Talazoparib interacts with oncolytic reovirus to enhance death-inducing signalling complex (DISC)-mediated apoptosis and immune response

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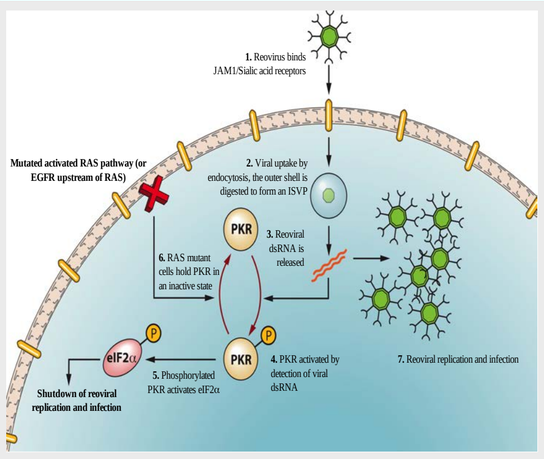
Background

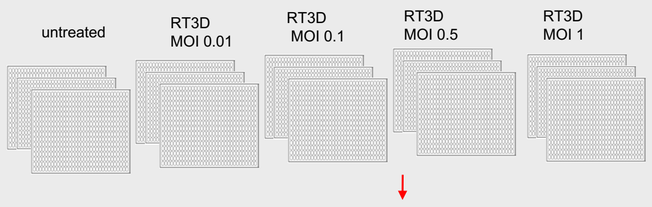


- Oncolytic viruses have shown potential in human clinical trials. Reovirus (RT3D), a naturally occurring human double-stranded RNA virus, has shown preclinical efficacy in the treatment of a wide range of tumour types and has now reached randomized phase II testing in clinical trials.
- Early clinical studies have shown that RT3D has modest monotherapy efficacy and it has been used in combination regimens with cytotoxic chemotherapy. However, not all patients benefit from these treatments, highlighting a need to identify therapeutic opportunities for combining oncolytic viruses with other novel anti-cancer drugs.
- We performed a high-throughput drug screen to employ a non-biased approach in determining any oncolytic viral-synergy interactions between RT3D and a range of different cancer drugs in the A375 melanoma cancer cell line.

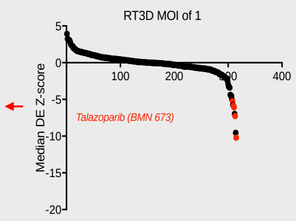
Identification of potential combination therapies with RT3D

A375 were seeded onto 384 wells at 500 cells/well

The next day, cells were treated with different targeted agents and chemotherapy drugs at concentrations ranging from 1-1000 nM and allowed to incubate for approximately 2 hours



Cell viability was measured 72 hours post-infection using CellTiter-Glo Luminescent Cell Viability Assay. The Z score (DE effect Z score = virus effect Z score) was obtained from the standardized value from (i) the median of triplicate samples normalised to virus only versus untreated (ii) the mean of virus only versus untreated and (iii) the median absolute deviation between (i) and (ii)" also known as Z-MAD. These were plotted on a waterfall plot as shown

