

Reviewer #1: Analysis of ctDNA is one of the most promising tool to monitor cancer diseases. For prostate cancer, ctDNA is a very interesting biomarker for the anticipation of PFS and OS, in response to therapies and for improving the clinical management of patients avoiding overtreatments. The high concordance between ctDNA genomic alterations and those found in tumor tissue biopsies strongly supports the potential of liquid biopsy to integrate clinical data and improve patient's management. However, how about its specific biomarker for prostate cancer?

Reply

Thank you very much for the observation. The question is a very interesting and it is an important matter to mention in the review.

PSA is the biomarker approved for men by FDA in 1986 and from then on, it has been widely used to predict incidence and recurrence of prostate cancer, despite its poor specificity. However, in mCRPC the PSA seems to be more specific as biomarker than in the onset of prostate cancer; substantially, its increase is related to cancer progression (74). The significance of PSA measurements in mCRPC is still interesting for the scientific community and a lot of studies have been published. For example, Aggarwal et al. have recently demonstrated that low PSA secretion levels can stratify mCRPC patients with treatment-emergent small-cell neuroendocrine prostate cancer (t-SCNC). In fact, low PSA secretors showed high t-SCNC, RB1 and TP53 loss and low AR transcription. In addition, OS and PFS were shorter in the low PSA secretor's group (75). In a retrospective study, Buttiglieri et al. have showed that early PSA drop was related to a better OS and PFS in mCRPC patients treated with abiraterone or enzalutamide (docetaxel-naïve or post-docetaxel setting) (76). Finally, a mathematical model of PSA dynamics has been just proposed to predict individual response to intermittent androgen deprivation therapy (77)

In our opinion, PSA can play an important role as biomarker for the management of mCRPC patients. However, PSA measurements could maintain some limitations due to the high individual variability. Liquid biopsy on circulating cell-free nucleic acids instead, offers the same low invasiveness, but also important molecular details on each specific tumor heterogeneity evolution. In conclusion, liquid biopsy on circulating cell-free nucleic acids along with PSA measurements and other clinical data, can assure the best treatment decision-making for mCRPC patients.

This comment has been added to the revised manuscript in the Future perspective section at pages 7-8 (written in red), with the appropriate references.

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75. Buttiglieri C, Tucci M, Sonetto C, Vignani F, Di Stefano RF, Pisano C, Turco F, Lacidogna G, Guglielmini P, Numico G, Scagliotti GV, Di Maio M. Prognostic role of early PSA drop in castration resistant prostate cancer patients treated with abiraterone acetate or enzalutamide. *Minerva Urol Nefrol.* 2020, 10: [PMID: 32284527 DOI: 10.23736/S0393-2249.20.03708-X]

76. Brady-Nicholls R, Nagy JD, Gerke TA, Zhang T, Wang AZ, Zhang J, Gatenby RA, Enderling H. Prostate-specific antigen dynamics predict individual responses to intermittent androgen deprivation. *Nat Commun.* 2020, 11:1750[PMID:32273504 DOI: 10.1038/s41467-020-15424-4]