Phase I study of PT-112, a novel pyrophosphate-platinum immunogenic cell death inducer, in advanced solid tumours

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Summary

Background PT-112, the first pyrophosphate-platinum conjugate, causes immunogenic cell death in experimental models, leading to recruitment of tumour-infiltrating lymphocytes. PT-112 also associates with bone (osteotropism), likely driven by its pyrophosphate moiety. This is the first-in-human study of PT-112 monotherapy, exploring its safety and efficacy in a patient population where standard of care therapies were exhausted and novel treatment options are needed.

Methods Patients with progressing, advanced solid tumours received PT-112 intravenously (1 h) on days 1, 8, 15 of a 28-day cycle in an open-label, multi-centre 3 + 3 dose-escalation trial, conducted at four US research sites. The primary objective was to assess safety and pharmacokinetics, and to identify a recommended phase 2 dose (RP2D). Eligibility criteria included: age ≥ 18 years, Eastern Collaborative Oncology Group (ECOG) Performance Status of 0−1, and disease evaluable by Response Evaluation Criteria in Solid Tumours (RECIST) v1.1 or by informative tumour markers. Patients receiving ≥ 1 dose of PT-112 were included in the safety and pharmacokinetic analyses, with the exploratory efficacy analysis including patients receiving ≥ 1 dose at 125 mg/m2. This study is registered at ClinicalTrials.gov, number NCT02266745, with the dose-escalation portion of the study closed.

Findings Between July 7th, 2014 and September 18th, 2018, 66 heavily pre-treated patients (median 4 prior lines, IQR 2−6) were enrolled and treated across 11 doses (12−420 mg/m2). Treatment-related adverse events included fatigue (23 patients, 35%), nausea (16 patients, 24%), and peripheral neuropathy (14 patients, 21%). Grade 3 events were experienced by 18 patients (27%), with no grade 4−5 events observed. The recommended phase 2 dose was determined to be 360 mg/m2. Nine (17%) of the 54 efficacy evaluable patients achieved progression-free survival ≥ 6 months. Durable partial responses were induced in non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), and thymoma. Radiographic and serum marker reductions were observed among ten patients with metastatic castration resistant prostate cancer, four of whom survived two years or longer.

Interpretation PT-112 is safe and well-tolerated in a heavily pre-treated population. Prolonged responses were noted against thymoma and lung cancer, along with radiographic and serum marker improvement in prostate cancer. Given the heterogeneous patient population, subsequent studies will be needed to characterize the risk/benefit ratio in more homogenous settings. Further development of PT-112 is ongoing, as single-agent and in combination with immune checkpoint inhibition.

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Research in context

Evidence before the study

To our knowledge, there is no prior example of cytotoxic pyrophosphate-containing agents in oncology therapeutic clinical development. We searched PubMed in April, 2021 using terms such as “pyrophosphate” “cytotoxic” “clinical trial” “platinum” and “oncology”, and found references to chelating agents, several entries referring to bisphosphonates, and one trial of a pyrophosphate-containing immune-agonist not known to possess direct anti-cancer effects. PT-112 is the first pyrophosphate-platinum conjugate in oncology clinical development, with a multimodal mechanism of action shown to promote immunogenic cancer cell death (ICD), including increases in relevant immune cell populations (i.e. dendritic cells and cytotoxic / helper T cells) in pre-clinical model systems.

Added value of the study

This study is the first-in-human clinical study assessing the safety, tolerability, and exploratory efficacy of PT-112 for the treatment of patients with advanced solid tumours. Within the limits of the Phase 1 study design, PT-112 was shown to be well tolerated with dose-proportional pharmacokinetics. It exhibited evidence of drug activity, including increases in thymic, small cell and non-small cell lung, and in prostate cancer patients, among a non-selected, heavily pre-treated population, including long-duration cases of disease stabilization and response.

Implications of all the available evidence

The Phase I study results suggest that use of PT-112 is feasible within an advanced solid tumour population and merit the continued clinical study of PT-112 in more homogeneous patient populations in more precisely defined treatment settings. Additionally, the use of additional correlative assessments is needed to determine the role of the immune system in PT-112-induced responses.