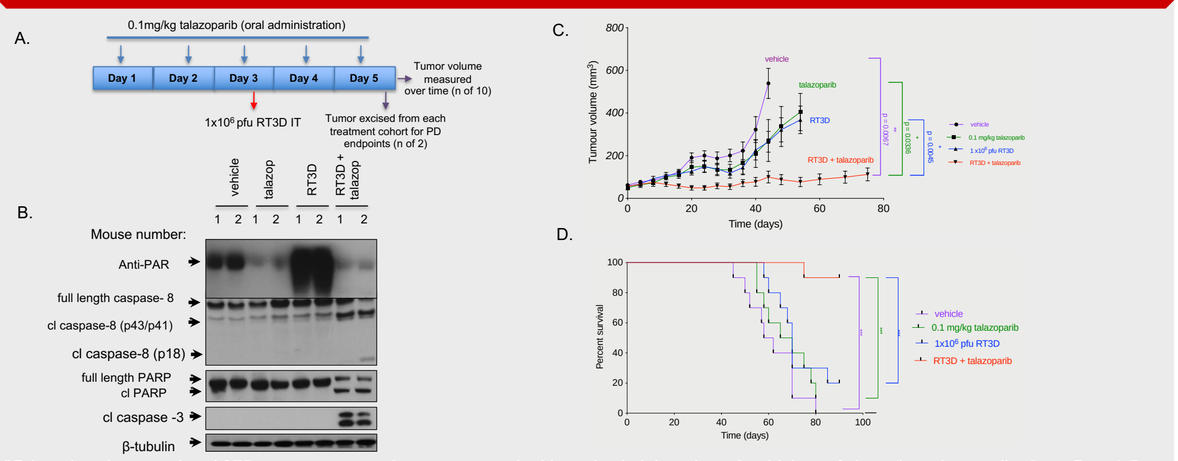


Potential oncolytic viral sensitizers were identified from the screen and validated. Highlighted in red are the Z scores of talazoparib (BMN-673) a potent PARP inhibitor at concentrations from 1-1000nM in combination with MOI of RT3D at 0.5 and 1. We chose to validate it in a panel of melanoma cell lines.

A375 MeWo D04

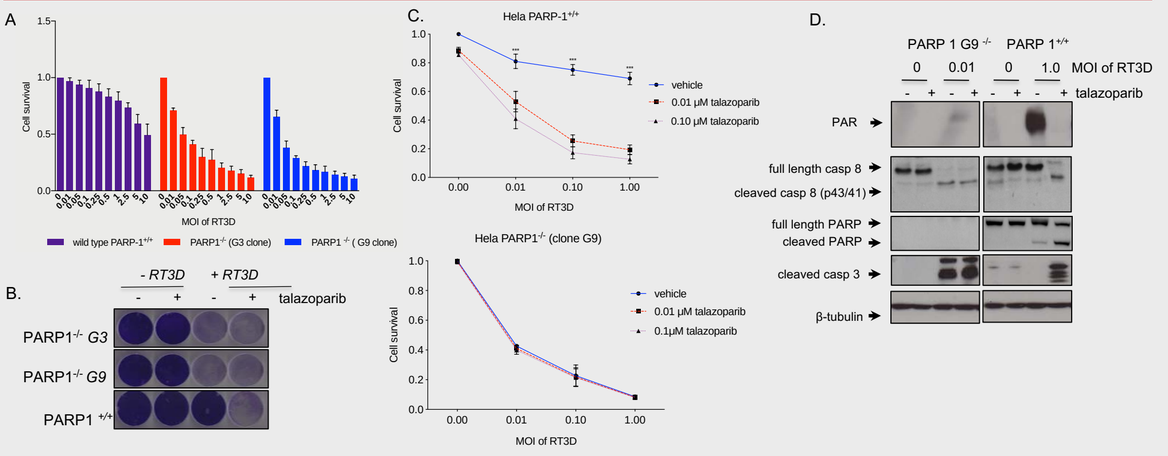
A375, MeWo and DO4 melanoma cells were treated with 0.1 μM of talazoparib and increasing doses of RT3D. At 72 hours post-infection, cell survival was assessed by SRB assay.

