HPV and p53 Status Determine Irradiation-related Responses to a Selective DNA-PK Inhibitor in Head and Neck Squamous Cell Carcinoma Models

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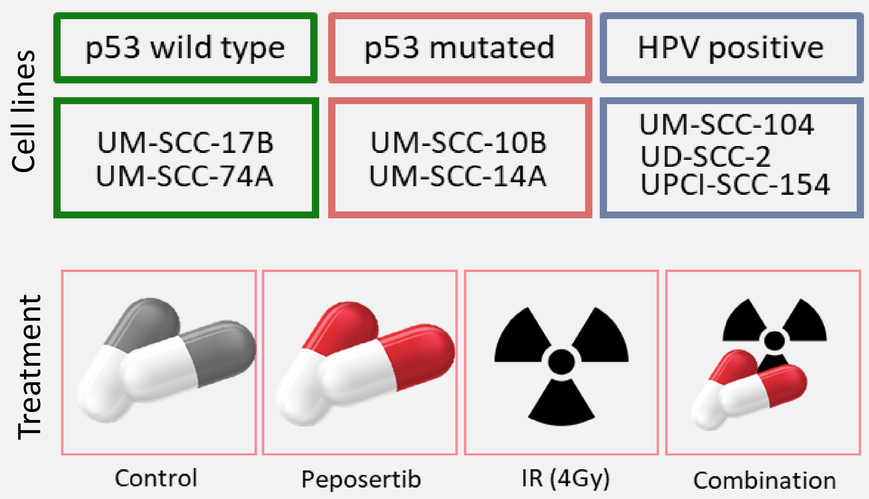
INTRODUCTION

Major risk factors of head and neck squamous cell carcinoma (HNSCC) development include tobacco use and infection with human papillomavirus (HPV). In HPV-positive cancers, E6 oncoprotein leads to the degradation of the p53 protein, whereas HPV-negative cancers are the results of non-repaired DNA and accumulation of defects in key tumor suppressor genes, including TP53. Irradiation (IR) causes DNA double-strand breaks (DSBs), which can be repaired by either nonhomologous end joining (NHEJ) or homologous recombination (HR). Peposertib (M3814) is a small-molecule inhibitor of DNA-PK, a key regulator of NHEJ. In combination with IR, inhibition of NHEJ along with induced DSBs can potentially increase treatment efficacy.

