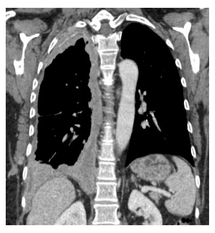
Inhibition of anti-apoptotic Bcl-xL mitigates the radioresistance of mesothelioma cells

Mark R. Jackson, Miranda Ashton, Anthony J. Chalmers
Institute of Cancer Sciences, University of Glasgow, UK

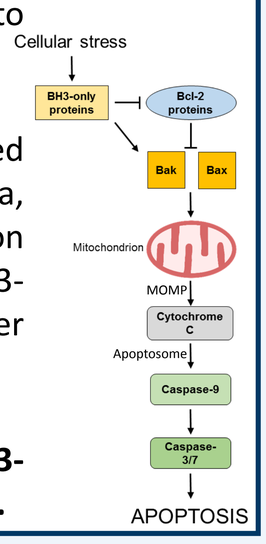
mark.jackson.2@glasgow.ac.uk
Abstract EP-2319

Introduction

Malignant pleural mesothelioma is a devastating disease with median survival of 12 months from diagnosis.



Radiotherapy (RT) shows some efficacy in the palliative setting, but complex anatomy and proximity of organs at risk limits its use with curative intent. Intensity-modulated radiotherapy has improved the accuracy of dose delivery but curative doses are unlikely to be achieved so additional strategies are required to overcome the intrinsic radioresistance of mesothelioma.



Apoptosis is a pathway by which stress signals, such as RT induced DNA damage, cause cell death. Cancers, including mesothelioma, exhibit resistance to apoptosis that is associated with upregulation of anti-apoptotic Bcl-2 proteins. Bcl-2 family inhibitors (BH3-mimetics) have shown therapeutic efficacy in many cancer indications but have not yet been tested in mesothelioma.

This worked aimed **to assess the therapeutic potential of BH3-mimetics as single-agents and radiosensitizers in mesothelioma.**

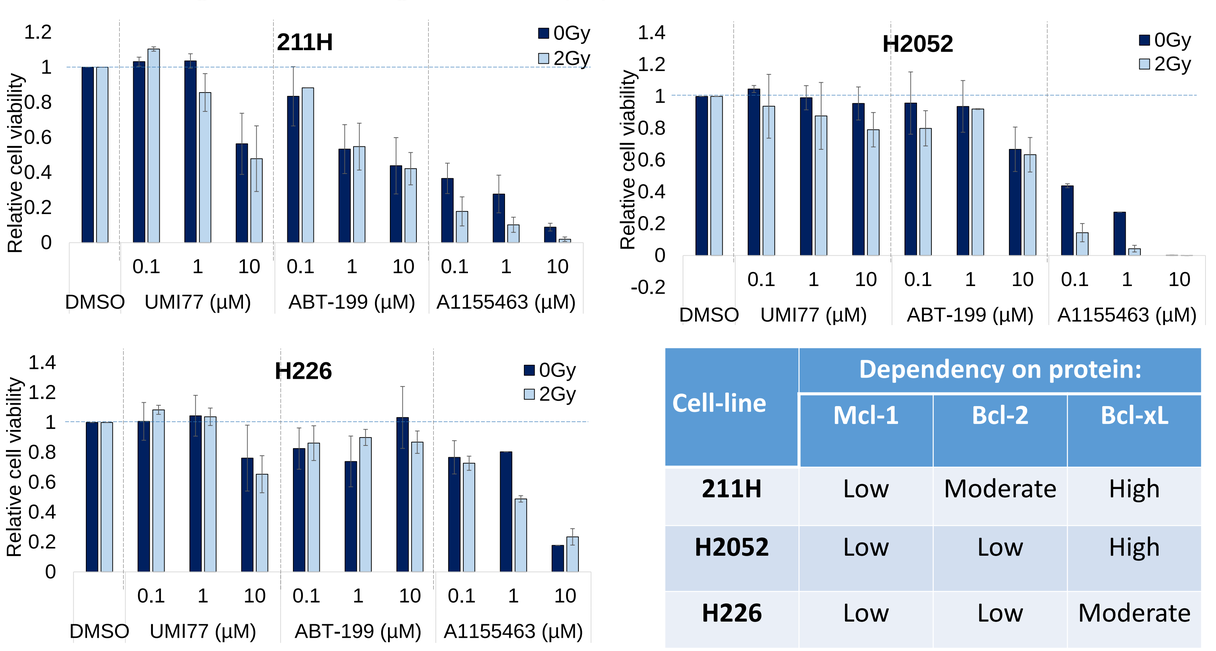
1. Mesothelioma cells depend on Bcl-xL for survival

• Specific inhibitors of the major anti-apoptotic Bcl-2 proteins (Mcl-1, Bcl-2, Bcl-xL) used to identify pathways of apoptosis-resistance in three mesothelioma cell lines.

