Clinical Cancer Research

A First-in-Human Phase I Study of the Anticancer Stem Cell Agent Ipafricept (OMP-54F28), a Decoy Receptor for Wnt Ligands, in Patients with Advanced Solid Tumors

Antonio Jimeno[1], Michael Gordon[2], Rashmi Chugh[3], Wells Messersmith[1], David Mendelson[2], Jakob Dupont[4], Robert Stagg[4], Ann M. Kapoun[4], Lu Xu[4], Shailaja Uttamsingh[4], Rainer K. Brachmann[4], and David C. Smith[3]

Abstract

Purpose: Wnt signaling is implicated in tumor cell dedifferentiation and cancer stem cell function. Ipafricept (OMP-54F28) is a first-in-class recombinant fusion protein with the extracellular part of human frizzled 8 receptor fused to a human IgG1 Fc fragment that binds Wnt ligands. This trial evaluated ipafricept in patients with solid tumors.

Experimental design: A 3+3 design was used; ipafricept was given intravenously every 3 weeks. The objectives were determination of dose-limiting toxicities (DLTs), recommended phase 2 dose (RP2D), safety, pharmacokinetics (PK), immunogenicity, pharmacodynamics (PD), and preliminary efficacy.

Results: 26 patients were treated in seven dose-escalation cohorts (0.5, 1, 2.5, 5, 10, 15, and 20 mg/kg). No further dose escalation was pursued as PK modeling indicated that the target efficacious dose was reached at 10 mg/kg, and fragility fractures occurred at 20 mg/kg. Most common related grade 1 and 2 adverse events (AEs; $\geq$20% of patients) were dysgeusia, decreased appetite, fatigue, and muscle spasms. Ipafricept-related grade 3 TEAEs included hypophosphatemia and weight decrease (1 subject each, 3.8%). Ipafricept half-life was $\sim$4 days and had low incidence of antidrug antibody formation (7.69%) with no impact on drug exposure. Six patients had β-C-terminal telopeptide (β-CTX) doubling from baseline, which was reversible. PD modulation of Wnt pathway genes in hair follicles occurred $\geq$2.5 mg/kg. Two desmoid tumor and a germ cell cancer patient experienced stable disease for >6 months.

Conclusions: Ipafricept was well tolerated, with RP2D of 15 mg/kg Q3W. Prolonged SD was noted in desmoid tumor and germ cell cancer patients. *Clin Cancer Res; 23(24); 7490–7. ©2017 AACR.*

Introduction

Wnt genes, defined for their sequence homology to Integration 1 (INT-1) in mice (1) and its homologue *Wingless (*Wg) in Drosophila (2), are critical for cell fate determination and cell polarity during development. The crucial role of Wnt signaling in development was demonstrated in Drosophila, where Wg loss led to segment polarity defects (3). Three Wnt signaling pathways have been identified, including the canonical, noncanonical planar cell polarity pathway, and the noncanonical Wnt/Ca^{2+} pathway. In the canonical Wnt pathway, a cysteine-rich Wnt ligand binds the receptor termed Frizzled (4, 5) and low-density lipoprotein (LDL) receptor-related protein 5/6 (LRP5/6) that acts as a coreceptor (6–8) leading to pathway activation. Nineteen Wnt ligands and 10 Fzd receptors have been identified (9).

Wnt signaling is important in stem cell maintenance and determination of stem cell fate (10, 11), and hematopoiesis (12). Aberrant Wnt signaling in the stem cell compartment contributes to tumorigenesis, as shown by the high levels of Wnt expression observed in cancer stem cells (CSC) from colon cancer grown as spheroids (13).

Ipafricept (OMP-54F28) is a truncated Fzd8 receptor fused to the IgG1 Fc region that blocks Wnt signaling (14). In a patient-derived model of pancreatic cancer treated with gemcitabine and ipafricept, ipafricept alone reduced tumor growth to a greater extent than gemcitabine alone and a combination of the two showed additive effects. Ipafricept reduced the frequency of CSCs, an effect that was maximal in combination with gemcitabine, and resulted in a decrease in both liver and lung metastases.

This preliminary data supported exploring ipafricept in humans as a novel anti-CSC agent. This first-in-human phase I study reports the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD) markers, and preliminary antitumor efficacy, and recommended phase 2 dose (RP2D) for the truncated Fzd receptor monoclonal antibody ipafricept administered every 3 weeks in patients with advanced solid tumors. Wnt

[1]University of Colorado School of Medicine, Aurora, Colorado. [2]Pinnacle Oncology Hematology, Scottsdale, Arizona. [3]University of Michigan, Ann Arbor, Michigan. [4]OncoMed Pharmaceuticals, Redwood City, California.

