Targeting the Interleukin-11 Receptor α in Metastatic Prostate Cancer: A First-in-Man Study

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BACKGROUND: Receptors in tumor blood vessels are attractive targets for ligand-directed drug discovery and development. The authors have worked systematically to map human endothelial receptors (“vascular zip codes”) within tumors through direct peptide library selection in cancer patients. Previously, they selected a ligand-binding motif to the interleukin-11 receptor alpha (IL-11Rα) in the human vasculature. METHODS: The authors generated a ligand-directed, peptidomimetic drug (bone metastasis-targeting peptidomimetic-11 [BMTP-11]) for IL-11Rα-based human tumor vascular targeting. Preclinical studies (efficacy/toxicity) included evaluating BMTP-11 in prostate cancer xenograft models, drug localization, targeted apoptotic effects, pharmacokinetic/pharmacodynamic analyses, and dose-range determination, including formal (good laboratory practice) toxicity across rodent and nonhuman primate species. The initial BMTP-11 dose-finding study is also reported based on a single-institution, open-label, first-in-class, first-in-man trial (National Clinical Trials number NCT00872157) in patients with metastatic, castrate-resistant prostate cancer. RESULTS: BMTP-11 was preclinically promising and, thus, was chosen for clinical development in patients. Limited numbers of patients who had castrate-resistant prostate cancer with osteoblastic bone metastases were enrolled into a phase 0 trial with biology-driven endpoints. The authors demonstrated biopsy-verified localization of BMTP-11 to tumors in the bone marrow and drug-induced apoptosis in all patients. Moreover, the maximum tolerated dose was identified on a weekly schedule (20–30 mg/m²). Finally, a renal dose-limiting toxicity was determined, namely, dose-dependent, reversible nephrotoxicity with proteinuria and casts involving increased serum creatinine. CONCLUSIONS: These biologic endpoints establish BMTP-11 as a targeted drug candidate in metastatic, castrate-resistant prostate cancer. Within a larger discovery context, the current findings indicate that functional tumor vascular ligand-receptor targeting systems may be identified through direct combinatorial selection of peptide libraries in cancer patients. Cancer 2015;121:2411-21.

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KEYWORDS: bone metastasis-targeting peptidomimetic-11, clinical trial, interleukin-11 receptor α, prostate cancer, vascular targeting.
INTRODUCTION
Several lines of evidence indicate that blood vessels from tumors express unique receptors that act as vascular “zip codes” and can be targeted with ligands.\(^1\)\(^-\)\(^3\) Although the identification of functional ligand receptors within the tumor vasculature is challenging, we recently demonstrated that screening peptide libraries directly in humans enables unbiased target identification.\(^4\)\(^-\)\(^7\) Nevertheless, whether these targets can be translated into therapies remains unclear.

Treatment options for metastatic castrate-resistant prostate cancer are limited, and the development of new therapeutic approaches is urgently needed. We identified a peptide motif (CGRRAGGSC) that binds to interleukin-11 receptor alpha (IL-11R\(_a\)) in the tumor vascular endothelium by administering a phage library to a brain-dead cancer patient.\(^4\)\(^-\)\(^6\) Human IL-11R\(_a\) was overexpressed and had expression profiles similar to those of IL-11R\(_a\) and cluster of differentiation 31 (CD31 [also called platelet endothelial cell adhesion molecule]), with colocalization during tumor progression and metastases in a large cohort of prostate cancer patients; specific drug binding to IL-11R\(_a\) and dose-dependent apoptosis induction of prostate cancer cells\(^8\) is mediated through a receptor-interacting site within IL-11.\(^9\) Independent groups have confirmed this ligand-receptor system by characterizing the binding of CGRRAGGSC.\(^10\)\(^-\)\(^11\)

On the basis of these findings, we designed a ligand-directed agent, bone metastasis-targeting peptidomimetic-11 (BMTP-11), which consists of the CGRRAGGSC motif synthesized in tandem to D(KLAKLAK)\(_2\), an apoptosis-inducing motif that is active on cell internalization and has been validated in preclinical models.\(^8\)\(^-\)\(^17\) Detailed methods are described in Supporting Experimental Procedures (see online supporting information). Preclinical efficacy studies in rodents, pharmacokinetic, safety/toxicology studies in nonhuman primates, and criteria for patient entry followed the procedures described in the Supporting Methods (see online supporting information).