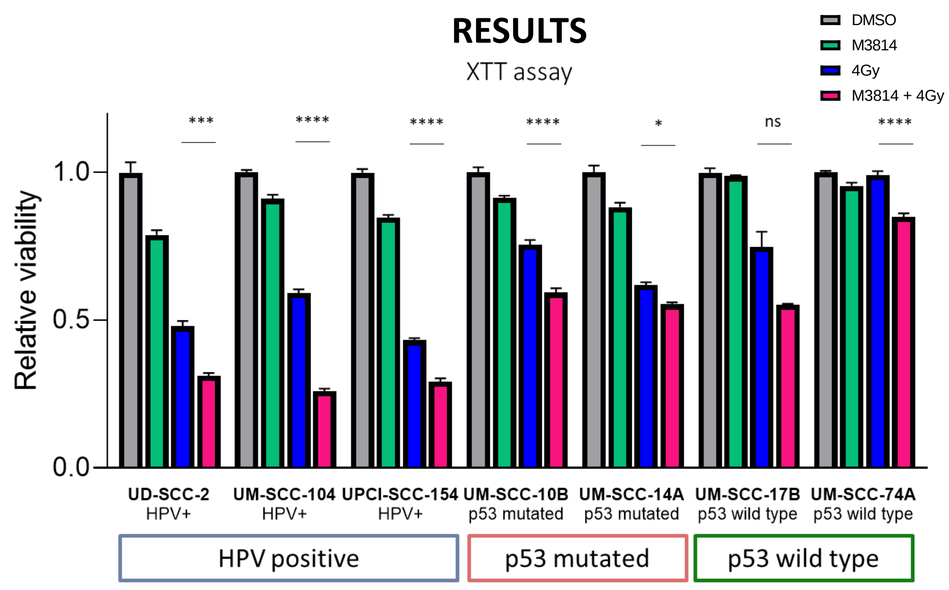
METHODS



All data is represented as mean ± SEM

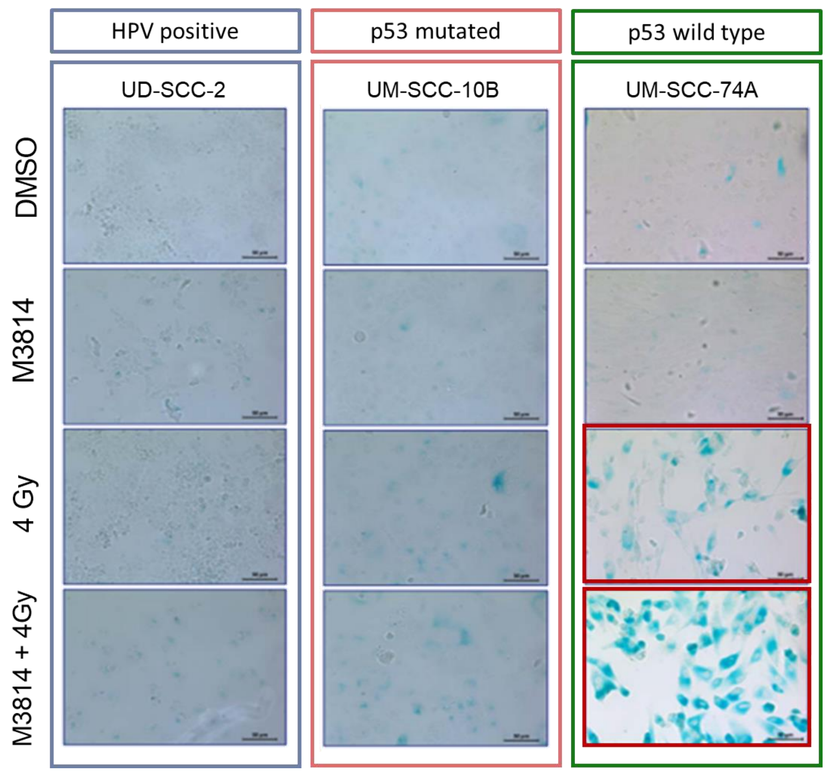
This study aims to investigate responses of HNSCCs with distinct HPV and p53 status to the treatment with IR, DNA-PK inhibition, and their combination.

RESULTS

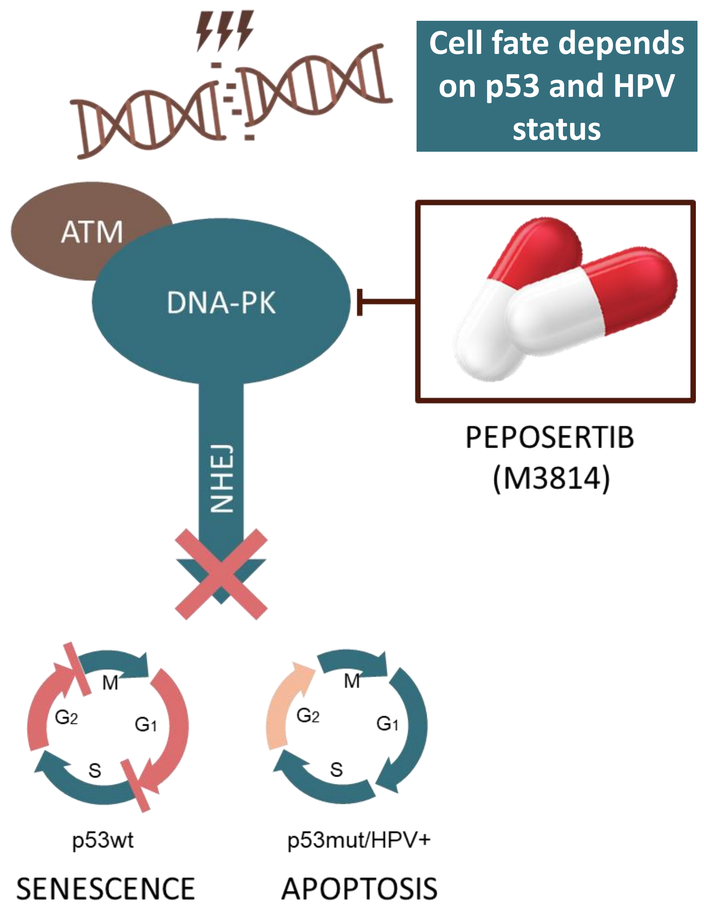


Viability and cell cycle analysis were performed to assess the response of cell lines to various treatment groups. Decreased number of viable cells after IR alone and particularly after combined treatment was observed in most of the cell lines with the most significant reduction in HPV+ cells. M3814 in combination with radiation therapy suppresses cell proliferation and viability

