

ANTILEUKEMIC EFFECTS OF THE NOVEL AGENT ELESCLOMOL

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Oxidative stress as a death effector in AML

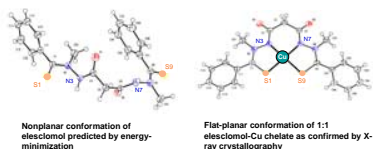
The respiratory chain is the most prominent source of cellular reactive oxygen generation, although many enzyme systems can contribute. Oxidative stress occurs when ROS production exceeds the capacity of cellular anti-oxidants.

Glutathione is the most abundant cellular anti-oxidant.

We previously showed that simultaneous monitoring of ROS production, GSH content, and mitochondrial membrane potential (MMP) is a powerful approach for studying the mechanisms of oxidative stress during apoptosis following treatment of AML blasts with Ara-C and γ -radiation, and that AML blasts are sensitized by the GSH-depleting agent BSO. Based on this earlier work, we hypothesized that AML blasts might be unusually sensitive to elesclomol, given the known ability of copper to catalyze ROS production via Fenton chemistry.

Elesclomol has a novel copper-dependent mechanism of action

Elesclomol is a first-in-class oxidative stress inducer that triggers apoptosis in cancer cells. In laboratory studies, elesclomol binds copper in plasma, facilitates its uptake into cells, and enables a transition between copper oxidation states once inside the cell. Elesclomol has been observed to increase the high level of ROS in cancer cells even further, leading to an increase in pro-apoptotic factors, a decrease in anti-apoptotic factors, the opening of the mitochondrial membrane pores, and ultimately to the initiation of programmed cell death via the mitochondrial apoptosis pathway. This mechanism of action, called oxidative stress induction, represents a novel way of selectively targeting and killing cancer cells. In preclinical models elesclomol showed potent anti-cancer activity against a broad range of cancer cell types, as well as an ability to enhance the efficacy of certain chemotherapy agents with minimal additional toxicity. In the clinic, the drug has been administered in clinical trials to >600 subjects, most recently in the phase 3 SYMMETRY trial for metastatic melanoma. It has displayed a satisfactory safety profile and favorable pharmacokinetics (Berkenblit et al. 2007).



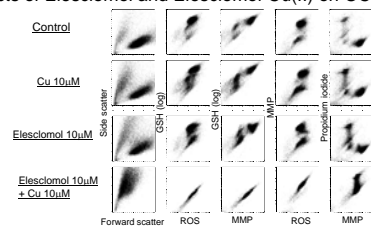
Materials and methods

Cell lines: OCI-AML2, OCI-AML5, OCI-M2 (p53 mutant M6), MV4-11 (FLT3-ITD) Patients were treated by the Princess Margaret Hospital Leukemia Program. Primary blasts from 10 AML patients isolated by density gradient or rbc lysis, maintained in α -MEM + 10%FBS \pm 10% 5637-conditioned medium.

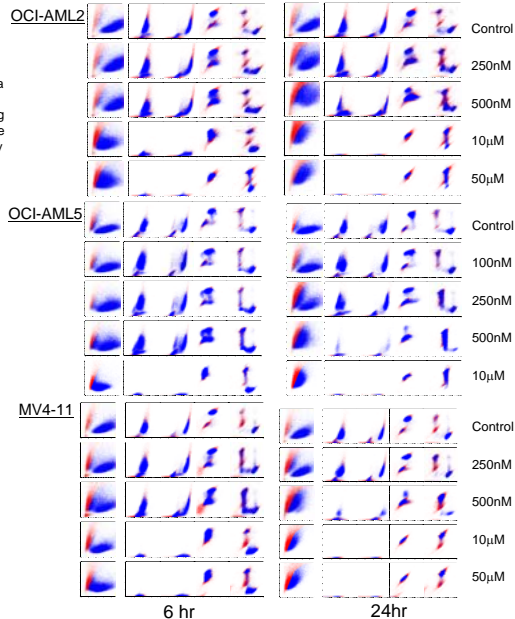
Flow Cytometry Protocol

Combined staining at 37° for:
MMP – DiIC(1,5) 40nM 30'
ROS – carboxy-dichlorofluorescein 5 μ M or dihydrorhodamine 123 1 μ M for 30'
GSH – monobromobimane 40 μ M for 5'
Surface membrane integrity – propidium iodide 1 μ g/ml for 5'
Samples run on 3 laser flow cytometer (Gallios, Beckman-Coulter)