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Corresponding Author: Antonio Jimeno, University of Colorado School of Medicine, 12801 East 17th Avenue Building RC-1 South; 8106, Aurora, CO 80045. Phone: 303-724-2478, Fax: 303-724-3892; E-mail: antonio.jimeno@ucdenver.edu

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Translational Relevance

This study reports a trial of ipafricept, a novel Wnt pathway inhibitor in patients with solid tumors. In addition to showing feasibility and adequate tolerability, pharmacodynamic studies revealed pathway inhibition in seriated normal tissue samples. Clinical efficacy was documented in tumors with Wnt and related pathway dependence.

pathway inhibition has effects in the bone, including bone remodeling (15), findings that were confirmed in the preclinical development of ipafricept (OncoMed, data on file). In the present study, bone assessments, including densitometry and the dynamics of β-C-terminal telopeptide (β-CTX), a marker of increased bone turnover, were closely assessed.

Patients and Methods

Patient eligibility

Eligible patients were ≥18 years, with histologically proven advanced solid malignancies, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, tumor at least 1 cm in a single dimension and radiographically apparent on CT or MRI, last chemotherapy, biologic, or investigational therapy ≥4 weeks prior to enrollment, and adequate organ function. Exclusion criteria included receiving other investigational anticancer agents; brain metastases; active/significant clinical issues; osteoporosis based on the total femur and L1–L4 T scores on screening DEXA bone density scan; bone metastases with a prior history of a fragility fracture, or with a lytic lesion requiring an orthopedic intervention, or not receiving a bisphosphonate or denosumab as per institutional guidelines; glucocorticoid therapy at the equivalent of ≥5 mg of oral prednisone for ≥4 weeks within the last 8 weeks; fasting β-CTX > 1,000 pg/mL; metabolic bone disease; or history of or newly diagnosed insufficiency or morphometric vertebral fracture. All patients provided written informed consent, and the study was approved by local Institution Review Boards and was conducted in accordance with the declaration of Helsinki, the International Conference on Harmonization Good Clinical Practice guidelines, and all applicable local regulatory requirements and laws.

Healthy volunteer selection

All volunteers for hair biomarker analysis provided informed consent and samples were anonymized whereby the volunteers' identifiers and the sample codes were deleted resulting in no traceability back to the volunteer.

Study design and treatments

This phase I, dose-escalation study (NCT01608867) was conducted at three centers in the United States. The primary objectives were to determine the safety, dose-limiting toxicities (DLT), maximum tolerated dose (MTD), andRP2D for ipafricept every 3 weeks (Q3W). Secondary objectives included PK, PD, immunogenicity, and antitumor activity of ipafricept. Enrollment was sequential in a "3+3" design.

Ipafricept was administered intravenously over 30 minutes Q3W with doses of 0.5, 1, 2.5, 5, 10, 15, and 20 mg/kg. Dose escalation was allowed if DLT occurred in 0/3 or ≤1/6 patients in each cohort during any time from the first dose to 28 days after the first dose (i.e., study days 0–28).

DLTs were defined during the first 28 days of treatment, as any possible treatment emergent grade ≥3 toxicities. A DLT was defined as any grade 3 or greater AE (except for grade 3 infusion reactions that resolve within 24 hours) as assessed using the CTCAE version 4.02 of the NCI, or any AE that results in the subject being discontinued from study that occurs any time from study days 0 to 28, unless the event can be clearly attributed to another cause. Study treatment continued until disease progression, unacceptable toxicity (including DLT criteria above), or withdrawal of consent.

Study assessments

Safety assessments were conducted weekly throughout the study and for 30 days after treatment. Adverse events (AE) were graded using Common Terminology Criteria for Adverse Events (CTCAE), version 4.02. In addition to β-CTX at screening, every 28 days while on study, and t treatment termination, a complete bone safety assessment by DEXA was performed at screening, every 56 days, and at treatment termination. Tumor response was assessed every 56 days, according to RECIST version1.1. Tumor markers were followed prospectively when elevated at baseline.

Pharmacokinetic analyses

Samples were obtained for PK analysis prior to and after infusion and 0.5, 1, 3, 6, 24, and 72 hours after infusion following the first and third infusions. Samples were also obtained prior to and at the end of the second infusion and a single sample was obtained on nondosing days 7, 14, 28, 35, 49, 56, and 63. Finally, samples were obtained weekly for the first 4 weeks following discontinuation of study drug and then at weeks 8 and 12 following termination of study drug. Serum was assayed for concentrations of ipafricept by ELISA.

For PK analyses, noncompartmental analysis was conducted for individual subjects with evaluable PK data. Summary statistics, including mean, standard deviation, median, minimum, and maximum of the PK parameters, were reported by dose group. A preestablished plasma level of 170 μg/mL of ipafricept was defined to inform and determine dose escalation and RP2D definition.

Immunogenicity

Blood samples were collected for analysis of anti-ipafricept antibodies using an electrochemiluminescence bridging immunoassay (ADA): predose on cycle 1 day 1, and on day 1 of every other cycle as well as at the 15-, 30- and 60-day follow-up visits.

