Tremendous progress made in recent times has changed the notion that tumors are simply proliferating cancer cells organized into invariable masses. Today, tumors are recognized as complex tissues composed of multiple cell types, including innate immune cells that have emerged as important defining features of anti-tumor therapy. Research has shown that, the immune system might play an important role in limiting formation of more than 80% of non-viral tumors. These trends have also heralded a change in anti-cancer therapeutics; oncologists now recognize that simply debulking the tumors via surgery and radio-/chemo-therapy is seldom enough. Thus, not surprisingly, bulk of their concentration localizes in the extranuclear (site of ‘off target’ effect) and only a fraction of it localizes in extranuclear compartments (Fig 1A) like the ER (site of ‘on target’ effects). Thus, the need to synergistically combine cancer cell killing (necrotic or apoptotic) with the activation of the anti-tumor immunity, has arose. Immunogenic apoptosis has all the major hallmarks of physiological apoptosis except that it can activate (rather than suppress) the immune system by emitting vital immunological signals, comprising damage-associated molecular patterns (DAMPs). Thus, cancer cells undergoing immunogenic apoptosis (or IA) have acquired the ability to communicate their antigenic memory to the immune system thereby leading to potent anti-tumor immunity. DAMPs that are vital for IA include surface-exposed calreticulin (ecto-CRT) and secreted ATP. IA tends to be stressor-dependent in that only selected chemotherapeutics induce it e.g., mitoxantrone, doxorubicin and oxaliplatin. This is because, reactive oxygen species (ROS)-based endoplasmic reticulum (ER) stress inducing agents are crucial for IA since ROS-based ER stress activates the specific danger signaling module required to emit immunogenicity-defining DAMPs. However, there are some technical glitches with these current inducers that limits the overall potential and applicability of IA.

Current inducers of IA, like anthra-cyclines, mitoxantrone and oxaliplatin, are primarily DNA-targeting agents and secreted ATP. IA tends to be stressor-dependent in that only selected chemotherapeutics induce it e.g., mitoxantrone, doxorubicin and oxaliplatin. This is because, reactive oxygen species (ROS)-based endoplasmic reticulum (ER) stress inducing agents are crucial for IA since ROS-based ER stress activates the specific danger signaling module required to emit immunogenicity-defining DAMPs. However, there are some technical glitches with these current inducers that limits the overall potential and applicability of IA.

Primary induction of photo-oxidative (phox)-ER stress in cancer cells evoked immunogenic apoptosis (IA) associated with pre-apoptotic emission of calreticulin and ATP, and protective antitumor immunity. This IA subroutine involved “cure” functions (e.g., PERK/PI3K-based modulation of secretory trafficking) rather than “private” ones (caspase-8 and eIF2α phosphorylation) engaged by other IA inducers.

The emergence of phox-ER stress induced immunogenic apoptosis

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the pre-apoptotic stage (stage preceding phosphatidylserine exposure) 788 (Fig. 1D).
In fact, the kinetics of their emission was quicker than those reported previously for these three DAMPs. This makes phox-ER stress-induced IA (in our knowledge) the first cell death subroutine with three crucial DAMPs following overlapping kinetics to be pre-apoptotically emitted. Moreover, by prompting the primary production of ROS at the ER, we fostered the relative amounts of these DAMPs “exposed” to the extracellular space as compared to those caused by other IA inducers, which are prevalently DNA-damaging in nature. 788 Thus, this form of “primary/on target” phox-ER stress-induced IA instigated a strong immunostimulatory pre-apoptotic stage before the immunosuppressive program that is innate to apoptosis, 788 commences (post-phosphatidylserine exposure and caspase activation). Phox-ER stressed/deadly killing cancer cells were also found to establish a highly productive interface with dendritic cells (on the levels of phagocytosis and phenotype/molecular maturation), 788 paving the way for IA that elicited a potent anti-tumor immune response. 788 Our results in a CT26 prophylactic immunization model have been recently substantiated in a CT26 therapeutic model, where it was shown that CT26 tumor-bearing mice whose tumors were eradicated with Hyp-PDT resisted formation of new tumors when subsequently re-challenged with live CT26 cells. 788 This further outlines the ability of Hyp-PDT to combine cancer cell killing with “revival/activation” of anti-tumor immunity within the same paradigm.

