Reviewer's report

Title: JS-K, a glutathione/glutathione S-transferase-activated nitric oxide releasing prodrug inhibits androgen receptor and WNT-signaling in prostate cancer cells

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Reviewer: Ralph Buttyan

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Minor Essential Revisions:
1) The article is fairly well-written; authors should correct minor spelling errors
First page of Background "....analoga/blocker" should be analog/blocker or analogue/blocker, the former being a US specific spelling whereas the latter is acceptable in all English spelling rules.
Page 13 Discussion: "dramatical" should be dramatic

2) In the second paragraph of Background the authors state: "Within a few years almost all PCa progress to a state of the disease termed castration resistant prostate cancer...". This is an exaggeration. While MOST treated patients do progress to CRPC, there is a significant fraction of patients for whom advanced disease is suitably controlled by hormone therapy and other patients in which hormone therapy can halt progression for many many years. The "almost all" statement is hyperbole and should be modified to reflect the clinical situation.

Discretionary Revisions:
1) The study represents very small preclinical evaluation of a novel NO generating pro-drug for prostate cancer. The limited yet divergent nature of the cell lines used in the study raise some concerns. Androgen-receptor- (AR-) less PC3 cells transfected with wildtype AR were used only for studies of effects of the drug on AR translocation whereas 2 other cell lines (LNCaP and CWR22rv1) were used to test for effects of drug on cell proliferation and AR expressions and activities and both of these cell lines express (different) mutant ARs. Given the highly divergent nature of these cell lines, one can not be sure that the observations made can be suitably generalized. Moreover, the investigators failed to test their drug for anti-proliferative effects on the AR-less PC3 parental cells. If PC3 cells are inhibited to the extent of the CWR22rv-1 cells then one might conclude that drug action on the AR is irrelevant to the growth suppressive effects of the drug for prostate cancer.

2) The investigators also test the effects of their drug on AR signaling and on "canonical" Wnt signaling through beta-catenin. In fact, beta-catenin is not only a co-activator of LEF-1/TCF (as was tested using the TOP/FOP reporters) it is also a well known co-activator of AR so, if the drug is affecting beta-catenin availability, the effects on AR activity may be mediated primarily by suppression
of beta-catenin activity. The authors should discuss this possibility.

3) Likewise, LEF-1/TCF (β-catenin) mediates primary expression of androgen receptor through interaction with LEF/TCF binding sites in the AR promoter (Yang, et al, Oncogene, vol 25, page 3436, 2006) and this certainly should be a point of discussion especially since the drug suppresses AR expression in CWR cells that has endogenous beta-catenin/LEF/TCF activity whereas LNCaP do not.

4) Given the nature of the drug as a potential effector of many nuclear transcription factor families, it will require carefully designed experimentation using cell lines of common origin with or without androgen receptor expression to identify the extent to which the effect of the drug on any particular prostate cancer cell line is specifically related to its effect on AR alone.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.