Peposertib-induced senescence primes irradiated p53 wild-type cancer cells for clearance by both immune cells and senolytic drugs

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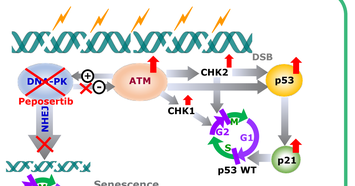
INTRODUCTION

Peposertib (M3814) is a potent and selective DNA-dependent protein kinase (DNA-PK) inhibitor in early-stage clinical development.[1,2] DNA-PK inhibition by peposertib effectively blocks the non-homologous end-joining (NHEJ) pathway of DNA double-strand break (DSB) repair and shows strong synergy with ionizing radiation (IR) and DSB-inducing chemotherapy. We have previously shown that M3814 overactivates the ATM/p53 signaling axis, reinforces cell cycle checkpoint arrest, and promotes durable premature senescence in irradiated p53 wild-type (WT) cancer cells. We have used this unique property of peposertib to generate nearly uniform senescent cell populations from irradiated cancer cells. Using this experimental model, we examined the immunological effects and potential therapeutic applications of IR+M3814-induced senescence.

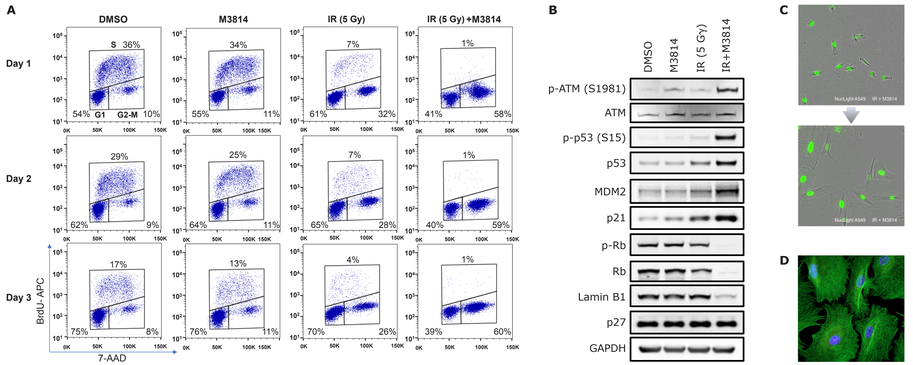


Adapted from Mol Cancer Res, 2019;17:2457-2468, Sun Q, et al. Therapeutic Implications of p53 Status on Cancer Cell Fate Following Exposure to Ionizing Radiation and the DNA-PK Inhibitor M3814, with permission from AACR

ATM, ataxia telangiectasia-mutated; **DNA-PK**, DNA-dependent protein kinase; **DSB**, double-strand break; **WT**, wild type

RESULTS

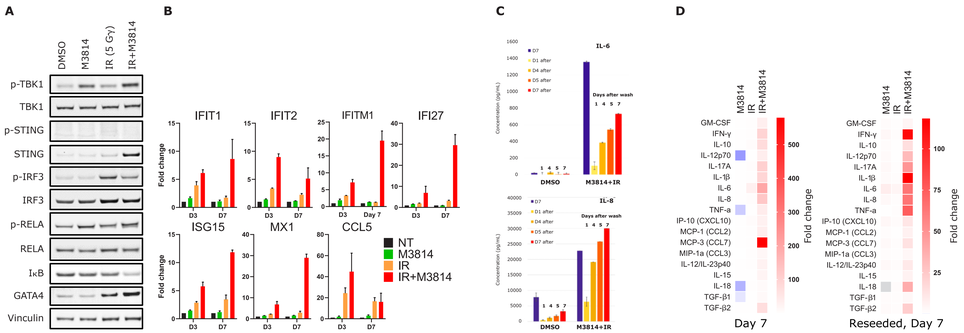
Figure 1. Peposertib blocks the cell cycle of irradiated A549 cells and induces durable premature senescence



A) Cell cycle analyses show peposertib potentiates IR-induced growth arrest in A549 lung epithelial carcinoma cells. **B)** Western blotting shows robust ATM/p53 pathway activation and further markers of senescence 6 days after IR. Morphological hallmarks of senescence were observed by live-cell imaging **C)** and immunofluorescence **D)**

RESULTS

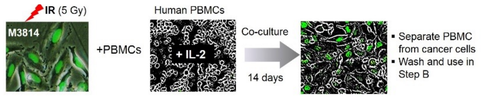
Figure 2. Peposertib enhances the activation and secretion of multiple inflammatory proteins in irradiated A549 cells



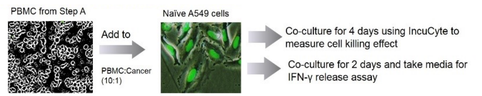
A) Western blotting and **B)** qPCR show Peposertib potentiates IR-induced inflammatory signaling. Peposertib potentiated senescent cells display a durable secretory phenotype when assayed by **C)** Enzyme-linked immunosorbent assay (ELISA) and **D)** Meso Scale Discovery (MSD) assay

