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**Adenovirus-directed enzyme prodrug therapy with *E. coli* nitroreductase (NTR) plus CB1954 enhances the effect of radiotherapy *in vitro* and *in vivo***

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We evaluated the ability of replication-defective adenovirus (E1/E3 deleted) expressing NTR under the control of either a CMV or tissue-specific (CTP1) promoter to sensitise cancer cells to the combined effects of CB1954 and radiation. Sensitivity of parental SKOV3 ( $IC_{50} > 100$  micromolar) and stably transduced SKOV3-NTR ( $IC_{50} = 0.58$  micromolar) cells to CB1954 was measured by MTT assay. To investigate a NTR-CB1954 mediated bystander effect with radiation, conditioned media from SKOV3 or SKOV3-NTR cells that had been incubated with CB1954 (0, 5, 12.5, 25 micromolar) overnight was added to SW480 cells that were subsequently irradiated (0, 3, 6, 9 Gy). Conditioned medium from SKOV3 cells did not affect radiosensitivity but that from SKOV3-NTR cells caused significant enhancement in a drug- and radiation-dose dependent fashion. The ability of CB1954 to sensitise SKOV3-NTR (but not SKOV3) cells to radiation-induced cell kill was shown by FACS with dual staining for propidium iodide/fluorescein diacetate, apoptotic staining with DAPI and measurement of double-stranded DNA breaks by FACS and confocal microscopy of gammaH2AX foci. AdCMV-NTR (MOI 10) in combination with radiation (0, 3 Gy) in the presence of CB1954 (1-500 micromolar) in SW480 and HCT-116 cells showed enhanced radiation- and drug-dose dependent cytotoxicity. The combination of AdCMV-NTR and AdCTP1-NTR, CB1954 and single fraction and fractionated radiation was assessed by clonogenic assay with evidence of enhanced activity of both viruses in SW480 and HCT-116 cells. AdCMV-NTR and AdCTP1-NTR and CB1954 also enhanced the effect of fractionated radiotherapy (12 Gy in 4 fractions) in SW480 xenograft tumours.