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Pharmacodynamic study of the oral hedgehog pathway inhibitor, vismodegib, in patients with metastatic castration-resistant prostate cancer

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Abstract

Purpose—Hedgehog (Hh) pathway signaling has been implicated in prostate cancer tumorigenesis and metastatic development and may be upregulated even further in the castration-resistant state. We hypothesized that antagonism of the Hh pathway with vismodegib in men with metastatic castration-resistant prostate cancer (mCRPC) would result in pathway engagement, inhibition and perhaps induce measurable clinical responses in patients.

Methods—This is a single-arm study of oral daily vismodegib in men with mCRPC. All patients were required to have biopsies of the tumor and skin (a surrogate tissue) at baseline and after 4 weeks of therapy. Ten patients were planned for enrollment. The primary outcome was the pharmacodynamic assessment of Gli1 mRNA suppression with vismodegib in tumor tissue. Secondary outcomes included PSA response rates, progression-free survival (PFS), overall survival (OS) and safety.

Results—Nine patients were enrolled. Gli1 mRNA was significantly suppressed by vismodegib in both tumor tissue (4/7 evaluable biopsies, 57%) and benign skin biopsies (6/8 evaluable

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Compliance with ethical standards

Conflict of interest ESA has served as a paid consultant/advisor for Janssen, Astellas, Essa, Sanofi, Dendreon, Merck and Medivation; has received research funding to his institution from Genentech, Novartis, Astellas, Sanofi, Dendreon, Bristol Myers Squibb, Janssen, Johnson & Johnson, Medivation, Merck and Tokai; and is a co-inventor of a biomarker technology that has been licensed to Tokai.

Human and animal rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from all individual participants included in the study.

biopsies, 75%). The median number of treatment cycles completed was three, with a median PFS of 1.9 months (95% CI 1.3, NA), and a median OS of 7.04 months (95% CI 3.4, NA). No patient achieved a PSA reduction or a measurable tumor response. Safety data were consistent with the known toxicities of vismodegib.

Conclusions—Hh signaling, as measured by Gli1 mRNA expression in mCRPC tissues, was suppressed with vismodegib in the majority of patients. Despite this pharmacodynamic response that indicated target inhibition in some patients, there was no apparent signal of clinical activity. Vismodegib will not be developed further as monotherapy in mCRPC.

Keywords

Vismodegib; Hedgehog; Gli; Metastatic castration-resistant prostate cancer

Introduction

Hedgehog signaling has been demonstrated to be crucial in normal tissue development, including organogenesis, through regulation of cell differentiation and cell proliferation [1, 2]. However, alterations to this pathway are common, leading to a wide range of human pathology including various cancers such as prostate cancer [3, 4]. The canonical signaling pathway is activated when the secretory protein sonic hedgehog binds to and inactivates Patched1 (PTCH1), a transmembrane protein responsible for inhibiting Smoothened (Smo). Smo leads to a diverse signaling cascade, ultimately activating the transcription factor Gli1, which positively regulates many genes including cyclin D1, c-Myc and Bcl-2.

Of particular importance to prostate cancer, the androgen receptor (AR) is an important regulator of the Hh pathway [5, 6]. Androgen deprivation therapy has been shown to upregulate Hh signaling. Efsthathiou and colleagues demonstrated this principle in prostatectomy tissue specimens, noting increased Hh expression in men receiving neoadjuvant ADT compared to controls [7]. Also, preclinical models suggest that targeting this pathway leads to important clinical changes to the AR axis. Ibuki et al. [8] demonstrated delayed progression to castration resistance and decreased Gli1 expression in xenograft models using a Smo antagonist.

Preclinical studies demonstrate Hh signaling may be an important target in treating prostate cancer. Multiple agents have demonstrated activity at inhibiting prostate cancer cell growth in cell culture and xenograft models by antagonizing the Hh pathway [8–10]. Itraconazole is one agent known to have antagonistic activity on Gli1 expression [11]. In the first prostate cancer-specific clinical trial targeting the Hh pathway, itraconazole demonstrated some clinical activity resulting in a median PFS of 35.9 weeks in the high-dose arm (600 mg daily) [12]. Additionally, pharmacodynamic evaluation of Gli1 demonstrated inhibition of the target in this clinical trial, suggesting that some of the clinical effects were mediated through antagonism of Hh signaling.

