcitabine combination. It is notable that the five patients who achieved a confirmed PR in our study achieved a mean (range) survival of 16.2 (6.8 to 27.3) months after receiving an average of 11.2 cycles on this trial, although we cannot exclude the possibility that these patients would have had similar benefit with gemcitabine mono-therapy. We were unable to identify any significant correlation between overall survival and tumor stroma, Ki-67, CSCs and SHH level in this sub-group of patients.

Recently, there has been increasing interest in understanding the contribution of pancreatic stem cells to cancer development and more importantly, the investigative possibility to specifically target these CSCs to improve on survival in PDA. We have shown previously that SHH expression is markedly up-regulated in pancreatic CSCs (19) and aberrant expression has been found to produce PanIN lesions and develop genetic changes similar to PDA (15). Here we demonstrated that following administration of single agent GDC-0449, the CSCs had a relative decrease by 35.4% in 22 evaluable patients compared to baseline. This difference in percentage of CSCs after 3 weeks of single agent GDC-0449, however, did not have significant correlation with survival.

We did not observe any significant correlation between expression of SHH, Ki-67, GLI1, and PTCH1 at baseline, or changes in expression following GDC-0449, and OS. Hwang and colleagues (27) showed that the efficacy of HH antagonists may be dependent on the tumor-

associated stromal compartment. However, on exploratory analysis, we did not observe any significant correlation between degree of stroma at baseline, or change in degree of stroma on serial biopsy, and baseline SHH expression, GLI1 inhibition, or OS. Perhaps in part due to a small number of patients, we also were unable to identify a predictive marker for the select group of patients who responded well to the combination and experienced prolonged clinical benefit.

Overall, GDC-0449 alone and in combination with gemcitabine has an acceptable safety profile with no new or unexpected toxicity. The most common AEs included long-term, low grade fatigue, dysgeusia, nausea/vomiting, hyponatremia, anorexia, alopecia and myalgia. This side effect profile is consistent with data obtained from the phase I trial in metastatic solid malignancies (21).

In conclusion, treatment with GDC-0449 in patients with metastatic pancreatic adenocarcinoma showed significant down-regulation of the HH target genes GLI1 and PTCH1 in vivo. However, no significant correlation was noted between CSCs, fibrosis, SHH, Ki-67, GLI1, PTCH1 (baseline values, or relative change on post-treatment biopsy) and individual survival. Moreover, the GDC-0449 and gemcitabine combination did not improve on the median PFS or OS compared to historical data for gemcitabine alone in this cohort of patients with pancreatic cancer arguing against pursuing the use of this treatment regimen in patients with metastatic pancreatic cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the patients and their families who participated in this study. We would like to acknowledge Dr. Dafydd Thomas and Dr. Thomas Giordano along with the University of Michigan Comprehensive Cancer Center Tissue Core for Ki-67 and SHH staining. Additionally, we would like to acknowledge Kevin Frank and Amy Tsai for data management support and the UMCCC Biostatistics Core for their support in the design stage, data analysis and scientific interpretation. GDC-0449 was supplied by the Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI) and was provided to the NCI under a collaborative Agreement between Genentech Inc. (San Francisco, CA) and DCTD, NCI.

Declaration of funding:

This research is supported (in part) by the National Institutes of Health through the University of Michigan's Cancer Center Support Grant (5 P30 CA046592) by the use of the Cancer Center Clinical Trials Office Core. It is also supported (in part) by a Michigan Institute for Clinical and Health Research Pilot Grant (ULRR024986).

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Statement of translational relevance

Pancreatic ductal adenocarcinoma (PDA) remains a disease with an exceptionally poor prognosis. The hedgehog (HH) signaling pathway is a key regulator in tumorigenesis of PDA. We have previously shown that sonic hedgehog (SHH) is up-regulated in pancreatic cancer stem cells (CSCs). This study assessed the effect of GDC-0449 (vismodegib), an oral small-molecule inhibitor, on HH pathway inhibition through paired biopsies, before and after GDC-0449 monotherapy, followed by GDC-0449 plus gemcitabine, in patients with metastatic PDA. We evaluated GLI1 and PTCH1 inhibition, change in CSCs, Ki-67, tumor stroma, and assessed tumor response, survival and toxicity. No significant correlation was noted between CSCs, fibrosis, SHH, Ki-67, GLI1, PTCH1 (baseline values, or relative change on post-treatment biopsy) and survival. We show that while GDC-0449 for 3 weeks leads to down-modulation of GLI1 and PTCH1, the sequential regimen of GDC-0449 and gemcitabine was not superior to gemcitabine alone in the treatment of metastatic PDA.