RESULTS
Preclinical Efficacy of BMTP-11 Against Prostate Cancer Xenografts
To evaluate the efficacy of BMTP-11 in preclinical settings, 2 classic nude mouse models were chosen: a prostate-specific antigen (PSA)-producing, androgen-dependent model (LNCaP-derived) and a non-PSA-producing, androgen-independent model (DU145-derived).\(^8\) In a third experimental system, we selected the bone-forming prostate cancer MDA-PCa-118b model,\(^18\) which forms osteoblastic lesions in severe combined immunodeficient (SCID) mice and uniquely recapitulates human prostate cancer.

In pilot experiments, nude mice bearing DU145-derived tumor xenografts received either BMTP-11 (5-15 mg/kg subcutaneously every other day; produced under good manufacturing practice conditions) or saline (controls) (Supporting Fig. 1; see online supporting information). The efficacy of BMTP-11 was assessed by measuring serial changes in tumor volume. Tumors were reduced (\(P<.0001\)) relative to controls in nude mice that received 10 to 15 mg/kg BMTP-11 (Supporting Fig. 1A; see online supporting information), whereas lower BMTP-11 doses were less effective (Supporting Fig. 1B,C; see online supporting information).

Next, we assessed the efficacy of BMTP-11 (10 mg/kg once weekly) by either intravenous or subcutaneous administration in DU145-derived (Fig. 1A-C) or LNCaP-derived (Fig. 1D-F) tumor xenografts. Differences in either DU145-derived or LNCaP-derived tumor volumes in treated nude mice (\(P<.0001\)) were observed relative to controls and, the optimal dose of BMTP-11 in tumor-bearing mice was determined at approximately 10 mg/kg per week.

Marked expression/localization of IL-11R\(_a\) was observed in MDA-PCa-118b tumors\(^18\) but not in nonmalignant stroma (Fig. 1G). Heterogenous IL-11R\(_a\) expression was measured using quantitative x-rays to substantiate the pretreatment cohort assignment of tumor-bearing SCID mice using the bone-to-soft tissue ratio within tumors (Fig. 1H,I). Intravenous BMTP-11 treatment (10 mg/kg weekly) had antitumor effects compared with controls (\(P<.0001\)) (Fig. 1J-L). The average control-treated tumors increased by 235% (range, 62%-520%); in contrast, the change in BMTP-11–treated, tumor-bearing SCID mice was only 10.6% (range, −40%...
to 74.3%), thus indicating nearly complete growth suppression (Fig. 1K).

**BMTP-11 Tissue Distribution**

To evaluate BMTP-11 tissue distribution, polyclonal antibodies (immunoglobulin G [IgG] antibodies [IgGs]) against the BMTP-11 proapoptotic domain were produced in rabbits using a single D(KLAKLAK) peptide (Supporting Fig. 2A; see online supporting information). The antibody revealed anti-BMTP-11 immunoreactivity as well as immunoreactivity to 2 other positive controls (Supporting Fig. 2B; see online supporting information), whereas there was no immunoreactivity against a negative control peptide (Supporting Fig. 2C; see online supporting information). Affinity-purified anti-D(KLAKLAK) IgGs detected BMTP-11 in a concentration-dependent...
manner (Supporting Fig. 2D; see online supporting information). BMTP-11 immunoreactivity was observed in nontumor-bearing mice injected with BMTP-11 (25 mg/kg) into the kidneys 20 minutes postinjection (Supporting Fig. 3A; see online supporting information) and was restricted to the proximal tubules (Supporting Fig. 3B; see online supporting information).