Vismodegib is an FDA-approved agent for the treatment of basal cell carcinoma. It potently inhibits Hh signaling without any known effect on the cholesterol/steroid pathway in

contrast to itraconazole. To our knowledge, this is the first published clinical trial of vismodegib in prostate cancer.

Materials and methods

Patients

This study enrolled men with mCRPC who had metastatic tumors accessible for biopsy. Patients were eligible for enrollment after disease progression on prior therapy based on Prostate Cancer Working Group 2 (PCWG2) criteria [13]. There were no exclusions based on number or type of prior therapies. Treatment with any prior anti-androgens, chemotherapy, immunotherapy or other investigational agents was permitted, with a 2- to 4-week washout, depending on the prior therapy. All patients were required to be at least 18 years of age. Patients must have documented a castrate level of serum testosterone (<50 ng/dL). All patients must have an ECOG of 0–1 at the time of enrollment. Patients must have adequate renal (creatinine clearance of ≥ 40 mL/min), liver (AST/ALT $\leq 2.5 \times$ ULN and total bilirubin $\leq 1.5 \times$ ULN) and bone marrow function (ANC $\geq 1500/\mu\text{L}$, platelet count $\geq 100,000/\mu\text{L}$). All patients were counseled to use condoms, even after a vasectomy, during sexual intercourse with female partners of reproductive potential during treatment with vismodegib and for 2 months after the last dose to avoid exposing a pregnant partner and unborn fetus to vismodegib. Patients were excluded if concurrently using systemic corticosteroids (>5 mg of prednisone or equivalent dose). Also, patients were excluded if they had known brain metastasis or untreated meningeal or dural disease, were being actively treated with other investigational agents, requiring ongoing treatment with anticoagulants or diagnosed with a bleeding diathesis, or had any documented grade 2 or higher treatment-related toxicity from any prior therapy. All patients were required to provide written, informed consent.

Study design

This is a single-institution, single-arm phase 2 clinical trial and pharmacodynamic study. At the time of study enrollment, all patients underwent a tumor biopsy and skin biopsy of an unaffected area. Blood parameters were obtained including complete blood count (CBC), complete metabolic profile (CMP), activated partial thromboplastin time (APTT) and prothrombin time with international normalized ratio calculation (PT/INR). A nuclear medicine bone scan and CT scan of the chest, abdomen and pelvis were performed to establish a baseline of the metastatic disease burden. Prostate-specific antigen (PSA) tests were obtained at the time of enrollment and every 4 weeks on study. All patients then were treated with oral, daily vismodegib (150 mg) until disease progression, unacceptable toxicity or patient consent withdrawal. After four weeks of therapy, all patients had repeat tumor and skin biopsies and collection of standard blood tests (PSA, CBC and CMP). Repeat CT and bone scans were performed at 3-month intervals for disease evaluation.

Statistics

The primary endpoint was the proportion of patients treated with vismodegib who achieved a pharmacodynamic (PD) response, defined as a decrease in GLI1 mRNA expression >1.2 times the standard deviation (SD) of the baseline values and at least a 50% (twofold)

reduction in GLI1 mRNA expression in metastatic tumor biopsies when comparing the paired post-treatment biopsies to pre-treatment biopsies. The trial planned to enroll 10 evaluable patients who had measurements of GLI1 levels from tumor biopsy samples both at baseline and after 4 weeks of treatment. Significant inhibition of GLI1 expression with vismodegib would be declared if at least five of 10 patients demonstrate a PD response. The design had 90% power to detect a true 65% PD response rate across patients, with a false-positive rate of 3.3% under the null hypothesis that vismodegib has no effect in downregulating Gli1 expression. Fold changes from baseline are reported using descriptive statistics and paired sample *t* tests.

The effect of vismodegib on PSA responses was assessed as the proportion of patients with PSA responses with $\geq 50\%$ PSA reductions at any time point. PSA response data were summarized with a proportion and presented with an exact 95% confidence interval.

