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DNA-PK OR ATM INHIBITION INHIBITS NON-HOMOLOGOUS END JOINING AND ENHANCES CHEMO- AND RADIO SENSITIVITY IN HEPATOCELLULAR CANCER CELL LINES

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Introduction
Hepatocellular carcinoma (HCC) is chemotherapy resistance possibly due to dysregulation of DNA damage signalling and repair. DNA double-strand breaks (DSBs) are the most cytotoxic lesions induced by ionising radiation (IR) and anticancer drugs such as topoisomerase II poisons (eg, doxorubicin). DSBs are repaired by non-homologous end joining (NHEJ), initiated by DNA-dependent protein kinase (DNA-PK), and homologous recombination (HR), reportedly initiated by Ataxia telangiectasia mutated (ATM). DNA-PK is up-regulated in HCC (GEO profile), and ATM and HR inhibition in cells. This novel vector, therefore, has the potential to treat. Surgical resection and liver transplantation are considered curative therapies but they are feasible for only a small number of patients. Other therapeutic options, such as radiofrequency ablation and arterial chemoembolization, are effective only in small tumours. Recombinant adeno-associated virus (rAAV) vectors are ideally suited for gene transfer-based therapeutic approaches for HCC because of their safety profile and remarkable tropism for the liver, with >90% of the vector particles being detectable in hepatocytes following a single systemic administration in non-human primates.

Methods
DNA-PK and ATM protein levels and activation by IR (Western blot), DSB levels (γ-H2AX foci), HR (RAD51 foci), cell dependent protein kinase (DNA-PK), and homologous recombination (HR), both inhibited in HCC cell lines, re

Results
DNA-PK protein concentration and activity were high in all HCC cell lines. In contrast, ATM expression was variably expressed in HCC cells. Cell-specific sensitivities to IR and doxorubicin correlated with ATM expression (highest in HepG2 and lowest in HepB). NU7441 sensitised all cells to doxorubicin (average PF90 4.3±3.0) and IR (average PF90 3.9±1.1), significantly increasing growth inhibition and reducing survival (4.8 to 3.3-fold; colony forming assays). KU55933 significantly potentiated cytotoxicity in HepG2 cells (eightfold) but had little effect on cytotoxicity in HepB cells. Following exposure to IR, both NU7441 and KU55933 delayed DSB repair (~50% clearance γH2AX foci at 4 h vs only 10%–15% in presence NU7441). NU7441 also enhanced HR (threelfold increase in RAD51 foci), while KU55933 had little effect.

Conclusion
DNA-PK levels were high in all HCC cells and its inhibition with NU7441 was associated with significant chemo- and radio-sensitisation. Potentiation by ATM inhibition varied in the cell lines, reflecting the level of ATM expression. Both inhibitors substantially impaired the rapid phase of DNA repair commonly attributed to NHEJ. Notably, KU55933 had little effect on HR, suggesting that ATM is not central to this repair pathway. We propose that these inhibitors will increase the effectiveness of lower safer doses of cytotoxic therapies, amplifying tumour toxicity, and that DNA-PK and ATM levels in tumour and normal-tumour will predict those patient likely to benefit.

Competing interests
None declared.

REFERENCES