Next, BMTP-11 metabolism was analyzed in tissues using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry19 (Supporting Fig. 4; see online supporting information). Although the absolute concentration of each BMTP-11 metabolite could not be quantified unequivocally, the relative abundance of individually identified metabolites was measured based on peak intensities relative to BMTP-11 and other internal standards. Kidneys from mice injected with BMTP-11 revealed that BMTP-11 disappearance correlated strongly with the appearance of GG-D(KLAKLAK)2, G-D(KLAKLAK)2, and D(KLAKLAK)2 metabolites (Supporting Fig. 4B; see online supporting information). We observed that D(KLAKLAK)2 was the predominant metabolite, and there were no detectable levels of the parent compound or metabolites containing any portion of CGRRAGGSC 4 hours postadministration (Supporting Fig. 4C; see online supporting information). These results indicate that the immunoreactivity in the kidney sections may represent a combination of the drug and its metabolic derivatives.

**BMTP-11 Stability and Interaction**

We established assays to evaluate BMTP-11 in vitro, in vivo, and ex vivo. BMTP-11 stability in saline was determined by mass spectrometry. BMTP-11 was solubilized and incubated either at room temperature or at 37°C, and serial aliquots were analyzed. Spectra analyses revealed only 2 major peaks, both corresponding to BMTP-11: the first peak was the single-charged form, and the second peak corresponded to the double-charged form (Supporting Fig. 5; see online supporting information). The latter peak was not present in samples that were incubated for 8 to 24 hours, thus establishing that BMTP-11 is stable in aqueous solutions (Supporting Fig. 5; see online supporting information). Finally, to evaluate the functional attributes of BMTP-11 in a preclinical setting, bone marrow supernates from human prostate cancer specimens were collected and incubated with the drug, and its activity against prostate cancer cells in vitro was unchanged.
TABLE 1. Bone Metastasis-Targeting Peptidomimetic-11 Pharmacokinetic Parameters in Nonhuman Primates

| Pharmacokinetic Parameter | BMTP-11 (mg/kg) | BMTP-11 (mg/kg) |
|---------------------------|----------------|----------------|
| Dose, mg                  | 20.1           | 71.1           |
| AUC, μg·h/mL              | 8.88 ± 1.04    | 26.81 ± 2.14   |
| Clearance, liter/h        | 2.26 ± 0.27    | 2.85 ± 0.21    |
| K10, 1/h                  | 0.78 ± 0.22    | 1.03 ± 0.22    |
| Half-life, h              | 0.89 ± 0.25    | 0.67 ± 0.14    |
| Vd, liter                 | 2.90 ± 0.59    | 2.58 ± 0.45    |
| MRT, h                    | 1.28 ± 0.35    | 0.97 ± 0.20    |

Abbreviations: AUC, area under the curve; BMTP-11, bone metastasis-targeting peptidomimetic-11; K10, elimination rate constant; MRT, mean residence time; SE, standard error; Vd, volume of distribution.

compared with BMTP-11 that was not pretreated (Supporting Fig. 6; see online supporting information).

**BMTP-11 Pharmacokinetics in Rodents**

To profile the biodistribution and pharmacokinetic properties of BMTP-11, first, we carried out pilot studies in mice using $^{125}$I-BMTP-11 (Supporting Fig. 7; see online supporting information). Mice received either $^{125}$I-BMTP-11 (15 mg/kg intravenously) or phosphate-buffered saline, and blood samples were serially collected postinjection. After 24 hours, the mice were killed, and their organs were collected and analyzed using a $\gamma$-scintillation counter. This iodination strategy determined a specific $^{125}$I-BMTP-11 activity of 0.27 millicuries per gram. On the basis of a standard curve (Supporting Fig. 7A; see online supporting information), we estimated that 0.05% of the dose would constitute a good signal-to-noise ratio (approximately 50-fold). Circulating $^{125}$I-labeled BMTP-11 levels dropped quickly, and, by 15 minutes postinjection, only approximately 25% of the dose was present in whole blood; radioactivity levels in whole blood no longer decreased after 4 hours (Supporting Fig. 7B; see online supporting information). After 24 hours, most of the radioactivity was in the kidneys, liver, spleen, and heart (Supporting Fig. 7C; see online supporting information). A pharmacokinetics profile was calculated using the standard curve, and a noncompartmental analysis was performed (Supporting Table 1, Supporting Fig. 7D; see online supporting information).