Talazoparib potentiates RT3D anti-tumour activity in an A375 xenograft model



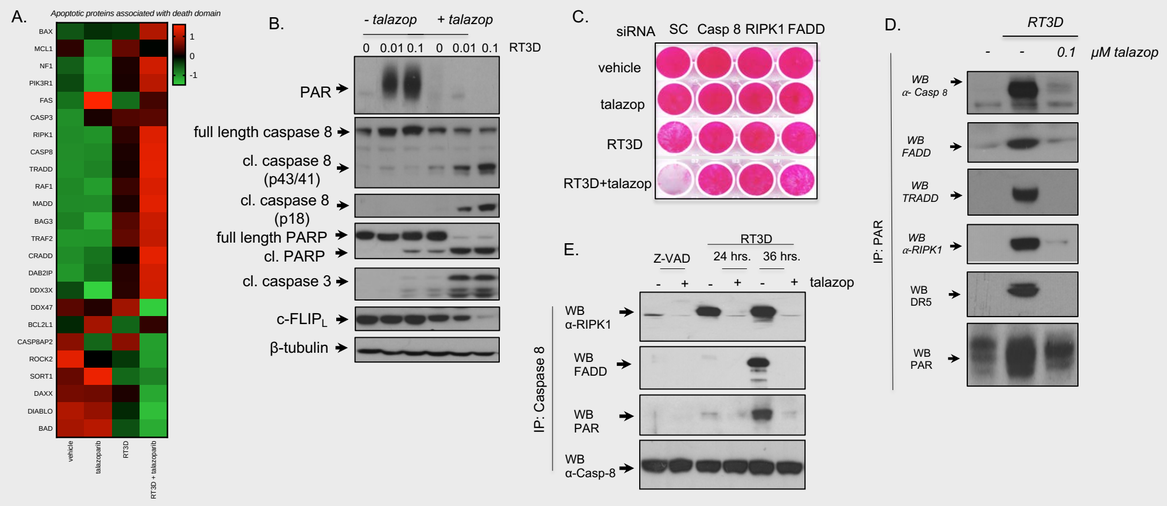
CD1 nude mice carrying A375 tumour xenografts were treated with oral administration of vehicle or 0.1 mg/kg talazoparib from Day 1-5. RT3D was injected intratumorally on Day 3 at $1x10^6$ pfu as shown in the cartoon depiction (A). Pharmacodynamic (PD) endpoints were assessed by immunoblotting. PAR antibody was used to assess effect of talazoparib and caspase 3, a marker of apoptosis was carried out following homogenization of A375 xenografts 5 days post-treatment from each treatment arm. Equal loading of proteins was assessed by probing for β-tubulin (B). Size of tumors was measured for each treatment cohort consisting of vehicle, talazoparib, RT3D at $1x10^6$ pfu or combination of RT3D and talazoparib (C). Each bar represents mean SE± of 10 replicates. Mice treated with RT3D plus talazoparib had significantly longer survival benefit compared to either agent alone (D).

Fig 3: Loss of PARP-1 is synthetically lethal with RT3D



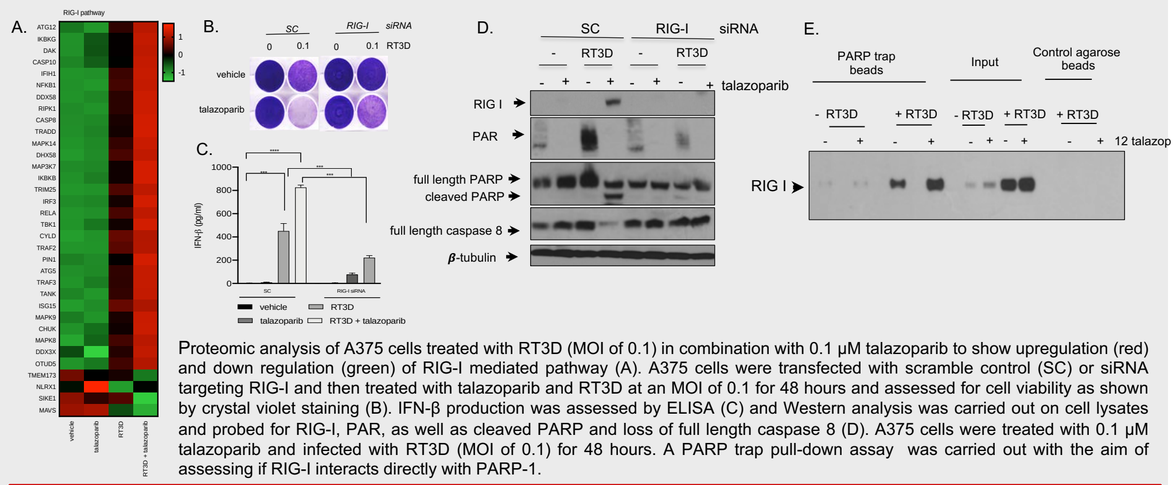
RT3D sensitivity was assessed in HeLa PARP-1 paired cells. PARP-1 wt and PARP-1 null (clone G3 and G9) and cytotoxicity carried out by MTT 72 hours post-infection (A). Cell viability was carried out to assess RT3D and talazoparib in HeLa PARP-1 paired models ($PARP1^{+/+}$ & $PARP1^{-/-}$) as shown by crystal violet assays (B) and SRB cell viability assays (C). Western analysis was carried out to assess apoptosis as shown by cleavage of caspase 3 and PARP as markers of apoptosis and PAR expression following RT3D infection. Equal loading was measured by probing for β-tubulin (D).

