OSU Research Progress Update: August 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.

Also, we have found that AR-42 induced cell-cycle arrest at the G1 phase in normal meningeal cells while it predominantly arrested Ben-Men-1 meningioma (NF2-deficient) cells at G2. We showed 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. In addition, we showed that AR-42 treatment substantially decreased the expression of Aurora kinases A and B, two mitotic spindle assembly checkpoint 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.

We presented this information at the annual *Children's Tumor Foundation* NF Conference in June 2012. Also, a manuscript describing these findings has been submitted to the journal *Cancer Research* and received favorable reviews. We have acknowledged the support from *AdvocureNF2* in this manuscript. In addition, we have submitted a grant proposal for a Phase I clinical trial of AR-42 in patients with vestibular schwannoma and meningioma to the Department of Defense NF Research Program.

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

To date, we have 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 with IC_{50} ~ 20 nM for primary meningioma cells and 10 nM for vestibular schwannoma and Ben-Men-1 cells.

Biochemical analysis revealed that silvestrol induced cell cycle arrest at G2/M in primary vestibular 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.

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Additionally, we found that bruceantin treatment decreased cyclin D1, cyclin A, and CDK inhibitors, p21 and p27. Also, we identified another natural compound, designated A2183D2F4K2, which effectively inhibited proliferation of schwannoma and meningioma cells at about 0.5 μM of IC_{50}.