Research Progress Update: May 2012

1. Progress on AR-42 (HDAC-42) as a potential therapy for NF2-associated schwannomas and meningiomas:

Previously, we reported that AR-42 showed a decrease in tumor volume of both vestibular schwannomas and meningiomas in our mouse models. To further evaluate the *in vivo* efficacy of AR-42, we have recently established a quantifiable intracranial model for NF2-deficient meningiomas, whose growth can be easily monitored over time using bioluminescence imaging (BLI). Using this model, we showed that AR-42 treatment reduced tumor size by about 80~98% after six months. MRI and immunohistochemistry confirmed inhibition of tumor growth. To determine whether the residual tumors in mice after six months of AR-42 treatment would resume growth in the absence of AR-42, we fed an AR-42-treated mouse normal diet for an additional six months and monitored tumor regrowth. Interestingly, after removal from AR-42 treatment, the tumor remained small with minimal regrowth (about two-fold increase in bioluminescence signal over the six months on normal diet). Although BLI detected a low level of bioluminscence signal from the tumor in the mouse removed from AR-42 treatment for six months, the tumor remained too small to be detected by MRI.

We have looked and found alteration in the cell cycle of growth in meningioma cells. AR-42 induced cell cycle arrest at the G1 phase in normal meningeal cells while it arrested Ben-Men-1 meningioma (NF2-deficient) cells at G2. Further studies revealed that AR-42 differentially affected cell-cycle progression of normal meningeal and Ben-Men-1 meningioma cells by regulating the expression of various CDK inhibitors and cyclins. To further examine the effect of AR-42 on cell-cycle progression through M phase, we analyzed the expression of various mitotic spindle assembly checkpoint kinases and found that AR-42 treatment substantially decreased the expression of some of these kinases.

Together with our previous studies in schwannoma models, our results suggest that AR-42 has potential to become a viable treatment for NF2-associated tumors. The findings that AR-42 differentially affects cell-cycle progression of normal meningeal and Ben-Men-1 meningioma cells may have implications for why AR-42 is well-tolerated while promoting tumor regression as prolonged G2 arrest may lead to cell death in proliferating tumor cells.

A manuscript describing these findings is being prepared for publication, and this information will be presented at the annual *Children's Tumor Foundation* NF meeting in June 2012. Unfortunately, our grant submission for a Phase 0 evaluation of AR-42 was not recommended for funding. In our NIH grant critique, reviewers felt that we should further investigate the mechanisms of action of AR-42, particularly in meningiomas, in order to qualify for funding. We are in the process of conducting further studies on AR-42's mechanisms of actions in the various cell types/models and plan to resubmit for funding of future clinical studies.

2. Progress on the investigation of natural compounds for the treatment of NF2-associated tumors:

To date, we have now screened 23 plant-derived natural compounds and found four of them *(silvestrol, episilvestrol, cucurbitacin D,* and *bruceantin)* potently inhibit the growth of primary vestibular schwannoma and meningioma cells and Ben-Men-1 cells at sub-micromolar concentrations. The 50% inhibitory concentration (IC_{50}) values for all four were less than 300 nM, with silvestrol being the most potent (IC_{50} ~ 20 nM for primary meningioma cells and 10 nM for vestibular schwannoma and Ben-Men-1 cells). Also, we found that silvestrol induced cell cycle arrest at G2/M in schwannoma and meningioma cells and resulted in a marked dose-dependent accumulation of cells in the G2/M phase in Ben-Men-1 cells. Consistently, Ben-Men-1 cells treated with silvestrol showed dramatically decreased levels of PCNA and cyclin A, molecules essential for progression through S and G2 phases. In addition, silvestrol treatment noticeably reduced the phosphorylation of ERK1/2 and AKT, two key mitogenic proteins.

This information will also be presented at the annual *Children's Tumor Foundation* NF meeting in June 2012.