625: PARP14 inhibition restores PD-1 immune checkpoint inhibitor response following IFNγ-driven adaptive resistance

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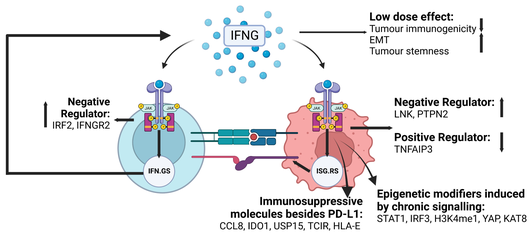
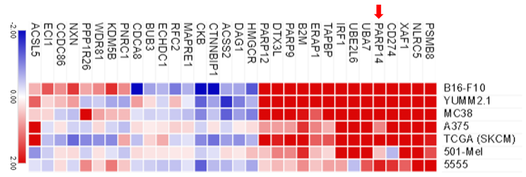
INTRODUCTION



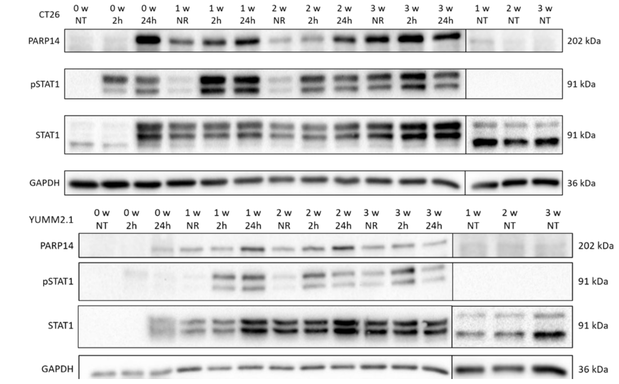
- Adaptive resistance limits immune checkpoint blockade therapy (ICBT) response duration and magnitude.
- Interferon γ (IFNγ), a critical cytokine that promotes cellular immunity, also induces adaptive resistance to ICBT.
- PARP14 has recently emerged as a promising therapeutic target in chronic inflammation.
- PARP14 polarises T-cell immune responses towards type 2 T helper (T_H2) mediation, by acting as a STAT6 co-activator.
- In IFNγ-treated macrophages, pro-inflammatory differentiation was suppressed by PARP14 through inhibition of STAT1 phosphorylation and down-regulation of STAT1 target genes.
- We hypothesised poly-ADP ribosyl polymerase 14 (PARP14) as a potential negative feedback inhibitor of IFNγ signalling in tumours.

RESULTS

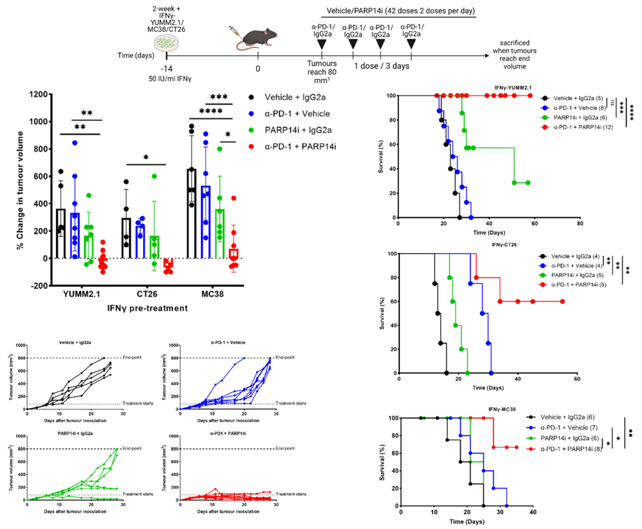


Chronic IFNγ exposure to tumour cells upregulates PARP14. We exposed human (A375 and 501-mel) and mouse (B16-F10, MC38, 5555, and YUMM2.1) tumour cell lines to IFNγ (20 IU/mL for human and 50 IU/mL for mouse cell lines) or BSA continuously for 2 weeks, and subsequently performed RNA sequencing (RNA-seq). In addition to well-established IFNγ target genes such as *CD274, IRF1,* and *B2M, PARP14* was consistently upregulated in all cell models as well as in expression data from *IFNG*^{high} patient melanoma (comparing top 15% by *IFNG* expression to lowest 15% in the TCGA SKCM dataset).

