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 cyto-toxic 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 I study design, PT-112 was shown to be well tolerated with dose-proportional pharmacokinetics. It exhibited evidence of drug activity, including 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[1,2] 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.[3,4] ICD induction was further validated *in vivo* in well-established vaccine and abscopal models,[4] 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 immuno-suppressive 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).[4] In separate models, synergy was also observed with the combination of PT-112 anti-CTLA-4 treatment in mice.[4] 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.[4−7]

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,[8,9] does not readily affect markers of DNA damage or repair,[6] and appears largely unaffected by DNA repair pathways.[6,7] Instead, PT-112 modulates apoptotic and cell cycle pathways,[10,11] 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.[3]

Pre-clinical pharmacokinetic studies demonstrated a high proportion of intact PT-112 parent molecule, and of free platinum unbound to blood plasma proteins,[6] likely due to the pyrophosphate ligand strength. PT-112 did not trigger acute or chronic neuropathy in mouse models, based on assessment of cold hyperalgesia, platinum accumulation in dorsal root ganglia, and nerve conduction velocity.[6] Rat models did not show evidence of renal toxicity, as assessed by blood urea nitrogen, serum creatinine, or glomerular filtration rate.[6]

Imaging studies in mice demonstrated that PT-112 is distributed at pharmacological concentrations *inter alia* 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,[12] 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 Vk*MYC mouse model of multiple myeloma and was found to synergize with IMiDs and proteasome inhibitors *in vitro*.[12,13] A single-agent Phase I study in relapsed or refractory multiple myeloma with PT-112 is ongoing (NCT03288480).[14]

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 treatment-related adverse event (TRAE) occurring within cycle 1 that met any of the following criteria: (1) grade 3-4 non-hematologic toxicity, except grade 3 nausea, vomiting, or diarrhoea (unless persistent >3 days despite optimal medical management); (2) grade ≥2 peripheral neuropathy that does not resolve to grade ≤1 within 7 days; (3) grade 4 anaemia; (4) grade 4 neutropenia persisting >5 days; (5) febrile neutropenia; (6) grade 4 thrombocytopenia persisting >5 days or thrombocytopenia with clinically significant bleeding; or (7) any TRAE precluding cycle 2 treatment for >7 days. Patients were surveyed by questionnaire after each infusion to assess 11 symptoms of acute neurotoxicity.[15]

Pharmacokinetics - Samples were collected on cycle 1 days 1 (C1D1) and 8 (C1D8) immediately pre-dose, and at 15, 29, 59, 63, 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 ($AUC_{0\text{-inf}}$), maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), plasma elimination half-life ($t_{1/2}$), clearance (CL/F), and volume of distribution (Vss/F) were calculated using non-compartmental analyses. Dose proportionality of C_{max} and $AUC_{0\text{-inf}}$ 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 = $A*dose^B$, 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,[16] or by modified International Thymic Malignancy Interest Group (ITMIG) RECIST[17] 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.