Bcl-2 protein:

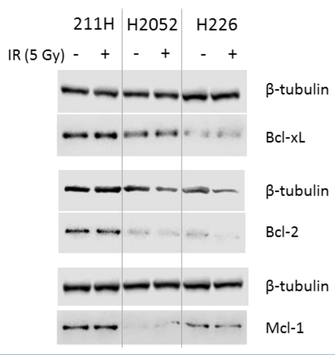
MCL-1	UMI-77
BCL-2	ABT-199
BCL-xL	A1155463 A1331852

Relative cell viability data normalized for effect of radiation-alone to highlight therapeutic interactions between drugs and ionizing radiation (IR).



Cell-line	Dependency on protein:		
	Mcl-1	Bcl-2	Bcl-xL
211H	Low	Moderate	High
H2052	Low	Low	High
H226	Low	Low	Moderate

Mesothelioma cells found to be dependent on Bcl-xL function for survival.

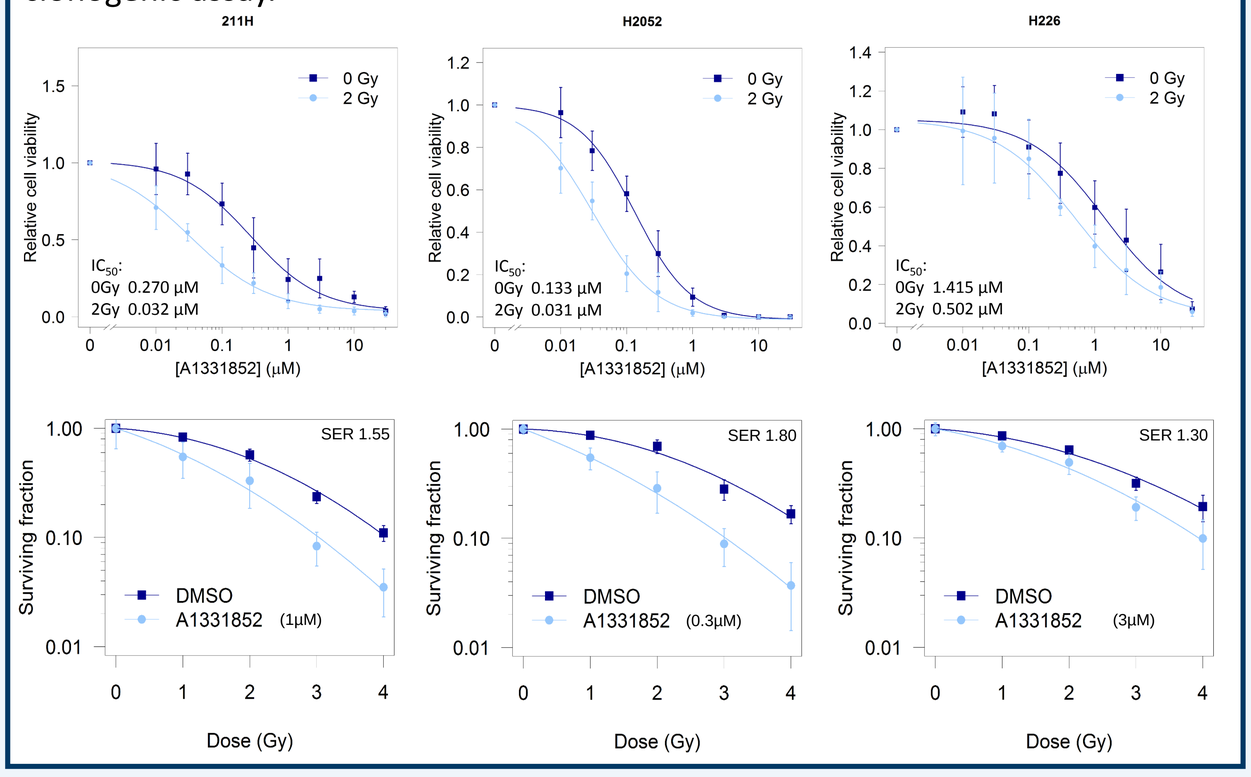


• Expression of Mcl-1/Bcl-2/Bcl-xL as proportion of total Bcl-2 protein family expression correlated with protein dependencies of cell lines.

