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Intravesical recombinant BCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial (SAKK 06/19)

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Article Summary

- Innovative immunotherapy protocol for muscle-invasive bladder cancer (MIBC)
- Multimodal therapy of BCG, PD-L1 blockade, cisplatin and gemcitabine in combination with radical cystectomy and pelvic lymphadenectomy
- Phase II study with the primary end point of pathological complete remission

Abstract

Introduction: The combination of checkpoint inhibition and cisplatin-based chemotherapy is investigated in muscle invasive bladder cancer (MIBC) and results from phase 2 trials have been presented. Intravesical Bacillus Calmette Guérin (BCG) has been used for non-muscle invasive bladder cancer (NMIBC) in patients with carcinoma in situ (CIS) and high-grade Ta/T1 tumors. BCG induces innate and adapted immune response and upregulation of PD-L1 in preclinical models. The proposed trial is intended to implement a new immuno-immuno-chemotherapy induction therapy for MIBC. The combination of BCG and checkpoint inhibition with chemotherapy aims higher intravesical responses and better local and systemic control of disease.

Methods and Analysis: SAKK 06/19 is an open label single arm phase II trial for patients with resectable MIBC T2-T4a cN0-1. Intravesical rBCG (VPM1002BC) is applied weekly for 3 instillations followed by 4 cycles of neoadjuvant cisplatin/gemcitabine every 3 weeks (q3w). Atezolizumab 1200mg q3w is started together with rBCG and given for 4 cycles. All patients then undergo restaging and radical cystectomy and pelvic lymphadenectomy. Atezolizumab is continued as maintenance therapy after surgery q3w for 13 cycles. Pathological complete remission is the primary endpoint. Secondary endpoints include pathological response rate (<ypT2N0), event-free survival, recurrence-free survival, overall survival, feasibility and toxicity. An interim safety analysis will be performed after the first 12 patients have completed neoadjuvant treatment specifically assessing toxicity possibly associated with intravesical rBCG application.

Ethics and dissemination: The study has received approval by ethical committee Zurich, Switzerland, BASEC-No. 2021-01872. Results will be made available by publication. Trial registration number: NCT04630730

Keyword

muscle-invasive, resectable, urothelial cancer, Bacillus Calmette-Guérin, neo-adjuvant, adjuvant, chemotherapy, checkpoint inhibition, radical cystectomy, pelvic lymphadenectomy

Strengths and limitations of this study

This study is based on the clinical results and experience from a completed predecessor study using the same therapeutic multimodality concept. The innovative concept of combining local immunotherapy with chemotherapy, immune checkpoint blockade and radical cystectomy will be performed in a patient population with a very high unmet medical need. The study is a single arm phase II study and will be therefore not practice changing and only hypothesis generating.

Introduction

Beside bladder sparing chemoradiation therapy, radical cystectomy is the accepted standard curative treatment modality for patients with muscle invasive bladder cancer (MIBC) without evidence of metastatic disease (cM0) (1). Despite the radical surgical approach, stage independent cure rates are however only around 50% at 5 years. Two phase III trials using cisplatin-based neoadjuvant chemotherapy demonstrated a significant improvement of overall survival of muscle-invasive bladder cancer of approximately 5% compared to radical cystectomy alone (2-3). These results were confirmed in a meta-analysis demonstrating that the addition of neoadjuvant cisplatin-based chemotherapy can improve overall survival (OS) by around 5% (4). Therefore, according to international guidelines, the use of cisplatin-based neoadjuvant chemotherapy is considered standard of care in all patients with localized MIBC with planned curative local treatment (1).

For a long time, there was no consensus which cisplatin-combination regimen (cisplatin/gemcitabine vs dose dense MVAC [ddMVAC, MVAC: methotrexate, vinblastine, adriamycin and cisplatin]) should be administered in the neoadjuvant setting. Recently, a phase III clinical trial (VESPER) suggested improved OS for the ddMVAC regimen compared to cisplatin/gemcitabine (5).

There remains a high unmet need to improve the cure rate for patients with localized MIBC. Moreover, establishment of a treatment with high local control omitting the need for either complete resection or irradiation of the bladder would substantially improve

quality of life for those patients. Early results from clinical trials support the feasibility of bladder preserving approaches after immune-chemo-therapy (HCRN GU16-257) (6) In recent years, immunotherapy using PD-1 or PD-L1 targeting immune checkpoint inhibitors (ICI) proved to be beneficial for patients with metastatic bladder cancer and a significant improvement in OS was shown for pembrolizumab in the second-line setting (7). The first results have been presented and published using ICIs as neoadjuvant treatment for localized MIBC. Two monotherapy studies using either pembrolizumab (PURE-01) or atezolizumab (ABACUS) demonstrated pCR of 30-40% (8, 9).

Atezolizumab is a human monoclonal antibody (mAb) of the immunoglobulin G (IgG) 1 kappa subclass that inhibits binding of PD-L1. Atezolizumab was the first ICI to be tested in patients with urothelial carcinoma (UC). The published study program of atezolizumab in UC is broad, comprising phase I to phase IV trials in metastatic pretreated patients (10 - 13) and a phase II trial in metastatic treatment naïve cisplatin-ineligible patients (14). In the phase I trial, 95 pretreated metastatic UC patients received atezolizumab achieving a 40% response rate (10). The phase II trial included 310 platinum-pretreated patients and achieved a response rate of 15% including 5% complete remissions (CR) (11). 931 patients were randomized in the phase III trial comparing atezolizumab against chemotherapy of physician's choice (either docetaxel, paclitaxel or vinflunine). While the primary endpoint of improved OS for patients with high PD-L1 expression was not reached, the OS was numerically higher in the intention to treat population (12). Atezolizumab had a better safety profile than chemotherapy with 20% grade 3/4 toxicity as compared to 43% on chemotherapy. The efficacy and safety were confirmed in a large real-world population (N=1004) safety trial also including patients usually not eligible for immunotherapy trials such as patients with brain metastasis, autoimmune disease, renal insufficiency, HIV positivity as well as frail patients (13). Moreover, atezolizumab monotherapy demonstrated interesting efficacy in the first line treatment of cisplatin-ineligible patients with a response rate of 23% (9% CR) and an OS of 15.9 months (14).

The combination of cisplatin/gemcitabine chemotherapy with atezolizumab has been demonstrated to be effective and safe in a large phase III trial (15). The trial was positive for the primary endpoint of progression free survival (PFS) without unexpected toxicity from the chemo-immunotherapy combination.

Intravesical instillation of Bacillus Calmette Guérin (BCG) is the recommended standard of care treatment for patients with intermediate/high risk for progression non-muscle invasive bladder cancer (NMIBC) after complete transurethral resection of the bladder tumor (TURB) (16). BCG was shown to cure carcinoma in situ (CIS) and prevent recurrence of high grade NMIBC and to prolong survival compared to TURB alone (16, 17). While the exact mechanism of BCG effect is not entirely understood, it is clear that intravesical BCG induces a local inflammation leading to induction of the innate immune system allowing for a tumor-specific immunity (adaptive immune response (18, 19). Several different BCG strains have been developed and used for intravesical therapy. It has been recognized that there might be differences in terms of immunogenicity and efficacy between strains (20). This has increased interest in developping novel BCG formulations.

