Author’s response to reviews

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

Authors:

Martin Laschak (martin.laschak@uni-ulm.de)
Klaus-Dieter Spindler (kmspindler@t-online.de)
Andres J Schrader (ajschrader@gmx.de)
Andrea Hessenauer (andrea.hessenauer@web.de)
Wolfgang Streicher (wolfgang.streicher@uni-ulm.de)
Mark Schrader (mark.schrader@uniklinik-ulm.de)
Marcus V Cronauer (marcus.cronauer@uni-ulm.de)

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Author’s response to reviews:

Dear Editor,

Please find attached our revised manuscript entitled > JS-K, a glutathione/glutathione S-transferase-activated nitric oxide releasing prodrug inhibits androgen receptor and WNT-signaling in prostate cancer cells < by Martin Laschak et al.

We have thoroughly revised our manuscript according to the reviewer’s comments. We hope that our manuscript is now suitable for publication in BMC Cancer and look forward to hearing from you soon,

Sincerely,

Marcus V. Cronauer, Ph.D
Ulm University
Department of Urology
e-mail: marcus.cronauer@uni-ulm.de

Reviewer 1

Changes in the manuscript are highlighted in red.

MAJOR COMPULSORY REVISIONS:
Comment 1: Statistical analysis in figures 2 und 6 is inappropriate. The authors need to take into account repeated measures.

Answer 1: Data are now the mean of 3 independent experiments performed in quadruplicates.

Comment 2: The WNT-studies are interesting but not justified in the Introduction and discussion. They are not additive to the story around androgenic function at least based on manuscript as written…….

Answer 2: We added an additional paragraph in the discussion linking the dramatic downregulation of AR and AR-V in 22Rv1 following JS-K treatment to the inhibition of the WNT-signalling pathway.

Comment 3: The authors need to comment on the rationale for using different cell lines........these studies (Fig 3 +4) should preferably be repeated in the other cell lines to confirm their suppositions on mechanism.

Answer 3: Dimerization and translocation experiments (Fig 3+4) were redone in an additional PCa cell line (DU-145) to rule out cell line i.e PC-3 specific effects – additional files Fig 1S and 2S

MINOR REVISIONS

Comment 4: reference 45 is described incorrectly with 29 patients involved not 17.

Answer 4: The number of patients in reference 45 (now ref. 51) is corrected to 29.

Comment 5: The authors should comment at least the limitations of the MTT assay....

Answer 5: Limitations of the MTT assay were added/mentioned in the results (see: Growth inhibitory effects of JS-K are most pronounced CPRC cells)

Reviewer 2

Changes in the manuscript are highlighted in red.

MINOR ESSENTIAL REVISIONS

Comment 1: First page of background:....(analog/a/blocker....page 13: Discussion...(dramatical)
Answer 1: analoga was replaced by analog……dramatical was changed to dramatic

Comment 2: within a few years (almost all patients) progess to a state of the disease….

Answer 2: The term >almost all< was replaced by >many<

DISCRETIONARY REVISIONS

Comment 1: ….the limited yet divergent nature of the cell lines used in the study raise some concerns…..AR negative PC were used for translocation whereas LNCaP and 22Rv1 were used on cell proliferation and AR-expression….given the highly divergent nature of these cell lines one cannot be shure that the observations made can be generalized……moreover the investigators failed to test their drug for anti-proliferative effects on the AR-less PC3……

Answer 1: Translocation as well as AR-dimerization experiments were additionally performed in DU-145 to rule out PC-3 specific effects. Additional proliferation studies using the AR-negative DU-145 as well as the AR-positive LNCaP (hormone-dependent) and LNCaP-SSR (castration resistant) were done showing that (a) AR-negative DU-145 are less sensitive to NO than AR-positive LNCaP and/or LNCaP-SSR and (b) the statement CRPC cells are more sensitive to the effects of NO (comparing LNCaP to 22Rv1) was extended by comparing the hormone dependent LNCaP to a castration resistant LNCaP-subline – LNCaP-SSR.

Comment 2: ….if the drug is affecting beta catenin availability, the effects of AR activity may be mediated primarily by suppression of beta catenin activity…the authors should discuss this possibility.

Answer 2: The fact that #-catenin is involved in AR-signalling is mentioned in the introduction to < JS-K inhibits WNT/#-catenin signaling in 22Rv1 cells>.

Comment 3: LEF/TCF mediates primary expression of AR…..this certainly should be a point of discussion

Answer 3: The canonical WNT-pathway was shown to modulate AR-mRNA (Yang et al, Oncogene 2006). Interestingly, JS-K suppresses the AR-protein particularly in 22rv1 cells that display a functional WNT/#-catenin signalling pathway and not in LNCaP where the pathway is malfunctioning. As suggested by the reviewer we added an additional paragraph in the discussion part.

Comment 4: ........using cell lines of common origin to identify which effect of the drug is due to a modulation of the AR
Answer 4: In proliferation studies we used the AR-negative DU-145 as well as the AR-positive LNCaP (hormone-dependent) and LNCaP-SSR (castration resistant) showing that:

(I) DU-145 are less sensitive to NO than the AR-positive LNCaP, LNCaP-SSR or 22Rv1 (this paper and Oncogene 26, 1875, 2007).

(II) the CRPC cell lines (LNCaP-SSR, 22Rv1) are more sensitive to JS-K than LNCaP.

We speculate that the higher susceptibility of CRPC cells towards JS-K can be explained by a modulation of AR-activity: (a) 22Rv1 express a constitutively active AR-V (b) in the absence of androgenic stimuli the full length AR of LNCaP-SSR is predominantly nuclear and probably active (PloS One 2011;6(9):e25341). Both cell lines 22Rv1 and LNCaP-SSR were shown to be dependent on their AR-V or AR for growth and survival in the absence of androgens. We hypothesize that the CRPC cells are more sensitive to the effects of NO because NO targets the zinc fingers of AR as well as AR-V.