Biomarker studies

Hair samples were used to study the PD of Wnt signaling. RNAs of the hair follicles were extracted using PicoPure RNA Isolation Kit (Life Technologies). RNAs were visualized on the Agilent 2100 Bioanalyzer, and integrity was confirmed by the presence of intact 28S and 18S ribosomal peaks. Affymetrix human gene chip U133 Plus 2.0 arrays were used for profiling the gene expression levels in hair follicle samples (Almac Diagnostics). GCRMA was used to normalize the arrays and to summarize the signals. Paired-sample empirical Bayes analysis was used to identify genes differentially expressed in the samples between predose and postdose time points and between different dosing groups. Genes were significantly modulated based on a p value less than 0.05 and an

absolute fold change greater than 1.5. In addition, paired-sample bootstrapping was used to access the significance of the fold changes (SAS, R). The 95% CI (Bias-Corrected adjusted, BCa) was calculated according to standard methods, applying a non-parametric bootstrap procedure. Genes were considered significant at a 95% confidence interval (CI) and a gene expression change of greater than 1.5-fold. The hair data from the control subjects without ipafricept treatment were analyzed using the same methods. Gene Set Enrichment Analysis was performed to obtain the biological processes affected by ipafricept in hair follicles.

Statistical methods

All analyses were conducted using SAS software version 9.1 or higher (SAS Institute). The general analytical approach for all endpoints was descriptive in nature. There were four analysis populations: intent to treat (ITT), safety, immunogenicity, and PK.

Demographic and analytical data were summarized using descriptive statistical methods. Summary statistics were presented for age, height, and weight by cohort and overall. Ethnicity, race, age category, and gender were summarized by cohort and overall. Duration of exposure in days, total number of infusions given, total dose received (mg), dose intensity, and number of subjects by cohort were summarized for all subjects. All safety analyses were conducted on the safety population. Treatment-emergent adverse events were coded using version 13.1 or higher of the Medical Dictionary for Regulatory Activities. Toxicity grade was defined according to the CTCAE v4.02. All laboratory tests, vital sign measurements, and ECG data were presented in data listings. The ECOG scores were summarized for the safety population as frequencies and percentages using a shift from baseline table by visit. Compliance was summarized as the number of subjects who had dose(s) interrupted.

Results

Patients

Between October 2012 and January 2014, 26 patients received at least one dose of ipafricept. Patients' baseline characteristics are summarized in Table 1.

Table 1. Characteristics of 26 enrolled patients

Characteristic	Number	%
Age, years		
Median	54	
Range	26–79	
Gender		
Female	9	35
Male	17	65
ECOG performance status		
0	11	42
1	15	58
Tumor type		
Colorectal	4	15
Pancreas	3	12
Cervix	2	8
Desmoid	2	8
Lung	2	8
Other	11	41

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Table 2. Dose escalation

Dose level (mg/kg)	Patients (n)	Cycles, median (range)
0.5	3	2 (1–4)
1	3	3 (1–18)
2.5	3	5 (2–6)
5	5	3 (2–7)
10	3	3 (3–4)
15	3	1 (3–5)
20	6	2 (1–9)

Abbreviation: DLT, dose-limiting toxicity.

Dose escalation

Dose escalation and the number of patients treated in each dose level are listed in Table 2. No DLTs were observed, and the MTD was not reached per protocol. Although subjects in the 20 mg/kg cohort did not experience protocol-defined DLTs during the first 28 days (i.e., DLT window), the trough levels achieved in this dose level more than doubled the preestablished threshold plasma level of 170 μg/mL predicted to be biologically effective and efficacious in preclinical models were exceeded, and dose escalation stopped.

Additionally, there were fragility fractures reported at 20 mg/kg in two subjects (grade 2 fragility fractures on days 175 and 253). Therefore, 20 mg/kg was not considered a safe dose to continue further clinical investigations, and the next lower dose level, 15 mg/kg once every 3 weeks, was considered tolerable and the RP2D of ipafricept.

The mean and median numbers of infusions administered across all treatment cohorts were 5.9 and 3, respectively (range, 2–47), and the median duration of exposure was 43 days.

Tolerability

All 26 patients are evaluable for toxicity. Table 3 summarizes treatment-emergent adverse events (TEAE), related to the study treatment. Treatment-related adverse events resolved or improved to grade $\leq$2 upon treatment discontinuation. Table 4 lists TEAEs occurring in $\geq$10% subjects in descending order of total frequency, regardless of attribution. All patients experienced at least one TEAE, and the most common were fatigue, decreased appetite, dysgeusia, nausea, constipation, muscle spasms, back pain, vomiting, peripheral edema, and decreased weight. Most events were grade 1 or 2. No patients died during the study.

