PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	A novel sequential treatment strategy for patients with muscle-invasive bladder cancer (MIBC): intravesical recombinant BCG, followed by neoadjuvant chemo-immunotherapy, radical cystectomy plus pelvic lymphadenectomy, and adjuvant immunotherapy. Protocol of a multicenter, single arm phase 2 trial (SAKK 06/19).
AUTHORS	Petrausch, Ulf; Spahn, Martin; Schneider, Martina; Hayoz, Stefanie; Rentsch, Cyrill A.; Rothschild, Sacha; Omlin, Aurelius; Cathomas, Richard

VERSION 1 – REVIEW

REVIEWER	Mancini, Mariangela University of Padua, Surgical, Oncological and Gastroenterological Sciences
REVIEW RETURNED	21-Oct-2022

GENERAL COMMENTS	I find this protocol really novel and very interesting. However, I would like to ask the authors to address a few points in the text, before accepting this paper for publication in BMJ Open. The points are detailed in the text below. TITLE: The most important novelty of this protocol, which is also its main strength, is that the sequential treatment strategy proposed by the Authors, has never been tried before for patients with non-metastatic MIBC. Moreover, this strategy responds to a clear and urgent need of patients with MIBC, which is to improve the results, in terms of survival, or radical cystectomy and pelvic lymphadenectomy, which is still the standard of care for patients with non-metastatic muscle invasive disease. Therefore, I think the correct sequence of the reported protocol should be clearly stated in the title. A proposed change for the title, which would make it more accurate and adherent to reality is: “A novel sequential treatment strategy for patients with muscle-invasive bladder cancer (MIBC): intravesical recombinant BCG, followed by neoadjuvant chemo-immunotherapy, radical cystectomy plus pelvic lymphadenectomy, and adjuvant immunotherapy. A multicenter, single arm phase 2 trial (SAKK 06/19).” I understand that the title proposed by the Authors is the same of their official protocol. But since they are proposing the manuscript for a peer-reviewed publication in BMJ open, the title should be more explicative and accurate for the general readers of the journal. The fact that radical cystectomy and pelvic lymphadenectomy (which kind of lymphadenectomy will be performed? Standard?

	Extended? This should also be clarified), are part of the sequential treatment, is not a secondary point. The fact that, as the Authors state later in the text, “the design of the trial is to pave a scientific way to avoid radical cystectomy in the future” does not have anything to do with the fact that radical cystectomy is still the center of care of MIBC worldwide, and it is a central step in the treatment sequence proposed in this trial. The first step in the protocol is induction with 3 does of intravesical recombinant BCG. A first dose of Atezolizumab is administered along with the first BCG instillation. Is there a particular reason why a short course of 3 doses of BCG was chosen? Is one reason the concern that more doses could postpone too much the starting of the neoadjuvant chemotherapy? If this is so, then it should be discussed in the text. The idea of starting a treatment protocol with intravesical BCG in MIBC is really intriguing and novel, but, if I understood it correctly, this will postpone the start of the neoadjuvant chemotherapy by three weeks, and the time from the start of any therapy to radical cystectomy will become longer accordingly. We do not know if this is completely safe for the patients. Also, we do not know how many of the patients treated with BCG + 1 dose of Atezolizumab will respond. We do know however, that a number of patients are nonresponsive to BCG and nonresponsive to immunotherapy. The importance of finding biomarkers of response to treatment (or of resistance to treatment) in this respect would be crucial, especially in trials, like the one proposed, where standard treatment is deferred to test novel combinations of treatments. This point should be mentioned by the Authors and discussed in the Introduction. Also, a recent study showing the possibility of utilizing upfront biomarkers of resistance to ICI therapy in bladder cancer should be added to the references as a possible solution, in the next future, to this problem (Mancini M. et al: Cancers, 2021, doi.org/10.3390/cancers13236016). Moreover, the Authors should discuss why neadjuvant chemotherapy is based on cisplatin/gemcitabine in the protocol, and not ddMVAC, which recently has shown better results in terms of OS as compared to the cisplatin/gemcitabile association. Is the reason the fact that gemcitabine reduces MDSCs and is therefore a better partner for association of chemotherapy with immunotherapy? If this is the reason, then it should be clearly stated in the rationale of the trial. Final remark: This trial is not meant to be a practice changing study, but a hypothesis generating study, and a very interesting one, as the Authors say. However, I think that careful critical thinking should be added at the end. The protocol proposed, novel, promising and really well designed, is indicated in very specific subsets of patients with MIBC: no small cells, < or = cN1, cM0, PS 0-1, suitable for curative multimodality treatment including radical surgery and toxic systemic and intravesical drugs, with no hematuria, eGFR >50 ml/min/1.73m2, able to retain BCG instillation for more than 1 hour, with a PVR<150 ml, with no prior treatments for bladder cancer, including BCG, no immunitary conditions or recent treatments decreasing immunitary capacities. There is obviously a large group of patients with MIBC who do not meet these requirements, and cannot be included in the protocol. For all these patients, standard treatments will have to be applied. The trial should at the end prove that quality of life and survival of the patients included in the study group are improved, as compared not to all the MIBC patients, but to a group of patients