The PSA progression-free survival (PSA-PFS) and progression-free survival (PFS) are defined by the Prostate Cancer Working Group 2 (PCWG2) criteria using RECIST 1.1 criteria for each patient. PSA-PFS and PFS are defined as the time of first dose (a) until PSA progression (by 25% increase in PSA from nadir) or death and (b) until any evidence of progression (by 25% increase in PSA from nadir, a new lesion on bone or CT scan, or physical examination) or death. PFS will be assigned to the earliest observed time. Patients who had not yet met the primary or secondary endpoint (PFS, OS) at the last follow-up were censored at the date when the last tumor assessment was made. Overall survival is defined as the time from study enrollment until either the time of death from any cause or the censor date from the last known follow-up. Results are summarized using the Kaplan–Meier method with 95% confidence intervals.

The correlation between change from baseline in Hh pathway gene expression levels in tumor tissue and skin biopsies is reported using Spearman's correlation coefficient. The relationship between the change of Hh pathway gene expression levels pre- and post-therapy and clinical response is reported using descriptive statistics in summary tables. For discrete clinical endpoints, the number of patients achieving the clinical endpoint is compared using regression. For survival endpoints, the relationship is explored through Cox proportional hazards models.

Study outcomes

The primary outcome was the proportion of patients who achieved both a $\geq 50\%$ (twofold) decrease in GLI1 mRNA expression and a decrease >1.2 times the standard deviation of the baseline value in GLI1 expression between the pre-treatment and post-treatment tumor biopsies. Secondary outcomes include PSA response rates as defined by the proportion of patients experiencing a $\geq 50\%$ PSA reduction at any time point during the study; determination of the objective tumor response rate as defined by RECIST 1.1 for each patient; determination of the PSA progression-free survival (PSA-PFS) as defined by PCWG2 criteria for each patient; and the correlation between Hh pathway gene suppression and clinical responses (if responses were observed). Finally, safety and tolerability, including any drug-related toxicities of vismodegib, were reported via CTCAE version 4.0.

Pharmacodynamic assessments

The tumor and skin biopsies were evaluated for changes to Hh-regulated transcripts, including GLI1, GLI2, PTCH1 and AKT1, between pre-treatment and post-treatment tumor and skin biopsies as evaluated by qRT-PCR. RNA from fresh-frozen tissue from either tumor or skin biopsies was isolated using the Qiagen RNeasy Micro Kit (Cat#774004). For skin biopsies, tissue was first processed utilizing the QIAshredder (Qiagen, 79654) as well. First-strand complementary DNA was synthesized from RNA with RT2 You Prime First Strand Beads (GE Healthcare, cat. No. 27926401) with random hexamer primers (Invitrogen, cat no. N808127). Quantitative RT-PCR was performed in quadruplicate for each specimen using Taqman reactions (Taqman Universal Master Mix II, Life Technologies (cat#4440040) with primers for Gli1 (Hs02800695_m1), Gli2 (Hs01119974_m1), PTCH1 (Hs00181117_m1) or AKT1 (Hs00178289_m1) and normalized to HPRT1 (Hs02800695_m1). Applied Biosystems software was used to calculate threshold cycle values for each gene of interest.

Results

Patient characteristics

In total, ten patients were screened and nine patients enrolled into the study. The study was closed by the sponsor after nine patients enrolled. Baseline characteristics are shown in Table 1. The median age at diagnosis of prostate cancer is 58 (range, 48–76 years), and median age at enrollment is 65 years (range 58–79 years). One patient was documented to have metastatic disease at diagnosis, and the other eight patients had localized disease at diagnosis that subsequently progressed. The median total number of prior systemic therapies excluding androgen deprivation therapy is 3 (range, 1–6). Five patients (56%) had bone pain at enrollment, and six patients (67%) had documented visceral metastatic disease at enrollment. All patients had an ECOG PS of 0–1. The median PSA at enrollment was 98.6 ng/mL. The median Gleason score is 9 (range 7–10 with one patient having an unknown Gleason score). Most patients had advanced, high-risk disease as noted by the high incidence of visceral metastatic disease and the high rate of prior docetaxel chemotherapy use in this study population.