Two subjects in the 20 mg/kg dose level experienced grade 2 fragility fractures (L3 pedicle and sacral insufficiency) during the study, that were attributed to ipafricept. Both events occurred late in the course of therapy (days 253 and 175, respectively), and were preceded by β-CTX elevations >50% postdose (191–308 pg/mL and 528–1,050 pg/mL, comparing days 0–56, respectively). Six of 26 subjects experienced a $\geq$2-fold increase of β-CTX (2 in cohort 1, 1 in cohort 5, and 3 in cohort 7). Five of these 6 subjects (1 subject each in cohorts 1 and 5, and 3 subjects in cohort 7) received zoledronic acid, and in all 5 cases, with follow-up, β-CTX returned to baseline. A third patient who had asymptomatic elevation of β-CTX did not receive zoledronic acid as the patient was transitioned to hospice care upon coming off study due to progression.

Pharmacokinetics

Ipafricept PK data are shown in Table 5 and Fig. 1. A total of 25 out of the 26 subjects had sufficient data for estimation of pharmacokinetic parameters. PK of ipafricept appeared to be

Table 3. Treatment-related TEAEs (all grade 1 or 2, except hypophosphatemia and an occurrence of weight loss in patients in 20 mg/kg levels, noted with an asterisk)

Cohort	1 ($n = 3$)	2 ($n = 3$)	3 ($n = 3$)	4 ($n = 5$)	5 ($n = 3$)	6 ($n = 3$)	7 ($n = 6$)	Total ($n = 26$)
Dose level (mg/kg)	0.5	1	2.5	5	10	15	20	
Dysgeusia	1	—	—	1	2	1	5	10 (38.5%)
Fatigue	1	1	1	—	1	1	4	9 (34.6%)
Muscle spasms	1	—	—	2	2	1	3	9 (34.6%)
Decreased appetite	—	—	2	2	1	—	3	8 (30.8%)
Alopecia	—	1	—	—	1	—	3	5 (19.2%)
Nausea	—	1	1	—	1	—	2	5 (19.2%)
Vomiting	—	—	1	1	—	1	1	4 (15.4%)
Weight decreased	—	—	—	—	—	1	3*	4 (15.4%)
Diarrhea	—	—	—	2	1	—	—	3 (11.5%)
Hypercalcemia	—	—	—	1	—	—	2	3 (11.5%)
Hypocalcemia	1	1	1	—	—	—	—	3 (11.5%)
Hypophosphatemia	1	—	—	1	—	—	1*	3 (11.5%)
Nail disorder	—	—	—	—	1	—	2	3 (11.5%)
Pruritus	1	—	—	—	1	—	1	3 (11.5%)
L3 pedicle fracture	—	—	—	—	—	—	1	1 (3.8%)
Sacral insufficiency fracture	—	—	—	—	—	—	1	1 (3.8%)

mostly linear in the dose and concentration ranges studied. Drug exposure parameters such as AUC, peak concentration, trough concentration increased proportionally with dose. Between the 1 and 20 mg/kg dose levels, clearance was slow as expected for protein therapeutics, ranging from 14.5 to 22.2 mL/day/kg with no notable dose dependency. Terminal half-life of ipafricept was about 4 days (ranging from 3.5 to 5.2 days). The steady state volume of distribution of ipafricept maintained relatively stable across the dose levels of 0.5 and 20 mg/kg, and ranged from approximately 71.1 to 112 mL/kg, which suggested the molecule distributes primarily in the vascular space with modest extravasation into the tissue space. The volume of distribution was larger than what would have been expected for a monoclonal antibody, probably due to the comparatively lower ipafricept molecular weight. Intersubject variability in clearance and volume of distribution was low for ipafricept, which is another hallmark of protein therapeutics when compared with small molecule therapeutics.

Table 4. TEAEs occurring in ≥15% of subjects in descending order of frequency ($n = 26$), regardless of attribution

Preferred term	Subjects, *N* (%)
Total number of TEAEs	496
Number of subjects with at least one TEAE	26 (100)
Fatigue	14 (53.8)
Decreased appetite	11 (42.3)
Dysgeusia	10 (38.5)
Nausea	10 (38.5)
Constipation	9 (34.6)
Muscle spasms	9 (34.6)
Back pain	8 (30.8)
Vomiting	8 (30.8)
Oedema peripheral	8 (30.8)
Weight decreased	8 (30.8)
Dehydration	6 (23.1)
Dyspnoea	6 (23.1)
Headache	6 (23.1)
Alopecia	5 (19.2)
Blood bilirubin increased	5 (19.2)
Blood creatinine increased	5 (19.2)
Hyponatremia	5 (19.2)
Pruritus	5 (19.2)
Pyrexia	5 (19.2)
Abdominal pain	4 (15.4)
Alanine aminotransferase increased	4 (15.4)
Anemia	4 (15.4)
Aspartate aminotransferase increased	4 (15.4)
Chills	4 (15.4)
Cough	4 (15.4)
Depression	4 (15.4)
Myalgia	4 (15.4)
Renal failure acute	4 (15.4)

Twenty-two out of 26 subjects had sufficient PK data on day 42, the presumed steady state, to support estimation of the exposure parameters. No notable drug accumulation was observed within the dose range of 0.5 to 10 mg/kg. On the dose levels of 15 and 20 mg/kg, the average AUC_{last} changes from day 0 to day 42 were 23% and 10%, respectively. Such lack of significant drug accumulation was to be expected given the approximately 4-day half-life of the molecule. The two subjects who experienced grade 2 fragility fractures were both in the highest dose cohort of 20 mg/kg. One subject had the second highest drug exposure (i.e. AUC, peak concentration, trough concentration etc.) in the cohort ($n = 6$), while the other subject had the lowest drug exposure in the cohort.