**BMTP-11 Pharmacokinetics in Nonhuman Primates**

Next, we assessed the pharmacokinetics of BMTP-11 in cynomolgus monkeys (Fig. 2, Supporting Fig. 8; see online supporting information). We detected BMTP-11 in plasma spiked with drug concentrations as low as 0.003 μg/mL (Supporting Fig. 8A,B; see online supporting information). Ratios between BMTP-11 and $^{125}$I(KLAKLAK)$_2$, the internal standard (Supporting Fig. 8C; see online supporting information), exhibited good linearity (Fig. 2A), indicating that CGRRAGGSC did not alter the stability of $^{125}$I(KLAKLAK)$_2$. Cynomolgus monkeys received intravenous infusions of BMTP-11 at doses of 1 mg/kg, 3 mg/kg, or 9 mg/kg over 2 hours to mimic the intended clinical application. Plasma samples were collected over the course of 24 hours to generate plasma concentration-time curves for each animal receiving each dose (Fig. 2B). Our results indicated that BMTP-11 levels increased during infusion and decreased exponentially to background levels at 8 hours. The area under the curve (AUC) from zero to infinity was calculated from each plasma concentration-time curve. In the dose ranges evaluated, the AUC increased proportionally with increased dose, indicating that BMTP-11 clearance mechanisms were neither saturated (Fig. 2C) nor concentration-dependent. The data were best fitted to a 1-compartment open-body model (Fig. 2D), and pharmacokinetic parameters were calculated (Table 1). The elimination rate constant for BMTP-11 was calculated using the terminal portion of the plasma concentration-time curves. The curve corresponding to the lowest BMTP-11 dose tested (1 mg/kg) was not used, because samples that were collected 2 hours after the start of infusion (before discontinuing infusion) and 5 minutes postinfusion at this dose were undetectable. Preliminary assessment of BMTP-11 metabolites in plasma from monkeys was based on their molecular mass (Fig. 2E).

**Dose-Range Determination and Toxicity Studies in Rodents and Nonhuman Primates**

Good laboratory practice (GLP) studies on mice, rats, and monkeys were conducted to identify the dose range and administration route for a formal assessment of the safety of BMTP-11 (Supporting Table 2; see online supporting information). Within the nonacute group (defined as the group of animals in which >50% survived to study termination), renal injury, such as hyperplastic and/or regenerative lesions (as determined by clinical pathology and/or gross necropsy), was the predominant toxicity, with concentration-dependent severity observed across rodents and primates. Clinical chemistry parameters related to renal function, such as serum creatinine and blood urea nitrogen, often were elevated but generally returned to baseline at the end of the study, indicating renal adaptive/
regenerative response. To rule out a neutralizing effect contributing to transient renal findings, we measured serum levels of anti-BMTP-11 (antidrug antibodies) from single-dose and multiple-dose studies in rodents and nonhuman primates (Supporting Fig. 9; see online supporting information). Our results indicated that none of the mice that received a single dose (3-10 mg/kg) and none of the monkeys that received multiple BMTP-11 doses (4 weekly doses at 30 mg/kg per dose) developed immunoglobulin M (IgM) or IgG anti-BMTP-11 antibodies; serum samples collected from animals in these studies did not exhibit immunoreactivity against BMTP-11 (Supporting Fig. 9A,B; see online supporting information). Because CGRAGGSC mimics native IL-11 binding to IL-11Rα, which activates signal transducer and activator of transcription 3 (STAT3),9 we measured serum anti-IL-11 levels to ascertain whether there was a humoral immunogenic response against BMTP-11. Neither IgM nor IgG was detected above background (Supporting Fig. 9C; see online supporting information), indicating that BMTP-11 is unlikely to induce a humoral response. Tumor toxicity results and the lack of an immunogenic response led to a formal, definitive GLP safety assessment study of BMTP-11 at doses of 1 mg/kg, 3 mg/kg, 6, and 9 mg/kg in nonhuman primates to complete preclinical requirements for clinical trials.