Pharmacodynamic assessment

Pharmacodynamic analysis of mRNA expression levels as measured by RT-PCR in tumor and benign skin tissue was only available in seven of the nine patients. One patient did not receive a post-treatment biopsy, and another patient had insufficient tissue for evaluation from the repeat biopsy. GLI1 expression was significantly reduced in tumor biopsies with a median decrease of 9.8-fold (range 8841-fold decrease to 122-fold increase), while the GLI1 expression in skin was reduced by a median of 5.7-fold (range 11.7-fold decrease to 1.1-fold decrease). In tumor tissue, four of seven patients (57%) were noted to have Gli1 levels decrease after vismodegib therapy. However, only two of seven met the primary endpoint of both a twofold decrease and 1.2 standard deviation decrease. Since the primary endpoint was a change in the pharmacodynamic endpoint in tumor tissue, the primary outcome was not met (see Fig. 1a: waterfall plot of Log2 mRNA expression fold change in tumor biopsies). Gli1 expression in the skin was reduced in all evaluable patients. However, only

five of the eight met the more robust criteria of a twofold decrease and at least a 1.2 standard deviation decrease. Gli2 expression was decreased in skin tissue biopsy samples in four of seven patients (57%), with a median decrease of 1.15-fold (range 2.01-fold decrease to 1.77-fold increase). However, Gli2 expression in tumor was only decreased in three of seven patients (42%) for a median increase of 2.64-fold (range 11.00-fold decrease to 13.83-fold increase) (see Fig. 1b). AKT expression in tumor biopsies was decreased in four of seven patients (57%) for a median decrease of 1.15-fold (range 5.28-fold decrease to 2.93-fold increase), and the mRNA levels from skin biopsies were decreased in five of seven patients (71%) for a median decrease of 1.23-fold (2.69-fold decrease to 1.21-fold increase) (see Fig. 1c). Finally, PTCH1 expression was decreased in four of seven patients (57%), and five of seven patients (71%) in tumor and skin biopsy tissue, respectively, for a median decrease of 1.87-fold (range 2.81-fold decrease to 23.10-fold increase) and 1.41-fold decrease (range 3.18-fold decrease to 1.32-fold increase) (see Fig. 1d). There was no statistically significant correlation between skin and tumor biopsy changes in any pharmacodynamic endpoint studied. See Table 2: correlation of mRNA expression in tumor and skin biopsies.

Clinical outcomes

Seven patients were evaluable for PSA response. One patient had no measurable PSA production at enrollment, and PSA remained <0.01 throughout the study. A second patient had disease progression prior to the first on-study PSA evaluation. All seven evaluable patients had PSA increases as the best response to therapy (see Fig. 2, PSA response). All patients progressed prior the pre-specified restaging interval of 3 months. The median PSA-PFS was 0.92 months (95% CI 0.9, NA). At the time of data cutoff, all patients have progressed and six patients have died. The median PFS was 1.87 months (95% CI 1.3, NA). The median OS was 7.04 months (95% CI 3.4, NA) (see Fig. 3, survival analysis). There was no association between Gli1 mRNA expression levels in either the tumor or skin biopsies and PSA-PFS, PFS or OS when analyzed by univariate Cox analysis (see supplementary information).

Safety

Vismodegib was well tolerated with few grade 3 and no grade 4 events. The majority of the grade 3 events were determined by the treating physician to be unrelated to the study drug. The most common adverse events included dysgeusia, nausea/vomiting, fatigue, musculoskeletal pain and anorexia, which is consistent with the known toxicity profile of vismodegib. There were no new safety concerns noted in this study. Several common toxicities noted in other trials of vismodegib, including alopecia, hyponatremia and liver dysfunction, did not occur in this study (see Table 3: adverse events, for a complete list of all the adverse events).

Discussion

This is the first published prostate cancer-specific clinical trial targeting the Hh pathway with a potent Hh inhibitor. The trial was designed with the primary endpoint of a pharmacodynamic response to Gli1 mRNA expression. Based on the biopsy results, this trial did not meet its primary endpoint. Even though all patients experienced a decrease in

intratumoral or cutaneous Gli1 expression from vismodegib therapy, it did not meet our pre-specified criteria. Despite the inhibition of the intended drug target, there does not appear to be any clinical benefit in this small phase II clinical trial in this patient population. All patients experienced disease progression as the best response to therapy as measured by all endpoints, including PSA and radiographic analysis.