Experiments were carried out in preclinical setting with the specific goal of identification of the pharmacokinetic driver and the target drug exposure for efficacy (unpublished data on file). Utilizing preclinical cancer models derived from human tumors, pharmacokinetic parameters such as AUC, peak serum concentration (i.e., C_{max}), steady state trough serum concentration (i.e., $C_{min,}$ss) were analyzed for correlation with efficacy. While maintaining the same dosing intensity, thus equal AUC, infrequent dosing at higher dose levels resulted in significantly better efficacy than frequent dosing at lower dose levels, revealing that C_{max} may be the pharmacokinetic driver for efficacy, while maintaining a high steady state trough concentration was not instrumental for improving efficacy. More experiments may be needed to clarify the role of AUC in driving efficacy. The C_{max} that correlated with maximum efficacy in the preclinical models was 170 μg/mL, while the corresponding AUC was 1400 day*μg/mL. At 10 mg/kg, ipafricept clinical C_{max} exceeded 170 μg/mL in all patients; at 20 mg/kg, ipafricept clinical AUC reached or exceeded 1400 day*μg/mL in most patients. It was estimated that the target efficacious dose was reached at 10 mg/kg.

Table 5. Summary pharmacokinetic parameters of ipafricept following the first i.v. infusion in patients with advanced solid malignancies

Day 1										
Dose (mg/kg)	***n***	**C_{max} (μg/mL)**	**AUC_{last} (day* μg/mL)**	**AUC_{0-21} (day* μg/mL)**	**AUC_{0-inf} (day* μg/mL)**	**%AUC_{Exp}**	**CL (mL/day/kg)**	**V_{ss} (mL/kg)**	**$t_{1/2}$ (day)**
0.5	3	11.5 ± 5.28	20.4 ± 13.6	21.5 ± 13.7	21.6 ± 13.9	6.11 ± 3.56	29.3 ± 14.6	76.5 ± 23.1	2.37 ± 0.894
1	3	18.6 ± 2.60	54.4 ± 6.39	62.7 ± 16.2	55.8[a]	5.64[a]	18.1[a]	93.8[a]	4.58[a]
2.5	3	61.5 ± 4.40	124 ± 47.6	125 ± 47.2	126 ± 47.9	1.69 ± 0.624	21.5 ± 6.68	76.2 ± 19.0	3.49 ± 1.11
5	5	115 ± 33.5	335 ± 139	335 ± 139	353 ± 154	4.24 ± 3.20	16.6 ± 7.74	86.1 ± 24.2	4.85 ± 1.15
10	3	266 ± 69.9	651 ± 166	651 ± 166	663 ± 170	1.77 ± 0.386	15.9 ± 4.79	71.1 ± 21.5	3.88 ± 0.257
15	3	273 ± 65.5	673 ± 122	673 ± 122	694 ± 129	2.92 ± 0.610	22.2 ± 4.48	112 ± 9.80	4.53 ± 0.302
20	6	474 ± 92.5	1380 ± 305	1380 ± 305	1450 ± 341	4.68 ± 2.11	14.5 ± 3.77	83.5 ± 11.2	5.12 ± 0.619
Day 42									
Dose (mg/kg)	***n***	**C_{max} (μg/mL)**	**AUC_{last} (day* μg/mL)**	**AUC_{0-21} (day* μg/mL)**	**AUC_{0-inf} (day* μg/mL)**	**%AUC_{Exp}**	**CL (mL/day/kg)**	**V_{ss} (mL/kg)**	**$t_{1/2}$ (day)**
0.5	2	13.2[a]	21.9[a]	24.2[a]	24.2[a]	8.39[a]	23.7[a]	60.5[a]	1.98[a]
1	3	21.1 ± 3.85	46.7 ± 14.1	50.6 ± 14.8	50.9 ± 15.2	7.79 ± 8.81	21.2 ± 7.39	64.6 ± 9.47	2.81 ± 1.44
2.5	2	59.0[a]	110[a]	112[a]	113[a]	1.79[a]	22.6[a]	62.8[a]	2.53[a]
5	4	120 ± 44.1	422 ± 295	391 ± 241	290 ± 134	1.72 ± 1.39	17.9 ± 12.7	91.5 ± 45.4	4.91 ± 3.20
10	3	254 ± 43.5	645 ± 191	634 ± 182	651 ± 191	0.889 ± 0.815	16.8 ± 5.55	78.3 ± 21.8	4.43 ± 0.535
15	2	302[a]	825[a]	811[a]	841[a]	1.77[a]	18.6[a]	98.9[a]	4.84[a]
20	6	461 ± 47.7	1520 ± 361	1490 ± 339	1590 ± 398	4.03 ± 3.09	14.1 ± 3.34	86.4 ± 14.0	5.34 ± 0.818