**Preclinical Safety of BMTP-11 in Nonhuman Primates**

A GLP-compliant safety study was carried out using cynomolgus monkeys to mimic the clinical regimen expected (intravenous weekly for 4 doses). The objectives of this nonclinical safety evaluation in primates were: 1) to identify target organs for toxicity and determine whether toxicity was reversible, 2) to determine the starting dose and dose-escalation, and 3) to identify parameters for safety monitoring in humans. A recovery group at the highest dose (9 mg/kg) was included. Clinical signs attributed to BMTP-11 included alopecia, vomiting, dehydration, increased urine output, and erythema. These clinical signs were dose-dependent and were observed at the highest doses (6 mg/kg and 9 mg/kg).

Hematologic findings included mild leukocytosis, anemia, and mild thrombocytopenia. Mild-to-marked azotemia was noted 1 week after the initial BMTP-11 dose. Azotemia lessened with continued administration and was completely resolved at the end of the recovery period, with the exception of 2 of 3 monkeys in the highest dose group.

Alterations in urinary analytes included glucosuria, proteinuria, leukocyturia, and increased transitional/renal epithelial cells. The magnitude of proteinuria and cell counts in the urine decreased with continued drug administration; most urinary abnormalities resolved by the end of the recovery period.

Primate necropsies 24 hours after the final BMTP-11 infusion revealed dose-dependent findings primarily in the monkeys that had received 3 to 9 mg/kg. Pale discoloration of the kidneys was grossly consistent with nephrosis. Kidney weights increased at the highest BMTP-11 dose. Histologic lesions were identified in the kidneys, stomach, and pancreas and at infusion sites. Similar dose-dependent lesions were described as degenerative/necrotic, regenerative/reparative, and fibrotic and were reported for BMTP-78,14 adipotide,17 and other D(KLAKLAK)2-containing peptidomimetics. Tubular

### Table 2. Baseline Patient Demographics and Clinical Features by Bone Metastasis-Targeting Peptidomimetic-11 Dose

| Variable                                      | BMTP-11 Dose |
|-----------------------------------------------|--------------|
|                                               | 18 mg/m²     | 36 mg/m²     | 27 mg/m²     |
|                                               | Patient 1    | Patient 2    | Patient 3    |
|                                               | Patient 4    | Patient 5    | Patient 6    |
| Age at diagnosis, y                          | 62           | 55           | 63           | 57           | 67           | 63           |
| Age at trial registration, y                 | 74           | 69           | 68           | 61           | 68           | 65           |
| Interval from androgen deprivation to CRPC, y| 5.3          | 7.0          | 2.0          | 0.2          | 0.4          | 0.5          |
| Interval from CRPC to trial registration, mo | 78           | 38           | 36           | 38           | 8            | 12           |
| No. of prior cytotoxic therapies             | 2            | 2            | 5            | 6            | 1            | 2            |
| Previous treatment with abiraterone          | Yes          | Yes          | No           | Yes          | No           | No           |
| Serum PSA at registration                    | 2141         | 334          | 384          | 711          | 321          | 53           |
| Serum alkaline phosphatase at trial registration | 259      | 293          | 520          | 443          | 447          | 388          |
| Hemoglobin at trial registration             | 11.5         | 11.9         | 10.5         | 8.2          | 8.9          | 9.2          |

Abbreviations: BMTP-11, bone metastasis-targeting peptidomimetic-11; CRPC, castrate-resistant prostate cancer; PSA, prostate-specific antigen.
necrosis and regeneration were noted in monkeys that had received BMTP-11 at any dose; however, these findings were graded minimal-to-mild at the 2 lowest dose levels, and mild-to-moderate fibroplasia was observed only at the 2 highest dose levels.