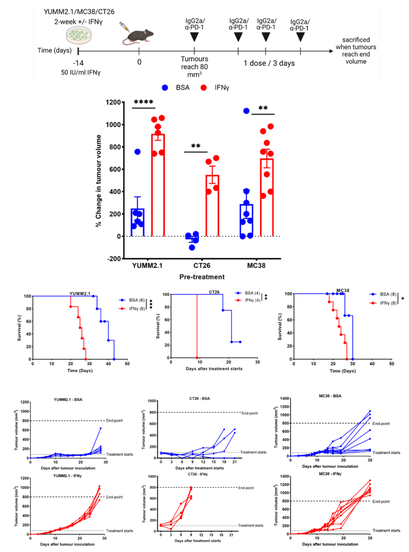
RESULTS



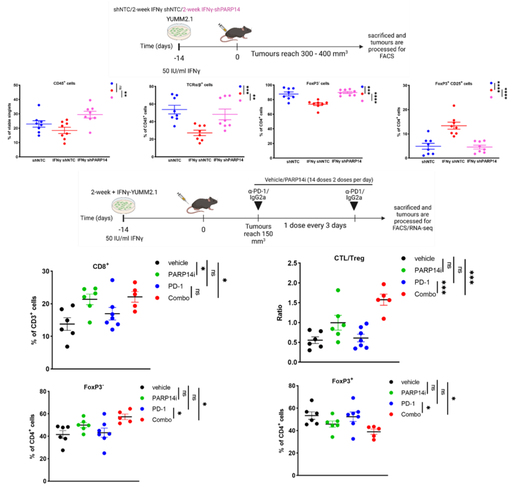
Tumour cells IFN-γ signalling pathway would persist by the same exposure of IFN-γ repeatedly and would reach to the highest level, which would no longer respond to further IFN-γ stimulation. CT26 and YUMM2.1 tumour cells were treated with 50 IU/mL IFN-γ for up to 3 weeks. PARP14, pSTAT1 and STAT1 protein expression were determined via western blot relative to a GAPDH loading control. Samples identifications were denoted from left to right: 0 w NT: 0-week no treatment (NT); 0 w 2h: IFN-γ treatment for 2-hour (2h) at 0-week; 0 w 24h: IFN-γ treatment for 24-hour (24h) at 0-week; 1 w NR: IFN-γ treatment for 1-week with no restimulation (NR); 1 w 2h: IFN-γ treatment for 1-week plus restimulation of IFN-γ for 2h; 1 w 24h: IFN-γ treatment for 1-week plus restimulation of IFN-γ for 24h; 2 w NR: IFN-γ treatment for 2-week with NR; 2 w 2h: IFN-γ treatment for 2-week plus restimulation of IFN-γ for 2h; 1 w 24h: IFN-γ treatment for 2-week plus restimulation of IFN-γ for 24h; 3 w NR: IFN-γ treatment for 3-week with NR; 3 w 2h: IFN-γ treatment for 3-week plus restimulation of IFN-γ for 2h; 3 w 24h: IFN-γ treatment for 3-week plus restimulation of IFN-γ for 24h; 1 w NT: 1-week NT; 2 w NT: 2-week NT; 3 w NT: 3-week NT.



PARP14 pharmacological antagonism reverse adaptive resistance to α-PD-1 therapy. Chronic IFN-γ pre-treated YUMM2.1/MC38/CT26 cells were subcutaneously implanted into 8–12-week-old wild-type syngeneic female mice. Treatment with either α-PD-1 or IgG2a antibody was initiated once tumour volume reached 80-100 mm³, with antibodies administered every three days for four doses. In parallel, the animals also received two daily doses of the PARP14 inhibitor (PARP14i) RBN012759 or vehicle for three weeks. The percentage of tumour volume change between the first dose of treatment and the administration of the final α-PD-1 dose of mice receiving implants of YUMM2.1, CT26, and MC38 pre-treated with IFN-γ.Kaplan-Meier plots of mice receiving implants of YUMM2.1, CT26, MC38 pre-treated with IFN-γ. Growth rate for each tumour of IFN-γ pre-treated YUMM2.1.

