PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Efficacy of Neoadjuvant Atezolizumab Treatment in Patients with Advanced Urothelial Bladder Cancer According to the BASQ Classification: Study Protocol for an Open-label, Two cohorts, Phase II trial
AUTHORS	Yuk, Hyeong Dong; Jeong, Chang Wook; Kwak, Cheol; Kim, Hyeon; Moon, Kyung Chul; Ku, Ja Hyeon

VERSION 1 – REVIEW

REVIEWER	Rafael Morales-Barrera Vall d' Hebron University Hospital Catalonia, Spain
REVIEW RETURNED	02-Dec-2019

GENERAL COMMENTS	The authors should add the reference of ABACUS trial (Nature 2019)

REVIEWER	Remco Molenaar Amsterdam UMC
REVIEW RETURNED	07-Feb-2020

GENERAL COMMENTS	The authors provide a protocol of an interesting clinical trial that investigates the efficacy of neoadjuvant atezolizumab in urothelial bladder cancer patients while attempting to associate this efficacy to the molecular cancer subtype (basal or luminal). While this protocol is of interest and potentially warrants publication, some issues need to be addressed: - It is unclear what the authors' hypothesis is with regard to the basal/luminal type and atezolizumab response. Based on the molecular and clinical features of these molecular subtypes, which subtype will have a better response after atezolizumab treatment? - This is a non-randomized study in which all patients will receive neoadjuvant atezolizumab. While this not necessarily complicates its statistical analysis and interpretation, the protocol deserves to include more information on the biological and clinical characteristics of the basal and luminal subtypes. Are they equally malignant, or is one subtype associated with a longer overall survival, longer progression-free survival and/or better response to the current standard of care? - The authors confusingly use the terms "cohort" and "arm" interchangeably in this protocol. In my opinion, an "cohort" is more appropriate here than "arm" because all patients receive the same treatment. - The term "UBC" should be defined in the abstract since this is not a specialized urology journal.

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	- In the strengths and limitations, the authors use the past tense. Perhaps the present tense is more appropriate? In general, the authors should carefully assess the appropriate use of past and present and future tense in the manuscript. - Strengths and limitations: “1. It was the first open label, two cohort, phase II trial to explore the effect of neoadjuvant immune check point inhibitors on molecular subtypes.” Is this statement true for just bladder cancer or for all malignancies? - Strengths and limitations: “The differences between the two groups allow you to compare the effects” This is a colloquialism. - Introduction: “Several treatments are currently being studied, and eventually one day, the first-line neoadjuvant treatment for advanced UBC will be immune check point inhibitors instead of cisplatin-based chemotherapy 6.” This statement seems premature because the clinical trials have not been analyzed yet. - In the sample size considerations, the authors should specify that they aim to include 20 patients with basal subtype and 20 patients with luminal subtypes (at least, that is what I understood from Figure 1). What was the proportion of basal and luminal tumours in the TCGA publication? Is this proportion applicable to the Korean population? Is it realistic to enroll 20 patients of each subtype within approximately the same time period? - The endpoint/inclusion criteria table is oddly placed in the middle of the references. - Why is the CT and X-ray performed after 2 cycles whereas the atezolizumab treatment period is 3 cycles?

REVIEWER	Haris Zahoor Norris Comprehensive Cancer Center of USC, Los Angeles, USA
REVIEW RETURNED	16-Feb-2020

GENERAL COMMENTS	I would like to congratulate authors for their efforts to answer an important research/clinical question regarding the role of BASQ classification in predicting response to IO therapy, in a prospective fashion. This is a phase II trial of neoadjuvant atezolizumab in MIBC. The spirit of this trial is exploratory as evident by statistical analyses section. Investigators have planned to enroll 40 patients and perform biomarker analyses. I have the following comments/suggestions/concerns for the authors. If the primary objective of the trial is evaluating treatment response to atezo, why authors chose primary endpoint of ORR in ITT population ? How is is this different than ABACUS or PURE 01 studies ? What is the rationale for sample size calculation ? It appears it is more of feasibility/pilot study. If so, what is the rationale for 40 patients and dividing into 2 groups ? Similarly, although its exploratory in nature, I would still prefer to have an underlying hypothesis for the study. For example, what do authors suspect in terms of differential response in BASQ subtypes ? Why authors think 3 cycles of atezo better are than 2 as compared to ABACUS study?