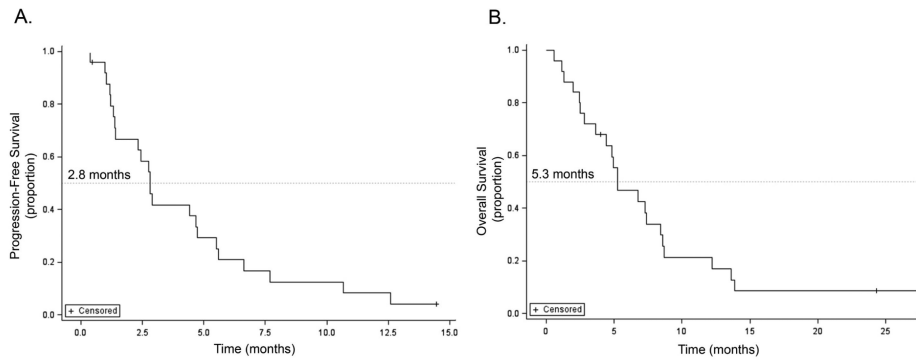


Figure 1. Median progression-free survival (A) and overall survival (B) in patients with metastatic pancreatic adenocarcinoma receiving 150 mg of GDC-0449 daily for 4 weeks followed by 150 mg GDC-0449 daily and 1000 mg/m² of gemcitabine on days 1, 8 and 15 every 28 days.

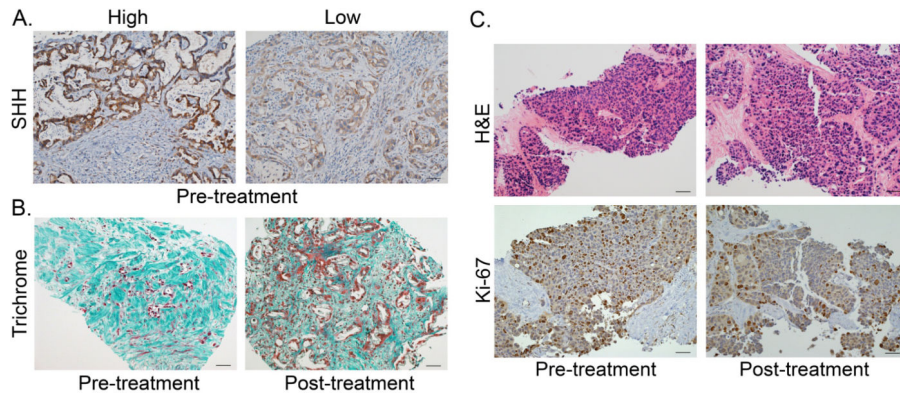


Figure 2.

A. Immunostaining for sonic hedgehog (SHH) shows high (>200) and low (<200) SHH score in patients with pancreatic adenocarcinoma (PDA). B. Trichrome staining shows decrease in fibrosis on repeat biopsy after 3 weeks of GDC-0449 monotherapy compared to baseline. C. PDA tissue from same patient immunostained for Ki-67 shows decrease in expression on repeat biopsy after 3 weeks of GDC-0449 monotherapy compared to baseline. The tissue was also stained with hematoxylin and eosin (H&E) to assess for morphology.

Table 1

Demographics and Baseline Characteristics

Variable	Total patients enrolled (N=25)	
	No. of patients	%
Age, years		
Median	65	
Range	46-80	
Sex		
Male	15	60
Female	10	40
ECOG performance status		
0	16	64
1	9	36
Race		
Caucasian	24	96
African-American	1	4
Histology		
Adenocarcinoma	24	96
Acinar	1	4
Location of biopsy		
Liver metastasis	17	68
Primary pancreatic mass	4	16
Omental metastasis	2	8
Other (perihepatic mass, neck lymph node)	2	8
Serum CA 19-9		
Median, U/mL	3211	
Range, U/mL	4-429,939	
> 150 U/mL	18	72
Biopsy		
Baseline	25	100
At 3 weeks	23	92