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,
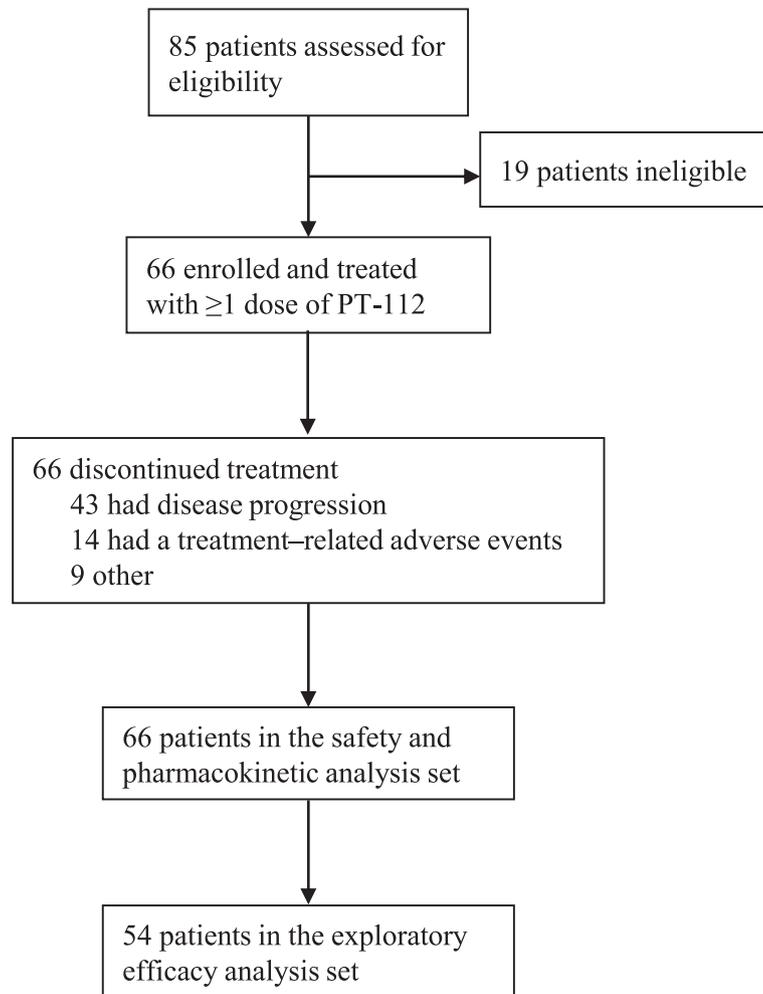
Figure 1. Trial profile.

and 420 mg/m²), 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, 65%), 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/m² (colorectal cancer), grade 3 pancytopenia at 150 mg/m² (ovarian cancer), delay of cycle 2 due to grade 2 renal toxicity at 250 mg/m² (cervical cancer), and grade 3 rash at 300 mg/m² (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/m² and subsequently to re-escalate to 150 mg/m², enrolling additional patients. No DLT was observed in either case, and the CSC authorized further dose escalation.

No DLT was observed at 420 mg/m², and dose escalation was discontinued. A maximum tolerated dose

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.

(MTD) was not reached. The R2PD was set at 360 mg/m^2 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/m^2 (IQR 3740−5040) and 5·1 months (IQR 4·0−6·7), 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 C_{max} and AUC_{o-inf} are graphically shown in Figure 2b and 2c. PT-112 was quickly absorbed across different dose levels, with T_{max} at 1 h on C1D1 and C1D8. The average terminal half-life, V_{SS}/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 C_{max} and AUC_{o-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 1·0, indicating these data are consistent with a proportional relationship between dose and both C_{max} and AUC_{o-inf} (Supplementary Table 4).

Fifty-four patients were evaluable for exploratory efficacy assessment following PT-112 treatment at ≥125 mg/m^2, deemed the lowest active dose: two non-indolent metastatic adenoid cystic head and neck cancer patients with progressive disease treated at 125 mg/m^2 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