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	I will encourage the authors to provide more concise discussion re BASQ classification and differential response to IO therapy reported in literature. Please provide exact details re the BASQ classification employed in this trial.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name
Rafael Morales-Barrera

Institution and Country
Vall d' Hebron University Hospital
Catalonia, Spain

Please state any competing interests or state 'None declared':
None declared

Please leave your comments for the authors below

Thank you for your letter and the reviewers' helpful comments regarding our manuscript. We were pleased to note the favorable comments in reviewers' opening remarks. We have carefully reviewed their comments and made the necessary changes to the original manuscript, which are indicated by underlined, blue font in the revised manuscript.

The authors should add the reference of ABACUS trial (Nature 2019)
▶Thank you for this helpful comment. We have added the reference 8 and citations

Reviewer: 2
Reviewer Name
Remco Molenaar

Institution and Country
Amsterdam UMC

Please state any competing interests or state 'None declared':
None declared

Please leave your comments for the authors below
The authors provide a protocol of an interesting clinical trial that investigates the efficacy of neoadjuvant atezolizumab in urothelial bladder cancer patients while attempting to associate this efficacy to the molecular cancer subtype (basal or luminal). While this protocol is of interest and potentially warrants publication, some issues need to be addressed:

Thank you for your letter and the reviewers' helpful comments regarding our manuscript. We were pleased to note the favorable comments in reviewers' opening remarks. We have carefully reviewed

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their comments and made the necessary changes to the original manuscript, which are indicated by underlined, blue font in the revised manuscript.

- It is unclear what the authors' hypothesis is with regard to the basal/luminal type and atezolizumab response. Based on the molecular and clinical features of these molecular subtypes, which subtype will have a better response after atezolizumab treatment?

▶Thank you for this helpful comment. We have added the sentence in page8, line 146

The basal type of MIBC patients is expected to have better efficacy and clinical response of the neoadjuvant atezolizumab treatment than the luminal type.

- This is a non-randomized study in which all patients will receive neoadjuvant atezolizumab. While this not necessarily complicates its statistical analysis and interpretation, the protocol deserves to include more information on the biological and clinical characteristics of the basal and luminal subtypes. Are they equally malignant, or is one subtype associated with a longer overall survival, longer progression-free survival and/or better response to the current standard of care?

▶Thank you for this helpful comment. The basal and luminal types can be said to be divided into subtypes based on keratin markers in the same malignant bladder cancer. As reported in the literature, the basal type is reported to have a relatively poor prognosis and poor chemotherapy response. It is also known to be relatively disadvantageous in survival.

- The authors confusingly use the terms "cohort" and "arm" interchangeably in this protocol. In my opinion, an "cohort" is more appropriate here than "arm" because all patients receive the same treatment.

▶Thank you for this helpful comment. We have corrected the use of the term in Figure 1

- The term "UBC" should be defined in the abstract since this is not a specialized urology journal.

▶Thank you for this helpful comment. We have added the full term of UBC in abstract

The atezolizumab has become the standard therapy for urothelial bladder cancer (UBC) patients who are not responding to cisplatin-based chemotherapy and is also used as a first-line treatment in cisplatin-ineligible patients.

- In the strengths and limitations, the authors use the past tense. Perhaps the present tense is more appropriate? In general, the authors should carefully assess the appropriate use of past and present and future tense in the manuscript.

▶Thank you for this helpful comment. We have revised the past tenses in the strengths and limitations. And once again we reviewed the present and past tense of the whole manuscript.

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- Strengths and limitations: “1. It was the first open-label, two cohort, phase II trial to explore the effect of neoadjuvant immune check point inhibitors on molecular subtypes.” Is this statement true for just bladder cancer or for all malignancies?

▶Thank you for this helpful comment. We have revised the sentence to bladder cancer only in page5, line 90

1. It is the first open-label, two cohort, phase II trial to explore the effect of neoadjuvant immune check point inhibitors on molecular subtypes in UBC.

- Strengths and limitations: “The differences between the two groups allow you to compare the effects” This is a colloquialism.