A far developed and promising new BCG-derived vaccine is the recombinant Mycobacterium bovis (M. bovis) BCGΔureC::hly. rBCGΔureC::hly which was formulated as VPM1002BC for intravesical immunotherapy against NMIBC. This recombinant BCG (rBCG) VPM1002BC leads to translocation of proteins to the cytosol of infected host or cancer cells by perforation of the phagosome (21, 22). In preclinical models, these changes induce macrophage apoptosis, T cell priming, and proinflammatory cytokine expression, leading to CD4+ and CD8+ T cell responses that are superior compared to the parental BCG subtype Prague. These observations are potentially leading to an improved immune response. VPM1002BC has been used for intravesical therapy in patients with BCG refractory NMIBC in a clinical phase I/II trial (SAKK06/14). The phase I part demonstrated very good tolerance of the compound without need for dose modifications or grade 3 or 4 adverse events (23). The phase II part including 42 patients clearly met the primary endpoint resulting in a recurrence-free survival (RFS) rate in the bladder at 60 weeks in 49.3% of patients (24), while historical data from second-line treatment with conventional BCG results in a RFS rate of 12.5% (25). Only two patients (5%) did tolerate less than 5 instillations and this was not directly related to VPM1002BC. Over the whole course of therapy, treatment related grade 1, 2 and 3 adverse events (AEs) were observed in 14.3%, 54.8%, and 4.8% of the patients, respectively.

Methods/Design

The trial aims to implement a new immuno-immuno-chemotherapy induction therapy for MIBC combining rBCG intravesical installations and ICI followed by neo-adjuvant ICI in combination with chemotherapy followed by radical cystectomy and adjuvant ICI (figure 1).

The trial is a single arm phase 2 trial including patients with histologically proven urothelial cell carcinoma of the bladder (pT2 or cT2, cT3 or cT4a and ≤ cN1 (defined as a solitary lymph node ≤ 2 cm in the greatest dimension) and cM0 and be considered suitable for curative multimodality treatment including radical cystectomy by a multidisciplinary tumor board. Furthermore, location of tumor must allow placement of catheter without risk of bleeding. All histological subtypes are eligible with the exception of small cell neuroendocrine carcinoma. The renal function must be estimated to reach a glomerular filtration rate of (eGFR) > 50 mL/min/1.73m² to allow the use of cisplatin. Patients with prior intravesical BCG, with macrohematuria and those unable to retain BCG instillation for less than 1 hour are excluded.

The protocol includes additional research questions such as preoperative assessment of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and correlation with the pathological outcome, the tumor immunome before and after neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy endpoints, the effect of the gut microbiota on the response to immunotherapy, immune parameters in urine samples and their relation to efficacy endpoints.

Patients receive intravesical rBCG (VPM1002BC) by 3 weekly instillations of rBCG with single dose of VPM1002BC, live, 1-19.2 x 10^8 colony forming units (CFU) on day 1, 8 and 15 of the protocol. Atezolizumab 1200mg fixed dose is started with the first instillation of rBCG (1/-1 day) and continued in combination with the chemotherapy every 3 weeks (q3w) for 4 cycles. Chemotherapy consists of cisplatin and gemcitabine for 4 cycles and is started on day 22 after the first rBCG instillation. Cisplatin is used at a dose of 70mg/m^2 iv on d1 q3w and gemcitabine is used at a dose of 1000mg/m^2 iv on d1 and d8 q3w. Radical cystectomy is performed 4 to 8 weeks after completion of the last chemo-immunotherapy cycle. Adjuvant atezolizumab is given 1200mg fixed dose q3w for 13 cycles starting 4-16 weeks after date of surgery.

Endpoints

The primary endpoint of the trial is pCR after neoadjuvant treatment defined as ypT0 ypN0 and no evidence of non-muscle invasive bladder cancer (low grade, high grade or CIS). The primary analysis will be based on the results from central pathology review. This endpoint will only be calculated for patients in the resected patients set.

The secondary endpoints are the following:

Event-free survival (EFS)

EFS is defined as the time from treatment start until one of the following events, whichever comes first:

- Progression during neoadjuvant treatment leading to inoperability
- Recurrence or progression (in case of disease persistence) of locoregional disease after surgery
- Appearance of metastases at any localization
- Death

Patients without event at the time of analysis and patients starting a subsequent treatment in the absence of an event will be censored at the date of the last available assessment showing no event before the start of the subsequent treatment, if any. This endpoint will be calculated for patients in the full analysis set (FAS).

Recurrence free survival (RFS)

RFS after R0 resection is defined as the time from surgery until one of the following events, whichever comes first:

- Recurrence of locoregional disease
- Appearance of metastases at any localization
- Death

Patients without event at the time of analysis and patients starting a subsequent treatment in the absence of an event will be censored at the date of the last available assessment showing no event before the start of the subsequent treatment, if any.
This endpoint will only be calculated for patients in the R0 resection set.

Overall survival (OS)

OS is defined as the time from treatment start until death from any cause. Patients not experiencing an event will be censored at the last date they were known to be alive.
This endpoint will be calculated for patients in the full analysis set (FAS).

Quality of resection

The quality of resection will be assessed in the following way:

- Complete resection (R0) defined as free resection margins proved microscopically
- Completeness of the lymphadenectomy and surgery using the photo documentation and histopathology
- Postoperative complications will be assessed using the Clavien-Dindo classification.

This endpoint will only be calculated for patients in the resected patients set.

Pathological response rate (PaR)

PaR is defined as pathological downstaging to <ypT2N0M0. The proportion of patients with PaR will be calculated for patients in the resected patients set. This endpoint will only be calculated for patients in the resected patients set.

Pattern of recurrence

Pattern of recurrence is defined as location of first tumor recurrence. Patterns can be locoregional or distant or any combination of these patterns.

Patients with secondary malignancies or patients with no recurrence will not be taken into consideration for this endpoint.

Feasibility

The following treatment feasibility criteria will be assessed:

- Completion of 3 instillations of intravesical VPM1002BC
- Completion of 4 cycles of neoadjuvant chemotherapy
- Completion of 4 cycles of neoadjuvant atezolizumab treatment
- Timely admission to and completion of planned surgery
- Timely initiation and completion of 13 cycles of adjuvant atezolizumab treatment

Adverse events (AE)

AEs will be assessed according to NCI CTCAE v5.0.

This endpoint will be calculated for patients in the safety set.

The protocol includes additional research questions such as preoperative assessment of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and correlation with the pathological outcome, the tumor immunome before and after neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy endpoints, the effect of the gut microbiota on the response to immunotherapy, immune parameters in urine samples and their relation to efficacy endpoints.

Statistics

The sample size is based on the primary endpoint pCR. The null hypothesis is a pCR rate ≤ 35% (based on reference 26) and the alternative hypothesis a pCR rate ≥ 55%. Using Simon's minimax two-stage design with a type I error of 5% and a power of 80%, 39 resected patients are needed. With an estimated drop-out rate of 15% (7 patients), we plan to recruit a total of 46 patients.