Moreover, we found that the ecto-CRT and immunogenicity incurred by phox-ER stress were caspase(-8) independent 788 thereby uncoupling cell death signaling and danger signaling, for the first time. In fact, the intracellular pathways controlling DAMP emission during phox-ER stress-induced IA, emerged predominantly from the advantageous off target ROS-based ER stress which causes pre-apoptotic surface exposure of CRT and defines the dying cell’s immunogenicity. While this secondary effect is capable of engaging apoptotic pathways on its own, its overall contribution to the apoptosis observed after treatment with these IA inducers is unknown. These processes are accompanied by early/initial apoptotic secretion of ATP and surface-exposed HSP70, both of which seem to be propelled as a result of general cellular stress. Overall, these processes lead to chemotherapeutics-induced IA. (C) Sub-cellular localization of hypericin. 150 nm of hypericin incubated with the T24 cancer cells for 16 h shows strong co-localization (merged image) with the ER Tracker Blue-White DTX dye. (D) Schematic of phox-ER stress-induced IA. Hypericin-based photochemical therapy (PDT) causes ROS-based ER stress as a part of a predominant “molecular effect” (result of predominant ER localization); this leads to the pre-apoptotic emission of crucial DAMPs like secreted ATP and surface-exposed CRT, HSP70. Overall, this “on target” ROS-based ER stress (on phox-ER stress) boosts IA.

References
1. Honamra D, Winstead RA. Hallmarks of cancer: the next generation. Cell 2011; 144:664-76; PMID: 21759528; http://dx.doi.org/10.1016/j.cell.2011.02.013
2. Gattick L, Korp O, Korsmo G. Diligentia the impact of immunogenic cell death in photodynamic cancer therapy. EMBO J 2012; 31:1061-79; PMID: 22252132; http://dx.doi.org/10.1038/emboj.2012.205
3. Gaz JD, Ventei D, Godil J, Vanrooijse P, Yuchoo DV, Agostini P. Immunogenic cell death, DAMPs and immunotherapeutic are emerging analogues. Biochim Biophys Acta 2010; 1805:53-71; PMID: 18703113
4. Obad M, Toman A, Chingkaling F, Finus GM, Agosti L, Peltokari J, et al. Catecholamine exposure dictates the immunogenicity of cancer cells. Nat Med 2010; 15:149-55; PMID:17720872; http://dx.doi.org/10.1038/nm.2120
5. Gaz JD, Yucel DV, Verbilko T, Kozmic A, Ferrara GB, Maynard T, et al. A novel pathway combining ubiquitin-regions and ATP secretion in immunogenic cancer cell death. EMBO J 2012; 31:1055-7; PMID:22252130; http://dx.doi.org/10.1038/emboj.2011.497
6. Cordoliani SA, Chemotherapeutics-associated oxidative stress impact on chemotherapy efflux. Onco Targets Ther 2010; 3:292-301; PMID:15320108; http://dx.doi.org/10.1177/1757862209318535
7. Vogely C, Dalmontino S, Gatta G, Rachele L. Preventing the cardio-protective effects of autophagy from basic concepts to clinical data Heart Med 2007; 3:151-7.
8. Gaz JD, Yucel DV, Vandervelde P, Agostini P. Hypericin-based photodynamic therapy induces surface exposure of damaged-associated molecular patterns like HSP70 and ubiquitin. Cancer Immunol Immunother 2012; 61:215-21; PMID:22125895; http://dx.doi.org/10.1007/s00262-011-1086-2
9. Saezic R, Vermeersch T, Hartl A, Krammer B. Does doxorubicin-PDT induce complete tumor regression in NALM-6 mice bearing CT26 colon carcinoma. Photodagmnos Therum 2011; 3:291-4; PMID:22621293; http://dx.doi.org/10.1016/j.pdpdt.2011.04.003