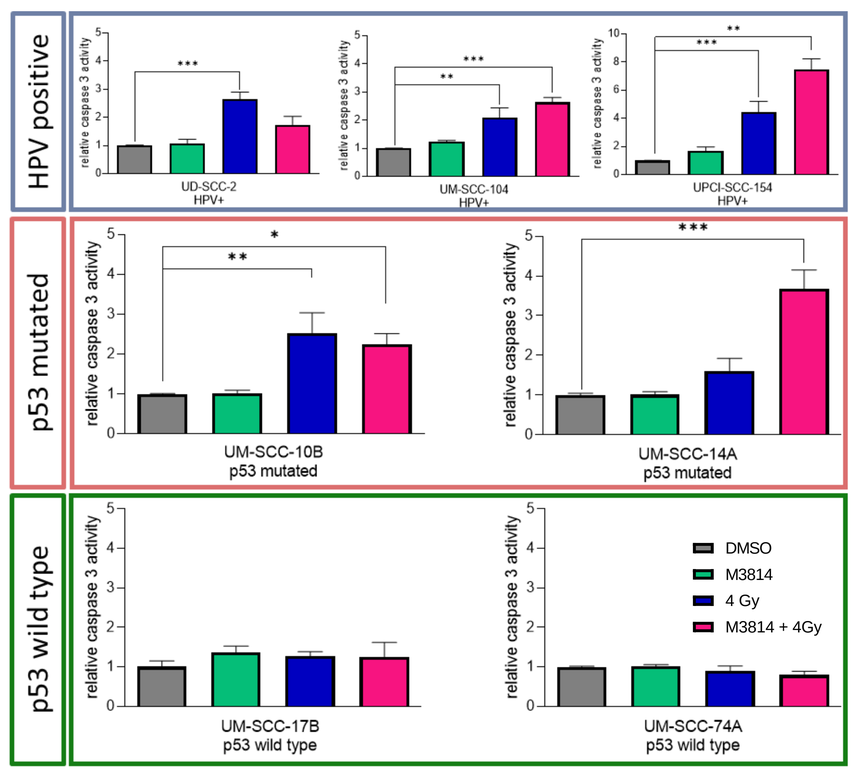
β-galactosidase assay - senescence



Senescence-associated β-galactosidase assay was used to evaluate the induction of senescence. β-galactosidase activity increased significantly in only p53-wt cells after irradiation and combination treatments.



