Cell death can be induced in cancer cells by physical or chemical treatments. Chemotherapeutic drugs kill tumor cells often by inducing apoptosis that can be accompanied by autophagy or, in a later stage, by cell necrosis. However, in order to completely eliminate cancer cells and avoid cancer recurrence, it is of fundamental importance the activation of the immune system. Dendritic cells (DC) are powerful antigen-presenting cells (APCs) that play a pivotal role initiating a specific immune response and in the eradication of apoptotic cancer cells by mediating the cross-presentation of tumor antigens to the cytotoxic T cells. Thus, one of the fascinating issues in oncoimmunology is inducing DC activation by tumor cell death to increase the effect of cancer therapy. Immunogenicity is dictated by dying cells’ exposure or release of several molecules able to activate the diverse components of the immune system, such as macrophages, natural killer cells (NK) and DC. The most characterized molecules involved in the immunogenicity of cell death are: calreticulin (CRT), heat shock protein (HSP) 70 and 90, ATP and high mobility group box 1 (HMGB1). HSPs and CRT are located in the cytoplasm or into the endoplasmic reticulum (ER) and their exposure or release seems to be linked to ER stress, although the underlying mechanisms have not been fully elucidated yet. More recently, it has been reported that autophagy can play a role in the immunogenic cell death, because during the autophagic process, that may precedes the cell death, molecules such as ATP are released to recruit and activate DC. HMGB1, another immunogenic molecule, is released when there is a severe damage of the plasmamembrane such as that induced by cell necrosis or necroptosis, that represents the final event of an apoptotic cell death.

We have recently shown that antican-
drugs Bortezomib (Velcade), a proteasome inhibitor and Tyrophostin AG 490, a Janus Activated Kinase 2/signal transducer and activator of transcription-3 (JAK2/STAT3) inhibitor, induce immunogenic cell death in primary effusion lymphoma (PEL). PEL is a non-Hodgkin’s lymphoma characterized by poor response to conventional chemotherapy and by an extremely aggressive clinical course. By coculturing Bortezomib- and AG 490-killed PEL cells with DC we found that they were able to activate DC, as indicated by the upregulation of CD83 and CD86 markers. However, nonetheless cell died by apoptosis, seen as Annexin V staining and higher percentage of sub G0/G1 DNA content, the use of broad-spectrum caspase-inhibitor zVAD-fmk only slightly reduced DC activation in the cocultures with killed-PEL cells. These results suggest that apoptosis itself in PEL is not responsible for the immunogenicity of both Bortezomib and AG 490. Rather, important for DC activation was PEL cell surface exposure of CRT, HSP90 and HSP70. Thus, reduction in DC maturation was achieved by a cocktail of antibodies directed against CRT, HSP90 and HSP70. Since caspase-inhibitor treatment only slightly reduced CRT, HSP90 and HSP70 expression, these findings indicate that additional mechanisms other than apoptosis are responsible for their exposure. Studies are in progress to elucidate how it occurs, exploring if and which pathways of ER stress response are induced in PEL cells by Bortezomib and AG 490 and if other cell death modalities are involved in the immunogenicity occurred with these drugs. Since PEL is a virus-associated lymphoma, it will be also interesting to explore if during the Bortezomib-induced cell death, viral antigens, hidden in live cells, become exposed on cell surface, possibly in association with HSPs.

The AG 490-induced cell death and its immunogenicity are less explored. AG 490 acts by inhibiting STAT3 phosphorylation and inducing apoptosis in cancer cells which display a constitutive activation of this molecule, such as PEL. STAT3 is also phosphorylated in the DC in the tumor environment and its activation correlates with their immunosuppression. We found that AG 490 is non-toxic for DC cocultured with PEL and several studies have shown that it can be also utilized to restore DC function in the tumor environment. Because of its action on both immune and tumor cells, the use of AG 490 as an inducer of immunogenic cell death could be very promising. Moreover, as STAT3 has been inversely correlated with autophagy, which has been reported...
To further analyze the molecular mechanisms responsible of DC activation, we found complete inhibition of DC maturation by using a neutralizing antibody directed against CD91 (Fig. 1), also known as LRP1, a polyfunctional molecule that has been reported to be the common receptor for CRT, HSP70 and HSP90.9 Similarly, strong reduction of DC activation was obtained following CD91 knockdown by small interference RNA.

