Augmentation of docetaxel anti-tumor activity by a stathmin-specific bi-functional shRNA.

Alex W. Tong[1], Yang Yu[1], Donald D. Rao[1], Phillip B. Maples[1], Neil Senzer[1,2], John Nemunaitis[1,2].

[1]Gradalis, Inc., Dallas, TX; [2]Mary Crowley Cancer Research Centers, Dallas, TX;

Stathmin (STMN1) is a 17-kDa cytosolic protein and the archetype member of a family of phosphoproteins (STMN1, SCG10, SCLIP, RB3/RB3′/RB3") that serves important signal transduction functions and regulates microtubule dynamics. Unphosphorylated STMN1 regulates rapid microtubule remodeling of the cytoskeleton through microtubule depolymerization, a critical process for mitotic spindle disassembly during cell cycle progression. We recently confirmed the overexpression of STMN1 mRNA transcripts in 86% (30/35) of tumor specimens across a variety of tumor histotypes at the Mary Crowley Cancer Research Centers (MCCRC). Using a proprietary bifunctional shRNA construct that mediates post-transcriptional mRNA knockdown by its interaction with cleavage-dependent and cleavage-independent RNA interference silencing complexes (RISCs), we demonstrated that STMN1 knockdown effectively reduced the growth of human colorectal CCL-247 cells by >80%, correspondingly arresting cycling cells at the G_2M phase. This treatment similarly reduced the growth of human breast cancer (MDA-MB-231) and melanoma (SK-MEL-28) lines by 45% and 48%, respectively. In vivo antitumor activity of STMN1 bi-shRNA was confirmed in xenograft models of CCL-247 cells with high STMN1 expression, and a primary osteosarcoma xenograft model derived from a low passage primary human osteosarcoma with moderate STMN1 expression. Previously studies by Seve and others have described an inverse relationship between elevated stathmin expression and chemoresponsiveness to microtubule-stabilizing taxanes. To characterize the interactive outcome of STMN1 knockdown with docetaxel, we carried out in vitro docetaxel (DOC) dose response assessments with or without co-treatment with bi-shRNA^{STMN1} in CCL-247 and SK-MEL-28 melanoma. BrdU determinations indicated that STMN1 knockdown significantly reduced DOC concentration needed to inhibit cancer cell growth by 50% (IC_{50}) of CCL-247 cells from 1.8$\pm$0.2 to 0.6$\pm$0.4 nm (n=3, $p<0.05$), and SK-Mel-28 cells from 1.7$\pm$0.2 nm to 0.1$\pm$0.0 (n=3, $p<0.05$). The 3- to >10-fold reduction in DOC IC_{50} suggest that bi-shRNA^{STMN1} can markedly enhance the effectiveness of docetaxel for human cancer cells, and may be potentially applicable as an experimental biotherapeutic approach in combination with docetaxel.