	with the same favorable characteristics, who have been treated with standard care. This fact should be conceptualized and discussed by the Authors at the end of the manuscript, in order to prevent overenthusiasm in the readers, and excessive simplification of the complex problem of improving effective treatment in the entire population of patients with MIBC.

REVIEWER	Satkunasivam, Raj Sunnybrook Health Sciences Centre
REVIEW RETURNED	22-Oct-2022

GENERAL COMMENTS	Intravesical recombinant BCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial (SAKK [Swiss Group for Clinical Cancer Research] 06/19) Summary • Open label Single arm Phase II clinical trial, sponsored by Roche • Population: Resectable MIBC T2-T4a cN0-1 M0 • Intervention: o BCG and Atezo are started at the same time o After x3 weekly cycles of BCG, GC x4 q3w is started o Atezo is given q3w x4 (concomitantly with GC) o After BCG-Atezo-GC treatment, all patients undergo restaging (TURBT?) followed by RC+PLND o After RC, Atezo is continued q3w x13 • 1ry endpoint is pCR • 2ry endpoints are pathological response rate (<ypT2N0), event-free survival, recurrence-free survival, overall survival, feasibility, and toxicity • Interim safety analysis (toxicity) after the first 12 patients have received neoadjuvant treatment • eGFR > 50 used as the cutoff for cisplatin eligibility • Additional research questions: MRI, ctDNA, PD-L1 expression, tumor immunome before and after NA therapy, gut microbiota, immune parameters in urine samples • GC is started on day 22 after the first BCG instillation • RC is performed 4-8 weeks after completion of last GC-Atezo cycle • Endpoints: o Primary: pCR defined as ypT0+ypN0, central pathology review o Secondary: □ Event-free survival (events defined as: progression leading to inoperability, recurrence or progression or locoregional disease after surgery, appearance of metastases, or death) □ Recurrence free survival: After R0, defined as the time from surgery until one of the following: recurrence of locoregional disease, appearance of metastasis, death □ OS defined as the time from treatment start until death from any cause □ Quality of resection assessed in the following way: complete resection (RO), LND completeness, postoperative complications using the CD classification (calculated only for resected patients) □ Pathological response rate (PaR) defined as pathological downstaging (<ypT2N0M0, calculated only for resected patients) □ Pattern of recurrence defined as location of first tumor recurrence: locoregional vs distant vs combination □ Feasibility defined as completion of the intervention receipt, including timely admission to and completion of planned surgery □ AEs will be assessed according to NCI CTCAE v5 • Statistics