Additional preclinical safety GLP studies were performed using a large cohort (n = 50) of primates (rhesus and cynomolgus monkeys) with D(KLAKLAK)2-containing drugs.14,17 Details on some multiple-dose studies have been reported.17 No lethality resulted from dose-dependent toxicity in monkeys that received single BMTP-11 doses up to 100 mg/kg. Preclinical safety studies identified renal tubules as the primary nontarget tissue for adverse events, with no irreversible toxicity encountered at the highest repeated doses tested. In the absence of an identified lethal dose, we proposed an allometrically estimated dose of 18 mg/m² in humans, corresponding to a BMTP-11 dose equivalent to 1.5 mg/kg in cynomolgus monkeys,20 as a conservative starting dose for a first-in-man clinical trial among castrate-resistant prostate cancer patients with high-volume osseous metastasis. This starting dose is equivalent to only 5% of the dose level associated with reversible renal toxicity in monkeys.

**BMTP-11 Clinical Trial**

The first-in-man study (NCT00872157; available at: http://clinicaltrials.gov/ct2/show/NCT00872157; accessed February 26, 2015) was designed to document ligand-
directed targeting of BMTP-11 and to evaluate a correlation of dose to activity and toxicity that would support a larger dose-selection trial. A patient cohort (n = 10) was identified and screened in the trial with “intent-to-treat.” During pre-enrollment screening, 4 patients were deemed ineligible for trial entry because of a low hemoglobin level (n = 1) or an absence of tumor metastases upon bone marrow biopsy (n = 3). The remaining patients (n = 6) were enrolled in a dose-escalation cohort and received BMTP-11 starting at 18 mg/m² intravenously weekly for 4 doses. All 6 enrolled patients had high-volume, castrate-resistant bone metastases for which no standard therapy options were available (Table 2). Patients had received a median of 2 previous chemotherapy regimens (range, 1-7 previous regimens), and 3 patients (50%) had received systemic radionuclide-based therapy. All patients had readily demonstrated biopsy-proven prostate cancer in bone before registration and underwent a repeat biopsy within 2 to 4 hours after the first BMTP-11 dose. All patients entered in this study had baseline IL-11Rα expression relative to a negative control IgG (Fig. 3).

Our trial required post-treatment bone metastasis biopsies for BMTP-11 drug localization (Fig. 4A). Patients 1 and 2 were treated at the lowest dose level (18 mg/m²), and each received all 4 planned BMTP-11 doses with no clinically apparent toxicity despite mild increases in serum creatinine, dipstick proteinuria, and urinary casts. Patient 3 was treated at the highest dose level (36 mg/m²) and had a grade 3 decrease in glomerular filtration: his serum creatinine level decreased from 0.7 mg/dL at baseline to 3.8 mg/dL on day 15. Consequently, he received only 2 BMTP-11 doses. The protocol was then modified to require more aggressive post-BMTP-11–forced diuresis and was reopened afterward at an intermediate dose level (27 mg/m²). Three patients were subsequently treated at the 27 mg/m² dose. Patient 4 completed the 4 planned doses with minimal renal toxicity. Patient 5 went off study after 2 doses because of disease progression: on day 15, his serum creatinine increased from a baseline of 0.6 mg/dL to 1.3 mg/dL, and his urine protein increased from 151 mg/24 hours to 1840 mg/24 hours. Patient 6 received 3 BMTP-11 doses and came off study on day 22 because his urine protein increased from 132 mg/24 hours to 2180 mg/24 hours.

A secondary objective was to define acute toxicity in patients. There were no treatment-related deaths and no grade 4 events. All grade ≥2 adverse events according to National Cancer Institute criteria were included regardless of attribution (Table 3). Most of the events (anemia, elevated alkaline phosphatase, and hypoalbuminemia) reflected metastatic prostate cancer affecting bone. Two patients (33%) came off study for dose-limiting renal toxicity. Consistently, proteinuria and increased serum creatinine levels were the most prominent toxicities identified in the formal preclinical evaluation of BMTP-11 in rodents and nonhuman primates.