	12−96 mg/m² (n = 12)		125 mg/m² (n = 4)		150 mg/m² (n = 9)		200 mg/m² (n = 5)		250 mg/m² (n = 16)		300 mg/m² (n = 10)		360 mg/m² (n = 7)		420 mg/m² (n = 3)		Total (n = 66)	
	G1−2	G3	G1−2	G3	G1−2	G3	G1−2	G3	G1−2	G3	G1−2	G3	G1−2	G3	G1−2	G3	G1−2	G3
All AEs	8(67)	1(8)	2(50)	1(25)	2(22)	4(44)	3(60)	1(20)	8(50)	4(25)	4(40)	2(20)	3(43)	3(43)	1(33)	2(67)	31(47)	18(27)
Blood And Lymphatic System Disorders	—	—	—	—	1(11)	1(11)	1(20)	1(20)	4(25)	3(19)	1(10)	1(10)	1(14)	1(14)	—	2(67)	8(12)	9(14)
Anaemia	—	—	—	—	1(11)	1(11)	1(20)	1(20)	2(12)	1(6)	—	—	1(14)	—	—	—	4(6)	3(5)
Neutropenia	—	—	—	—	—	1(11)	—	1(20)	—	1(6)	1(10)	—	—	1(14)	—	2(67)	1(2)	6(9)
Thrombocytopenia	—	—	—	—	—	1(11)	1(20)	—	4(25)	2(12)	—	1(10)	1(14)	—	2(67)	—	8(12)	4(6)
Gastrointestinal Disorders	3(25)	—	1(25)	—	3(33)	—	4(80)	—	6(38)	—	3(30)	—	5(71)	—	1(33)	—	26(39)	—
Constipation	—	—	—	—	1(11)	—	2(40)	—	3(19)	—	—	—	1(14)	—	—	—	7(11)	—
Diarrhoea	1(8)	—	—	—	1(11)	—	1(20)	—	1(6)	—	1(10)	—	2(29)	—	—	—	7(11)	—
Nausea	2(17)	—	—	—	2(22)	—	4(80)	—	2(12)	—	2(20)	—	3(43)	—	1(33)	—	16(24)	—
Paraesthesia oral	—	—	1(25)	—	—	—	—	—	2(12)	—	—	—	2(29)	—	—	—	5(8)	—
Vomiting	2(17)	—	—	—	1(11)	—	4(80)	—	1(6)	—	1(10)	—	1(14)	—	1(33)	—	11(17)	—
General Disorders And Administration Site Conditions	5(42)	—	—	—	3(33)	—	3(60)	—	4(25)	—	4(40)	1(10)	4(57)	1(14)	1(33)	—	24(36)	2(3)
Fatigue	5(42)	—	—	—	3(33)	—	3(60)	—	3(19)	—	4(40)	1(10)	2(29)	1(14)	1(33)	—	21(32)	2(3)
Investigations	2(17)	1(8)	1(25)	1(25)	—	—	1(20)	—	—	—	—	—	3(43)	—	—	—	7(11)	2(3)
Metabolism And Nutrition Disorders	3(25)	—	—	—	1(11)	1(11)	2(40)	—	3(19)	1(6)	1(10)	—	1(14)	1(14)	1(33)	—	12(18)	3(5)
Decreased appetite	2(17)	—	—	—	1(11)	—	2(40)	—	2(12)	—	1(10)	—	2(29)	—	—	—	10(15)	—
Musculoskeletal And Connective Tissue Disorders	2(17)	—	1(25)	—	—	—	—	—	2(12)	—	1(10)	—	1(14)	—	—	—	7(11)	—
Nervous System Disorders	2(17)	—	2(50)	—	1(11)	1(11)	3(60)	—	2(12)	1(6)	2(20)	—	3(43)	1(14)	2(67)	—	17(26)	3(5)
Dysgeusia	1(8)	—	—	—	1(11)	—	1(20)	—	—	—	—	—	2(29)	—	—	—	5(8)	—
Neuropathy peripheral	1(8)	—	1(25)	—	—	1(11)	1(20)	—	2(12)	1(6)	1(10)	—	3(43)	1(14)	2(67)	—	11(17)	3(5)
Respiratory, Thoracic And Mediastinal Disorders	1(8)	—	—	—	—	—	—	—	1(6)	—	2(20)	—	2(29)	—	1(33)	—	7(11)	—
Skin And Subcutaneous Tissue Disorders	1(8)	—	—	—	—	—	2(40)	—	—	—	—	—	1(14)	—	—	—	4(6)	—
Date are in n (%). No Grade 4 or 5 treatment-related adverse events occurred.																		