PARP-1 interacts with the DISC components following RT3D infection and loss of this interaction in the presence of talazoparib leads to enhanced apoptosis



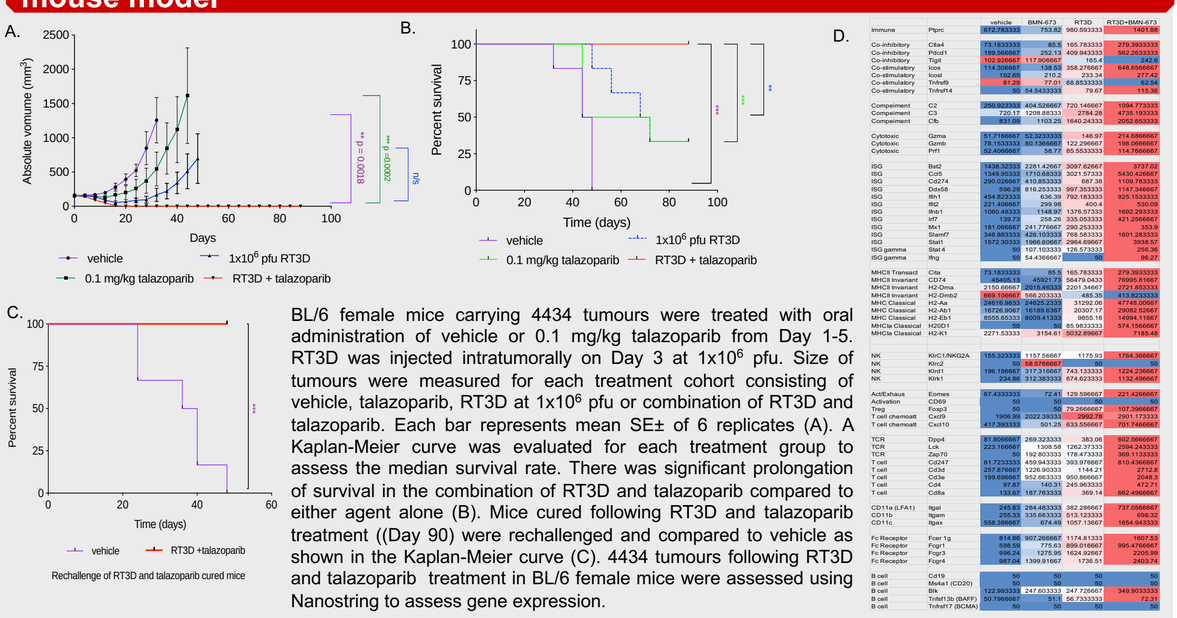
Proteomic analysis of A375 cells treated with RT3D (MOI of 0.1) in combination with 0.1 μM talazoparib to show upregulation (red) and down-regulation (green) of the apoptotic death domain pathway 48 hours post-treatment. Data are an average of 3 independent experiments (A). A375 cells were pre-treated with talazoparib and infected with RT3D. Western analysis was carried out to assess apoptosis as shown by caspase 8 cleavage, (an effector of apoptosis), caspase 3 and PARP cleavage (markers of apoptosis) and c-$FLIP_L$, an inhibitor of apoptosis. Equal loading of proteins was assessed by probing for β-tubulin (B). A375 cells were transfected with scramble control (SC) or siRNA targeting caspase-8, RIPK-1 and FADD and subsequently treated with talazoparib and RT3D at an MOI of 0.1 for 48 hours and cell viability assessed using SRB assay (C). Immunoprecipitation assay with PAR antibody in A375 cell lines following treatment with RT3D and BMN-673 was carried out and western analysis done to assess interaction between PAR and the DISC components (D) Likewise interaction between Caspase 8 and PARylated proteins was assessed and RIPK1 and FADD interaction used, which are known to interact with Caspase 8 (E).