CD91 is one of the receptors for damage associated molecular patterns (DAMPs) able to mediate the phagocytosis and subsequent cross-presentation of foreign antigens. It can also bind the human defensins that, with an autocrine regulatory loop, upregulate its expression and activate DC. Its importance in the immune response is also underlined by the finding that it is the only molecule upregulated on the DC surface of long-term, non-progressor HIV-infected patients.10

Our results demonstrate a pivotal role of CD91 in the DC activation mediated by Bortezomib- and AG-490-killed PEL. It will be interesting to unveil the molecular pathways activated in DC after CD91 engagement and whether CD91 upregulation, for example by using defensins, might be exploited to enhance the immune response and increase the efficacy of the immunogenic chemotherapies.

References
1. Ma Y, Conforti R, Aymeric L, Locher C, Kepp O, Kroemer G, et al. How to improve the immunogenicity of chemotherapy and radiotherapy. Cancer Metastasis Rev 2011; 30:71-82; PMID:21298323; http://dx.doi.org/10.1007/s10555-011-9283-2.
2. Garg AD, Nowis D, Golab J, Vandenabeele P, Krysko DV, Agostinis P. Immunogenic cell death, DAMPs and anticancer therapeutics: an emerging amalgamation. Biochim Biophys Acta 2010; 1805:53-71; PMID:20720113.
3. Griffith TS, Ferguson TA. Cell death in the maintenance and abrogation of tolerance: the five Ws of dying cells. Immunity 2011; 35:456-66; PMID:22035830; http://dx.doi.org/10.1016/j.immuni.2011.08.011.
4. Kepp O, Galluzzi L, Martins I, Schlemmer F, Adjemian S, Michaud M, et al. Molecular determinants of immunogenic cell death elicited by anticancer chemotherapy. Cancer Metastasis Rev 2011; 30:61-9; PMID:21249425; http://dx.doi.org/10.1007/s10555-011-9273-4.
5. Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegrini P, et al. Autophagy-dependent anticancer immune responses induced by chemotherapeutic agents in mice. Science 2011; 334:1573-7; PMID:22174255; http://dx.doi.org/10.1126/science.1208347.
6. Cirione M, Di Renzo L, Lorio LV, Conte V, Trivedi P, Santarelli R, et al. Primary effusion lymphoma cell death induced by bortezomib and AG 490 activates dendritic cells through CD91. PLoS One 2012; 7:31732; PMID:22412839; http://dx.doi.org/10.1371/journal.pone.0031732.
7. Melillo JA, Song L, Bhagat G, Blazquez AB, Plumlee CR, Lee C, et al. Dendritic cell (DC)-specific targeting reveals Stat3 as a negative regulator of DC function. J Immunol 2010; 184:2638-45; PMID:20212410; http://dx.doi.org/10.4049/jimmunol.0902960.
8. Zhang Y, Morgan MJ, Chen K, Choksi S, Liu ZG. Induction of autophagy is essential for monocyte-macrophage differentiation. Blood 2012; 119:2895-905; PMID:22223827; http://dx.doi.org/10.1182/blood-2011-08-372383.
9. Basu S, Binder RJ, Ramalingam T, Srivastava PK. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70 and calreticulin. Immunity 2001; 14:303-13; PMID:11290339; http://dx.doi.org/10.1016/S1074-7613(01)00111-X.
10. Stobbing J, Gazard B, Kim L, Portousmith M, Wildfire A, Tao L, et al. The heat-shock protein receptor CD91 is upregulated in monocytic of HIV-1-infected “true” long-term nonprogressors. Blood 2003; 101:4000-4; PMID:12531796; http://dx.doi.org/10.1182/blood-2002-11-3353.