Table 2: **Treatment-related adverse events occurring in ≥5% of patients.**
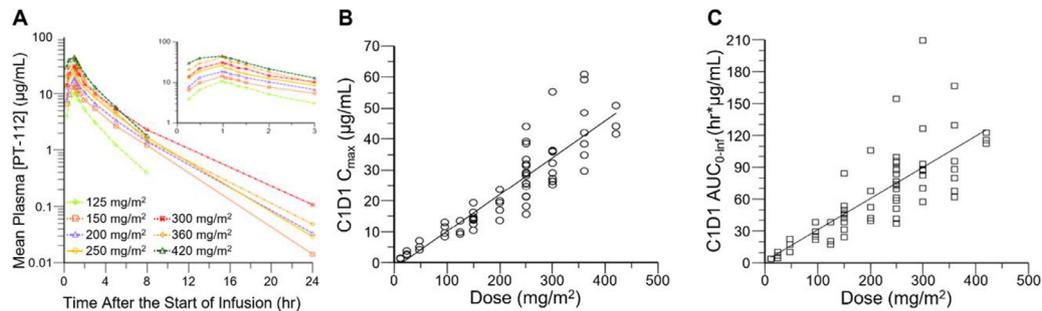
Figure 2. PT-112 Pharmacokinetics. (a) Mean plasma concentration *versus* nominal time after start of PT-112 infusion on C1D1 (dose levels ≥125 mg/m²). Inset shows earlier time points to visualize curve separation and C_{max}. Dose levels are indicated by colour and line style as indicated in the legend. (b) C_{max} and (c) AUC_{0-Inf} for PT-112 in plasma at all dose levels on C1D1.

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 who had previously received four lines of systemic therapy, including most recent prior anti-PD-1 antibody (best response, SD). The response was durable, with an overall tumour reduction of 39% (Figure 3a) and PFS 6·8 months. At six months, complete metabolic responses were observed by fluoro-deoxyglucose (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, confirmed PR at 360 mg/m² with tumour reduction of 47% (Figure 3b). Previous treatment included a platinum doublet with external beam radiation therapy (best response, PR); and progressive disease with new lesions to most recent anti-CTLA-4 and anti-PD-1 combination therapy, terminating 2·2 months prior to PT-112 treatment. After 5·3 months' treatment the patient discontinued due to grade 2 peripheral neuropathy. As of data cut-off, the patient remained progression-free (38 months), including >32 months with no further anti-cancer treatment; at most

recent imaging, the original RECIST target lesion was no longer detectable.

A 31-year-old male metastatic thymoma patient experienced durable PR at 360 mg/m². He was previously treated with four lines of cytotoxic therapy and was immunotherapy-naÿve. A 22% reduction after 1 cycle and 32% after 2 cycles on PT-112 therapy were assessed by ITMIG criteria for pleural disease (Figure 3c). Following a prolonged treatment holiday for personal reasons, re-staging showed further tumour regression 4·2 months after treatment start (61% overall reduction). After five cycles, therapy was held due to grade 3 neutropenia, and the patient remained progression-free with no further therapy for a total of 17 months. Symptoms from pre-existing myasthenia gravis were stable. Upon progression, the patient was re-challenged with PT-112 (300 mg/m² q2wk), experienced a second PR, and remained progression-free for a further 10 months as of data cut-off.

Of 10 mCRPC patients, nine were treated at 200 and 250 mg/m², and one at 420 mg/m². In the seven patients with RECIST-measurable disease, tumour reductions were seen in two (21% and 30%). Reductions in PSA and alkaline phosphatase (ALP) were observed in 4/10 and 9/10 patients, respectively, with one patient experiencing PSA reduction >50% (not shown). Clinical benefit in this sub-population included pain improvement and lack of skeletal-related events (not shown). Five patients achieved stable disease with PFS ≥3 months. Additionally, three mCRPC patients remained alive 47, 44, and 31 months since treatment start, as of data cut-off. Median survival was 15·1 months (IQR 3·3 −31·3), assessed retrospectively (see Supplemental Figure 3).