Introduction

PT-112 (chemical structure in Supplementary Figure 1) is the first pyrophosphate-containing anti-cancer agent under clinical development. PT-112 induces immunogenic cell death (ICD), a mode of cancer cell death that provokes an anti-cancer adaptive immune response and is characterized by the release of damage-associated molecular patterns (DAMPs) that interact with dendritic cells, among other immune cell types. In vitro work demonstrates that PT-112 triggers release of hallmark DAMPs (HMGB1, calreticulin, ATP), which may in part relate to marked generation of mitochondrial reactive oxygen species (ROS) observed.

ICD induction was further validated in vivo in well-established vaccine and abscopal models, wherein immune effects of PT-112 were clearly observed. PT-112 alone or in combination with anti-PD-(L)1 antibodies elicited accumulation of dendritic and effector T-cells and depletion of immunosuppressive cells in murine allograft models, and the addition of PT-112 to anti-PD-1 treatment amplified complete responses five-fold (5/7 mice vs. 1/7). In separate models, synergy was also observed with the combination of PT-112 anti-CTLA-4 treatment in mice. PT-112 also inhibited growth and/or caused regression in single-agent allograft and xenograft mouse models of solid tumours and multiple myeloma, at well-tolerated doses.

Identifying a novel agent with reduced neuro- and nephro-toxicity, and not subject to DNA repair drug-resistance pathways, was an important objective of the early discovery program. PT-112’s pleiotropic mechanism of action does not require significant binding to nuclear DNA, does not readily affect markers of DNA damage or repair, and appears largely unaffected by DNA repair pathways. Instead, PT-112 modulates apoptotic and cell cycle pathways, may exhibit selectivity to cancer cells with mitochondrial dysfunction and/or a glycolytic phenotype, and appears to exert its mechanistic effects within cellular organelles including mitochondria.

Pre-clinical pharmacokinetic studies demonstrated a high proportion of intact PT-112 parent molecule, and of free platinum unbound to blood plasma proteins, likely due to the pyrophosphate ligand strength. PT-112 did not trigger acute or chronic nephropathy in mouse models, based on assessment of cold hyperalgesia, platinum accumulation in dorsal root ganglia, and nerve conduction velocity. Rat models did not show evidence of renal toxicity, as assessed by blood urea nitrogen, serum creatinine, or glomerular filtration rate.

Imaging studies in mice demonstrated that PT-112 is distributed at pharmacological concentrations in kidney, lung, and liver, with highest concentrations in bone, using doses known to be safe and active in multiple mouse efficacy models. This distinct biodistribution profile is likely driven primarily by the pyrophosphate moiety, and offers a rationale to target diseases such as bone-metastatic solid tumours (e.g., metastatic castration-resistant prostate cancer, mCRPC) or multiple myeloma. Supporting this, PT-112 was active in the orthotopic VknMYC mouse model of multiple myeloma and was found to synergize with IMiDs and proteasome inhibitors in vitro.

A single-agent Phase I study in relapsed or refractory multiple myeloma with PT-112 is ongoing (NCT03288480).

Here, we report the first-in-human dose-escalation study of PT-112 in advanced solid tumours.

Methods

Study Design - An open-label, multi-centre 3 + 3 dose-escalation design was used, which allowed...
supplemental enrolment at doses already deemed safe. PT-112 was administered via 1 h IV infusion on days 1, 8, and 15 of a 28-day cycle. The starting dose (12 mg/m²) and schedule were based on animal toxicology and pharmacokinetic studies, wherein the highest no-adverse event level (NOAEL) and the highest non-severely toxic dose (HNSTD) in two species were evaluated and a safety factor applied to human equivalent doses. The study was conducted across four clinical research centres in the United States of America, each of which obtained ethical approval for the study from their local institutional review board or from the Western Institutional Review Board (WIRB®).

Patients - Eligible patients were ≥18 years of age and had (1) an advanced solid tumour without available effective standard of care but evaluable by RECIST v1.1 or by informative tumour markers; (2) ECOG Performance Status of 0–1; (3) documented disease progression; and (4) sufficient bone marrow, renal, and liver function. Patients with stable brain metastases (no lesion >2 cm) were eligible if active treatment was not required at time of screening.

Patient Consent - Before enrolling, patients signed an informed consent form, documenting their willingness to participate in the study and all the necessary assessments, understanding of the potential risks associated with participating in the study, alternatives to participation in the study, and agreement to use de-identified data in the context of academic papers and regulatory documents.

