Apoptosis: a clinical perspective

Dissection of the signalling pathways mediating apoptosis — controlled cell death — in cancer, inflammation and neurodegenerative disorders has stimulated the discovery of a wealth of potential drugs to target this process.

More than 140,000 articles have been published on molecules that mediate the intrinsic and extrinsic apoptotic cascades, such as BCL-2, TNF, NF-κB and p53. This interest in part stems from the substantial potential to therapeutically intervene in these pathways, as highlighted in this issue.

However, despite initial excitement over the potential of TNF as an agonist for treating cancer, clinical modulation of apoptosis has largely been limited to antagonizing TNF activity in autoimmune diseases — a US$12 billion market nevertheless. But what if we could augment T-cell apoptosis in multiple sclerosis, or inhibit stress-induced death from hypoxia in heart cells or neurons? Conversely, what if it was possible to overcome cellular survival signals in cancers overexpressing BCL-2, for example?

Promisingly, initial clinical results suggest that targeted pro-apoptotic agents such as inhibitors of BCL-2 and agonists of TRAIL receptors could be beneficial in cancer. However, while we hope to be proven wrong, so far, it does not seem that these agents will have the dramatic clinical effects and rapid approval of the prototypical molecularly targeted anticancer drug imatinib. Indeed, an antisense inhibitor of BCL-2, oblimersen, has been in clinical development for a decade without yet being approved.

Improved application of biomarkers seems likely to be the key to promoting the further development of apoptosis-targeted therapies — as it pivotally was in the measurement of C-reactive protein after anti-TNF therapy in rheumatoid arthritis. The best biomarkers are those for which there is an established physiological, pathological or pharmacological framework, which exists for proteins of the apoptotic signalling cascade. Unfortunately, biomarker studies have too infrequently been incorporated into past Phase III trials. For example, oblimersen was given with dacarbazine in a Phase III trial in melanoma, with only limited pharmacodynamic data on a relatively small number of metastatic melanoma samples that suggested BCL-2 was overexpressed. Tumour samples were not collected from patients, and although subset analyses suggest some benefit from oblimersen, no retrospective analysis to verify an association between BCL-2 expression and response can be undertaken. Subsequent studies showed that BCL-2 was not overexpressed in all melanomas and may be more abundant in melanomas of patients with a better prognosis.

Identification and assaying of the best target may not always be easy or obvious. For instance, *O*-glycosylation patterns of the TRAIL receptors may be more predictive than expression levels. Alternatively, assessment of other linked proteins such as the pro-apoptotic BAX in the case of BCL-2 or in the dual modulation of p53 and NF-κB targeting may be necessary. Nevertheless, progress in assessments is being made both by careful molecular studies of the targets, as described in this issue, and by development of more sophisticated, quantitative multi-marker technologies, such as multi-gene probe quantitative RT-PCR and automated fluorescent immunohistochemistry-based analyses (AQUA). These quantitative assays can identify patient subsets not distinguishable by more traditional methodologies such as standard immunohistochemistry, and have been applied to retrospective cohorts of patients assessing apoptotic pathways. Indeed, AQUA is now being used in Phase III clinical trials with targeted therapies.

Given an appropriate marker measurement, choice of the proper cut-off point for positivity may be critical. Setting the bar too high may restrict the population unduly and omit patients who would benefit, while setting the bar too low may dilute the treatment effect, resulting in a negative trial. One way to overcome this uncertainty is to randomize all patients, but to hedge by splitting the type I error — or false-positive rate — between the marker 'positive' patients and the entire group[1]. Another viable alternative is to initiate a trial to first define a good cut-off point and then continue with validation in subsequent patients, again by splitting the type I error.

Agents that specifically target mediators of apoptosis are most likely to be active when the mediators are both abundantly present and active in the target cells. Avoiding dismissal of potentially effective agents will need association between biomarkers and drug response in preclinical models and in Phase I trials, establishment of the prevalence of biomarker expression/activity in the disease population, subsequent selective treatment of patients most likely to respond, and identification of changes in treated patients. Selecting and applying biomarkers wisely with analytically and biostatistically insightful trial designs will be vital in realizing the full potential of targeted apoptosis modulators for cancer, inhibition of stress-induced cell death or elimination of unwanted T-cell responses.

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1. Hoering, A., LeBlanc, M. & Crowley, J. Randomized phase III trial designs for targeted agents. *Clin. Cancer Res.* **14**, 4358–4367 (2008).

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