Compounds of natural origin as promising inducers of immunogenic cell death

E. Turrini[1], E. Catanzaro[1], P. Hrelia[2], C. Fimognari[1]

[1]Dept. for Life Quality Studies, *Alma Mater Studiorum* – University of Bologna, Italy
[2]Dept. of Pharmacy and Biotechnology, *Alma Mater Studiorum* – University of Bologna, Italy

Successful cancer treatment might depend on the use of cytotoxic drugs able to induce apoptotic death, but also to promote tumor-specific immune responses, potentially leading to tumor eradication (Zitvogel, 2008). Some anticancer drugs, such as anthracyclines, are able to induce immunogenic cell death (ICD) through the activation of a variety of antigen presenting cells. ICD is induced by agents provoking both endoplasmic reticulum (ER) stress and the production of reactive oxygen species (ROS) and is associated with the expression and/or release of damage associated molecular patterns (DAMPs), crucial for the immunogenicity of the dying cancer cells (Krysko, 2012). There is a growing interest in the evaluation of products of natural origin, able to simultaneously interact with a variety of targets and to influence different pathways, as promising candidate to contrast the complexity of cancer. In this context, *Hemidesmus indicus* (H.i.), belonging to the family of *Asclepidaceae*, is an Indian weed widely used in the traditional medicine and extensively investigated for its *in vitro* and *in vivo* pharmacological properties, such as anticancer, anti-inflammatory and immunomodulatory properties (Das and Singh Bisht, 2012). We explored the ability of H.i. to induce ICD.
H.i. decoction modulated many components of intracellular signaling pathway and was able to induce apoptotic death in a panel of leukemic cell lines. The highest apoptotic effects were observed after 24 h in Jurkat cells at the concentration of 0.93 mg/mL (36.9% *vs* 5.0% of untreated cells). Additionally, treatment of Jurkat cells with H.i. 0.93 mg/mL for 24 h resulted in a significant break-down of the mitochondrial membrane potential and a 4-time increase in Bax/Bcl-2 ratio. H.i. induced a significant $[Ca^{2+}]_i$ increase through the intracellular mobilization of Ca^{2+} stores that, following 24 h H.i. treatment, was found to be five times higher than that of the control. Interestingly, thapsigargin, a sesquiterpene lactone promoting the release of intracellular ER Ca^{2+} stores, significantly up-regulated H.i.-induced $[Ca^{2+}]_i$ release. Furthermore, H.i. induced ROS production upon short time treatment of Jurkat cells, and co-treatment with N-acetylcysteine significantly reduced the apoptosis induced by the decoction. In fact, the percentage of apoptotic cells at H.i. 0.93 mg/mL dropped to 12.7% in presence of N-acetylcysteine. These *in vitro* results and in particular the ability of H.i. to induce apoptosis via increasing intracellular Ca^{2+} mobilization, imputable also to ER stress, and producing ROS, strongly support that H.i. is a promising ICD inducer. These data will be strengthen by further analysis on H.i. ability to provoke DAMPs trafficking, thereby promoting the activation of antigen presenting cells and the generation of tumor specific immune responses.

Das and Bisht (2012). *Phytother Res*. 27, 791-801.
Krysko et al. (2012). *Nat Rev Cancer*. 12, 860-75.
Zitvogel et al. (2008). *J Clin Invest*. 118, 1991-2001.