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Interfering with programmed cell death in neurodegenerative diseases: insights from experimental animal models

In a recent issue of *Drug Discovery Today*, Waldmeier and Tatton discuss the possibility of interfering with molecular pathways of programmed cell death (PCD) as a potential therapeutic strategy to treat neurodegenerative diseases [1]. The demonstration of the actual role of PCD in neuronal cell death mainly comes from experimental animal models of neurodegenerative diseases, in which key PCD molecules have been manipulated (either in transgenic or knockout studies), or inhibited by pharmacological agents or viral vectors. This set of studies has not only identified PCD components that either promote or prevent neuronal death, but also revealed molecular targets for the development of therapeutic avenues for preventing and treating neurodegenerative disorders [2].

According to these studies, it appears that targeting PCD upstream of its execution phase consistently results in marked neuroprotection, whereas interfering at a more downstream level, such as at caspase activation, produces variable results. Thus, once the caspase executioner program is in place, its inhibition seems insufficient to actually stop the degenerative process and it can only, at best, delay cell death. Therefore, pharmacological targeting of executioner caspases, which was once regarded as a promising therapeutic strategy to potentially slow down neuronal cell death in neurodegenerative diseases, has not completely fulfilled its initial expectations. These results rather encourage the development of novel neuroprotective strategies aimed at targeting critical upstream PCD molecular events.

In this context, activation of Bax has emerged as a culprit of the demise of neurons in some experimental models of neurodegeneration [3]. Bax exists in an inactive state in the cytosol of many cells, including neurons. In response to death stimuli, the Bax protein undergoes posttranslational changes that expose membrane-targeting domains, resulting in its translocation to mitochondrial membranes, into which Bax inserts and causes a release of diverse apoptogenic factors such as cytochrome *c*, which, in turn, activates downstream executioner caspases. Thus, if Bax activation represents the critical molecular event that regulates neurodegeneration, pharmacological targeting of Bax activation should result in significant neuroprotection. However, the lack of molecular tools specifically aimed at targeting Bax activation has so far impeded the testing of this hypothesis.

Recently, two new molecules have been identified that are capable of inhibiting Bax activation by preventing its mitochondrial translocation: Bax-inhibiting peptide (BIP) and Humanin (HN) [4–6]. BIP is a membrane-permeable peptide that is comprised of five amino acids designed from the Bax-binding domain of Ku70, a protein that interacts with Bax in the cytosol and prevents its mitochondrial translocation [4]. BIP has been shown to inhibit Bax-mediated PCD induced by staurosporine, UVC irradiation and anti-cancer drugs in several types of cells [5]. By contrast, the 24-amino acid peptide HN has also been indicated as preventing the translocation of Bax from cytosol to mitochondria [6]. In particular, it has been shown that: (1) reduction of HN expression by small interfering RNAs sensitizes cells to Bax and increases Bax translocation to membranes; (2) HN peptides block Bax association with isolated mitochondria and suppress cytochrome *c* release *in vitro* [5]; and (3) HN prevents death of neuronal cells caused by multiple types of familial Alzheimer's disease genes and by Aβ amyloid [7]. BIP and HN thus represent unique tools for pharmacologically targeting Bax activation, and several studies are currently in place to assess the effect of these molecules on neuronal cell death in experimental models of neurodegeneration.

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Miquel Vila
Department of Neurology
Movement Disorders Division
Neuroscience Research
Columbia University
650 West 168th Street, BB-307
New York, NY 10032.
e-mail: mv199@columbia.edu

Multistage adaptive designs for clinical trials

In their recent article in *Drug Discovery Today* [1], Bauer and Brannath provide an excellent and comprehensive overview on several issues that evolve from the application of adaptive designs in practice.

Their focus is on two-stage designs and they mention that the generalization to multi-stage designs is straightforward. Although the essential characteristics of adaptive designs become obvious in two-stage designs, it might nevertheless be advantageous to perform more than one interim analysis. For example, it is well known from the theory of group sequential designs that the implementation of four or five stages reduces the expected number of observations more distinctly than the implementation of just two stages [2]. Adaptive designs can be regarded as a generalization of group sequential designs, therefore, this property is brought forward to adaptive designs.

The extension of group sequential tests to adaptive designs was suggested by Lehmacher and Wassmer [3] in the context of multi-stage test designs where, in principle, the weighted inverse normal method was applied within a multi-stage group sequential test design [4]. An analogous approach was independently proposed by Cui *et al.* [5], who extended the error spending approach [6] to adaptive designs. It is emphasized that the procedure of Cui *et al.* coincides with the (weighted) inverse normal method and both procedures might be used to conduct an adaptively designed clinical trial.

In multi-stage designs, it is even more difficult to assess adaptive strategies. This is particularly due to the intrinsic arbitrariness of the adaptations performed when using this methodology. However, it looks intuitively advantageous to permit more than one interim stage. For example, a design adaptation might only be performed if interim results repeatedly deviate from assumptions made in the study protocol. Interim information might mislead the experimenter in changing the design, therefore, keeping the original design and awaiting the issue of the next stage in a multi-stage design looks more attractive than having just one opportunity to change the design. Clearly, practicability limits the actual number of stages and too many changes might compromize the integrity of the study.

It is also important to keep in mind that adaptive interim analyses usually require unblinding the data and hence the integrity of the study could be jeopardized from this as well. However, when criticising adaptive designs on these grounds, one should distinguish carefully between issues that also apply to classical group sequential designs ('inherited' issues) and problems that are intrinsic to adaptive designs.

There is still little published work on clinical trials with adaptive designs. Nevertheless, many pharmaceutical companies have conducted such trials in recent years to gain practical experience with this new class of statistical designs. Obviously, it is important that suitable software packages are available for performing these designs. Among others, a comfortable software package for group sequential designs is EaSt [7]. In the most recent version, EaSt also enables the flexible monitoring of clinical trials, but a different approach than the one considered here is used. By contrast, the software ADDPLAN [8] is particularly developed for the use of the combination test principle including group sequential designs. This makes a promising methodology available to the user.

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Gernot Wassmer
Department for Medical Statistics, Informatics and Epidemiology
University of Cologne
Germany
e-mail: gernot.wassmer@medizin.uni-koeln.de

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