	o Sample size based on the primary endpoint: pCR (null hypothesis is ≤35%) o Type I error 5% o Power 80% o 39 resected patients are needed: 46 recruited patients after a projected 15% drop-out rate o Interim safety analysis after the first 12 patients o Interim efficacy analysis after NA therapy and resection of the first 21 patients, futility defined as pCR in <8 patients o 2yr analysis after a minimum follow up of at least 2 years (max. 5 years) o Supportive analyses based on: local pathology, MRI, cystoscopy and bx prior to surgery, ctDNA o Subgroup analyses: high PD-L1 expression (using a ≥5% cutoff), ypT0, ypN0, resection status of TURBT • Rationale of using BCG in addition to GC+ICI: o Gemcitabine is known to reduce myeloid derived suppressor cells (MDSCs), thus becoming the ideal partner for chemo-IO. ICIs are intended to block negative feedback loop to allow for a prolonged T-cell response. However, prior chemo-IO studies in MIBC have reported pCR rates around 30-40% o BCG causes T-cell exhaustion (as evidenced in granulomas), ICIs are supposed to reactivate the immune response provoked by BCG o Overall: "BCG enhances the local inflammation by IFN release resulting in increased number of immune suppressive immune cells (MDSC), and upregulation of PD-L1, C: chemotherapy diminishes MDSC, checkpoint inhibition blocks PD1-PD-L1 axis, D: due to blocked immune suppressive network immune effector cells (T cells) expand and kill tumor cells, additional cytotoxic effect of chemotherapy kills tumor cells, activated T cells can cause systemic anti-tumor immune response." REVIEW: I commend the authors on a well-designed protocol for a phase II clinical trial evaluating the safety and efficacy of perioperative BCG plus chemoimmunotherapy in the setting of muscle-invasive bladder cancer. This study seeks to improve the complete response rates (pCR) seen with the current standard of care. In discussing their rationale, the authors mention the ongoing phase III trials assessing the efficacy and safety of BCG plus ICI combinations in both BCG-naïve and unresponsive settings. They mention the 34% pCR rates seen in their SAKK 06/17 trial, which used perioperative chemoimmunotherapy. Using BCG's immunostimulatory effect, they are hoping to further increase pCR rates. Overall, their rationale is scientifically sound, and their statistical plan makes sense. The primary endpoint is pCR with numerous oncologic secondary endpoints that are worth evaluating. I agree with their null hypothesis cutoff of ≤35% and their planned interim efficacy analysis after the first 12 patients have undergone radical cystectomy. Their planned subgroup analyses are also worth exploring as PD-L1 expression has been shown to be associated with response to immune checkpoint inhibition. Although not stated in their protocol, I hope that post-hoc subgroup analyses of their secondary endpoints are eventually performed. They have sought to decrease their potential bias in measurement of outcomes as pathology and imaging review will be performed centrally. They also have uniform and explicit outcome definitions which should decrease the bias in the reporting of outcomes. I appreciate that

	they will not be performing missing data imputation and have planned to do complete case analysis. Criticism: • We need to consider the current BCG shortages and the need for better stewardship of BCG. VPM1002BC comes from India and a potential disruption in the supply-chain is concerning. • Multiple CHEMO-IO vs CHEMO trials in first line metastatic Urothelial Carcinoma have been negative o Why do the authors believe that BCG would overcome the potential barrier? • There is a potential for a high rate of adverse events given the three-drug combination of perioperative therapy • The adjuvant use of atezolizumab in the setting of radical cystectomy is concerning. Its main AEs include urinary tract infection, anemia, and decreased appetite. This is concerning as radical cystectomy is a highly morbid surgery and adding these AEs to already debilitated patients could translate into worse quality of life outcomes. o This is not supported from IMVIGOR 010 o Why is adjuvant necessary for this trial design? • They will not assess quality of life outcomes, which are important in the setting of radical cystectomy • Regarding the writing, there are some typos throughout that will need to be corrected

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Mariangela Mancini, University of Padua

A proposed change for the title, which would make it more accurate and adherent to reality is: “A novel sequential treatment strategy for patients with muscle-invasive bladder cancer (MIBC): intravesical recombinant BCG, followed by neoadjuvant chemo-immunotherapy, radical cystectomy plus pelvic lymphadenectomy, and adjuvant immunotherapy. A multicenter, single arm phase 2 trial (SAKK 06/19).”
We thank for the suggestion and adapted the title accordingly.

The fact that radical cystectomy and pelvic lymphadenectomy (which kind of lymphadenectomy will be performed? Standard? Extended? This should also be clarified), are part of the sequential treatment, is not a secondary point.
Radical cystectomy with extensive lymph node dissection according to actual EAU guidelines will be performed. See clarification on Page 6. The secondary endpoint refers to the quality of the lymphadenectomy to serve as quality assurance not to the resection itself.