Talazoparib potentiates the RIG-I/IFNβ pathway following RT3D infection



Proteomic analysis of A375 cells treated with RT3D (MOI of 0.1) in combination with 0.1 μM talazoparib to show upregulation (red) and down regulation (green) of RIG-I mediated pathway (A). A375 cells were transfected with scramble control (SC) or siRNA targeting RIG-I and then treated with talazoparib and RT3D at an MOI of 0.1 for 48 hours and assessed for cell viability as shown by crystal violet staining (B). IFN-β production was assessed by ELISA (C) and Western analysis was carried out on cell lysates and probed for RIG-I, PAR, as well as cleaved PARP and loss of full length caspase 8 (D). A375 cells were treated with 0.1 μM talazoparib and infected with RT3D (MOI of 0.1) for 48 hours. A PARP trap pull-down assay was carried out with the aim of assessing if RIG-I interacts directly with PARP-1.

RT3D plus talazoparib enhance immunogenicity in an immunocompetent mouse model



BL/6 female mice carrying 4434 tumours were treated with oral administration of vehicle or 0.1 mg/kg talazoparib from Day 1-5. RT3D was injected intratumorally on Day 3 at $1x10^6$ pfu. Size of tumours were measured for each treatment cohort consisting of vehicle, talazoparib, RT3D at $1x10^6$ pfu or combination of RT3D and talazoparib. Each bar represents mean SE± of 6 replicates (A). A Kaplan-Meier curve was evaluated for each treatment group to assess the median survival rate. There was significant prolongation of survival in the combination of RT3D and talazoparib compared to either agent alone (B). Mice cured following RT3D and talazoparib treatment ((Day 90) were rechallenged and compared to vehicle as shown in the Kaplan-Meier curve (C). 4434 tumours following RT3D and talazoparib treatment in BL/6 female mice were assessed using Nanostring to assess gene expression.

Summary

- Talazoparib, a potent PARP inhibitor, was identified as one of the top hits from the screen and was investigated in combination with RT3D in a panel of melanoma cell lines.
- RT3D in combination with talazoparib had a significantly enhanced effect both *in vitro* and *in vivo*.
- Death-inducing signalling complex (DISC) mediates apoptotic cell death following RT3D and talazoparib treatment where interaction between the DISC and poly-ADP ribosylation (PAR) chains following RT3D infection is abrogated in the presence of talazoparib.
- Talazoparib enhances IFN-β signaling pathways through RIG-I through PARP-1 trapping on RIG-I which leads to enhanced signaling via this pathway.
- We saw anti-tumour efficacy in a 4434 immunocompetent mouse model following RT3D and talazoparib treatment and this correlated with an increase in an immune response

Conclusion

Our data provide a strong rationale for the combination of oncolytic viruses with PARP1 inhibitors to exploit immunogenic response in cancer treatment

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