Background Tigilanol Tiglate (TT) is a novel small molecule under development for local treatment of solid tumours via intratumoral (I.T.) injection. TT is a protein kinase C (PKC)/C1 domain activator that disrupts tumour vasculature, leading to haemorrhagic necrosis of the lesion. Strikingly, in both preclinical syngeneic mouse models and cutaneous/subcutaneous tumours presenting in the veterinary clinic, I.T. injection of TT results in complete and enduring ablation of target tumours in >70% of patients. TT has completed a Phase I/IIa dose-escalation study in humans to determine the safety, tolerability, preliminary efficacy and signs of abscopal effects in some patients. TT has also demonstrated cancer efficacy and signs of abscopal effects in some patients. TT has completed a Phase I dose-escalation trial in humans (ACTRN12614006856178), with strong evidence of local anticancer efficacy and signs of abscopal effects in some patients. However, the underlying mechanism of action (MOA) of TT, together with its immunotherapeutic potential in oncology, is not fully understood.

Methods A combination of microscopy, immunofluorescence, immunoblotting, subcellular fractionation, intracellular ATP assays, LDH release assays and mixed lymphocyte reactions were used to probe the MOA of TT in vitro. TT-mediated damage associated molecular pattern (DAMP) release/externalization was assessed using luciferase (ATP), ELISA (HMGB1), flow cytometry and immunohistochemical (calreticulin) approaches. In vivo experimentation with TT utilized CT-26 and B16-F10-OVA tumor bearing mice, with or without anti-PD1/anti-CTLA4 treatment.

Results Our data demonstrates that therapeutic concentrations of TT induce death of cancer and endothelial cell lines, both in vitro and in vivo, via oncosis. Whilst largely PKC-independent, PKC/C1 domain signaling appears necessary for timely oncolysis in vitro and efficacious tumor ablation in vivo. Our results also show that TT binds to ER membranes, causing ER stress with subsequent activation of the integrated stress response. This is followed by mitochondrial membrane potential loss, ATP depletion, organelle swelling, oncosis and terminal necrosis. We also found that TT treatment promoted the release/externalization of DAMPs (HMGB1, ATP, calreticulin) from cancer cells in vitro and in vivo. Characteristics indicative of immunogenic cell death (ICD). Confirmation of ICD in vivo was obtained through rechallenge experiments using CT-26 tumour bearing mice, which also demonstrated that TT promoted the development of tumour-specific T cells. In addition to stimulating immune cell infiltration into tumours, TT significantly improved treatment response in the B16-F10-OVA mouse melanoma model when combined with immune checkpoint blockade.

Conclusions These data indicate that TT is an oncolytic small molecule with the potential to enhance responses to immunotherapy. TT is currently undergoing Phase II trials in head and neck cancers (ACTRN12619001407189), soft tissue sarcomas, Stage III melanoma in-transit (NCT05234437) and non-resectable Stage IIIIB to IV M1c melanoma (TT/pembrolizumab combination: NCT04834973).

REFERENCES
5. QBiotics Group Ltd. website. https://qbiotics.com/