Abbreviations: AUC_{last}, area under the concentration–time curve from the first to the last observation; AUC_{inf}, area under the concentration–time curve from the first observation to the extrapolated time infinity; %AUC_{exp}, percentage of AUC_{inf} that was extrapolated; CL, clearance; C_{max}, maximum serum concentration after administration; *n*, number of subjects with C_{max} and AUC_{last} reported, may be higher than the number of subject that had the terminal phase based PK parameters (i.e., CL, $T_{1/2}$ etc.) summarized due to %AUC_{exp} larger than 20% in some subjects; $t_{1/2}$, half-life; T_{max}, time to maximum (peak) concentration; V_{ss}, volume of distribution at steady state.

[a]SD not reported due to $n < 3$.

Immunogenicity

Of the 26 subjects with samples for ADA testing, 2 subjects had treatment-induced ADA with no correlation to ipafricept dose level. One subject was in the 0.5 mg/kg q3w cohort, positive at day 28 and forward, with very low titer of 10. This subject had multiple PK data points available postday 28 and had no evidence of ADA impact on PK. The second subject was in the 10 mg/kg q3w cohort, positive 3 to 8 weeks after treatment termination with low titers of 10 to 20. At 3 weeks after treatment termination, the serum concentration of this subject was below detection limit, as expected. In conclusion, the overall ADA incidence was low in 2 of 26 subjects (7.69%) with no impact on drug exposure in this study.

Biomarkers studies

The PD of ipafricept was explored in pre- and posttreatment hair follicles from treated patients, as well as in control subjects (healthy volunteers). Supporting the mechanism of action, a decrease in the expression of Wnt pathway target genes such as *LGR6* and *DKK1* was documented in patients receiving 2.5 mg/kg and higher doses, as well as increase in expression of differentiation genes such as *NRCAM* (Fig. 2). Control subjects had no significant changes in these genes.

Antitumor activity

All 26 patients were evaluable for response (Fig. 3). Seven subjects (26.9%) had SD per RECIST 1.1 as best response. Partial or complete responses were not observed. Three patients (2 with desmoid tumor and one with germ cell cancer) were on study over 6 months. One of the desmoid tumor patients stayed on study for 98 months and came off due to personal preference but still maintaining SD. The patient with germ cell cancer self-referred to the study because his tumor had a β-catenin exon 3 mutation; he was previously progressing with a subcarinal adenopathy that went from 2 to 4 cm in the year before enrolling in the study, remained free of progression for 9 months, and came off study due to an asymptomatic L3 pedicle fracture. While not reaching conclusive criteria this may indicate a biological effect.

Discussion

The Wnt pathway is critical in many cancers, promoting tumor growth potentially through CSC proliferation, creating a favorable microenvironment for tumor growth and metastasis and contributing to therapy resistance. Several approaches are under development to target this pathway in human cancer, including the use of natural compounds, small molecule inhibitors, viral-based inhibitors and antibody-based inhibitors (16). Ipafricept is a novel fusion protein targeting Fzd8-interacting Wnt ligands that decreases Wnt signaling pathway arresting tumor growth.

This first-in-human phase I study reports the first-in-human results of ipafricept in patients with refractory solid tumors. While most patients experienced at least one treatment-related TEAE, the majority of ipafricept-related toxicities were mild, and included taste alterations, fatigue, muscle cramps, and decreased appetite. No DLTs were observed, and the RP2D was defined when dose escalation achieved and significantly surpassed the predetermined peak serum concentration threshold.

The identification of peak serum concentration as driver for efficacy, rather than steady state trough concentration, was unconventional at first glance. However, considering that Wnt signaling