BMTP-11 localization in treated patients was determined by mass spectrometry analyses (Fig. 4B) and/or immunohistochemistry (Fig. 4C). It is noteworthy that,
in all 6 patient biopsies (100%; 95% confidence interval, 54%-100%), BMTP-11 accumulated at bone metastasis sites, indicating binding to prostate cancer after intravenous infusion and consistent with the preclinical data. An analysis of all tissue samples by terminal deoxynucleotidyl transferase dUTP nick-end labeling revealed tumor apoptosis and BMTP-11 colocalization (Fig. 4D).

With respect to clinical activity, we observed no responses defined according to Prostate Cancer Working Group 2 criteria. Moreover, a heavily pretreated patient (Patient 4) (Table 2) who received treatment at the 27 mg/m² dose level had marked symptomatic improvement and experienced transient, simultaneous declines in serum PSA, alkaline phosphatase, and lactate dehydrogenase levels coincidentally during BMTP-11 treatment, but his tumor progressed rapidly when the candidate drug was discontinued (Fig. 5).

DISCUSSION

Although the biology of castrate-resistant prostate cancer remains poorly understood, most patients have osteoblastic bone metastases. With the exception of bone-seeking radiopharmaceuticals, the development of drugs targeting the bone metastasis tumor microenvironment has lagged behind new hormonal agents, immunotherapy, and cytotoxics.

The preclinical evaluation of BMTP-11 activity and its translation into an early clinical application in patients included targeted efficacy, the development of ligand-directed drug-detection methodology, and safety/toxicology studies in rodents and primates. All of these data were used to design and conduct a first-in-man clinical trial. The primary endpoints of the study in patients with prostate cancer were to document physical targeting of BMTP-11 and to evaluate the relation of dose to toxicity and efficacy in humans that would support a full phase 1 trial. The clinical dose-limiting toxicity was somewhat predictable from our preclinical tissue-distribution and GLP safety/toxicology studies; weekly BMTP-11 administration in the dose range studied was associated with renal toxicity. Preclinical safety study alterations in serum biochemical and urinary analytes were attributed primarily to nephrotoxicity and altered proximal tubular function, and minimal changes were identified in the animals that received the lowest doses. Renal toxicity is attributable to the D-enantiomer proapoptotic moiety. Our studies revealed that the targeting moiety (composed of L amino acid residues only) was no longer present in the plasma 4 hours postinjection. Thus, all targeted D(KLAKLAK)₂ peptidomimetics present a very similar toxicity profile regardless of the targeted receptor within selected tissues. Hypophosphatemia, hypokalemia, hyponatremia, hypochloremia, and glucosuria were attributed to decreased reabsorption at the proximal tubule. Given the absence of glomerular lesions, proteinuria also was considered a likely consequence of decreased endocytic uptake in the proximal tubule.

In the first-in-man trial, all patients experienced increased serum creatinine and proteinuria, usually with casts, and these nephrotoxic changes precluded the delivery of a full cycle in 2 of 6 patients (33%). These changes were largely reversible without intervention beyond drug withdrawal, and no patient developed chronic renal dysfunction. No other clinical adverse events were observed.

It is noteworthy that we demonstrated selective BMTP-11 localization in bone marrow involvement in 6 of 6 patients (100%) with prostate cancer. This observation leaves no doubt that BMTP-11 reliably binds to prostate cancer after infusion and is consistent with its original identification in cancer patients. Furthermore, colocalization of tumor apoptosis and BMTP-11 in this setting confirms the preclinical activity of this ligand-directed drug candidate and other D(KLAKLAK)₂-containing agents reported in animal models. We hope that future studies with lead-optimized linkers, specific blockers, new formulations, and dose or schedule changes may mitigate the reversible kidney toxicity resulting from renal proximal tubule uptake. Because no lesions were observed in the glomeruli, we concluded that the toxicity was not caused by renal clearance.