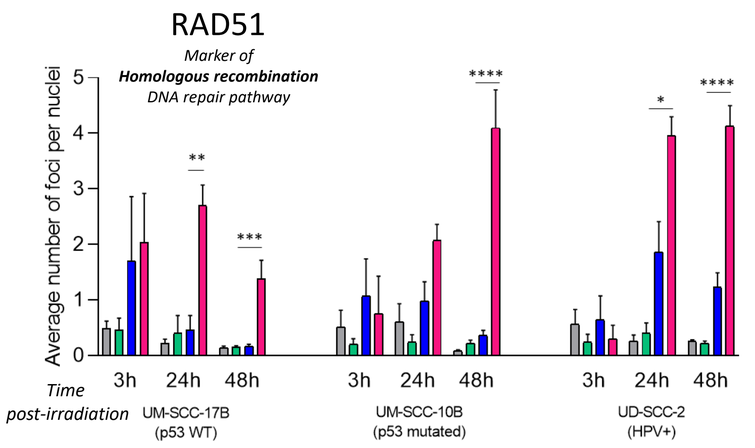
Caspase-3 activity assay - apoptosis

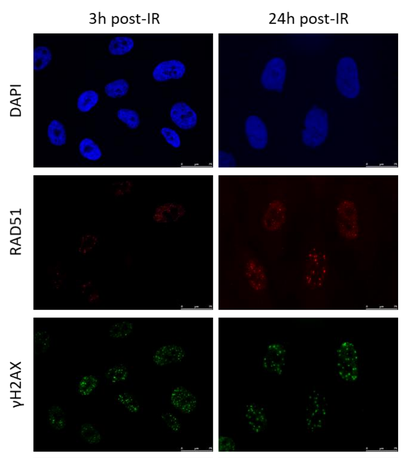


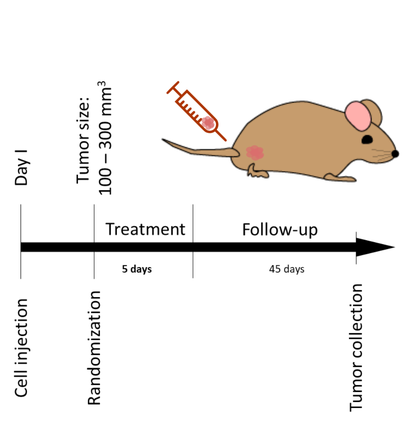
Caspase 3 activity assay was used to evaluate the induction of apoptosis in all treatment groups. IR and combined treatment promoted apoptosis in HPV+ and p53-mutated but not p53 wild type cell lines.

Inhibition of NHEJ combined with IR induces an abrogation of proliferation with different cell fates.
Whereas HPV+ and p53-mutated cells undergo apoptosis due to a common alteration in the p53 pathways, p53-wt cells are preferentially eliminated through senescence.

