Establishing the role of arginine deprivation in cancer treatment through pre-clinical activity of the recombinant human arginase PEG-BCT-100 in gliomas and soft-tissue sarcomas (STS)

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Background: Arginine deprivation is a novel approach to limit arginine-dependent tumour growth. The presence of enzymes involved in the *de novo* synthesis of arginine from citrulline, argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL) and ornithine transcarbamylase (OTC), can influence the sensitivity of tumour to arginine depletion. Gliomas and STS are two malignancies with poor prognoses in advanced disease. We studied the preclinical efficacy of PEG-BCT-100 (also known as rhArg1peg5000), a PEGylated recombinant human arginase 1 on glioma and STS cell lines. Predictive properties of ASS, ASL and OTC expression to PEG-BCT-100 treatment were examined.

Methods: Cells from 5 representative glioma cell lines (A172, M059J, M059K, T98G and U-87 MG) and 5 representative soft-tissue sarcoma (STS) cell lines (RD, GCT, HT-1080, SW872, SW982) were either incubated in full culture medium (control), arginine free medium or PEG-BCT-100 (1U/ml) treated medium. They were monitored by live cell imaging for 72 hours. Their corresponding IC_{50} were determined by cell viability assay. Additionally, an analysis of ASS1, ASL and OTC expression in adult gliomas determined from 72 archival samples.

Results: All cell lines were sensitive to PEG-BCT-100 and demonstrated significant cell proliferation inhibition. Their IC_{50}, as determined by cell viability assay were 0.156 U/ml, 0.300 U/ml, 0.312 U/ml, 0.415 U/ml, and 0.522 U/ml for glioma cell lines T98G, M059J, A172, U-87 MG and M059K respectively. In particular, cell death with both apoptosis and necrosis were observed in T98G, M059K and A172 cells. When cultured in arginine free medium, growth inhibition were only observed in M059J, M059K and T98G cells, indicating that these cells may be more auxotrophic to arginine. For STS cell lines, their IC_{50}, as determined by cell viability assay were 0.023 U/ml, 0.028 U/ml, 0.023 U/ml, 0.026 U/ml, 0.20 U/ml, 0.053 U/ml, and 0.03 U/ml for GCT, HT-1080, RD, SJSA-1, SW872 and SW982 respectively. Western blot analysis confirmed strong basal protein expression of ASL in all sarcoma cell lines, suggesting an insignificant role for ASL expression as a predictor for PEG-BCT-100 efficacy. Moreover, expression levels of ASS and OTC of cells derived from the cell lines were shown to be inversely correlated with their sensitivity towards arginine deprivation. Specifically, all sarcoma cell lines showed low levels of OTC expression, which suggests OTC expression as an important predictive biomarker for PEG-BCT-100 treatment efficacy. Determination of basal ASS1 and OTC expression in archival samples of adult gliomas showed complete absence of ASS1 (0%) and low incidence of OTC (8.3%) over-expression.

Conclusions: Arginine deprivation by PEG-BCT-100 is effective in suppressing glioma cell growth in vitro, suggesting arginine auxotrophism in gliomas and STS. Low expression of OTC, instead of ASS and ASL, may be a more important predictive biomarker for response to treatment. Confirmed absence of ASS1 and low-levels of OTC expression in archival glioma patients' samples confirm a potential target population for future clinical development. Synergism studies with commonly used cytotoxics in gliomas and STS are currently underway. Further in-vivo and clinical studies are warranted.