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.

After neoadjuvant therapy and resection of the first 21 patients an interim efficacy analysis will be performed. If the number of patients with pCR is 8 or less, the trial will be stopped for futility. If, however, the number of patients with pCR is 9 or more, the trial will continue to stage 2.

The primary analysis will take place after all patients have completed neoadjuvant therapy and had surgery, if applicable. The secondary analysis will be performed when all patients have reached a follow-up of at least 2 years.

For the primary endpoint, the point estimate of the pCR rate will be calculated using the uniformly minimum variance unbiased estimator (UMVUE) and the corresponding two-sided 90% confidence interval will be calculated using the "stage-wise ordering" based-method. If the lower bound of the confidence interval is above 35%, the null hypothesis can be rejected.

For all other binary endpoints the point estimate and exact 95% Clopper-Pearson confidence interval of the proportion will be calculated.

For the primary analysis of the primary endpoint the results from the central pathology review will be used. Supportive analyses are planned based on the following results:

- Local pathology
- MRI (local and central assessment) before surgery
- Cystoscopy and biopsy before surgery
- ctDNA

The following subgroup analyses are planned for the primary endpoint:

- high PD-L1 expression (assessed by standardized immunohistochemistry on tumor cells (TC) and tumor-associated immune cells (IC) using a ≥5% positivity on IC (i.e. IC2) as cutoff) versus no or low expression
- ypT0 vs rest
- ypN0 vs rest
- resection status of TUR-B (complete versus incomplete)

All time-to-event endpoints will have the median value estimated using the Kaplan-Meier method. The number and type of events of each endpoint will be presented descriptively by frequency and percentage.

Categorical variables will be summarized with frequency and percentage. The denominator for percentages will be the number of patients within the set of interest, unless otherwise specified. Continuous variables will be summarized using median and range.

Laboratory values will be expressed as the absolute values and as grading according to NCI CTCAE v5.0. AE grading will be presented by type, grade, and relation showing frequency and percentage of the within-patient worst grade. In addition, grade ≥ 3 AEs and AEs with relation to treatment ≥ 3 will be summarized separately.

Patient and Public Involvement

The protocol was developed within the SAKK network involving multiple stakeholders including physicians specialized in uro-onocology, nurses and the patient advisory board. The design of the trial is aimed to improve cure rates and to pave a scientific way to avoid radical cystectomy in the future, both clear aims to improve quality of live. Patients will be recruited within the SAKK network and the trial is accessible to the public via the SAKK webpage (https://www.sakk.ch/en/news/new-trial-patients-bladder-cancer-sakk-0619). After closing and analysis of the trial results will be published in scientific journals. A lay abstract will be uploaded on the SAKK webpage.

Discussion

The herein presented clinical trial SAKK 06/19 is the further development of immuno-chemotherapies for MIBC within the SAKK network. SAKK has performed a predecessor single arm phase II trial using neoadjuvant chemo-immunotherapy with cisplatin/gemcitabine in combination with the PD-L1 inhibitor durvalumab (SAKK 06/17). In this trial a total of 61 patients were included in Switzerland and in one German center between 5/2018 and 9/2019. We presented the primary analysis at ASCO 2022 (26) as first trial in MIBC to report a primary endpoint of EFS (manuscript in preparation).

The rationale of the SAKK 06/17 trial was the addition of neo-adjuvant chemotherapy with cisplatin and gemcitabine to checkpoint inhibition to support the development of a therapeutic immune response by reducing the influence of the chronic inflammation caused by the immune suppressive innate cell network. Predominantly myeloid derived suppressor cells (MDSCs, including macrophages and neutrophils) are responsible for chronic inflammation hampering the immune response. Gemcitabine is known to reduce MDSCs and is therefore the ideal partner for an immuno-chemotherapy (29). As a consequence of immune activation, IFN-gamma is released resulting in TH1 T cell response. However, IFN-gamma also induces PD-1 expression on TH1 T cells leading to adaptive immune suppression aiming to stop the T-cell response (30). The use of ICIs is intended to block this negative feedback loop to allow a prolonged T-cell response.

Several similar neo-adjuvant studies in MBIC using immunotherapy or the combination of immuno-chemotherapy have reported pCR rates in the same range of 30-40% and in addition, residual NMIBC can be found in approximately 15-20% (8, 9, 27, 28). Therefore there is hardly any improvement in the pCR rate compared to cisplatin-based chemotherapy, especially when compared to the more active regimen of ddMVAC (5).

In view of these rather modest results so far, strategies to further augment the immune response need to be evaluated. Beside concomitant application of radiotherapy and immune checkpoint blockade, BCG appears to be a promising combination partner. BCG has been used for treatment of NMIBC for decades with very good success. It induces initial CR in 70-75% of patients with CIS and prevents recurrence in 55-65% of patients with high-risk papillary tumors (16, 17). However, 25-45% of patients don't respond initially and up to 40% experience relapse after initial response. BCG induces an intense local inflammatory response that mediates tumor immunity. Several steps

are involved in mounting the inflammatory response including attachment to the urothelium with uptake by antigen presenting cells (APC) and putative internalization into urothelial cells followed by a boost of the innate immune response and induction of adaptive responses (18). Preclinical experiments demonstrated that intravesical BCG instillations induce a robust infiltration of T cells (CD4+ and CD8+) in the bladder wall (31). Moreover, a systemic immune response arises following intravesical BCG demonstrated by increased levels of different cytokines and chemokines including IFNγ, IL-1, IL-2, Il-8, TNF, CCL2, CCL5 (32).

Resistance mechanisms to BCG are not entirely understood but interestingly, granulomata found in patients not responding to BCG were found to be highly expressing PD-L1 (30) suggesting a T-cell exhaustion resulting from checkpoint activation. Patients with ARIDA1A mutation and CCNE1 amplification also appear to be at higher risk of relapse after BCG treatment (33). The immune response induced by intravesical BCG is, however, not solely restricted to the superficial urothelial layer but affects the whole bladder wall and also induces a systemic immune response (20). Therefore, the next logical step appears to use intravesical BCG also in patients with muscle-invasive bladder cancer as an adjuvant to prime and boost the immune response (both innate and adaptive) when using systemic immunotherapy with checkpoint inhibitors (figure 2). This intended priming of the immune system might be better achieved by using the novel rBCG strain VPM1002BC which appears to have improved safety (21) immunogenicity (22). This is mediated by the exchange of the urease C gene with the lsteriolysin gene in rBCG VPM1002BC leading to a stronger adoptive and innate immune response. Furthermore, increased autophagy likely contributes to more rapid elimination of rBCG in the host and because listeriolysin is only active at acidic pH it is rapidly degraded in the cytosol of the host cell and it's effects are short-lived.

This trial tests the hypothesis 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.

Trial status

Recruitment started May 2022, estimated closure of accrual April 2025.

