Background PT-112 is a novel platinum-pyrophosphate conjugate\(^1\) under clinical development for cancer therapy.\(^2\) Besides mediating cytostatic and cytotoxic effects in numerous human and mouse cancer cells, PT-112 elicits various danger signals that are linked to immunogenic cell death (ICD), such as calreticulin exposure, as well as ATP and HMGB1 secretion.\(^3\)\(^-\)\(^6\) Accordingly, mouse cancer cells succumbing to PT-112 in vitro efficiently protect immunocompetent, tumor-naïve mice from challenge with living cancer cells of the same type.\(^7\)\(^-\)\(^9\) Moreover, PT-112 synergizes with PD-1 or PD-L1 blockade to control mouse tumors developing in immunologically competent hosts.\(^7\)\(^-\)\(^9\) In some tumor models, robust type I interferon (IFN) signaling is required for ICD.\(^10\) However, the role of type I IFN signaling in the immunogenicity of PT-112 remains unclear.

Methods We used wild-type, mitochondrial DNA (mtDNA) depleted (rho\(^0\)) as well as Casp2\(^{-/-}\) and Casp3\(^{-/-}\) mouse TS/A cells.\(^11\) ELISA, flow cytometry and immunofluorescence microscopy were employed to monitor type I IFN levels, reactive oxygen species (ROS) generation, mitochondrial polarization, cell death, and cytosolic dsDNA accumulation driven by PT-112 and underlying regulatory mechanisms.

Results In cultured TS/A cells, PT-112 induced mitochondrial dysfunction, as demonstrated by ROS generation and mitochondrial hyperpolarization, as well as cytosolic accumulation of dsDNA that was abrogated in rho\(^0\) TS/A cells. Type I IFN secretion by wild-type TS/A responding to PT-112 was not observed. Both Casp2 and Casp3 deletion provided TS/A cells with some protection from PT-112-driven ROS generation and mitochondrial hyperpolarization, but only Casp3 deletion afforded robust protection against the acute cytotoxicity of PT-112. Importantly, both Casp2 and Casp3 deletion augmented cytosolic dsDNA accumulation driven by PT-112.

Conclusions PT-112 causes pronounced mitochondrial dysfunction in cancer cells coupled with cytosolic mtDNA accumulation, which generally leads to type I IFN secretion.\(^12\) PT-112-driven caspase activation, however, possibly prevents mtDNA-driven type I IFN secretion, likely reflecting CGAS cleavage by active CASP3\(^12\) and/or the rapid CASP3-dependent transition of dying cancer cells into metabolically inert corpses.\(^13\)\(^-\)\(^14\) In line with this possibility, both Casp2\(^{-/-}\) and Casp3\(^{-/-}\) TS/A cells exhibited increased cytosolic mtDNA accumulation upon PT-112 treatment. As PT-112 is a potent inducer of ICD in vaccination assays,\(^7\) CASP3 activation elicited during the cytotoxic response to PT-112 may not influence the ability of PT-112 to drive prophylactic anticancer immunity in tumor-naïve hosts. Additional studies with established tumor models are needed to clarify the impact of caspases on the immunogenicity of PT-112 in clinically relevant settings.

REFERENCES