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 7·1 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 5); as well as individual biomarker reductions, whereby
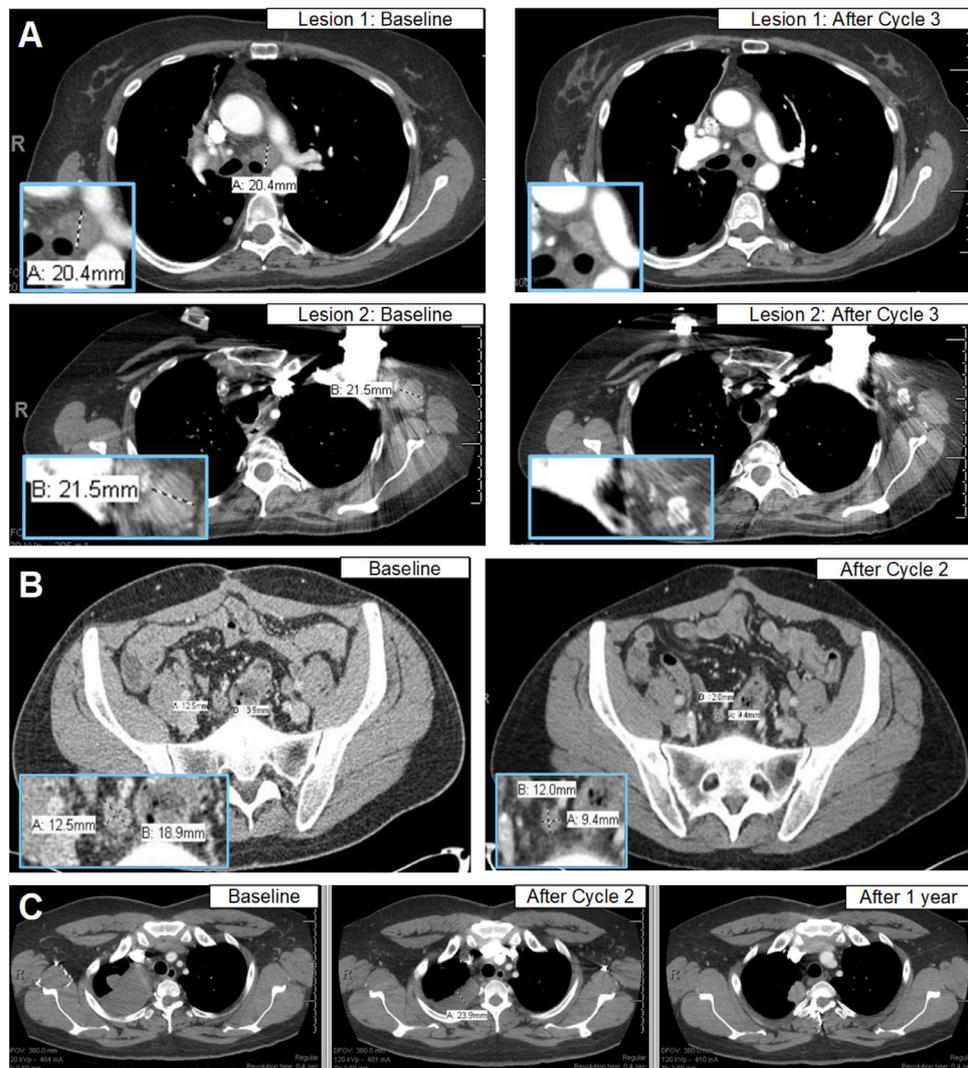
Figure 3. CT Scans Showing RECIST Responses in Three Patients Treated with PT-112. (a) Target NSCLC lesions at baseline (left) and after 3 cycles (right); insets are zoomed views of target lesions. (b) Target SCLC lesion at baseline (left) and after 2 cycles (right); insets are zoomed views of target 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).