Outcomes - The primary endpoint was to determine safety, tolerability, pharmacokinetics, and the RP2D. The secondary endpoint was to collect exploratory evidence of anti-tumour activity. All outcomes were assessed locally at the clinical sites.

Safety - Adverse events (AEs) were evaluated according to Common Terminology Criteria for Adverse Events v4.0. A clinical safety committee (CSC) adjudicated dose-limiting toxicities (DLTs), dose escalations and de-escalations, RP2D, and maximum tolerated dose (MTD). A DLT was defined as a clinically significant dose-limiting toxicities (DLTs), dose escalations, RP2D, and maximum tolerated dose. Given the exploratory nature of efficacy analysis, this dose threshold was selected retrospectively on the basis of the first evidence of tumour control being observed at this dose level. Descriptive statistics were used to characterize the central tendency, frequency, and variability of various data; focus (other than in the PK analysis section) was on use of methods that were robust regardless of whether data were normally distributed.

Pharmacokinetics - Samples were collected on cycle 1 days 1 (C1D1) and 8 (C1D8) immediately pre-dose, and at 15, 29, 59, 70, 80, 90 min, and 2, 3, 5, 8, and 24 h after infusion start. Plasma samples were analysed for parent PT-112 and for elemental Pt in both plasma and plasma ultrafiltrate (PUF). PK parameters, including area under the curve (AUCₒ_INF), maximum plasma concentration (Cₘₐₓ), time to Cₘₐₓ (Tₘₐₓ), plasma elimination half-life (tₒ½), clearance (CL/F), and volume of distribution (Vss/F) were calculated using non-compartmental analyses. Dose proportionality of Cₘₐₓ and AUCₒ_INF on days 1 and 8 were assessed using a non-linear power model using Phoenix WinNonlin software. Briefly, the proportional relationship between each PK parameter and dose were written as a power function: PK parameter = A*dose⁰, where A is a constant and B is the proportionality coefficient. The relationship was considered dose proportional if 95% confidence intervals (CIs) for B included 1.0.

Efficacy - Patients were assessed for exploratory PT-112 anti-cancer effects every two cycles by physical examination, medical scans, and informative tumour markers. Best response to treatment was assessed by RECIST v1.1, or by modified International Thymic Malignancy Interest Group (ITMIG) RECIST² in cases of thymoma with pleural involvement. Survival among mCRPC patients was analysed retrospectively.

Statistics - Patients who received ≥1 dose of PT-112 were included in safety analysis. Efficacy analyses were exploratory in nature, conducted in patients who received ≥1 dose of PT-112 at ≥125 mg/m², excluding doses deemed sub-therapeutic. Given the exploratory nature of efficacy analysis, this dose threshold was selected retrospectively on the basis of the first evidence of tumour control being observed at this dose level. Descriptive statistics were used to characterize the central tendency, frequency, and variability of various data; focus (other than in the PK analysis section) was on use of methods that were robust regardless of whether data were normally distributed.

Study registration - This trial was registered at ClinicalTrials.gov under the number NCT02266745.

Role of the Funding Source – The funder of the study had a role in the study design, data analysis, data interpretation, and writing of this report. Authors DK and TA had access to the full dataset, and authors DK, TA, MP, JJ, and AB were primarily responsible for the decision to publish.

Results - Between July 7th, 2014 and September 18th, 2018, 66 patients were enrolled and treated with at least one infusion of PT-112 (Figure 1). The dose escalation spanned 11 dose levels (12, 24, 48, 96, 125, 150, 200, 250, 300, 360,
and 420 mg/m$^2$), with cohorts consisting of 3–16 patients (Table 1). Sixteen supplemental patients were enrolled at doses previously deemed safe by the CSC to collect additional safety and efficacy data. Median age was 62.5 years (range 24–83, IQR 49–69). Forty-seven patients (71%) had an ECOG performance status of 1 at entry; thirty-seven (56%) had multiple sites of disease (21 [32%] with liver and 22 [33%] with bone metastasis). The median number of prior systemic therapies was four (range 0–11, IQR 2–6), including platinum salts (47 patients, 71%), taxanes (37 patients, 56%), kinase inhibitors (20 patients, 30%), and immune checkpoint inhibitors (11 patients, 17%) (not shown). The mCRPC subpopulation was more heavily pre-treated than the overall population (median 6 lines of prior systemic therapy, range 2–10, IQR 4–8, not shown).

