ADDENDUM

Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury

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In this Article[1], we described a small-molecule inhibitor of necroptosis, termed Necrostatin-1 (Nec-1). Since the original publication, additional data regarding the properties of Nec-1 have been reported, including off-target activity and metabolic stability in mice, that are important in designing *in vitro* and, especially, *in vivo* experiments with Nec-1.

Teng *et al.*[2] reported an optimized derivative of Nec-1, termed 7-Cl-O-Nec-1 (**66** in ref. 2), that was used in ref. 1 to demonstrate the protection in an ischemic brain injury model. This molecule showed higher activity in inhibiting necroptosis in Jurkat cells than Nec-1 (EC_{50} = 210 nM versus EC_{50} = 490 nM), no nonspecific cytotoxicity at high concentrations (~100 μM) and reasonable pharmacokinetic characteristics following intravenous administration in mice. Degterev *et al.*[3] subsequently reported that Nec-1 shows limited metabolic stability, which is substantially improved with 7-Cl-O-Nec-1. Takahashi *et al.*[4] also reported that Nec-1 showed paradoxical toxicity at lower, but not higher, doses in a mouse model of systemic inflammatory stress syndrome (SIRS). No such toxicity was observed with 7-Cl-O-Nec-1. Thus, for in-cell and *in vivo* experiments, we recommend the use of 7-Cl-O-Nec-1.

Muller *et al.*[5] reported that Nec-1, also known by its chemical name of methylthiohydantoin-tryptophan, is a micromolar inhibitor of indolamine 2,3-deoxygenase (IDO) with EC_{50} = 11.4 μM in a cell-based assay. Thus, given the ~20-fold higher activity of Nec-1 in a necroptotic assay, the use of lower concentrations of this molecule could be helpful in distinguishing between inhibition of necroptosis and IDO-related processes. Another known inhibitor of IDO, 1-methyl-DL-tryptophan, lacks activity against necroptosis as reported by both Degterev *et al.*[3] and Takahashi *et al.*[4] Notably, both reports show that optimized 7-Cl-O-Nec-1 lacks activity against IDO. Overall, potential nonspecific toxicity, inhibition of IDO and limited stability of Nec-1 should be taken into account when the molecule is used *in vivo*, whereas 7-Cl-O-Nec-1 lacks these liabilities and thus represents a superior choice for *in vivo* studies.

References

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CORRIGENDUM

Conformational stabilization of ubiquitin yields potent and selective inhibitors of USP7

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In the version of this article initially published, the authors neglected to acknowledge an important collaborator and to include a citation of their related work. The error has been corrected in the HTML and PDF versions of the article.