List of abbreviations

APC	Antigen presenting cells
BCG	Bacillus Calmette-Guerin
CIS	Carcinoma in situ
CR	Complete response
EFS	Event-free survival
eGFR	Estimated glomerular filtration rate
mAB	Monoclonal antibody
MDSC	Myeloid-derived suppressor cells)
MIBC	Muscle invasive bladder cancer
NCI CTCAE	NCI Common Terminology Criteria for Adverse Events
NMIBC	Non-Muscle invasive bladder cancer
OS	Overall survival
PaR	Pathological response
pCR	Pathological complete remission
PD-L1	Programmed cell death-ligand 1
PD1	Programmed cell death protein 1
PFS	Progression-free survival
RFS	Recurrence-free survival
SAKK	Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung (Swiss Group for Clinical Cancer Research)

Ethics approval

Ethics approval 25.03.2022, ethical committee Zurich, Switzerland, BASEC-No. 2021-01872

Consent for publication

All authors have no objections for publication.

Availability of data and materials

Not applicable

Conflict of interest declaration

UP: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS, Merck, Pfizer, Roche, MSD, Janssen, Novartis

RC: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS, Merck, Pfizer, Roche, MSD, Ipsen, Janssen, Novartis; Honoraria (compensated, institutional) for Janssen, Astellas
MS: None
SH: None
CR: None
SR: Honoraria (compensated, institutional) from Roche, Astra Zeneca, BMS, Boehringer Ingelheim, MSD, Novartis, Amgen, Eli Lilly, Eisai, Merck Serono, Pfizer, Takeda, Bayer, Janssen, Otsuka, PharmaMar, Sanofi; Advisory role (institutional,compensated): Astea Zeneca, Boehringer Ingelheim, BMS, Pfizer, Eisai, Eli Lilly, Merck Serono, MSD, Roche, Novartis, Takeda, Amgen, Otsuka; Research Funding (institutional): Abbvie, BMS, Astra Zeneca, Boehringer Ingelheim, Merck Serono, Roche
MS: None
AO: Advisory role (compensated, institutional): Astra Zeneca, Astellas, Bayer, Janssen, Molecular Partners, MSD, Pfizer, Roche, Sanofi Aventis (compensated, institutional). Novartis, Janssen, Bayer, MSD, AstraZeneca, Merck, Astellas (compensated). Research support (institutional): TEVA, Janssen. Travel support Astellas, Bayer, Janssen, Sanofi Aventis. Speakers Bureau (compensated, institutional): Astellas, Bayer, Janssen

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Author contributions
UP study design & manuscript writing, RC study design & manuscript writing, MS study design, SH study design and statistical planning, CR study design, SR translational research, AO study design and coordination.

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Figures

Figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

Figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFNγ 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.

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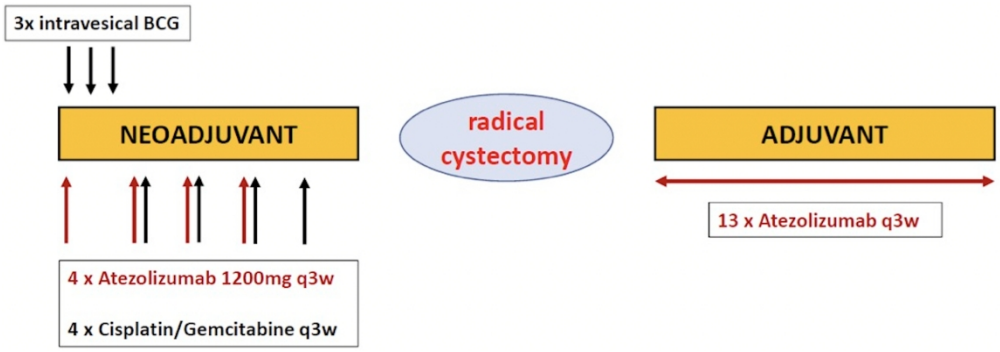

figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

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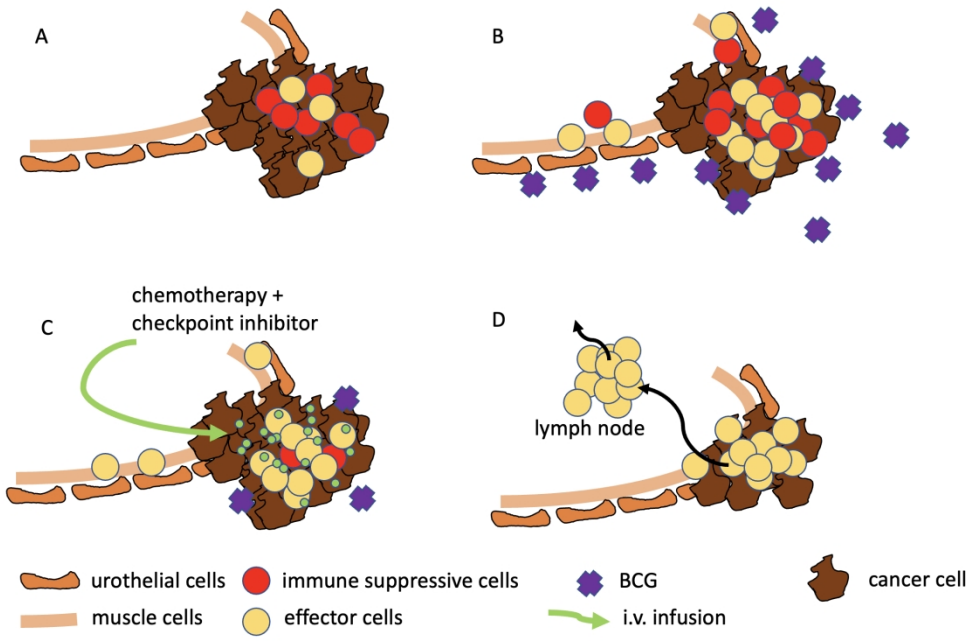

figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFN□ release resulting in increased number of immune suppressive immune cells (MDSC), and upregulation of PD-L1, C: chemotherapy diminishes MDSC, checkpoint inhibition 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.

416x267mm (144 x 144 DPI)

BMJ Open

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).

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Abstract

Introduction: The combination of checkpoint inhibition and cisplatin-based chemotherapy is investigated in muscle invasive bladder cancer (MIBC) and results from phase 2 trials have been presented. Intravesical Bacillus Calmette Guérin (BCG) has been used for non-muscle invasive bladder cancer (NMIBC) in patients with carcinoma in situ (CIS) and high-grade Ta/T1 tumors. BCG induces innate and adapted immune response and upregulation of PD-L1 in preclinical models. The proposed trial is intended to implement a new immuno-immuno-chemotherapy induction therapy for MIBC. The combination of BCG and checkpoint inhibition with chemotherapy aims higher intravesical responses and better local and systemic control of disease.

Methods and Analysis: SAKK 06/19 is an open label single arm phase II trial for patients with resectable MIBC T2-T4a cN0-1. Intravesical rBCG (VPM1002BC) is applied weekly for 3 instillations followed by 4 cycles of neoadjuvant cisplatin/gemcitabine every 3 weeks (q3w). Atezolizumab 1200mg q3w is started together with rBCG and given for 4 cycles. All patients then undergo restaging and radical cystectomy and pelvic lymphadenectomy. Atezolizumab is continued as maintenance therapy after surgery q3w for 13 cycles. Pathological complete remission is the primary endpoint. Secondary endpoints include pathological response rate (<ypT2N0), event-free survival, recurrence-free survival, overall survival, feasibility and toxicity. An interim safety analysis will be performed after the first 12 patients have completed neoadjuvant treatment specifically assessing toxicity possibly associated with intravesical rBCG application.

