**ABSTRACT**

IT-139, sodium trans-[tetrachlorobis(1H-indazole)ruthenate(III)] is a first in class small molecule that has successfully completed a phase I clinical trial against solid refractory cancer. 11 of 41 patients achieved stable disease with manageable side effects and 8 patients showed tumor size reduction including a durable partial response. Preclinical studies suggest IT-139 down-regulates the chaperone GRP78, but the mechanism of action is not fully understood. GRP78 (also known as BiP and HSPA5) is the primary sensor and regulator of the endoplasmic reticulum (ER) stress response. In cancer cells it is preferably expressed on the cell surface, but also translocates to the mitochondria and cytoplasm where it regulates critical oncogenic signaling pathways, thus making GRP78 an excellent target for therapy. Here we show in vitro results of IT-139 treatment in a panel of cancer cell lines. IC50 levels were measured at 72 hours ranging from 1 µM to 167 µM. Annexin V staining by flow cytometry in HCT116, HT-29, A549 and LnCAP cell lines showed early and late apoptosis, which corresponds to their relative IC50s. UPR induction and overexpression of GRP78 has been reported to cause cell cycle arrest in G1. But in IT-139 treated cell lines (HCT116, HT-29, A549 and LC57) cell cycle arrest occurred in G2, whereas in A375 cells IT-139 induced cell cycle arrest at G1. These data suggest an inhibition of GRP78 levels and that cell cycle arrest status is dependent upon the cell line. Electron microscopic (EM) images of HCT116 and HT-29 cells treated with IT-139 for 24 hours showed significant vacuolisation, ER expansion and degeneration of intracellular organelles, strongly suggesting ER stress. Also, immunofluorescent analysis displayed no LC3-II punctate distribution, although unstressed cells suggest that IT-139 inhibits GRP78 mRNA expression. These results suggest that IT-139 downregulates GRP78 leading to an increase in ER stress, mitochondrial damage, decreased apoptosis, increased apoptosis and cell death.

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