▶Thank you for this helpful comment. We have revised the sentence in page 5, line 95

It compares the effects of neoadjuvant atezolizumab treatment on molecular subtypes through the differences between the two cohorts.

- Introduction: “Several treatments are currently being studied, and eventually one day, the first-line neoadjuvant treatment for advanced UBC will be immune check point inhibitors instead of cisplatin-based chemotherapy 6.” This statement seems premature because the clinical trials have not been analyzed yet.

▶Thank you for this helpful comment. We have revised the sentence in page 6, line 107

Several treatments are currently being studied, and in addition to cisplatin-based chemotherapy as the first-line neoadjuvant treatment for advanced UBC, it is reported that the benefits of immune check point inhibitors are positive

- In the sample size considerations, the authors should specify that they aim to include 20 patients with basal subtype and 20 patients with luminal subtypes (at least, that is what I understood from Figure 1). What was the proportion of basal and luminal tumours in the TCGA publication? Is this proportion applicable to the Korean population? Is it realistic to enroll 20 patients of each subtype within approximately the same time period?

Until recently, the number of patients registered was 23. 12 Basal, 11 luminal. We are enrolled in almost similar rate. It's not a large amount of prospective research, so we don't know the proper proportion of the Korean population. Based on our institutional data, in a previous study retrospectively analyzed of 100 patients with bladder cancer, the ratio of the basal type was relatively high with a ratio of 6: 4. However, it is not possible to exclude the bias in the selection of the distribution by selecting and performing samples with good staining. (Yuk et al. Clinical outcomes of muscle invasive bladder Cancer according to the BASQ classification. BMC Cancer 19, 897 (2019). DOI: 10.1186/s12885-019-6042-1)

Recent multicenter studies in western population show luminal papillary (24%), luminal nonspecified (8%), luminal unstable (15%), stroma-rich (15%), basal / squamous (35%), and neuroendocrine-like (3%). (Kamoun et al. A Consensus Molecular Classification of Muscle-invasive Bladder Cancer. Eur urology Eur Urol. 2019 Sep 26. DOI: 10.1016/j.eururo.2019.09.006)

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In another study, luminal ratios are around 34%. (de Jong et al. A Genomic Classifier for Predicting Clinically Aggressive Luminal Bladder Tumors with Higher Rates of Pathological Upstaging. J Urol. 2020 Feb 14, DOI: 10.1097/JU.0000000000000798)

- The endpoint/inclusion criteria table is oddly placed in the middle of the references.

▶Thank you for this helpful comment. There seems to have been a mistake. Fixed it behind the reference.

- Why is the CT and X-ray performed after 2 cycles whereas the atezolizumab treatment period is 3 cycles?
▶Thank you for this helpful comment. The effectiveness of neoadjuvant immunotherapy can be judged by pCR or downstaging or upstaging after surgery. Therefore, we planned a CT scan 2 cycles later, in case it would help to judge patients who did not respond to neoadjuvant immunotherapy earlier. In the final analysis, we planned to analyze whether CT, which is performed after 2 weeks, helps to exclude the ineffective group.

Reviewer: 3
Reviewer Name
Haris Zahoor

Institution and Country
Norris Comprehensive Cancer Center of USC, Los Angeles, USA

Please state any competing interests or state ‘None declared’:
NONE

Please leave your comments for the authors below
I would like to congratulate authors for their efforts to answer an important research/clinical question regarding the role of BASQ classification in predicting response to IO therapy, in a prospective fashion.

This is a phase II trial of neoadjuvant atezolizumab in MIBC. The spirit of this trial is exploratory as evident by statistical analyses section. Investigators have planned to enroll 40 patients and perform biomarker analyses. I have the following comments/suggestions/concerns for the authors.

Thank you for your letter and the reviewers’ helpful comments regarding our manuscript. We were pleased to note the favorable comments in reviewers’ opening remarks. We have carefully reviewed their comments and made the necessary changes to the original manuscript, which are indicated by underlined, blue font in the revised manuscript.