Keyword

muscle-invasive, resectable, urothelial cancer, Bacillus Calmette-Guérin, neo-adjuvant, adjuvant, chemotherapy, checkpoint inhibition, radical cystectomy, pelvic lymphadenectomy

Strengths and limitations of this study

- Completed predecessor study using the same therapeutic multimodality backbone
- Combination of local immunotherapy with chemotherapy, immune checkpoint blockade and radical cystectomy
- Open label, single arm phase II study
- Primary endpoint: pathological complete remission
- Population: MIBC cT2-T4a cN0-1 cM0

Introduction

Beside bladder sparing chemoradiation therapy, radical cystectomy is the accepted standard curative treatment modality for patients with muscle invasive bladder cancer (MIBC) without evidence of metastatic disease (cM0) (1). Despite the radical surgical approach, stage independent cure rates are however only around 50% at 5 years. Two phase III trials using cisplatin-based neoadjuvant chemotherapy demonstrated a significant improvement of overall survival of muscle-invasive bladder cancer of approximately 5% compared to radical cystectomy alone (2-3). These results were confirmed in a meta-analysis demonstrating that the addition of neoadjuvant cisplatin-based chemotherapy can improve overall survival (OS) by around 5% (4). Therefore, according to international guidelines, the use of cisplatin-based neoadjuvant chemotherapy is considered standard of care in all patients with localized MIBC with planned curative local treatment (1).

For a long time, there was no consensus which cisplatin-combination regimen (cisplatin/gemcitabine vs dose dense MVAC [ddMVAC, MVAC: methotrexate, vinblastine, adriamycin and cisplatin]) should be administered in the neoadjuvant setting. Recently, a phase III clinical trial (VESPER) suggested improved OS for the ddMVAC regimen compared to cisplatin/gemcitabine (5).

There remains a high unmet need to improve the cure rate for patients with localized MIBC. Moreover, establishment of a treatment with high local control omitting the need for either complete resection or irradiation of the bladder would substantially improve quality of life for those patients. Early results from clinical trials support the feasibility of bladder preserving approaches after immune-chemo-therapy (HCRN GU16-257) (6) In recent years, immunotherapy using PD-1 or PD-L1 targeting immune checkpoint inhibitors (ICI) proved to be beneficial for patients with metastatic bladder cancer and a significant improvement in OS was shown for pembrolizumab in the second-line setting (7). The first results have been presented and published using ICIs as neoadjuvant treatment for localized MIBC. Two monotherapy studies using either pembrolizumab (PURE-01) or atezolizumab (ABACUS) demonstrated pCR of 30-40% (8, 9).

Atezolizumab is a human monoclonal antibody (mAb) of the immunoglobulin G (IgG) 1 kappa subclass that inhibits binding of PD-L1. Atezolizumab was the first ICI to be tested in patients with urothelial carcinoma (UC). The published study program of atezolizumab in UC is broad, comprising phase I to phase IV trials in metastatic pretreated patients (10 - 13) and a phase II trial in metastatic treatment naïve cisplatin-ineligible patients (14). In the phase I trial, 95 pretreated metastatic UC patients received atezolizumab achieving a 40% response rate (10). The phase II trial included 310 platinum-pretreated patients and achieved a response rate of 15% including 5% complete remissions (CR) (11). 931 patients were randomized in the phase III trial comparing atezolizumab against chemotherapy of physician's choice (either docetaxel, paclitaxel or vinflunine). While the primary endpoint of improved OS for patients with high PD-L1 expression was not reached, the OS was numerically higher in the intention to treat population (12). Atezolizumab had a better safety profile than chemotherapy with 20% grade 3/4 toxicity as compared to 43% on chemotherapy. The efficacy and safety were confirmed in a large real-world population (N=1004) safety trial also including patients usually not eligible for immunotherapy trials such as patients with brain metastasis, autoimmune disease, renal insufficiency, HIV positivity as well as frail patients (13). Moreover, atezolizumab monotherapy demonstrated interesting efficacy in the first line treatment of cisplatin-ineligible patients with a response rate of 23% (9% CR) and an OS of 15.9 months (14).

The combination of cisplatin/gemcitabine chemotherapy with atezolizumab has been demonstrated to be effective and safe in a large phase III trial (15). The trial was

positive for the primary endpoint of progression free survival (PFS) without unexpected toxicity from the chemo-immunotherapy combination.

Intravesical instillation of Bacillus Calmette Guérin (BCG) is the recommended standard of care treatment for patients with intermediate/high risk for progression non-muscle invasive bladder cancer (NMIBC) after complete transurethral resection of the bladder tumor (TURB) (16). BCG was shown to cure carcinoma in situ (CIS) and prevent recurrence of high grade NMIBC and to prolong survival compared to TURB alone (16, 17). While the exact mechanism of BCG effect is not entirely understood, it is clear that intravesical BCG induces a local inflammation leading to induction of the innate immune system allowing for a tumor-specific immunity (adaptive immune response (18, 19). Several different BCG strains have been developed and used for intravesical therapy. It has been recognized that there might be differences in terms of immunogenicity and efficacy between strains (20). This has increased interest in developping novel BCG formulations.

A far developed and promising new BCG-derived vaccine is the recombinant Mycobacterium bovis (M. bovis) BCGΔureC::hly. rBCGΔureC::hly which was formulated as VPM1002BC for intravesical immunotherapy against NMIBC. This recombinant BCG (rBCG) VPM1002BC leads to translocation of proteins to the cytosol of infected host or cancer cells by perforation of the phagosome (21, 22). In preclinical models, these changes induce macrophage apoptosis, T cell priming, and proinflammatory cytokine expression, leading to CD4+ and CD8+ T cell responses that are superior compared to the parental BCG subtype Prague. These observations are potentially leading to an improved immune response. VPM1002BC has been used for intravesical therapy in patients with BCG refractory NMIBC in a clinical phase I/II trial (SAKK06/14). The phase I part demonstrated very good tolerance of the compound without need for dose modifications or grade 3 or 4 adverse events (23). The phase II part including 42 patients clearly met the primary endpoint resulting in a recurrence-free survival (RFS) rate in the bladder at 60 weeks in 49.3% of patients (24), while historical data from second-line treatment with conventional BCG results in a RFS rate of 12.5% (25). Only two patients (5%) did tolerate less than 5 instillations and this was not directly related to VPM1002BC. Over the whole course of therapy, treatment related grade 1, 2 and 3 adverse events (AEs) were observed in 14.3%, 54.8%, and 4.8% of the patients, respectively.

Methods/Design

The trial aims to implement a new immuno-immuno-chemotherapy induction therapy for MIBC combining rBCG intravesical installations and ICI followed by neo-adjuvant ICI in combination with chemotherapy followed by radical cystectomy and adjuvant ICI (figure 1).