Abbreviations: ECOG, Eastern Cooperative Oncology Group; U, units

Table 2

Summary of Efficacy

Variable	No. of Patients	%
Number of Evaluable Patients	25	
Progression free survival		
At 3 months	10	40
Median, months	2.8	
95% CI, months	1.4 to 4.7	
Overall survival		
Median, months	5.3	
Range, months	3.6 to 8.4	
Number of Evaluable Patients	23	
Radiological response, after 4 weeks on GDC-0449 alone		
Complete response	0	0
Partial response	0	0
Stable disease	14	60.9
Progressive disease	9	39.1
Objective response rate	0	0
Radiological response, best overall		
Complete response	0	0
Partial response	5	21.7
Stable disease	10	43.5
Progressive disease	8	34.8
Objective response rate	5	21.7
CA 19-9 response, after 4 weeks on GDC-0449 alone		
No. of evaluable patients *	20	87
> 20 % decrease	0	0
20 % decrease	1	5
Primary increase	19	95
CA 19-9 response, best overall		
No. of evaluable patients *	19	82.6
> 50 % decrease	6	31.6
50 % decrease	2	10.5
Primary increase	11	57.9

Abbreviations: CI, confidence interval

* Number of patients is less than the 23 (=overall evaluable patients) due to normal values throughout course of therapy (n=3) and missing values at follow-up (n=1).

Table 3

Impact of Clinical and Correlative Variables on Overall Survival

Variable	Evaluable patients (N=23)			
	No. of patients *	Hazard Ratio	95 % CI	P
Age				
>65 years	9	2.72	0.98-7.58	0.05
≤65 years	14			
Gender				
Male	14	0.63	0.25-1.60	0.33
Female	9			
Baseline CA 19-9				
> 1000 U/mL	15	2.15	0.82-5.63	0.12
≤ 1000 U/mL	8			
Baseline SHH				
> 200	12	0.76	0.29-1.99	0.76
≤ 200	8			
Baseline Ki-67				
> 30 %	13	0.68	0.26-1.79	0.43
≤ 30 %	7			
Baseline <i>GLII</i>				
> 0.5	9	1.01	0.42-2.46	0.98
≤ 0.5	14			
Baseline <i>PTCHI</i>				
> 2.0	11	0.79	0.33-1.89	0.60
≤ 2.0	12			
Baseline fibrosis				
> 50 %	14	0.44	0.18-1.10	0.08
≤ 50 %	9			
Baseline cancer stem cells				
> 5 %	9	0.75	0.30-1.86	0.54
≤ 5 %	13			
Change in Ki-67, after 3 weeks				
Decrease	9	0.87	0.32-2.35	0.79
Increase	8			
Change in <i>GLII</i>, after 3 weeks				
> 75 % decrease	15	0.85	0.32-2.26	0.74
≤ 75 % decrease, or increase	6			
Change in <i>PTCHI</i>, after 3 weeks				
Decrease	19	0.79	0.26-2.39	0.68

Variable	Evaluable patients (N=23)			
	No. of patients *	Hazard Ratio	95 % CI	P
Increase	4			
Change in fibrosis, after 3 weeks				
Decrease	10	1.07	0.44-2.61	0.89
Increase	12			
Change in cancer stem cells, after 3 weeks				
Decrease	11	0.62	0.24-1.60	0.32
Increase	10			
CA 19-9 response, best overall				
> 50 % decrease	6	0.13	0.03-0.60	0.01
50 % decrease, or increase	13			

Abbreviations: CI, confidence interval; Shh, sonic hedgehog.

* Number of patients may be less if both pre-treatment and post-treatment samples were not available.

Table 4

Summary of Correlative Studies

Relative change in variable between baseline and repeat biopsy after 3 weeks on GDC-0449	Evaluable patients (N=23)	
	No. of patients	%
Ki-67 index, IHC		
No. of evaluable patients	17	68
> 20 % decrease	3	17.65
20 % decrease	6	35.29
Increase	8	47.06
Median		-1.07
Cancer stem cells, % FACS		
No. of evaluable patients	21	84
> 20 % decrease	10	47.62
20 % decrease	1	4.76
Increase	10	47.62
Median		-16.08
Fibrosis, trichrome		
No. of evaluable patients	22	88
> 50 % decrease	3	13.64
0-50 % decrease	7	31.82
Increase	12	54.54
Median		0
GLII, qRT-PCR		
No. of evaluable patients	23	100
> 50 % decrease	19	82.61
0-50 % decrease	3	13.04
Increase	1	4.35
Median		-93
PTCH1, qRT-PCR		
No. of evaluable patients	23	100
> 50 % decrease	9	39.13
50 % decrease	10	43.48
Increase	4	17.39
Median		-38

Abbreviations: IHC, immunohistochemistry; FACS, fluorescence activated cell sorting; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction.