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Cell Death Identification in Anticancer Therapy – Letter

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In their recent review Rello-Varona et al (1) emphasize that “The correct evaluation of cell death in every experimental setting related to cancer research is a must”. Accordingly they discuss the variety of methods to identify dead cells and to distinguish the various forms of cell death from one another. While we agree with their descriptions of the different forms of cell death and appreciate that the mechanism of cell death is often worth characterizing in cancer research, we would emphasize three aspects of cell killing that make the precise characterization of the type of cell death less important than the quantification of cell *survival* using clonogenic endpoints when evaluating cancer therapies.

- 1) The mode of cell death, though often not the eventual level of killing (or survival), resulting from the majority of cancer therapies (typically DNA damaging agents), is highly dependent on the genetics of the tumor cell. For example, the amount of apoptosis occurring in cells *in vitro*, or in solid tumors or in certain normal tissues can be markedly affected by Bcl-2 (2) Bax or Bak1(3), p53 or p21 (4) status, without affecting the overall level of cell killing as determined by clonogenic survival *in vitro* or by animal survival. An important consequence of this is that any particular cancer therapy could be judged to be effective or ineffective if assessed only using assays for cell death (such as apoptosis) as opposed to those measuring long term survival.
- 2) The genetic background (and mode of cell death) strongly influences the *rate* at which cells die. For example the rate of loss of viability is very different for p53 wild-type and knockout cells, a fact that can render a 24-hour trypan blue staining assay highly misleading for overall cell killing (5).
- 3) Cell viability assays will not detect cells made senescent by the treatment. Senescence, although not technically a form of cell death can be an important contributor to the effectiveness of anti-cancer agents. Senescence, along with all other forms of cell death will be captured using assays that measure cell survival.

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We believe that the above considerations are succinctly summarized by the Nomenclature Committee on Cell Death 2012 (6)“During the process of functional characterization, great attention should be paid to ensure that genetic and chemical interventions truly modify the incidence of cell death (as assessed by clonogenic cell survival), rather than activate alternative lethal pathways”.

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