The trial is a single arm phase 2 trial including patients with histologically proven urothelial cell carcinoma of the bladder (pT2 or cT2, cT3 or cT4a and ≤ cN1 (defined as a solitary lymph node ≤ 2 cm in the greatest dimension) and cM0 and be considered suitable for curative multimodality treatment including radical cystectomy by a multidisciplinary tumor board. Furthermore, location of tumor must allow placement of catheter without risk of bleeding. All histological subtypes are eligible with the exception of small cell neuroendocrine carcinoma. The renal function must be estimated to reach a glomerular filtration rate of (eGFR) > 50 mL/min/1.73m² to allow the use of cisplatin. Patients with prior intravesical BCG, with macrohematuria and those unable to retain BCG instillation for less than 1 hour are excluded.

The protocol includes additional research questions such as preoperative assessment of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and correlation with the pathological outcome, the tumor immunome before and after neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy endpoints, the effect of the gut microbiota on the response to immunotherapy, immune parameters in urine samples and their relation to efficacy endpoints.

Patients receive intravesical rBCG (VPM1002BC) by 3 weekly instillations of rBCG with single dose of VPM1002BC, live, 1-19.2 x 10^8 colony forming units (CFU) on day 1, 8 and 15 of the protocol. Atezolizumab 1200mg fixed dose is started with the first instillation of rBCG (1/-1 day) and continued in combination with the chemotherapy every 3 weeks (q3w) for 4 cycles. Chemotherapy consists of cisplatin and gemcitabine for 4 cycles and is started on day 22 after the first rBCG instillation. Cisplatin is used at a dose of 70mg/m^2 iv on d1 q3w and gemcitabine is used at a dose of 1000mg/m^2 iv on d1 and d8 q3w. Radical cystectomy with extensive lymph node dissection according to actual EAU guidelines is performed 4 to 8 weeks after completion of the last chemo-immunotherapy cycle. Adjuvant atezolizumab is given 1200mg fixed dose q3w for 13 cycles starting 4-16 weeks after date of surgery.

Endpoints

The primary endpoint of the trial is pCR after neoadjuvant treatment defined as ypT0 ypN0 and no evidence of non-muscle invasive bladder cancer (low grade, high grade or CIS). The primary analysis will be based on the results from central pathology review. This endpoint will only be calculated for patients in the resected patients set.

The secondary endpoints are the following:

Event-free survival (EFS)

EFS is defined as the time from treatment start until one of the following events, whichever comes first:

- Progression during neoadjuvant treatment leading to inoperability
- Recurrence or progression (in case of disease persistence) of locoregional disease after surgery
- Appearance of metastases at any localization
- Death

Patients without event at the time of analysis and patients starting a subsequent treatment in the absence of an event will be censored at the date of the last available assessment showing no event before the start of the subsequent treatment, if any. This endpoint will be calculated for patients in the full analysis set (FAS).

Recurrence free survival (RFS)

RFS after R0 resection is defined as the time from surgery until one of the following events, whichever comes first:

- Recurrence of locoregional disease
- Appearance of metastases at any localization
- Death

Patients without event at the time of analysis and patients starting a subsequent treatment in the absence of an event will be censored at the date of the last available assessment showing no event before the start of the subsequent treatment, if any.
This endpoint will only be calculated for patients in the R0 resection set.

Overall survival (OS)

OS is defined as the time from treatment start until death from any cause. Patients not experiencing an event will be censored at the last date they were known to be alive. This endpoint will be calculated for patients in the full analysis set (FAS).

Quality of resection

The quality of resection will be assessed in the following way:

- Complete resection (R0) defined as free resection margins proved microscopically
- Completeness of the lymphadenectomy and surgery using the photo documentation and histopathology
- Postoperative complications will be assessed using the Clavien-Dindo classification.

This endpoint will only be calculated for patients in the resected patients set.

Pathological response rate (PaR)

PaR is defined as pathological downstaging to <ypT2N0M0. The proportion of patients with PaR will be calculated for patients in the resected patients set. This endpoint will only be calculated for patients in the resected patients set.

Pattern of recurrence

Pattern of recurrence is defined as location of first tumor recurrence. Patterns can be locoregional or distant or any combination of these patterns.

Patients with secondary malignancies or patients with no recurrence will not be taken into consideration for this endpoint.

Feasibility

The following treatment feasibility criteria will be assessed:

- Completion of 3 instillations of intravesical VPM1002BC
- Completion of 4 cycles of neoadjuvant chemotherapy
- Completion of 4 cycles of neoadjuvant atezolizumab treatment
- Timely admission to and completion of planned surgery
- Timely initiation and completion of 13 cycles of adjuvant atezolizumab treatment

Adverse events (AE)

AEs will be assessed according to NCI CTCAE v5.0.
This endpoint will be calculated for patients in the safety set.

The protocol includes additional research questions such as preoperative assessment of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and correlation with the pathological outcome, the tumor immunome before and after neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy endpoints, the effect of the gut microbiota on the response to immunotherapy, immune parameters in urine samples and their relation to efficacy endpoints.

Statistics

The sample size is based on the primary endpoint pCR. The null hypothesis is a pCR rate ≤ 35% (based on reference 26) and the alternative hypothesis a pCR rate ≥ 55%. Using Simon's minimax two-stage design with a type I error of 5% and a power of 80%, 39 resected patients are needed. With an estimated drop-out rate of 15% (7 patients), we plan to recruit a total of 46 patients.

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.

After neoadjuvant therapy and resection of the first 21 patients an interim efficacy analysis will be performed. If the number of patients with pCR is 8 or less, the trial will be stopped for futility. If, however, the number of patients with pCR is 9 or more, the trial will continue to stage 2.

The primary analysis will take place after all patients have completed neoadjuvant therapy and had surgery, if applicable. The secondary analysis will be performed when all patients have reached a follow-up of at least 2 years.

For the primary endpoint, the point estimate of the pCR rate will be calculated using the uniformly minimum variance unbiased estimator (UMVUE) and the corresponding two-sided 90% confidence interval will be calculated using the "stage-wise ordering" based-method. If the lower bound of the confidence interval is above 35%, the null hypothesis can be rejected.

For all other binary endpoints the point estimate and exact 95% Clopper-Pearson confidence interval of the proportion will be calculated.

For the primary analysis of the primary endpoint the results from the central pathology review will be used. Supportive analyses are planned based on the following results:

- Local pathology
- MRI (local and central assessment) before surgery
- Cystoscopy and biopsy before surgery
- ctDNA

The following subgroup analyses are planned for the primary endpoint:

- high PD-L1 expression (assessed by standardized immunohistochemistry on tumor cells (TC) and tumor-associated immune cells (IC) using a ≥5% positivity on IC (i.e. IC2) as cutoff) versus no or low expression
- ypT0 vs rest
- ypN0 vs rest
- resection status of TUR-B (complete versus incomplete)

All time-to-event endpoints will have the median value estimated using the Kaplan-Meier method. The number and type of events of each endpoint will be presented descriptively by frequency and percentage.

Categorical variables will be summarized with frequency and percentage. The denominator for percentages will be the number of patients within the set of interest, unless otherwise specified. Continuous variables will be summarized using median and range.