The fact that, as the Authors state later in the text, “the design of the trial is to pave a scientific way to avoid radical cystectomy in the future” does not have anything to do with the fact that radical cystectomy is still the center of care of MIBC worldwide, and it is a central step in the treatment sequence proposed in this trial.
We agree with this statement, however, in future our endpoint complete pathological response could be meaningful to guide the development of neo-adjuvant treatment schedules allowing deep responses. These protocols could then be used to design trials without cystectomies, which has to be the ultimate aim. Actually certain groups are already performing trials with such designs and first

results have been presented

Is there a particular reason why a short course of 3 doses of BCG was chosen? Is one reason the concern that more doses could postpone too much the starting of the neoadjuvant chemotherapy? If this is so, then it should be discussed in the text. The idea of starting a treatment protocol with intravesical BCG in MIBC is really intriguing and novel, but, if I understood it correctly, this will postpone the start of the neoadjuvant chemotherapy by three weeks, and the time from the start of any therapy to radical cystectomy will become longer accordingly. We do not know if this is completely safe for the patients. Also, we do not know how many of the patients treated with BCG + 1 dose of Atezolizumab will respond. We do know however, that a number of patients are nonresponsive to BCG and nonresponsive to immunotherapy.
To avoid clinically relevant delay three installations of BCG were considered to be enough to prime and boost. Page 13. After the first 12 patients have completed neoadjuvant treatment, an interim safety analysis will be performed. AEs and SAEs will be analyzed descriptively. Special focus will be given to CTCAE grade ≥3 directly related to intravesical rBCG. Page 9

The importance of finding biomarkers of response to treatment (or of resistance to treatment) in this respect would be crucial, especially in trials, like the one proposed, where standard treatment is deferred to test novel combinations of treatments. This point should be mentioned by the Authors and discussed in the Introduction. Also, a recent study showing the possibility of utilizing upfront biomarkers of resistance to ICI therapy in bladder cancer should be added to the references as a possible solution, in the next future, to this problem (Mancini M. et al: Cancers, 2021, doi.org/10.3390/cancers13236016).
This clinical trial is the built and designed on a completed predecessor study, which showed no detrimental results. The manuscript is in preparation, results were presented in a poster discussion at ASCO 2022 annual meeting (Cathomas et al Journal of Clinical Oncology 40, no. 16_suppl (June 01, 2022) 4515-4515.). The herein presented clinical trial is accompanied by a clinical and scientific program. Page 9

Moreover, the Authors should discuss why neadjuvant chemotherapy is based on cisplatin/gemcitabine in the protocol, and not ddMVAC, which recently has shown better results in terms of OS as compared to the cisplatin/gemcitabile association. Is the reason the fact that gemcitabine reduces MDSCs and is therefore a better partner for association of chemotherapy with immunotherapy? If this is the reason, then it should be clearly stated in the rationale of the trial.
The ddMVAC protocol was avoided to not allow methotrexate to built up its known T cell suppressive capacity counteracting the immune activating intention of this protocol. Page 11.
Cisplatin/Gemcitabine is still considered one of the standards of care for neoadjuvant chemotherapy in this setting.

Final remark: This trial is not meant to be a practice changing study, but a hypothesis generating study, and a very interesting one, as the Authors say. However, I think that careful critical thinking should be added at the end. The protocol proposed, novel, promising and really well designed, is indicated in very specific subsets of patients with MIBC: no small cells, < or = cN1, cM0, PS 0-1, suitable for curative multimodality treatment including radical surgery and toxic systemic and intravesical drugs, with no hematuria, eGFR >50 ml/min/1.73m2, able to retain BCG instillation for more than 1 hour, with a PVR<150 ml, with no prior treatments for bladder cancer, including BCG, no immunitary conditions or recent treatments decreasing immunitary capacities. There is obviously a large group of patients with MIBC who do not meet these requirements, and cannot be included in the protocol. For all these patients, standard treatments will have to be applied. The trial should at the end prove that quality of life and survival of the patients included in the study group are improved, as compared not to all the MIBC patients, but to a group of patients with the same favorable characteristics, who have been treated with standard care. This fact should be