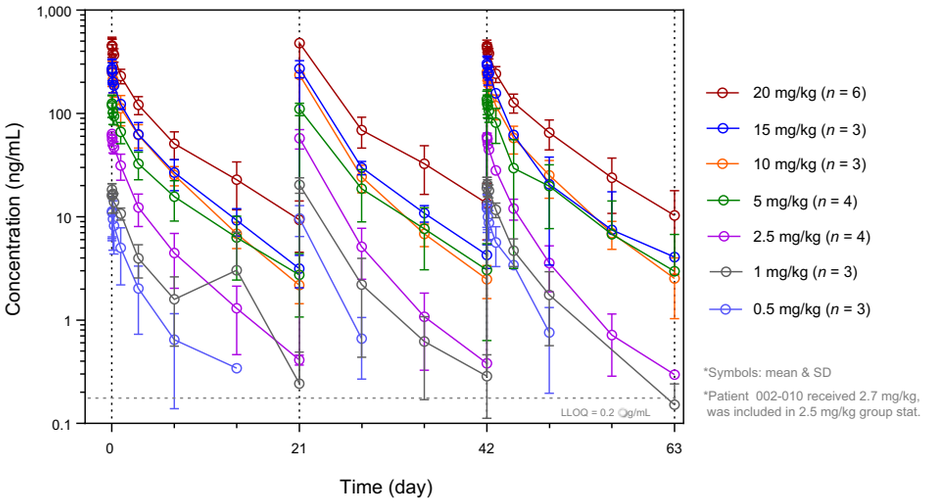

Figure 1.
Plasma concentration–time curves for ipafricept during cycle 1.

is critical for CSC fate determination and the mechanism of action of ipafricept is reduction of CSC frequency in tumors, it is conceivable that a high dose of ipafricept exposure is more effective in the alteration of cell fate in tumors, which leads to a cascade of antitumor effects over a period of time that outlasts the pharmacokinetic residence time.

Ipafricept appears to have a toxicity profile consistent with other embryonic pathway modulators, such as hedgehog pathway inhibitors, including dysgeusia and fatigue (17, 18). The most characteristic and unique toxicity was alterations in bone remodeling; 6 patients had doubling of β-CTX that was reversible upon zoledronic acid administration in 5 patients, and 2 subjects experienced fragility fractures at the highest dose of 20 mg/kg Q3W. While these are potentially significant toxicities, their reversibility and/or potential preventability with bisphosphonate therapy can help contextualize their risk/benefit ratio. Adaptative

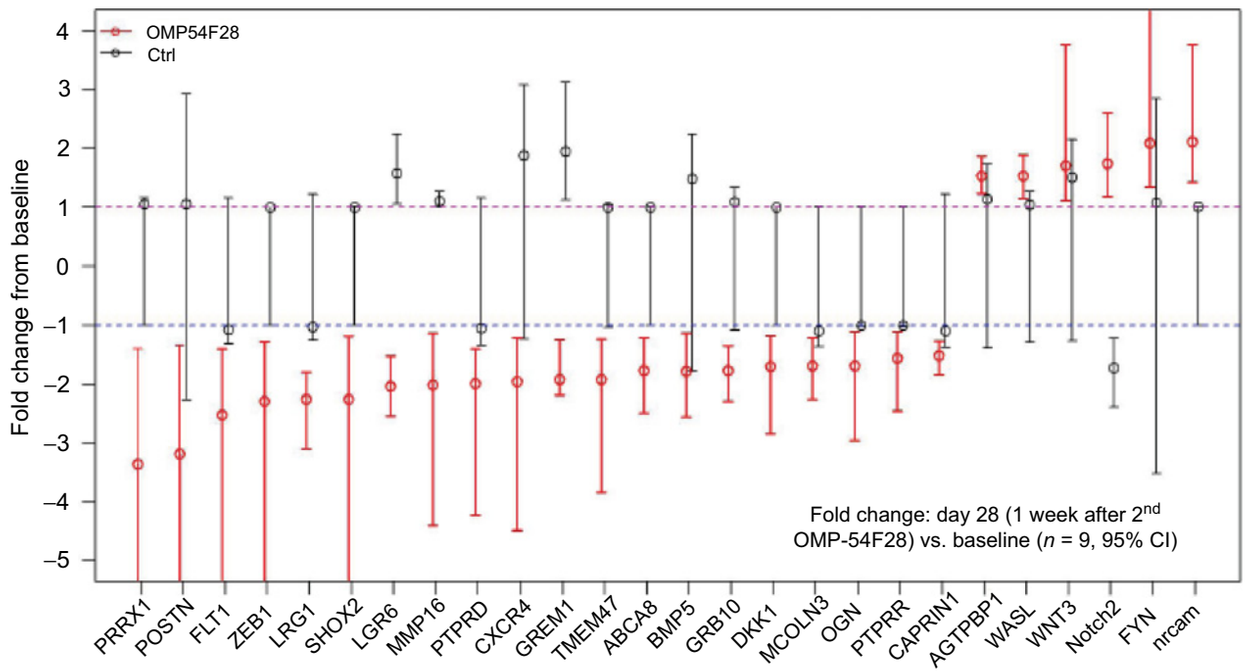

Figure 2.
Hair follicle PD results in treated and healthy volunteer subjects. Ipafricept significantly affected expression of genes associated with Wnt signaling and cellular fate. RNAs were isolated from hair follicles from each of 9 patients, and gene expression profiles determined by microarray analysis. The fold change represents the gene expression ratio comparing posttreatment (day 28) with pretreatment (day 0) samples. The 9 patients represented (shown in red) were dosed as follows: 0.5 mg/kg Q3W ($n = 1$), 1.0 mg/kg Q3W ($n = 2$), 2.5 mg/kg Q3W ($n = 3$), and 5.0 mg/kg Q3W ($n = 3$). Hair follicle harvest occurred 1 week following the second dose of ipafricept. Gray bars indicate fold change in control hair follicle samples collected from healthy volunteers not treated with ipafricept.