2. Validation of a Bcl-xL inhibitor as a radiosensitizer

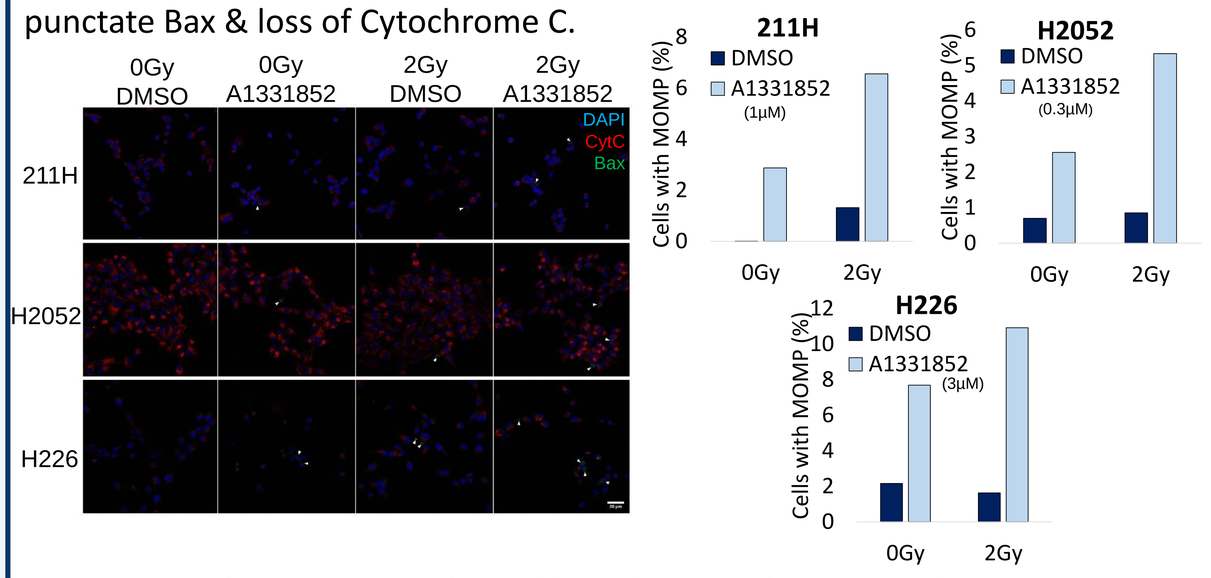
• Orally-available second generation Bcl-xL inhibitor (A1331852) selected as lead candidate.

Viability assays demonstrated potent single-agent activity. Following normalization for radiation-alone, combination of drug + IR significantly reduced IC_{50} in 211H & H2052 cells. Radiosensitization was further confirmed by clonogenic assay.