Laboratory values will be expressed as the absolute values and as grading according to NCI CTCAE v5.0. AE grading will be presented by type, grade, and relation showing frequency and percentage of the within-patient worst grade. In addition, grade ≥ 3 AEs and AEs with relation to treatment ≥ 3 will be summarized separately.

Patient and Public Involvement

The protocol was developed within the SAKK network involving multiple stakeholders including physicians specialized in uro-onocology, nurses and the patient advisory board. The design of the trial is aimed to improve cure rates and to pave a scientific way to avoid radical cystectomy in the future, both clear aims to improve quality of live. Patients will be recruited within the SAKK network and the trial is accessible to the public via the SAKK webpage (https://www.sakk.ch/en/news/new-trial-patients-

bladder-cancer-sakk-0619). After closing and analysis of the trial results will be published in scientific journals. A lay abstract will be uploaded on the SAKK webpage.

Discussion

The herein presented clinical trial SAKK 06/19 is the further development of immuno-chemotherapies for MIBC within the SAKK network. SAKK has performed a predecessor single arm phase II trial using neoadjuvant chemo-immunotherapy with cisplatin/gemcitabine in combination with the PD-L1 inhibitor durvalumab (SAKK 06/17). In this trial a total of 61 patients were included in Switzerland and in one German center between 5/2018 and 9/2019. We presented the primary analysis at ASCO 2022 (26) as first trial in MIBC to report a primary endpoint of EFS (manuscript in preparation).

The rationale of the SAKK 06/17 trial was the addition of neo-adjuvant chemotherapy with cisplatin and gemcitabine to checkpoint inhibition to support the development of a therapeutic immune response by reducing the influence of the chronic inflammation caused by the immune suppressive innate cell network. Predominantly myeloid derived suppressor cells (MDSCs, including macrophages and neutrophils) are responsible for chronic inflammation hampering the immune response. Gemcitabine is known to reduce MDSCs and is therefore the ideal partner for an immuno-chemotherapy (27). As a consequence of immune activation, IFN-gamma is released resulting in TH1 T cell response. However, IFN-gamma also induces PD-1 expression on TH1 T cells leading to adaptive immune suppression aiming to stop the T-cell response (28). The use of ICIs is intended to block this negative feedback loop to allow a prolonged T-cell response. Furthermore, 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.

Several similar neo-adjuvant studies in MBIC using immunotherapy or the combination of immuno-chemotherapy have reported pCR rates in the same range of 30-40% and in addition, residual NMIBC can be found in approximately 15-20% (8, 9, 29, 30). Therefore there is hardly any improvement in the pCR rate compared to cisplatin-based chemotherapy, especially when compared to the more active regimen of ddMVAC (5).

In view of these rather modest results so far, strategies to further augment the immune response need to be evaluated. Beside concomitant application of radiotherapy and

immune checkpoint blockade, BCG appears to be a promising combination partner. BCG has been used for treatment of NMIBC for decades with very good success. It induces initial CR in 70-75% of patients with CIS and prevents recurrence in 55-65% of patients with high-risk papillary tumors (16, 17). However, 25-45% of patients don't respond initially and up to 40% experience relapse after initial response. BCG induces an intense local inflammatory response that mediates tumor immunity. Several steps are involved in mounting the inflammatory response including attachment to the urothelium with uptake by antigen presenting cells (APC) and putative internalization into urothelial cells followed by a boost of the innate immune response and induction of adaptive responses (18). Preclinical experiments demonstrated that intravesical BCG instillations induce a robust infiltration of T cells (CD4+ and CD8+) in the bladder wall (31). Moreover, a systemic immune response arises following intravesical BCG demonstrated by increased levels of different cytokines and chemokines including IFNγ, IL-1, IL-2, Il-8, TNF, CCL2, CCL5 (32).

Resistance mechanisms to BCG are not entirely understood but interestingly, granulomata found in patients not responding to BCG were found to be highly expressing PD-L1 (28) suggesting a T-cell exhaustion resulting from checkpoint activation. Patients with ARIDA1A mutation and CCNE1 amplification also appear to be at higher risk of relapse after BCG treatment (33). The immune response induced by intravesical BCG is, however, not solely restricted to the superficial urothelial layer but affects the whole bladder wall and also induces a systemic immune response (20). Therefore, the next logical step appears to use intravesical BCG also in patients with muscle-invasive bladder cancer as an adjuvant to prime and boost the immune response (both innate and adaptive) when using systemic immunotherapy with checkpoint inhibitors (figure 2). To avoid clinically relevant delay three installations of BCG were considered to be enough to prime and boost. This intended priming of the immune system might be better achieved by using the novel rBCG strain VPM1002BC which appears to have improved safety (21) immunogenicity (22). This is mediated by the exchange of the urease C gene with the lsteriolysin gene in rBCG VPM1002BC leading to a stronger adoptive and innate immune response. Furthermore, increased autophagy likely contributes to more rapid elimination of rBCG in the host and because listeriolysin is only active at acidic pH it is rapidly degraded in the cytosol of the host cell and it's effects are short-lived.

This trial tests the hypothesis 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.

Ethics and dissemination: The study has received approval by ethical committee Zurich, Switzerland, BASEC-No. 2021-01872. Results will be made available by publication. Trial registration number: NCT04630730

Trial status

Recruitment started May 2022, estimated closure of accrual April 2025.

List of abbreviations

APC	Antigen presenting cells
BCG	Bacillus Calmette-Guerin
CIS	Carcinoma in situ
CR	Complete response
EFS	Event-free survival
eGFR	Estimated glomerular filtration rate
mAB	Monoclonal antibody
MDSC	Myeloid-derived suppressor cells)
MIBC	Muscle invasive bladder cancer
NCI CTCAE	NCI Common Terminology Criteria for Adverse Events
NMIBC	Non-Muscle invasive bladder cancer
OS	Overall survival
PaR	Pathological response
pCR	Pathological complete remission
PD-L1	Programmed cell death-ligand 1
PD1	Programmed cell death protein 1
PFS	Progression-free survival
RFS	Recurrence-free survival
SAKK	Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung (Swiss Group for Clinical Cancer Research)

Ethics approval

Ethics approval 25.03.2022, ethical committee Zurich, Switzerland, BASEC-No. 2021-01872

Consent for publication

All authors have no objections for publication.