conceptualized and discussed by the Authors at the end of the manuscript, in order to prevent overenthusiasm in the readers, and excessive simplification of the complex problem of improving effective treatment in the entire population of patients with MIBC.
We agree with the reviewer and we have emphasized the fact that this is a clinical study testing a hypothesis that needs further exploration.
This trial tests the hypothesis in a clearly defined patient group if a new recombinant BCG can enhance the local and systemic immune response in the context of immune checkpoint inhibition and chemotherapy and thereby increase pCR rate and consequently also event-free survival. Improving pCR rate would be a next step to the ultimate goal of omitting radical surgery or extensive local radiotherapy to the bladder for these patients. In case of encouraging results further studies have to establish quality of life and superiority about standard of care. Page 13

Reviewer: 2
Dr. Raj Satkunasivam, Sunnybrook Health Sciences Centre

We need to consider the current BCG shortages and the need for better stewardship of BCG. VPM1002BC comes from India and a potential disruption in the supply-chain is concerning.
We appreciate the critical comment, but we see VPM1002BCG as an advantage from a scientific (increased cytokine release) and logistically (independent of conventional supply chain) point of view. In fact VPM1002BCG has the potential to overcome such shortages.

Multiple CHEMO-IO vs CHEMO trials in first line metastatic Urothelial Carcinoma have been negative
We agree on that statement, however the only positive trial in metastatic urothelial cancer (JAVELIN 100) had a sequential schedule, which is in part recapitulated in our trial.

Why do the authors believe that BCG would overcome the potential barrier?
We have discussed the biological effect on page 11 and 12.

There is a potential for a high rate of adverse events given the three-drug combination of perioperative therapy
We also see the risk of a new combination and therefore implemented a safety phase. Page 9

The adjuvant use of atezolizumab in the setting of radical cystectomy is concerning. Its main AEs include urinary tract infection, anemia, and decreased appetite. This is concerning as radical cystectomy is a highly morbid surgery and adding these AEs to already debilitated patients could translate into worse quality of life outcomes.
We do not quite agree with the reviewer in this point. Atezolizumab is a checkpoint inhibitor and the main side effects are of immune-related toxicity, usually mild a demonstrated in the ajduvnat trial IMvigor 010. Urinary tract infections, anemia and decreased appetite are not more common with atezolizumab but most likely associated in this trial to the extensive surgery performed. Therefore we feel that safety for atezolizumab in this setting is acceptable.

This is not supported from IMVIGOR 010
We agree with the reviewer in this point. Indeed IMvigor did not demonstrate a significant benefit for the use of adjuvant atezolizumab. However, our trial setting is somewhat different since we do give perioperative therapy as a multimodality treatment and aim to benefit from enhanced immunogenicity after BCG-induction and cisplatin-based neoadjvuant chemotherapy. In our view the use of atezolizumab within a clearly defined trial setting is justified.

Why is adjuvant necessary for this trial design?

See answer to previous question. Moreover, as mentioned before the JAVELIN 100 study seems to get its effect from an sequential and longer application of checkpoint inhibition.

They will not assess quality of life outcomes, which are important in the setting of radical cystectomy
We will focus on the safety and feasibility with this trial. We included a critical statement in this context at the end of the discussion. Page 13.

VERSION 2 – REVIEW

REVIEWER	Mancini, Mariangela University of Padua, Surgical, Oncological and Gastroenterological Sciences
REVIEW RETURNED	15-Feb-2023

GENERAL COMMENTS	I think the Authors responded adequately to the raised points and made good changes to the text of the manuscript. They did not raise the point as required, to include, in the future, biomarkers of chemo or immune resistance upfront, in the pre-clinical phase, in order to avoid unnecessary delay of surgery in non.respondents. This conceptualization, which I think is a key point in any innovative trial including neoadjuvant strategies, would make the manuscript more up-to-date and the Authors should include it. I think that this manuscript is suitable for publication in BMJ Open.