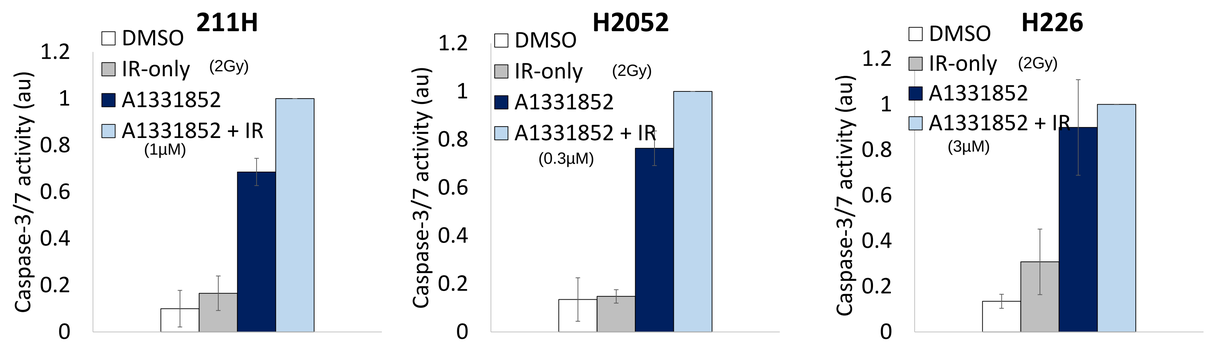


3. A1331852 promotes apoptosis following IR

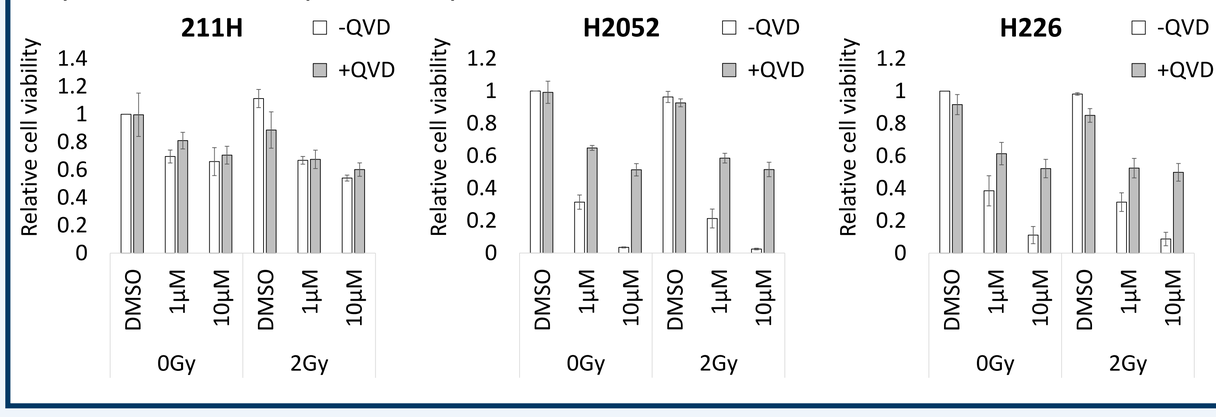
• To confirm observed reduction in survival resulted from specific activation of apoptosis, cells treated with drug + radiation and assayed for induction of mitochondrial outer-membrane permeabilization (MOMP) by presence of punctate Bax & loss of Cytochrome C.



• Activation of apoptosis confirmed by induction of caspase-3/7 activity.

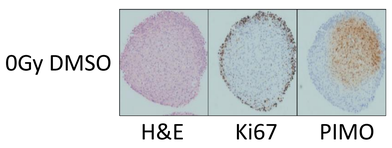


• In H2052 & H226 cells, pre-treatment with caspase inhibitor (QVD) partially rescued A1331852-induced loss of viability in a 24 hour assay, indicating caspase-dependent and caspase-independent mechanisms.



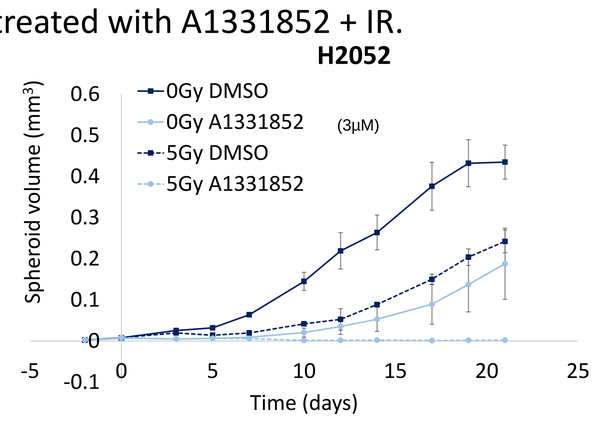
4. Combination therapy promotes regression of spheroids

• H2052 cells grown as 3D spheroids and treated with A1331852 + IR.



A1331852 or IR caused growth-delay but failed to control spheroid growth.

Combination treatment induced complete spheroid regression.



Conclusions

- **Mesothelioma cells dependent on Bcl-xL for survival. Bcl-xL inhibition exhibited therapeutic activity.**
- **Relative expression of Bcl-xL acts as a predictive biomarker of sensitivity to inhibition.**
- **A1331852 validated as a potential single-agent therapeutic and radiosensitizer for mesothelioma in 2D and 3D culture.**
- **A1331852 mechanism involves induction of apoptosis and subsequent caspase-dependent and -independent cell death.**

ESTRO37 EP-2319
Radiobiology track: Radiobiology of cancer (others)
Mark Jackson
DOI: 10.3252/pso.eu.ESTRO37.2018
Poster presented at: ESTRO 37