The most frequent reason for treatment withdrawal was disease progression (43 patients, 63%), followed by TRAEs (14 patients, 21%). Remaining patients withdrew due to treatment-emergent AEs (TEAEs), consent withdrawal, study non-compliance, death, or at investigator discretion (Figure 1).

Reversible DLTs occurred in four patients: grade 3 hypersensitivity reaction at 150 mg/m$^2$ (colorectal cancer), grade 3 pancytopenia at 150 mg/m$^2$ (ovarian cancer), delay of cycle 2 due to grade 2 renal toxicity at 250 mg/m$^2$ (cervical cancer), and grade 3 rash at 300 mg/m$^2$ (squamous head and neck cancer), which upon further investigation was deemed unrelated to study drug. Additional details are provided in Supplementary Table 1. Platinum-containing agents are known to cause a low rate of hypersensitivity reactions, and the CSC deemed this case unrelated to the PT-112 dose level. As such, the CSC decided to de-escalate to 125 mg/m$^2$ and subsequently to re-escalate to 150 mg/m$^2$, enrolling additional patients. No DLT was observed in either case, and the CSC authorized further dose escalation.

No DLT was observed at 420 mg/m$^2$, and dose escalation was discontinued. A maximum tolerated dose

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**Figure 1.** Trial profile.
(MTD) was not reached. The R2PD was set at 360 mg/m² on the basis of the alternate protocol mechanism, whereby tolerability and partial responses (PR) in two of three patients fulfilled selection criteria for the RP2D, once safety was validated in four additional patients followed for a minimum of two cycles.

The safety profile is summarized in Table 2 by dose level. The most common TRAEs were fatigue (23 patients, 35%), nausea (16 patients, 24%), peripheral neuropathy (14 patients, 21%) and thrombocytopenia (12 patients, 18%). Grade 3 TRAEs were reported in 18 (27%) patients; no grade 4−5 TRAEs were observed. At RP2D, 1/7 patients required dose reduction (not shown). Treatment-related thrombocytopenia was more frequent in the more heavily pre-treated prostate cancer patients, and haematological growth factors were not routinely used (not shown). All TEAEs are listed by dose level in Supplementary Table 2.

Seven (11%) had grade 2 peripheral neuropathy following a median cumulative dose and duration of treatment of 4320 mg/m² (IQR 3740−5040) and 5.1 months (IQR 4−6.7), respectively (not shown); three worsened to grade 3. Acute neuropathy, as assessed in part by a validated multi-symptom patient questionnaire, was sparse (21 instances in eight patients over 596 total infusions) and mostly limited to grade 1 (19/21) (not shown).

Mean PT-112 concentration-time plots for C1D1 are shown in Figure 2a, and the relationship between dose and the PK parameters Cmax and AUC0-inf are graphically shown in Figure 2b and 2c. PT-112 was quickly absorbed across different dose levels, with Tmax at 1 h on C1D1 and C1D8. The average terminal half-life, VSS/F, and CL/F were 2.15 h, 16.5 L, and 6.8 L/hr, respectively, with no clear differences across dose levels. There was no evidence of significant residual PT-112 or elemental Pt in plasma or PUF between C1D1 and C1D8 (not shown). Mean PK parameters for PT-112 and Pt in plasma and PUF are listed by dose level in Supplementary Table 3. The majority of the Pt in plasma was accounted for by the parental PT-112 molecule, demonstrating the stability of PT-112 in plasma. The dose proportionality assessments of Cmax and AUC0-inf for PT-112, total Pt in plasma, and total PT in PUF on C1D1 and C1D8 all yielded 95% CIs for the proportionality coefficients that included 1, indicating these data are consistent with a proportional relationship between dose and both Cmax and AUC0-inf (Supplementary Table 4).