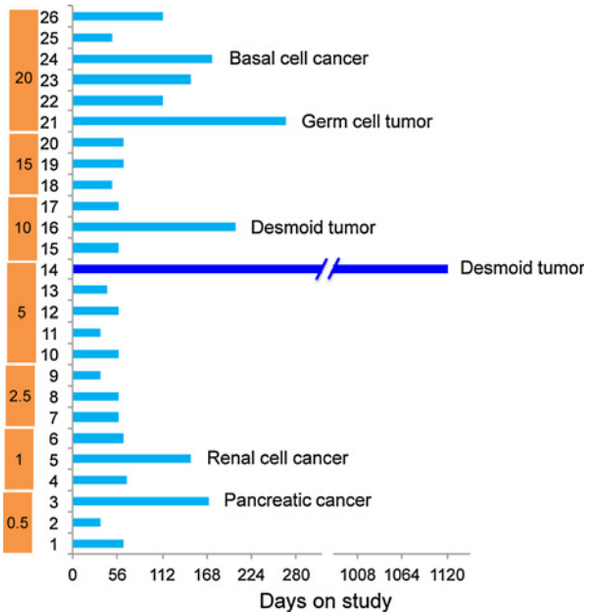

Figure 3.
Swim plot of time on study, with dose levels (in mg/kg, Q3W) by the left axis.

dosing strategies based on β-CTX levels and dynamic variations should be considered for this and other agents targeting this pathway.

Desmoid tumors have Wnt over expression and a recent phase I study of a gamma-secretase inhibitor (targeting the Notch pathway) showed sustained PR and SD in a majority of treated patients (19). The cross-talk between Notch and Wnt-β catenin pathways is known to contribute to tumorigenesis (20). Constitutive activation of β-catenin due to mutations in β-catenin or the adenomatous polyposis coli (APC) genes may predict benefit from Wnt inhibition. We documented prolonged SD during ipafricept treatment in a germ cell cancer patient whose tumor had a β-catenin activation. Tumors relying on dysregulated β-catenin signaling may still require other components of the canonical and/or noncanonical Wnt pathway to sustain growth, and depriving these tumors from further ligand activation may diminish intracellular signaling.

Wnt is a valid target for tumor inhibition, but further research needs to identify the cancer types most likely to benefit. We assessed the expression of target genes and biomarkers in pre- and posttherapy hair follicles. As expected, Wnt-dependent genes were suppressed, and genes indicating increased cell differentiation increased. Preclinical models suggest that Wnt is critical in mediating chemotherapy-resistant phenotypes, and while we did not see marked responses with single agent ipafricept, it is possible that combining Wnt inhibitors with chemotherapeutic or biological agents could enhance activity.

In summary, ipafricept can be safely administered with manageable toxicities, including several bone events. Several phase Ib studies have been completed assessing ipafricept combined with nab-paclitaxel and gemcitabine in pancreas cancer, with carboplatin and paclitaxel in ovarian cancer, and with sorafenib in hepatocellular cancer.

Disclosure of Potential Conflicts of Interest

R. Chugh is a consultant/advisory board member for EMD Serano and Epizyme Inc., and reports receiving commercial research grants from AADi, Advenchen, Epizyme, Lilly, Mabvax, Medivation, and Morphotek Novartis. W. Messersmith reports receiving commercial research grants from OncoMed. J. Dupont and R. Stagg have ownership interests (including patents) at OncoMed. D.C. Smith reports receiving commercial research grants from OncoMed. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A. Jimeno, M. Gordon, J. Dupont, R. Stagg, L. Xu, R.K. Brachmann, D.C. Smith
Development of methodology: J. Dupont, R. Stagg, L. Xu, R.K. Brachmann
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Jimeno, M. Gordon, R. Chugh, W. Messersmith, D. Mendelson, J. Dupont, A.M. Kapoun, L. Xu, R.K. Brachmann, D.C. Smith
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Jimeno, M. Gordon, R. Chugh, W. Messersmith, D. Mendelson, J. Dupont, R. Stagg, A.M. Kapoun, L. Xu, S. Uttamsingh, R.K. Brachmann, D.C. Smith
Writing, review, and/or revision of the manuscript: A. Jimeno, M. Gordon, R. Chugh, W. Messersmith, R. Stagg, A.M. Kapoun, L. Xu, S. Uttamsingh, D.C. Smith
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Dupont
Study supervision: A. Jimeno, J. Dupont, R. Stagg, L. Xu, S. Uttamsingh, R.K. Brachmann

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