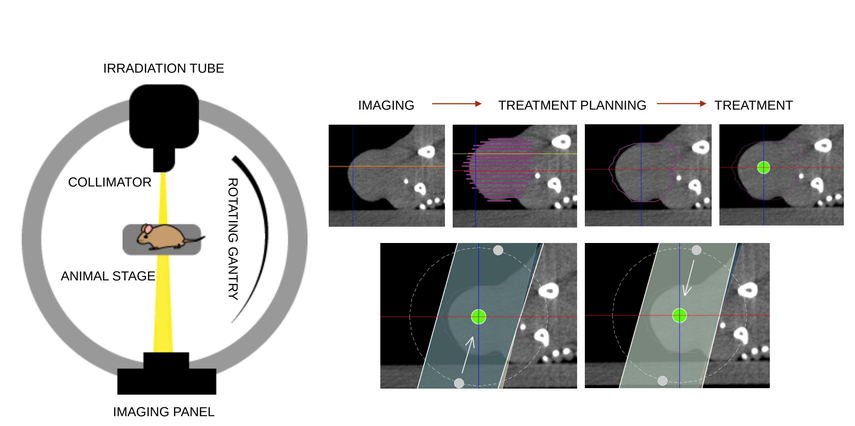
DNA DAMAGE AND REAPAIR

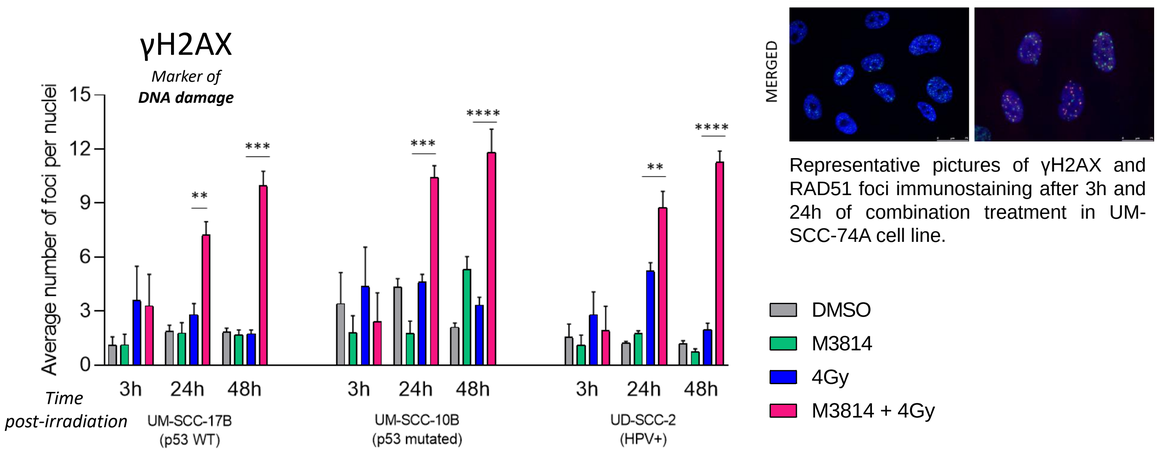






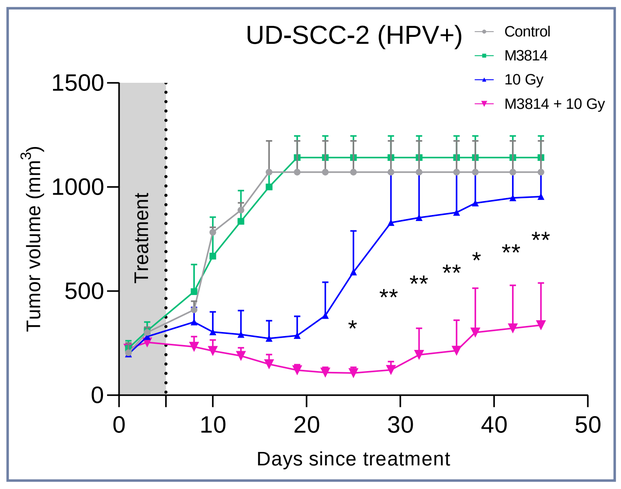
IN-VIVO XENOGRAFT MODEL

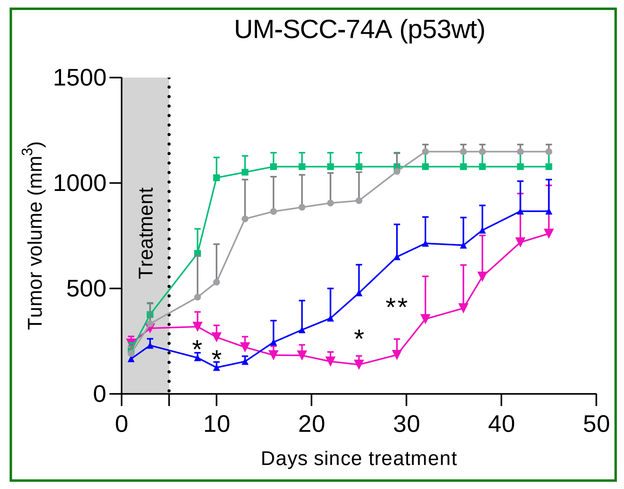




Representative pictures of γH2AX and RAD51 foci immunostaining after 3h and 24h of combination treatment in UM-SCC-74A cell line.

γH2AX (DNA damage marker) and RAD51 (marker of homologous recombination) foci are significantly increased after combination therapy after 24h and 48h in all cell lines. This indicates a delayed DNA repair when non homologous end joining (NHEJ) is inhibited along with irradiation. The absolute RAD51 foci number is increasing in combination treatment between 3h and 48h in p53 mutated and HPV+ but not in p53 wild type cell line (UM-SCC-17B). However, the increase of γH2AX is still observed in UM-SCC-17B after 48h in combination group. The difficult-to-repair breaks that are not able to be repaired by homologous recombination (HR) can be the driving force leading the p53wt cells to senescence.





NMRI-nu mice with subcutaneous xenografts of HPV+ (UD-SCC-2) and p53 wild type (UM-SCC-74A) cell lines were treated with either fractionated 10Gy (2Gy*5days) IR or in combination with orally distributed M3814 (100mg/kg*5days). IR was delivered with the small animal radiation therapy system SmART, which is a state-of-the-art focal precision irradiation system that mimics clinical radiotherapy and imaging. We have observed a positive tumor response upon treatment with M3814 and irradiation in-vivo. Significant effects of combination treatment on tumor growth control was observed particularly in HPV+ xenografts.

1. Inhibition of NHEJ combined with IR causes decrease in viability, delay in DNA repair, different cell fates and tumor growth control in-vivo
2. Peposertib efficiently radiosensitizes tumor cells resulting in better response in HPV+/p53 mutated cells both in-vitro and in-vivo
3. p53 in addition to HPV screening can be considered as a biomarker for DNA-PK-targeted treatment and become an important guiding point for a precision medicine

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