Fifty-four patients were evaluable for exploratory efficacy assessment following PT-112 treatment at ≥125 mg/m², deemed the lowest active dose: two non-indolent metastatic adenoid cystic head and neck cancer patients with progressive disease treated at 125 mg/m² achieved progression-free survival (PFS) of ≥6 months (19.5 and 7.3 months, Supplementary Figure 2). The best response was stable disease (SD) in 17 patients and

| Characteristic                              | Values |
|---------------------------------------------|--------|
| Total Number of Patients                    | 66     |
| Age (years)                                 |        |
| Median                                      | 62.5   |
| Range                                       | 24–83  |
| IQR                                         | 49–69  |
| Gender                                      |        |
| Male                                        | 38     |
| Female                                      | 28     |
| Ethnicity                                   |        |
| Caucasian                                   | 54     |
| Black                                       | 9      |
| Asian                                       | 2      |
| Other                                       | 1      |
| ECOG PS                                     |        |
| 0                                           | 19     |
| 1                                           | 47     |
| Tumour Types                                |        |
| Appendiceal                                 | 2      |
| Basal Cell                                  | 1      |
| Bladder                                     | 1      |
| Bone Sarcoma                                | 2      |
| Breast                                      | 2      |
| Cervical / Uterine                          | 3      |
| Colorectal                                  | 6      |
| Endometrial                                 | 3      |
| Esophageal                                  | 1      |
| Gastric                                     | 1      |
| Head and Neck (adenocarcinoma, squamous)    | 13 (10.3) |
| Lung (NSCLC, SCLC)                          | 6 (4.2) |
| Melanoma                                    | 2      |
| Mesothelioma                                | 1      |
| Neuroendocrine                              | 1      |
| Ovarian                                     | 5      |
| Pancreatic                                  | 2      |
| Prostate                                    | 10     |
| Cutaneous Squamous Cell Carcinoma           | 1      |
| Thymoma                                     | 2      |
| Thyroid                                     | 1      |
| Site of Disease                             |        |
| Lung                                        | 33     |
| Liver                                       | 21     |
| Lymph Node                                  | 19     |
| Bone                                        | 22     |
| Other*                                      | 25     |
| Number of Sites                             |        |
| Single                                      | 29     |
| Multiple                                    | 37     |
| Prior Lines of Systemic Therapy             |        |
| Median                                      | 4      |
| Range                                       | 0–13   |
| IQR                                         | 2–6    |

Table 1: Patient characteristics.

*Includes Abdomen, Adrenal, Bladder, Brain, Breast, Cervix, Chest, Colon, Head/Neck, Kidney, Muscle, Ovary, Pancreas, Pleura, Peritoneum, Prostate, Soft Tissue, Thymus, Thorax, and Uterus.
| Dose Level | All AEs | Blood And Lymphatic | System Disorders | Gastrointestinal Disorders | General Disorders And Administration Site Conditions | Metabolism And Nutrition Disorders | Nervous System Disorders | Skin And Subcutaneous Tissue Disorders |
|------------|---------|---------------------|------------------|------------------------|---------------------------------------------------|----------------------------------|-----------------------|-------------------------------------|
| 12–96 mg/m² | 4/12 | 10/12 | 4/12 | 4/12 | 4/12 | 4/12 | 4/12 | 4/12 | 4/12 |
| 125 mg/m²  | 2/4 | 4/4 | 2/4 | 2/4 | 2/4 | 2/4 | 2/4 | 2/4 | 2/4 |
| 150 mg/m²  | 1/9 | 2/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 | 1/9 |
| 200 mg/m²  | 2/10 | 4/10 | 2/10 | 2/10 | 2/10 | 2/10 | 2/10 | 2/10 | 2/10 |
| 250 mg/m²  | 1/16 | 2/16 | 1/16 | 1/16 | 1/16 | 1/16 | 1/16 | 1/16 | 1/16 |
| 300 mg/m²  | 1/10 | 2/10 | 1/10 | 1/10 | 1/10 | 1/10 | 1/10 | 1/10 | 1/10 |
| 360 mg/m²  | 1/14 | 2/14 | 1/14 | 1/14 | 1/14 | 1/14 | 1/14 | 1/14 | 1/14 |
| 420 mg/m²  | 2/67 | 4/67 | 2/67 | 2/67 | 2/67 | 2/67 | 2/67 | 2/67 | 2/67 |

Table 2: Treatment-related adverse events occurring in ≥5% of patients.