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. Hae-mato-toxicities 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_{max}, terminal half-life, V_{SS}/F, CL/F, C_{max} and AUC_{o-inf} were generated. The data were consistent with a proportional relationship between dose and the C_{max} and AUC_{o-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
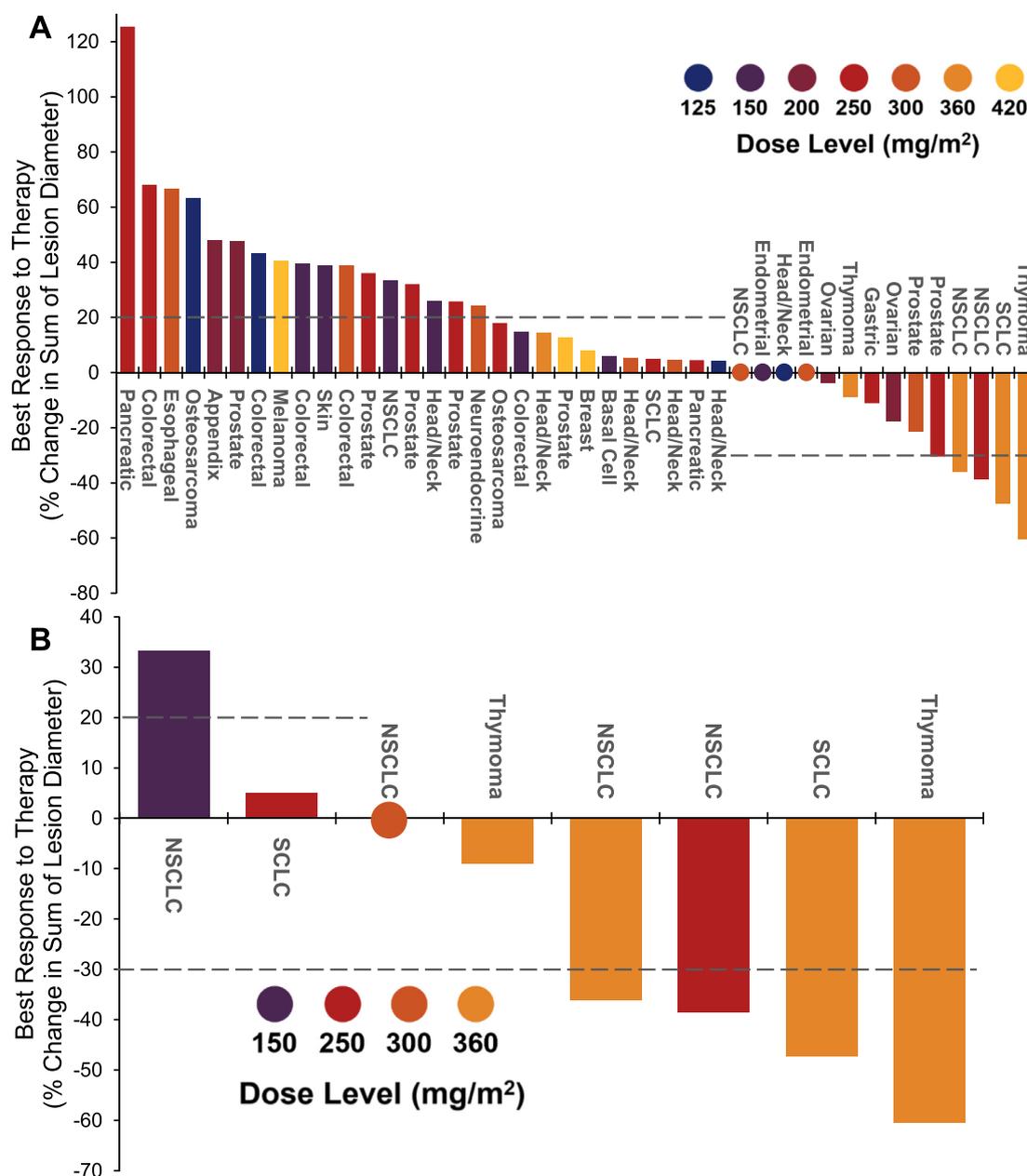
Figure 4. Waterfall Plot for Patients with Evaluable Disease and Treated with ≥ 125 mg/m² PT-112. Data are best response to treatment colourby dose level, with dotted lines representing progression (+20%) and response (−30%). ITMIG-modified RECIST criteria were used where applicable for patients with thymoma. Data are shown for (a) all patients and (b) only patients with thoracic cancers.