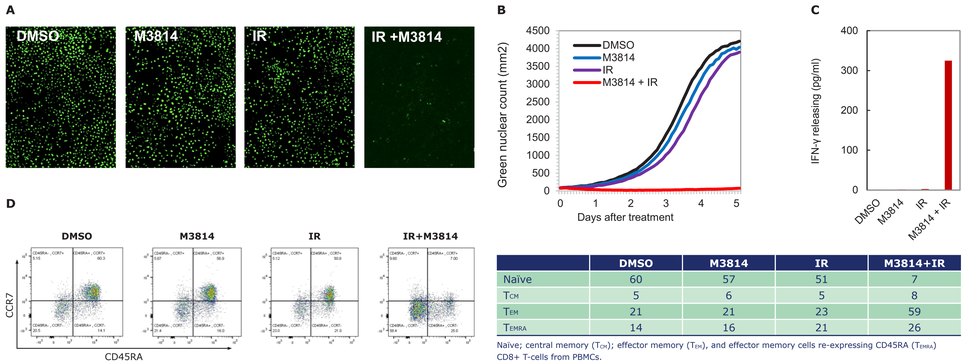
Figure 3. Human PBMCs co-cultured with senescent A549 cells show activation of T cells and accelerated killing of naïve cancer cells

Step 1: Priming and activation of T cells



Step 2: Cancer cell killing/INFγ release





	DMSO	M3814	IR	M3814+IR
Naïve	60	57	51	7
T_{CM}	5	6	5	8
T_{EM}	21	21	23	59
T_{EMRA}	14	16	21	26

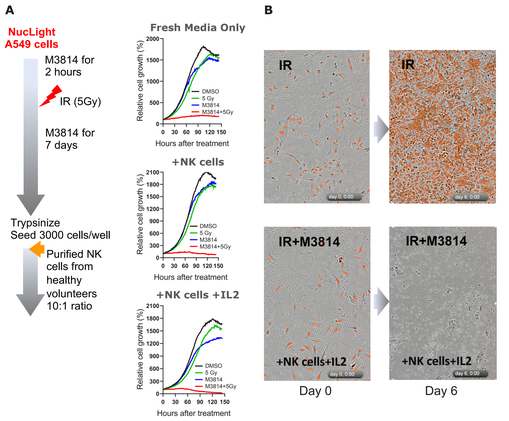
Naïve; central memory (T_{CM}); effector memory (T_{EM}), and effector memory cells re-expressing CD45RA (T_{EMRA}) CD8+ T-cells from PBMCs.

A) Images and **B)** graph from live-cell imaging show increased clearance of naïve Nuclight A549 cells, upon co-culture with PBMCs previously cultured with IR+peposertib exposed cells. **C)** MSD assay shows IFN-γ in co-culture media correlates with cell clearance. **D)** Flow cytometry analysis of CD8+ T-cell subsets

CONCLUSIONS

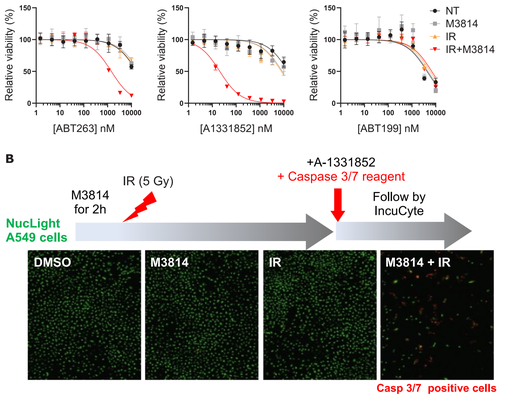
- **Our experiments reveal that selective inhibition of DNA-PK in irradiated p53 wild-type cancer cells provides a powerful mechanism for induction of premature cancer cell senescence with an immunostimulatory secretory phenotype *in vitro***
- **Peposertib-induced senescent cancer cells are sensitized to clearance by both immune cells and senolytic agents *in vitro*, suggesting that triple combination of radiotherapy with DNA-PK inhibitors and senolytics could offer a potential new approach to the treatment of locally advanced tumors**

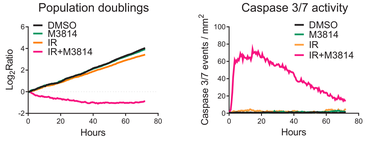
Figure 4. Senescent A549 cells induced by combined radiation and peposertib treatment are selectively killed by isolated NK cells *in vitro*



A) Graphs and **B)** images from live-cell imaging of treated Nuclight A549 cells co-cultured with isolated NK cells

Figure 5. IR+M3814-induced senescent A549 cells are selectively targeted by Bcl-xL inhibition





A) Dose-response following 48 hours of Bcl-2 family inhibitor treatment of A549 cells pretreated 5 days with peposertib, IR, and combination. **B)** Images (48 hours post-senolytic) and **C)** graphs from live-cell analysis of Nuclight A549 cells treated as in A) with A-1331852

1. Van Bussel MT, et al 2021. Br J Cancer 2021;124:728-735; 2. Sun Q, et al. Mol Cancer Res 2019;17:2457-2468

MC, QS, C-FL, and LTV are employees of EMD Serono, Billerica, MA, USA. FTZ, and AB are employees of the healthcare business of Merck KGaA, Darmstadt, Germany.

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