Effects of Elesclomol and Elesclomol-Cu(II) on OCI-AML2 cells



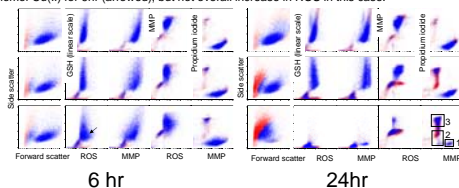
Elesclomol-Cu(II) depletes GSH and depolarizes the mitochondrial membrane in AML cell lines



Elesclomol-Cu(II) has potent effects on AML patient blast cells

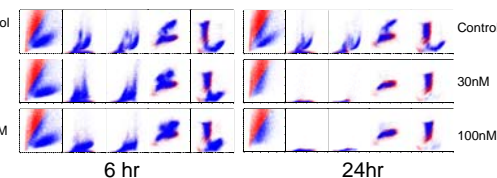
Patient #123

The dual MMP/PI plots show that loss of mitochondrial membrane potential precedes the loss of outer membrane integrity (numbered boxes, bottom right panel). Reduced GSH is lost co-incident with the loss of MMP. Pattern is similar to that seen in AML cell lines, but the primary patient blasts are more drug sensitive. There is a slight increase in ROS seen in the sample treated with 100nM elesclomol-Cu(II) for 6hr (arrowed), but not overall increase in ROS in this case.



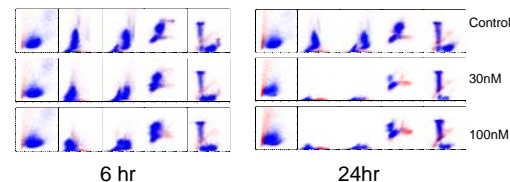
Patient #126

Has a more heterogeneous distribution of GSH and ROS at baseline, also highly sensitive to elesclomol-Cu(II)



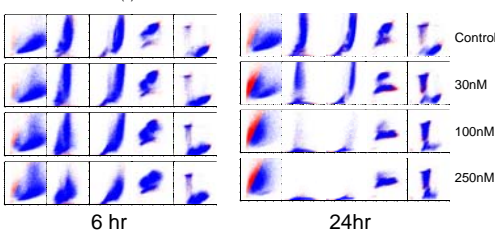
Patient #127

Pattern is similar to Patient #123, although the baseline GSH level is lower, and this example shows loss of GSH following exposure to 30 nM elesclomol-Cu(II), which is already evident at 6hr.



Patient #131

Overall pattern is similar to the other two cases, with loss of GSH co-incident with loss of MMP and increased ROS (most evident at the 30nM 24hr point). Blasts have higher baseline GSH and are less sensitive to elesclomol-Cu(II)



Conclusions

- Elesclomol is toxic to all primary AML blast cells tested to date
- Elesclomol-Cu(II) is more potent than the parent compound, consistent with novel action involving copper uptake
- Depletion of GSH and dissipation of mitochondrial membrane potential occurred in all cases
- Consistent with previously reported effects of copper toxicity on glutathione metabolism
- Reactive oxygen generation is less prominent - suggests not primary mechanism of action in AML - further investigation is ongoing
- Results support testing elesclomol in AML patients

References

- Berkenblit A, Eder JP, Jr., Ryan DP, Seiden MV, Tatsuta N, Sherman ML, Dahl TA, Dezuze BJ, Supko JG (2007) Phase I clinical trial of STA-4783 in combination with paclitaxel in patients with refractory solid tumors. *Clin Cancer Res* 13: 584-590
- Krishnar JR, He S, Balasubramanyam V, Kapoor J, Yang CY, Zhang M, Du Z, Baroum J, Berlin J (2008) Elesclomol induces cancer cell apoptosis through oxidative stress. *Mol Cancer Ther* 7: 2319-2327
- Sheng-Tanner X, Bump EA, Hedley DW (1998) An oxidative stress-mediated death pathway in irradiated human leukemia cells mapped using multilaser flow cytometry. *Radiat Res* 150: 636-647
- Hedley DW, McCulloch EA, Minden MD, Chow S, Curtis J (1998) Antileukemic action of buthionine sulfoximine: evidence for an intrinsic death mechanism based on oxidative stress. *Leukemia* 12: 1545-1552
- Backway KL, McCulloch EA, Chow S, Hedley DW (1997) Relationships between the mitochondrial permeability transition and oxidative stress during ara-C toxicity. *Cancer Res* 57: 2446-2451
- Hedley DW, Chow S (1994) Evaluation of methods for measuring cellular glutathione content using flow cytometry. *Cytometry* 15: 349-358

Conflicts of interest

RKB and VV are full time employees of Synta Pharmaceuticals. The work done in Dr Hedley's laboratory received financial support from Synta.