Despite the strong preclinical evidence identifying the Hh pathway as an important target in the pathogenesis of prostate cancer, vismodegib did not appear to produce any clinical benefits in this study population. It is important to recognize that these patients had aggressive, advanced disease as noted by the high Gleason scores and were heavily pre-treated with multiple hormonal and chemotherapy agents. It is possible that a single, targeted agent in this population is insufficient to produce meaningful clinical benefits. However, the lack of any apparent clinical activity despite the significant pharmacodynamics activity in many of these patients makes further pursuit of monotherapies selectively targeting the Hh pathway difficult to justify. Combination approaches may possibly hold value. For example, potential synergies have been demonstrated between inhibition of the Hh pathway and the epidermal growth factor (EGFR) pathway [14]. It is also possible that Hh-targeted therapies may have more activity in a different patient population, such as patients with localized hormone-sensitive prostate cancer or in those with biochemical (PSA only) recurrence following local therapy. The lack of clinical benefit with vismodegib suggests that the efficacy seen with prior clinical trials using other agents was in part due to off-target effects. For instance, in the previously reported study using itraconazole [12], the therapeutic efficacy was likely mediated through other pathways such as antagonism of the steroid synthesis pathway or angiogenesis.

Our study demonstrates the significant value of biopsy-based clinical trials in studying the potential efficacy of novel therapeutic targets using relatively small patient populations in short time frames. It also teaches us that robust target inhibition may not lead to clinical responses in patients. In this case, it is unlikely that Hh pathway activation is a dominant driver of disease biology in the castration-resistant state, and alternative therapeutic strategies should continue to be pursued.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Waterfall plot of Log2 mRNA expression fold change in tumor biopsies. **a** Gli1 tumor biopsy, **b** Gli1 skin biopsy, **c** Gli2 tumor biopsy, **d** Gli2 skin biopsy, **e** AKT tumor biopsy, **f** AKT skin biopsy, **g** PTCH1 tumor biopsy, **h** PTCH1 skin biopsy

Fig. 2.
Waterfall plot of best PSA response

Fig. 3. Kaplan-Meier survival curves. **a** The median PSA-PFS is 0.92 months (0.90, NA); **b** the median PFS is 1.87 months (1.3, NA); **c** the median OS is 7.04 months (3.4, NA)

Table 1

Baseline patient characteristics

Patient ID	Age at enrollment	Gleason	PSA	Prior docetaxel	Total number of prior therapies (excluding ADT)	Bone pain	ECOG PS	Visceral metastatic disease
2	66	9	21.4	Yes	5	Yes	0	No
3	65	9	46.7	Yes	2	Yes	0	No
4	65	9	1615.7	Yes	5	Yes	1	Yes
6	78	Unknown	155.2	Yes	3	No	1	Yes
7	58	8	98.6	No	3	Yes	1	Yes
8	79	9	74.5	No	2	Yes	1	No
9	71	7	4	Yes	1	No	1	Yes
10	70	8	1491.8	Yes	5	No	1	Yes
11	60	10	0	No	3	No	1	Yes

Table 2

Correlation of mRNA expression in tumor and skin biopsies

mRNA	Correlation	CI	<i>P</i> value
Gli1	0.35	−0.54, 0.87	0.44
Gli2	0.22	−0.64, 0.83	0.64
AKT	0.15	−0.68, 0.81	0.75
PTCH1	0.64	−0.22, 0.94	0.12

Table 3

Adverse events

Adverse event	All grades	%	Grade 3–4	%
AST elevation	2	22		
ALT elevation	2	22		
ALK Phosphatase elevation	2	22		
Anemia	3	33	1	11
Anorexia	4	44		
Constipation	1	11		
Creatinine elevation	2	22		
Dehydration	1	11	1	11
Diarrhea	2	22		
Dysgeusia	3	33		
Dyspnea	2	22	1	11
Fatigue	7	77		
Headache	1	11		
Hematocrit decrease	1	11		
Hyperlipidemia	1	11		
Hypocalcemia	1	11		
Lymphocyte decrease	1	11		
Muscle cramps	2	22		
Nausea	6	66	1	
Pain, musculoskeletal	5	55	1	11
Pain, other	5	55	2	22
Paresthesia	1	11		
Platelet count decrease	1	11		
Pneumonia	1	11	1	11
Sore throat	1	11		
Vomiting	4	44	1	11
Weakness	2	22		