Peer Review File

Review Comment
The authors are congratulated on a well written overview of the topic. I have only minor points for attention:

Comment 1: in the Introduction and abstract, I miss the rarity of the disease as cause for lack of translational research. While it is stated as a cause for lack of pharma interest, it also impairs tissue collection and correlation with clinical outcomes as it is difficult to gather enough data to generate statistically meaningful outcomes. (correction: is discussed under molecular profile, but would be good to shift up as general remark)

Response: As suggested we have added the following paragraph in the Abstract on page 2:
Preclinical experimental models are indispensable for the evaluation of tumor biology and identification of genomic alterations. However, since neither commercial PeSCC cell lines are available nor xenograft models are sustainably established, such analyses are challenging in the field of research. In addition, systemic therapies are less effective and toxic without decisive breakthroughs for years. Current systemic management of PeSCC is based on protocols that have been investigated in small series of up to 30 patients. Thus, there is an unmet medical need for new approaches necessitating research efforts to develop more efficacious systemic strategies.

In the Introduction section, we have included:
Besides causing a lack of awareness, the rarity of the disease impedes an adequate conduct of translational research. It almost seems impossible to gather enough patients ensuring pertinent tissue biobanking and providing studies with sufficient statistical power, both compulsory requirements for identification and approval of new therapeutic regimes.

Comment 2: "Overall, these studies showed significant differences in the genomic profiles of PeSCC positive and negative for HPV infection, highlighting the message that these tumors should be treated differently. " this is quite a bold statement not backed up by solid data. Yes there obviously is a different genomic profile, and this may (probably will) require a different treatment strategy. Please play it down somewhat.
Response: As suggested, we modified this paragraph accordingly:
Overall, these studies showed significant differences in the genomic profiles of PeSCC according to HPV infection status, suggesting that these tumors probably require different treatment strategies.

Comment 3: "However, the results obtained to date are encouraging, showing that patients can benefit from target-specific treatments", again, a statement is made on treatment based on expression/mutation data only. Please reduce to hypothetical statement.

Response: We agree with the reviewer's point of view. The sentence was modified, as suggested:

However, the results obtained to date are encouraging, leading to the reasoning that patients potentially can benefit from target-specific treatments.

Comment 4: Under the models section, this recent reference has been overlooked and should be discussed in the xenograft discussion: *Thomas A, Vanthoor J, Himmelreich U, et al. Establishment, Characterization, and Imaging of a First Platinum-resistant Penile Cancer Patient-derived Xenograft in Nude Mice: A eUROGEN Project. Eur Urol. 2020;78(2):294-296. doi:10.1016/j.eururo.2020.05.033.* Also the following reference has been quoted but warrants more elaborate discussion (first use of single cell in PeSCC etc, first preclinical report on immune, drawbacks of monoclonal model) *Huang T, Cheng X, Chahoud J, et al. Effective combinatorial immunotherapy for penile squamous cell carcinoma. Nat Commun. 2020;11(1):2124. Published 2020 May 1. doi:10.1038/s41467-020-15980-9*

Response: We modified this section, as suggested:

The first platinum-resistant penile cancer-patient derived xenograft (NOD/SCID/ IL2λ-receptor null mice) was recently established and characterized (52). Small animal imaging was used as proxies for therapeutic efficacy providing further output on tumor perfusion and metabolic activity. Humanized mice models are exciting alternatives to testing immune checkpoint blockade (52).

The reference of Huang T, et al. from Nat Commun was further elaborated in the same paragraph, as follows:

A pioneer study described the generation and characterization of the first genetically engineered mouse models of PeSCC (SA: PB-Cre4+ Smad4L/L ApcL/L and SAP: PB-Cre4+ Smad4L/L ApcL/L PtenL/L mice) (55). The authors showed that a single knock-out model was insufficient to drive penile tumorigenesis, only achieving success by applying Smad4 and Apc co-deletion in the androgen-responsive epithelium of the penis (55). The murine PeSCC presented gene signatures comparable with those described in humans. The single-cell analysis revealed an intratumoral immunosuppressive myeloid cell infiltration in the SA mice. A randomized pre-clinical trial using these models and immune-checkpoint inhibitors with or without targeted therapy showed that tumor eradication was achieved only upon combining different drugs. This study presented a valuable platform for testing and discovering treatment strategies, and results obtained by the authors suggested that combined target therapy and immunotherapy could be used in the treatment of PeSCC patients.

Comment 5: Rather than a clinical discussion of results in different chemo regimen, a discussion on why penile cancer responds so poorly would be of great interest to the readership.

Response: In order to address this issue, we have added the following sentence on pages 13-14:

Platinum-based agents, in particular cisplatin, are undoubtedly the mainstay of the current systemic treatment strategies of PeSCC alongside a wide range of other solid neoplasms. One of the most pertinent mechanisms of its anticancer activity is the induction of DNA lesions promoting activation of the DNA damage response and induction of mitochondrial apoptosis and subsequent cell death (60). Hereby, cisplatin resistance can rest upon alterations 1) in processes that predate its binding to DNA and cytoplasmic structures (pre-target resistance), 2) directly related to the molecular damage caused by cisplatin (on-target resistance), 3) in the lethal signaling pathways triggered by such molecular lesions (post-target resistance) and 4) influencing molecular mechanisms not associated with cisplatin-elicited signals (off-target resistance) (61). Notably, cancers with TP53 mutated respond worse to cisplatin than those harboring TP53 wild type as the post-target mode of cisplatin resistance (60). In turn, overactivation of ERBB2, consequently stimulating PI3K/AKT signaling, has been identified as the off-target mechanism of chemoresistance in several malignancies, e.g., non-small cell lung and gastric cancer (62, 63). Additionally, PTEN deficiency has been reported to confer cisplatin resistance in PeSCC (55). As outlined above, these molecular alterations are frequently encountered in PeSCC, at least partially elucidating its poor responsiveness to cisplatin and emphasizing the need for effective combination protocols targeting different drug resistance pathways.

Additional Comments: We changed the reference numbers in two pages (highlighted in grey).