Date are in n (%). No Grade 4 or 5 treatment-related adverse events occurred.
confirmed PR in three. RECIST PRs were observed at 250−360 mg/m² (Figure 3). Two additional patients (NSCLC, CRPC) experienced ≥30% reduction in tumour burden but did not have responses confirmed. All confirmed RECIST responders had metastatic thoracic malignancies (NSCLC, SCLC, and thymoma). Forty-two patients are included in a waterfall plot of drug-related effects on tumour size (Figure 4a). Twelve patients were not included due to absence of measurable disease or of baseline and/or follow-up scans. Six of eight thoracic cancer patients experienced clinical benefit from PT-112: three achieved SD, three achieved long-lasting PRs, one had progressive disease (PD), and another withdrew before follow-up (see Figure 4b for a waterfall plot of treatment effects in this sub-population, and Supplementary Figure 2 for a comparison to prior treatment outcomes in the PT-112 sub-population reaching PFS ≥6 months).

A PR was achieved in a 53-year-old female with stage IV NSCLC (adenocarcinoma, no actionable mutations) treated at a dose level of 250 mg/m² (Figure 3). The response was durable, with an overall tumour reduction of 39% and PFS 6−8 months. At six months, complete metabolic responses were observed by fluorodeoxyglucose (FDG)-positron emission tomography (PET) at metastatic sites in the lung, liver, and bone. The patient subsequently withdrew due to fatigue and peripheral neuropathy.

A 49-year-old male with extensive-stage (ES) SCLC experienced durable PR at 360 mg/m² (Figure 3b). Previous treatment included platinum doublet with external beam radiation therapy (best response, PR); and progressive disease with new lesions to most recent anti-CTLA-4 antibody (best response, SD). The response was durable, with an overall tumour reduction of 47% and PFS 6−8 months. At six months, complete metabolic responses were observed by fluorodeoxyglucose (FDG)-positron emission tomography (PET) at metastatic sites in the lung, liver, and bone. The patient subsequently withdrew due to fatigue and peripheral neuropathy.

Additional examples of anti-tumour activity include metabolic (FDG-PET) response in multiple metastatic sites, including in bone, in a basal-cell carcinoma patient with PFS of 7−11 months (150 mg/m², not shown); FDG-PET response in a pancreatic cancer patient with liver metastases, with possible immune-inflammatory features (250 mg/m², see Figure 3); as well as individual biomarker reductions, whereby
reductions ≥30% were observed in 7/33 evaluable patients (i.e., those with a measurable serum tumour biomarker, not shown).

Discussion
This study suggests the safety and tolerability of PT-112 single-agent over a range of doses in a heavily pre-treated solid tumour population. The most prevalent side effect was fatigue, largely grade 1 and resolved within 48 h. DLTs observed during escalation displayed no consistent trend and resolved to grade 0−1 within 3 weeks. Only isolated nephro- or acute neuro-toxicity, and no alopecia, mucositis, or severe constitutional side effects were reported, with no grade 4-5 TRAEs. Haematotoxicities were generally of low grade, with no febrile neutropenia or thrombocytopenia with bleeding. While cytopaenias were observed more frequently in mCRPC patients, who were generally more heavily pre-treated and had bone disease involvement, there was a lack of skeletal-related events. Parameter estimates from the PK data including $T_{\text{max}}$, terminal half-life, $V_{SS}/F$, $CL/F$, $C_{\text{max}}$ and $AUC_{0-\text{inf}}$ were generated. The data were consistent with a proportional relationship between dose and the $C_{\text{max}}$ and $AUC_{0-\text{inf}}$ parameters, and no evidence was found of meaningful accumulation of PT-112 or related Pt after multiple administrations. Future work on population PK modelling will be conducted and will include

Figure 3. CT Scans Showing RECIST Responses in Three Patients Treated with PT-112. (a) Target NSCLC lesions at baseline (left) and after 3 cycles (right); insets are zoomed views of target lesions. (b) Target SCLC lesion at baseline (left) and after 2 cycles (right); insets are zoomed views of target lesions. (c) Target thymoma lesion at baseline (left), after 2 cycles (center), and after 1 year from start of therapy (7.1 months since last infusion, right).
compartmental analyses and searches for covariates that may influence PT-112 absorption and elimination.

Preliminary evidence of single-agent activity was observed in heavily pre-treated patients with thoracic malignancies and mCRPC, including tumour responses and durable PFS. Cases of ongoing PFS and survival after treatment discontinuation may be indicative of immune involvement, consistent with the pre-clinical evidence of PT-112’s ICD induction.\(^4\) Such considerations will require well-designed investigation in subsequent clinical and correlative studies.