If the primary objective of the trial is evaluating treatment response to atezo, why authors chose primary endpoint of ORR in ITT population ? How is this different than ABACUS or PURE 01 studies ?
Unlike Abacus and PURE01 study,

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▶Thank you for this helpful comment. Unlike Abacus and PURE01 study, first, the purpose of the study is different. ABACUS and PURE-01 studies are about the effects of neoadjuvant immunotherapy. The purpose of our study is to select a target group for which neoadjuvant chemotherapy may be more effective. Secondly, it is a two cohort study that is prospectively divided into molecular subtypes. The ABACUS and PURE-01 studies are single-arm studies. In the ABACUS, it is classified retrospectively as TCGA to suggest effective molecular subtype. Third, there is a difference between the drug used and the regimen used. PURE01 study is pembrolizumab 3cycle, ABACUS study is atezolizumab 2cycle.

What is the rationale for sample size calculation ? It appears it is more of feasibility/pilot study. If so, what is the rationale for 40 patients and dividing into 2 groups ?

▶Thank you for this helpful comment. This study is an exploratory concept. To maximize the ease and feasibility of the research process, To detect a difference with 80% power and 5% significance level, we needed at least about 15 patients per cohort.

Similarly, although its exploratory in nature, I would still prefer to have an underlying hypothesis for the study. For example, what do authors suspect in terms of differential response in BASQ subtypes ?
▶Thank you for this helpful comment. We have added the sentence in page8, line 146

The basal type of MIBC patients is expected to have better efficacy and clinical response of the neoadjuvant atezolizumab treatment than the luminal type.

Why authors think 3 cycles of atezo better are than 2 as compared to ABACUS study?
▶Thank you for this helpful comment. The PURE 01 study used pembrolizumab 3 cycles and showed 42% pCR. And the ABACUS study used atezolizumab 2 cycles and showed 31% pCR. We can think that the effect is more increase as the cycle increases due to these two results and the characteristics of immunotherapy, and we expected that 3 cycles will be more effective than 2 cycles. And, as additional data accumulates, cisplatin-based neoadjuvant chemotherapy and immunotherapy are planned for comparative analysis. To determine which patient group is suitable for neoadjuvant chemotherapy or immunotherapy and whether it is advisable to perform immediately cystectomy. For this reason, it is suitable to compare after the execution in the same cycle, so we implemented it in 3 cycles.

I will encourage the authors to provide a more concise discussion re BASQ classification and differential response to IO therapy reported in the literature.
▶Thank you for this helpful comment. We revised the discussion concisely.

Please provide exact details re the BASQ classification employed in this trial.
▶Thank you for this helpful comment. We added the sentence in study population Pge13, Line 277

Atezolizumab is a humanized monoclonal anti-PD-L1 antibody and has demonstrated efficacy and safety as a first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic bladder cancer in the Phase II IMvigor210 trial 16. The primary outcome was an objective response rate of 23.5% (95% confidence interval [CI]: 16.2%-32.2%) in the group receiving atezolizumab 16. Researchers also performed a subgroup analysis of the effect of atezolizumab on the molecular UBC

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subtype14. Nivolumab showed the most effective drug response in the basal I subtype in the CheckMate 275 trial17. The luminal I subtype was low in PD-L1 and low in response to atezolizumab and nivolumab in tumor cells 16 17.

Genetic analysis of urothelial carcinoma found TCGA subtypes such as luminal and basal subtypes and TCGA clusters I to IV 10 11. However, the classification of molecular subtyping presents various criteria for each study, making it difficult to standardize the classification of TCGA subtypes 10-13 15. In the discussion of this classification, several researchers have defined Basal/Squamous-Like (BASQ) as a molecular subtype classification of bladder cancer.

The basal type is associated with a high resistance to chemotherapy and poor prognosis 14. This molecular subtype of urothelial carcinoma is related to cell differentiation and is distinguished as basal and luminal type by a keratin marker 11 18. The basal type is characterized by a high molecular weight keratin, which represents the basal and stem cell compartment, and the luminal type keratin represents the umbrella cell compartment 11 18.

VERSION 2 – REVIEW

REVIEWER	Remco Molenaar Amsterdam University Medical Centres, Amsterdam, The Netherlands
REVIEW RETURNED	31-Mar-2020

GENERAL COMMENTS	The authors have satisfactorily included the comments that were raised by me and the other reviewers.

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