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[12] 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
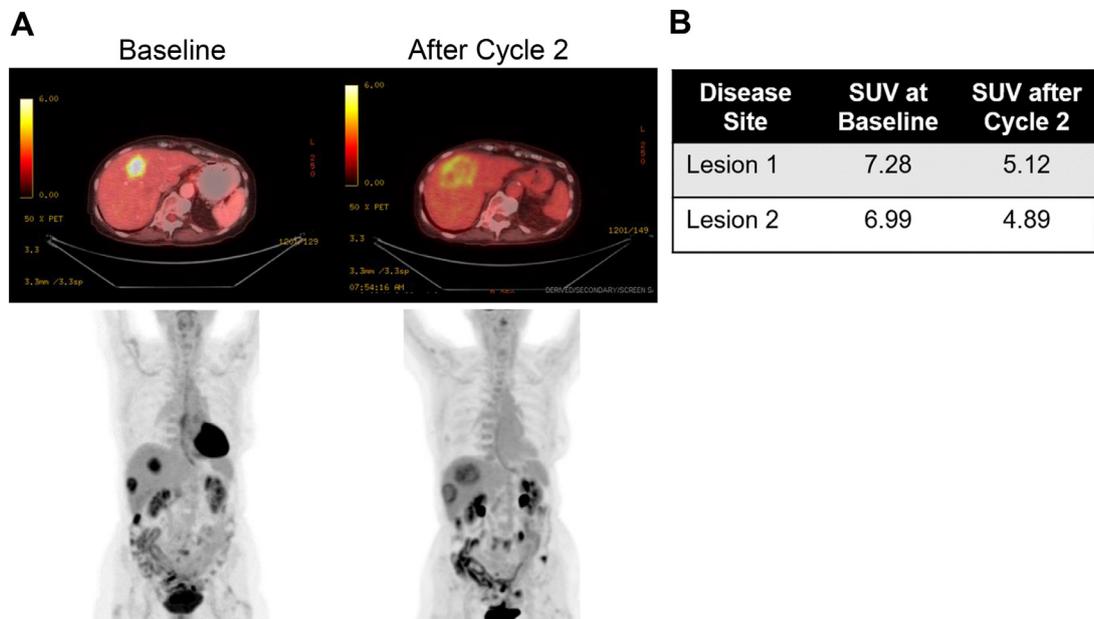
Figure 5. PET response in a liver lesion in a patient with metastatic pancreatic cancer. (a) Comparative PET images at baseline (left) and after cycle 2 (right) with the corresponding CT images at baseline (lower left) and after cycle 2 (lower right) (b) Corresponding standard uptake values (SUV) of FDG-18 of liver lesions.

Prostate Cancer Working Group 3),[18] 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, homogenous 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.

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

manuscript, and verifying the underlying data. RC, JI, 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. JJ was involved with literature searches, study design, data interpretation, and reviewing and editing the manuscript.