Reductions in PSA, ALP, tumour burden and bone pain in the mCRPC sub-population were observed predominantly at doses below the RP2D. We postulate that PT-112’s osteotropism\(^1\) may be linked to activity in patients with metastatic disease to the bone. Future studies will incorporate bone scans and bone-specific assessment criteria (e.g., as recommended by the

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*Figure 4.* Waterfall Plot for Patients with Evaluable Disease and Treated with \(\geq 125\) mg/m\(^2\) PT-112. Data are best response to treatment colour by dose level, with dotted lines representing progression (+20%) and response (~30%). ITMIG-modified RECIST criteria were used where applicable for patients with thymoma. Data are shown for (a) all patients and (b) only patients with thoracic cancers.
Prostate Cancer Working Group 3),

as well as pain scales, to measure more systematically PT-112’s effects on bone site of disease.

Separate investigation into the mechanism by which PT-112 initiates ICD is ongoing: evidence of mitochondrial ROS generation and ATP release in PT-112 sensitive cancer cells is consistent with the literature surrounding ICD.19 Mitochondria have been shown to play a role in the immunogenicity of radiation therapy, which itself can induce ICD 20; we hypothesize this could be the case with PT-112 as well.

Given that PT-112 induces ICD crossing in vitro and in vivo model systems, and synergy with immune checkpoint inhibitors,4 and that ICD can drive immune responses in a fashion separate from and potentially complementary to immune checkpoint inhibitors, we postulated that PT-112 is a viable combination candidate with immune checkpoint therapy and/or could help address the growing unmet medical need among non-responders to such therapy. PT-112 is combined with the PD-L1 antibody avelumab in an ongoing study (NCT03409458), which involves tools for measuring therapy-induced changes in T cell lineages.21,22 Additionally, PT-112 might provide benefit in combination with other standards of care, as certain ICD-inducing agents (e.g., anthracyclines, cyclophosphamide, proteasome inhibitors, taxanes) are frequently used as part of combination therapies with other chemotherapeutic, targeted, or radiological interventions.

In summary, PT-112’s tolerability profile, along with evidence of durable single-agent activity in individual cases, supports multi-cycle use and validation of the RP2D in subsequent clinical study. The present patient population was heterogenous with respect to tumour type and prior therapies received, elevating the importance of subsequent studies in well-defined, homogeneous populations to understand more precisely the risk/benefit profile of PT-112 in specific disease types and treatment settings. Notwithstanding the diversity of the study population, individual cases of response persisting long after treatment discontinuation, including in non-responders to prior immune checkpoint inhibitors, suggest the possibility that PT-112 may have triggered an adaptive immunity consistent with ICD. Methods are in place to characterize prospectively PT-112’s effects on patients’ immune profile in follow-on clinical studies. Effects on bone metastases and ALP may be due to PT-112’s osteotropism, and support investigation in cancers with bone involvement, including mCRPC. Collectively, these findings warrant further development of PT-112.

Funding
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Contributors
DK was involved with the study design, data collection, data interpretation, reviewing and editing the

Figure 5. PET response in a liver lesion in a patient with metastatic pancreatic cancer. (a) Comparative PET images at baseline (left) and after cycle 2 (right) with the corresponding CT images at baseline (lower left) and after cycle 2 (lower right) (b) Corresponding standard uptake values (SUV) of FDG-18 of liver lesions.
manuscript, and verifying the underlying data. RC, JJ, and AB were involved with data collection, data interpretation, and reviewing and editing the manuscript. TA was involved with literature searches, study design, data analysis, drafting of the manuscript and building figures, reviewing and editing the manuscript, and verifying the underlying data. MP was involved with literature searches, study design, data analysis, reviewing and editing the manuscript, and funding acquisition. JI was involved with literature searches, study design, data interpretation, and reviewing and editing the manuscript.

Data sharing statement
Individual participant data will not be made available, and other data/documents will not be shared or made available. Additional information about the study can be found on ClinicalTrials.gov under the number NCT02266745.

Declaration of interests
Promontory Therapeutics, as the study sponsor, paid for most aspects of the trial outside those billable to patient insurance and provided study drug (all authors). Promontory in the past has covered meeting and travel expenses for TA, MP, JJ, and DK. TA and MP are employees of Promontory Therapeutics and are paid by/own equity in Promontory. JJ and DK have non-remunerated roles on Promontory’s Scientific Advisory Board. JJ is a former employee of Janssen Oncology. RC has no conflicts to report.

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