1091 TIGILANOL TIGLATE IS A SMALL MOLECULE ONCOLYTIC THAT INDUCES IMMUNOGENIC CELL DEATH AND PROMOTES IMMUNE CELL INFILTRATION INTO HUMAN TUMORS

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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 and causes direct oncolysis of tumour cells. Together, these activities lead to haemorrhagic necrosis of injected tumours with enduring ablation of >70% of target tumours in both pre-clinical xenograft/syngeneic mouse models and cutaneous tumours presenting in the veterinary clinic.[1–3] TT has completed a Phase I/IIa dose-escalation trial in humans (ACTRN12614000685617), with strong evidence of local anti-cancer efficacy and signs of abscopal effects in some patients.[4] 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 (HMGB1, calreticulin) approaches. *In vivo* experimentation with TT utilized CT-26 and B16-F10 tumor bearing mice. Analysis of DAMP release and immune cell infiltration into TT treated human head and neck tumours (ACTRN12619001407189) was performed by immunohistochemistry.

Results Our data reveal that therapeutic concentrations of TT induce the death of cancer and endothelial cell lines via a pathway involving caspase activation and cleavage of the pore forming protein gasdermin E. TT promotes this mechanism of cell death by interacting with ER membranes, causing an ER stress response that results in loss of mitochondrial membrane potential, ATP depletion, organelle swelling and oncosis/pyroptosis. Treatment of cells with TT also led to the release of damage associated molecular patterns (DAMPs), indicative of an immunogenic cell death (ICD) pathway that also resulted in the generation of tumour-specific T cells in CT-26 tumor bearing mice. Whilst the induction of ICD is largely PKC-independent *in vitro*, PKC/C1 domain signaling appears necessary for efficacious tumour ablation *in vivo*. Consistent with our pre-clinical data, immunohistochemical analysis of TT-treated head and neck tumors found that drug stimulated DAMP release/externalisation and the recruitment of immune cells, principally $CD8^+$ T cells, into remnant tumour mass.

Conclusions These data indicate that TT is an oncolytic small molecule with the potential to ablate target tumours and enhance immunotherapy combinations through promoting immune cell infiltration. TT is currently undergoing Phase II trials in head and neck cancer (NCT05234437) and soft tissue sarcoma (NCT05755113).

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Ethics Approval The study obtained ethics approval from the following commitees and boards: Metro South Human Research Ethics Committee, 199 Ipswich Road, Woolloongabba, QLD, 4102, Australia. Ethics approval number: HREC/2019/QMS/54004. Bellberry Limited, 123 Glen Osmond Road, Eastwood, SA 5063, Australia. Ethics approval number: 2019–10-846 (REGIS 2019/ETH13063). Tata Memorial Hospital - Institutional Review Board, IRB Office, Dr. E. Borges Marg, Parel, Mumbai - 400 012, India. Ethics approval number: IEC/1219/3370/001. Tata Medical Center - Institutional Review Board, 14 Major Arterial Road (EW), New Town, Rajarhat, Kolkata - 700 160, India. Ethics approval number: 2019/PHARMA/57/IRB39.

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