

Abstract # **2736**

## Antileukemic Effects of the Novel Agent Elesclomol.

Sue Chow<sup>1</sup>, Masazumi Nagai<sup>2</sup>, Suqin He<sup>2</sup>, Ronald K Blackman, PhD<sup>2</sup>, James Barsoum, PhD<sup>2</sup>, Vojislav Vukovic, MD, PhD<sup>2</sup> and David Hedley, MD<sup>3</sup>

1. Medical Oncology, Princess Margaret Hospital, Toronto, ON, Canada, 2. Synta Pharmaceuticals Inc., Lexington, MA, USA, 3. Medical Oncology and Hematology, Princess Margaret Hospital, Toronto, ON, Canada

Elesclomol (N-malonyl-bis (N'-methyl-N'-thiobenzoyl hydrazide)) is an investigational first-in-class oxidative stress inducer that triggers apoptosis in cancer cells (Kirshner et al., *Mol Cancer Ther* 2008;7:2319–27). In the clinic, elesclomol is well tolerated in humans and showed activity in combination with paclitaxel in patients with refractory solid tumors (Berkenblit et al., *Clin Cancer Res* 2007;13:584–90). The aims of the current study are to examine the activity of elesclomol against a range of AML cell lines, including primary patient blast cultures, to investigate the mechanisms of drug action and the potential to combine elesclomol with other agents, and to identify candidate biomarkers for monitoring effects during treatment of leukemia patients with elesclomol. Here we describe the effects of elesclomol treatment in 4 AML cell lines selected based on their varying molecular attributes.

Effects on cellular redox state and mitochondrial function were monitored using a flow cytometry incorporating the glutathione (GSH) probe monobromobimane, the reactive oxygen species (ROS) probe carboxy-dichlorofluorescein and the mitochondrial membrane potential stain DiIC(1)5. In addition, outer cell membrane integrity was determined by propidium iodide exclusion. Dual staining of fixed, permeabilized cells with phospho-specific antibodies to p38 and SAPK/JNK was used to determine if elesclomol treatment results in activation of the stress-activated MAP kinase pathways.

Elesclomol showed potent anti-leukemic effects in vitro at concentrations as low as 10nM, which is well below the concentrations achieved in cancer patients, and greater toxicity was achieved with prolonged drug exposure. In OCI-AML2, a factor-independent, poorly differentiated AML cell line, toxicity was associated with loss of reduced GSH that coincided with a large increase in ROS generation and depolarization of the mitochondrial inner membrane, and later with loss of surface membrane integrity. A similar pattern was seen in OCI-M2, a p53-deficient erythroblastic leukemia cell line, except that during the early stages of drug effect these cells showed a large increase in reduced GSH, suggesting that initially they are able to compensate for drug-induced oxidative stress through enhanced cellular antioxidant production. In contrast, the factor-dependent line OCI-AML5, which appeared most sensitive to elesclomol, showed loss of outer membrane integrity without obvious prior oxidative stress while the Flt-3 ITD mutant line MV4-11 showed an initial loss of mitochondrial membrane potential without accompanying oxidative stress. Strikingly, we did not observe activation of the stress-responsive p38 or SAPK/JNK pathways in any of these 4 cell lines tested, suggesting that

this is not a prominent response to elesclomol activity in AML and that additional mechanisms may be at work for the activity of elesclomol in these cells. Further investigations are ongoing and additional studies, including evaluation of elesclomol activity in primary leukemic cells from AML patients, will be presented.

In summary, elesclomol is a potent novel compound that exerts anti-leukemic effects in tissue culture at drug concentrations that are well below those achieved in patients, suggesting that it might be active in leukemia patients.