Predicting Neuroblastoma Sensitivity to $^{211}$AtMM4, an $\alpha$-emitting PARP Inhibitor.


**Background**: Many cases of neuroblastoma are found to have MYCN amplification, which upregulates a variety of genes that participate in the low fidelity alternative NHEJ DNA repair pathway. PARP-1 is one of the proteins in this pathway, and can serve as a target for $\alpha$-emitting small molecules, such as $^{211}$AtMM4. PARP-1 expression has already been shown to correlate to neuroblastoma patient prognosis, and may be used to predict sensitivity to $^{211}$AtMM4.

**Methods**: We investigated a panel of aggressive high-risk neuroblastoma cell lines including both MYCN amplified and non-MYCN amplified cells. Western blots were performed on cell lines to analyze protein levels of enzymes in the classical NHEJ pathway and the alternative NHEJ pathway at baseline and following $^{211}$AtMM4 treatment. 0.2 μCi $^{211}$AtMM4 and 2.0 μCi $^{211}$AtMM4 in cell media for 4 hours were the two treatment doses used. Proportion of PARP-1 fragments of a certain length measured by Western blot was used to quantify the apoptosis. γ-H2AX levels were used to measure DNA damage.

**Results**: Western blot of proteins involved in the alt-NHEJ and c-NHEJ pathway did not show a strong correlation with $^{211}$AtMM4 sensitivity. γ-H2AX levels were seen to show a persistent increase following 0.2 μCi $^{211}$AtMM4 treatment, while these levels initially increased then decreased following the 2.0 μCi $^{211}$AtMM4 treatment.

**Conclusion**: No individual protein expression was found to correlate directly to $^{211}$AtMM4 sensitivity, although further analysis of combinations of protein expressions might be useful. The increase in γ-H2AX levels at low $^{211}$AtMM4 dose indicates continual repair of accrued DNA damage, while the decrease in γ-H2AX levels at high $^{211}$AtMM4 dose indicates cell death. Apoptotic cleavage fragments of PARP-1 show an increase in apoptosis after $^{211}$AtMM4 treatment.