In summary, this limited phase 0 trial with biology-driven endpoints 1) demonstrates ligand-directed drug

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**TABLE 3. Adverse Events**

| Adverse Event                  | No. of Patients<sup>a</sup> |
|-------------------------------|----------------------------|
|                               | Grade 3 | Grade 2 |
| Proteinuria                   | 0       | 6       |
| Back pain                     | 0       | 1       |
| Pain, not otherwise specified | 0       | 2       |
| Low serum albumin             | 0       | 1       |
| Alkaline phosphatase          | 2       | 2       |
| Anemia                        | 2       | 2       |
| Constipation                  | 0       | 1       |
| Creatinine                    | 0       | 1       |
| Fatigue                       | 0       | 2       |
| Glomerular filtration rate    | 1       | 1       |
| Hyperglycemia                 | 1       | 0       |
| Hypokalemia                   | 1       | 0       |
| Hypocholesterolemia           | 1       | 0       |
| Joint effusion                | 0       | 1       |
| Depression                    | 0       | 1       |

<sup>a</sup> Values are the number of patients who had an adverse event of the indicated grade.
localization in human tumor samples—data that validate vascular targeting observations in preclinical models, 2) narrows the dose-range and schedule for a formal phase 1 study, 3) defines the acute toxicity profile, and 4) suggests the possibility for clinical activity in patients with prostate cancer who have osteoblastic bone metastases. Despite our very small patient cohort in a small first-in-man study, anatomic localization has been clearly demonstrated, an upper limit on BMTP-11 dose has been established for this initially used “weekly × 4” schedule, and hints of drug efficacy have been observed. The current results provide justification for consideration of BMTP-11 as a targeted prototype drug against human prostate cancer and for the exploration of lower doses and/or alternative schedules to evaluate whether there might be a threshold below which the renal toxicity is minimized or abrogated.

Figure 5. Serial changes in serum tumor markers are illustrated in a patient before and after treatment with bone metastasis-targeting peptidomimetic-11 (BMTP-11). Serum prostate-specific antigen (PSA), alkaline phosphatase (Alk. Phos.), and lactate dehydrogenase (LDH) levels are indicated. 5-FU indicates infusional 5-fluorouracil plus concomitant external-beam radiotherapy; CVD, intravenous cyclophosphamide and vincristine plus oral dexamethasone; DES/Dex, oral diethylstilbestrol plus oral dexamethasone; G/S, gemcitabine intravenously plus oral sunitinib; KA/VE, oral ketoconazole plus doxorubicin intravenously alternating on a weekly basis with oral estramustine plus vinblastine intravenously; T/C, paclitaxel plus carboplatin intravenously every 3 weeks; T/S, docetaxel intravenously plus oral sunitinib; VP-16/CDDP, etoposide plus cisplatin intravenously every 3 weeks.
From a wider perspective, the translation from human-based discovery to a first-in-man clinical trial provides an integrated paradigm for streamlined targeted drug development in human cancer.

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**CONFLICT OF INTEREST DISCLOSURES**

At the time of the study, The University of Texas MD Anderson Cancer Center and Drs. Pasqualini, Arap, and Lobb owned equity stock in Alvos Therapeutics (Arrowhead Research Corporation, Pasadena, Calif). Dr. Christianson is a full-time employee of Arrowhead Research Corporation, which has licensed rights to technologies described in this article. Dr. Logothetis reports grants, personal fees, and nonfinancial support from Astellas, Novartis Pharmaceuticals, Bristol-Myers Squibb, Johnson & Johnson, Exelixis, and Pfizer. Dr. Arap reports research sponsorship, including grants, personal fees, and other support, from Arrowhead Research Corporation and is a shareholder in the company; he also reports grants, personal fees, and other support, from Alvos Therapeutics, Ablaris Therapeutics, and AAVP Biosystems and personal fees from AMP Pharmaceuticals, Ceramide Therapeutics, APAvadis, and Merck; in addition, he has a patient (8,846,859) with royalties paid to Arrowhead Research Corporation.

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The authors report on the development of a new ligand-directed peptidomimetic (termed bone metastasis-targeting peptidomimetic-11) for interleukin-11 receptor-based human vascular targeting, including the translation from preclinical studies to a first-in-class, first-in-man clinical trial in patients with metastatic, castrate-resistant prostate cancer.