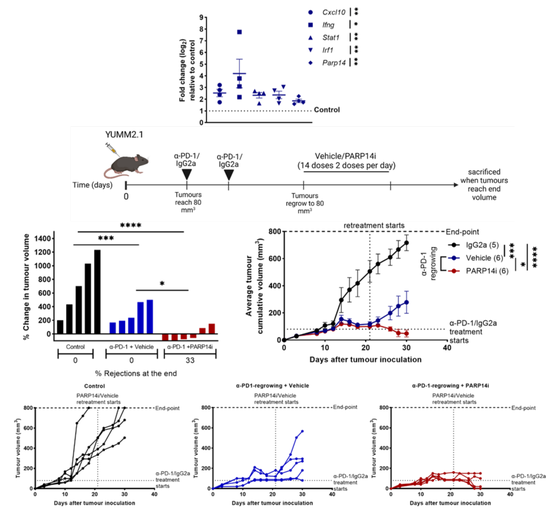


Chronic IFN-γ exposure drives resistance to α-PD-1 therapy. YUMM2.1, CT26, and MC38 cells were implanted into 8–12 weeks old wild-type syngeneic female mice after two-week pre-treatment with IFN-γ (50 IU/mL) or BSA. Treatment with α-PD-1 antibody was initiated once tumour volume reached 80-100 mm³, with dosing every three days for four doses. The percentage tumour volume change between the start of treatment and one week after the last α-PD-1 dose in YUMM2.1 and MC38 and the day that final dose was administered in CT26. Kaplan-Meier survival plots of each animal receiving YUMM2.1, CT26, and MC38 implants. The growth curve of each YUMM2.1, CT26, and MC38 tumour after α-PD-1 antibodies treatment starts or tumour inoculation.



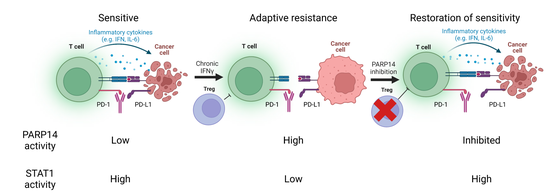
PAPR14 depletion or inhibition reverses chronic IFN-γ driven immune regulatory effects. 8–12-week-old wild-type C57BL/6J mice were subcutaneously implanted with IFNγ-naïve YUMM2.1 cells expressing shNTC or chronic IFNγ pre-treated YUMM2.1 cells expressing shNTC or shPARP14. Tumours were allowed to grow to 300–400 mm³ and then dissected and disaggregated for analysis by flow cytometry. Populations of total immune cells, T cells (TCRαβ+), effector CD4+ T cells, regulatory T cells (Treg cells; CD25+FoxP3+). 8–12-week-old wild-type C57BL/6J mice were subcutaneously implanted with chronic IFN-γ-pre-treated YUMM2.1 cells. Treatment with either α-PD-1 or IgG2a antibody was initiated once tumour volume reached 150 mm³ (two doses, three days apart) and PARP14i or vehicle (two doses daily for a week). At the end of the treatment period, tumours were dissected and disaggregated for analysis by flow cytometry and RNA-seq. Populations of cytotoxic CD8+ T cells, the ratio of CD8+GzmB+ cytotoxic lymphocytes (CTLs) to Treg cells, CD4+ T cells (CD4+ FoxP3-), Treg cells (CD4+ FoxP3+) in the tumour immune infiltrate.

RESULTS



PARP14 level was augmented and mediate resistance in tumours spontaneously relapsing after α-PD-1 treatment. RT-qPCR analysis of *Cxcl10, Ifng, Stat1, Irf1,* and *Parp14* mRNA expression levels in YUMM2.1 tumours regrowing after treatment with α-PD-1 antibody compared to tumours treated with IgG2a as the control group. 8–12-week-old wild-type C57BL/6J mice were subcutaneously implanted with YUMM2.1 cells. α-PD-1 antibody treatment (two doses, three days apart) was initiated once tumour volume reached ~80 mm³. Tumours regrew and upon reaching ~80 mm³, the tumours were treated with two daily doses of PARP14i or vehicle for a week. The percentage of tumour volume change between the start of α-PD-1 treatment and day 30 post-tumour-implantation (left), average tumour cumulative volume growth curve (right). (D–F) Growth curves for each tumour per condition; Control; α-PD-1-regrowing + Vehicle; α-PD-1-regrowing + PARP14i.

CONCLUSION



Proposed model of response to PD-1 immune checkpoint inhibition restoration by PARP14 inhibition in tumours with resistance driven by IFNγ