References

1 Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. *Nat Rev Immunol*. 2017;17:97–111.
2 Galluzzi L, Vitale I, Warren S, et al. Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. *J Immunother Cancer*. 2020;8: e000337.
3 Soler R, Ames T, Marco-Brualla J, et al. PT-112, a first-in-class pyrophosphate-platinum conjugate, selectively targets highly glycolytic tumor cells. *Eur J Cancer*. 2020;138:S53–S54.
4 Yamazaki T, Buqué A, Ames TD, Galluzzi L. PT-112 induces immunogenic cell death and synergizes with immune checkpoint blockers in mouse tumor models. *Oncoimmunology*. 2020;9: 1721810.
5 Moghaddas S, Majmudar P, Marin R, et al. Phosphaplatins, next generation platinum antitumor agents: a paradigm shift in designing and defining molecular targets. *Inorg Chim Acta*. 2012;393:173–181.
6 Ames T, Slusher B, Wozniak K, et al. Findings across pre-clinical models in the development of PT-112, a novel investigational platinum-pyrophosphate anti-cancer agent. *Eur J Cancer*. 2016;69:S153.
7 Bose RN, Moghaddas S, Belkacemi L, et al. Absence of activation of DNA repair genes and excellent efficacy of phosphaplatins against human ovarian cancers: implications to treat resistant cancers. *J Med Chem*. 2015;58:8387–8401.
8 Bose RN, Maurmann L, Mishur RJ, et al. Non-DNA-binding platinum anticancer agents: cytotoxic activities of platinum−phosphato complexes towards human ovarian cancer cells. *PNAS*. 2008;105:18314–18319.
9 Corte-Rodríguez M, Espina M, Sierra LM, et al. Quantitative evaluation of cellular uptake, DNA incorporation and adduct formation in cisplatin sensitive and resistant cell lines: comparison of different Pt-containing drugs. *Biochem Pharmacol*. 2015. https://doi.org/10.1016/j.bcp.2015.08.112. published online Sept 6.
10 Wang JQ, Ames T, Arciero E, Hehenberger MT, Chatterjee D. Characterization of molecular targets of the novel platinum agent PT-112 in human colon cancer cells. *Mol Cancer Ther*. 2015;14. C32−C32.
11 Tosi D, Perez-Gracia E, Pourquier P, et al. A kinome analysis of the molecular pharmacodynamics of PT-112 in a human cancer cell line. *Cancer Res*. 2017;77:2378.
12 Ames TD, Sharik ME, Rather GM, et al. Translational research of PT-112, a clinical agent in advanced phase I development: evident bone tropism, synergy *in vitro* with bortezomib and lenalidomide, and potent efficacy in the Vk*MYC mouse model of multiple myeloma. *Blood*. 2017;130. 1797−1797.
13 Chesi M, Robbiani DF, Sebag M, et al. AID-dependent activation of a MYC transgene induces multiple myeloma in a conditional mouse model of post-germinal center malignancies. *Cancer Cell*. 2008;13:167–180.
14 Kourelis T, Ailawadhi S, Vogl DT, et al. A phase I dose escalation study of PT-112 in patients with relapsed or refractory multiple myeloma. *Blood*. 2020;136:9–10.
15 Leonard GD, Wright MA, Quinn MG, et al. Survey of oxaliplatin-associated neurotoxicity using an interview-based questionnaire in patients with metastatic colorectal cancer. *BMC Cancer*. 2005;5:116.
16 Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45:228–247.
17 Kim HS, Lee JY, Lim SH, et al. Assessment of objective responses in thymic epithelial tumors using ITMIG modified criteria. *Lung Cancer*. 2016;96:48–51.
18 Scher HI, Morris MJ, Stadler WM, et al. Trial design and objectives for castration-resistant prostate cancer: updated recommendations from the prostate cancer clinical trials working group 3. *J Clin Oncol*. 2016. https://doi.org/10.1200/JCO.2015.64.2702. published online Feb 22.
19 Radogna F, Dicato M, Diederich M. Natural modulators of the hallmarks of immunogenic cell death. *Biochem Pharmacol*. 2019. https://doi.org/10.1016/j.bcp.2018.12.016. published online Jan 5.
20 Yamazaki T, Kirchmair A, Sato A, et al. Mitochondrial DNA drives abscopal responses to radiation that are inhibited by autophagy. *Nat Immunol*. 2020;21:1160–1171.
21 Bryce AH, Dronca RS, Costello BA, et al. PT-112 in advanced metastatic castrate-resistant prostate cancer (mCRPC), as monotherapy or in combination with PD-L1 inhibitor avelumab: findings from two phase I studies. *JCO*. 2020;38:83.
22 Karp DD, Dronca RS, Camidge R, et al. 1026MO Phase Ib dose escalation study of novel immunogenic cell death (ICD) inducer PT-112 plus PD-L1 inhibitor avelumab in solid tumours. *AnnOncol*. 2020;31:S708.