Availability of data and materials

Not applicable

Conflict of interest declaration

UP: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS, Merck, Pfizer, Roche, MSD, Janssen, Novartis

RC: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS, Merck, Pfizer, Roche, MSD, Ipsen, Janssen, Novartis; Honoraria (compensated, institutional) for Janssen, Astellas

MS: None

SH: None

CR: None

SR: Honoraria (compensated, institutional) from Roche, Astra Zeneca, BMS, Boehringer Ingelheim, MSD, Novartis, Amgen, Eli Lilly, Eisai, Merck Serono, Pfizer, Takeda, Bayer, Janssen, Otsuka, PharmaMar, Sanofi; Advisory role (institutional,compensated): Astea Zeneca, Boehringer Ingelheim, BMS, Pfizer, Eisai, Eli Lilly, Merck Serono, MSD, Roche, Novartis, Takeda, Amgen, Otsuka; Research Funding (institutional): Abbvie, BMS, Astra Zeneca, Boehringer Ingelheim, Merck Serono, Roche

MS: None

AO: Advisory role (compensated, institutional): Astra Zeneca, Astellas, Bayer, Janssen, Molecular Partners, MSD, Pfizer, Roche, Sanofi Aventis (compensated, institutional). Novartis, Janssen, Bayer, MSD, AstraZeneca, Merck, Astellas (compensated). Research support (institutional): TEVA, Janssen. Travel support Astellas, Bayer, Janssen, Sanofi Aventis. Speakers Bureau (compensated, institutional): Astellas, Bayer, Janssen

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Provision of drugs

Atezolizumab by Roche

VPM1002BC by VPM/BBIO/SIIPL

Author contributions

UP study design & manuscript writing, RC study design & manuscript writing, MS study design, SH study design and statistical planning, CR study design, SR translational research, AO study design and coordination.

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Figures

Figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

Figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFNγ 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.

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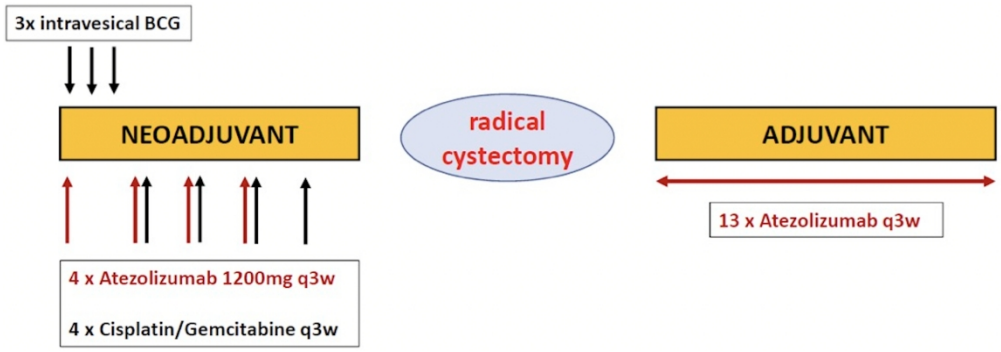

figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

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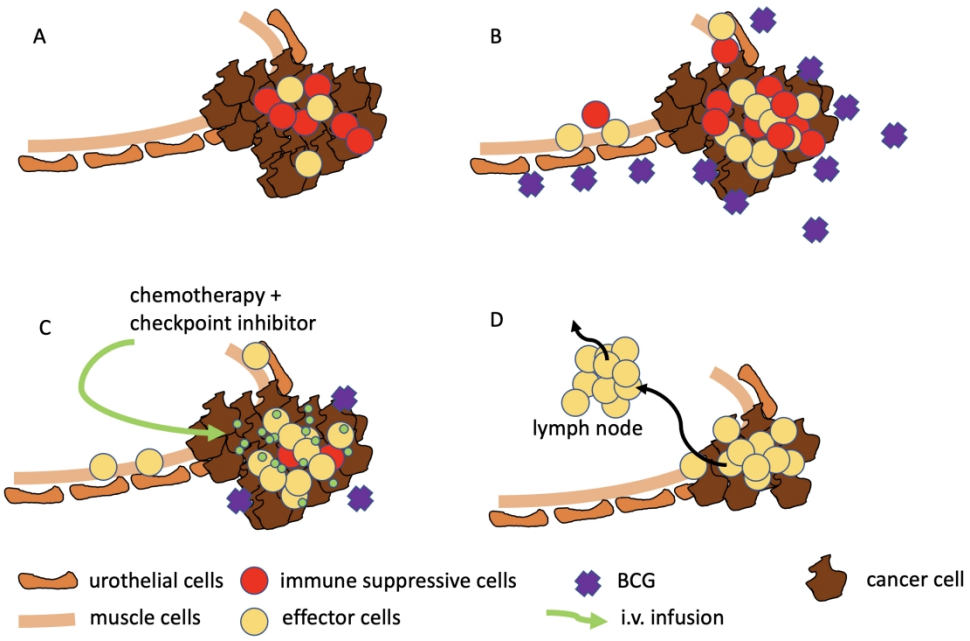

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416x267mm (144 x 144 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	11 -24 of 85
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	76 of 85
	2b	All items from the World Health Organization Trial Registration Data Set	11-24 of 85
Protocol version	3	Date and version identifier	1 of 85
Funding	4	Sources and types of financial, material, and other support	74 of 85
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	2 of 85
	5b	Name and contact information for the trial sponsor	1 of 85
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	74-85 of 85
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	74-85 of 85

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	25-27 of 85
	6b	Explanation for choice of comparators	63 of 85
Objectives	7	Specific objectives or hypotheses	28 of 85
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	63 of 85
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	76 of 85
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	30-31of 85
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	37–42 of 85
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	41 of 85
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	55-59 & 78 of 85
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	59 of 85
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	31 of 85
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	32 of 85

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	21 of 85
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	22 of 85
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n.a. phase II
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n.a. phase II
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n.a. phase II
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n.a. phase II
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n.a. phase II
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	55 – 59 of 85
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	75 of 85

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	62 of 85
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	63-64 of 85
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	63-64 of 85
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	64 of 85
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	74-75 of 85
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	63 of 85
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	43-54 of 85
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	74 of 85
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1 of 85
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	76 of 85

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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

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).

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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).

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Abstract

Introduction: The combination of checkpoint inhibition and cisplatin-based chemotherapy is investigated in muscle invasive bladder cancer (MIBC) and results from phase 2 trials have been presented. Intravesical Bacillus Calmette Guérin (BCG) has been used for non-muscle invasive bladder cancer (NMIBC) in patients with carcinoma in situ (CIS) and high-grade Ta/T1 tumors. BCG induces innate and adapted immune response and upregulation of PD-L1 in preclinical models. The proposed trial is intended to implement a new immuno-immuno-chemotherapy induction therapy for MIBC. The combination of BCG and checkpoint inhibition with chemotherapy aims higher intravesical responses and better local and systemic control of disease.

Methods and Analysis: SAKK 06/19 is an open label single arm phase II trial for patients with resectable MIBC T2-T4a cN0-1. Intravesical rBCG (VPM1002BC) is applied weekly for 3 instillations followed by 4 cycles of neoadjuvant cisplatin/gemcitabine every 3 weeks (q3w). Atezolizumab 1200mg q3w is started together with rBCG and given for 4 cycles. All patients then undergo restaging and radical cystectomy and pelvic lymphadenectomy. Atezolizumab is continued as maintenance therapy after surgery q3w for 13 cycles. Pathological complete remission is the primary endpoint. Secondary endpoints include pathological response rate (<ypT2N0), event-free survival, recurrence-free survival, overall survival, feasibility and toxicity. An interim safety analysis will be performed after the first 12 patients have completed neoadjuvant treatment specifically assessing toxicity possibly associated with intravesical rBCG application.

Keyword

muscle-invasive, resectable, urothelial cancer, Bacillus Calmette-Guérin, neo-adjuvant, adjuvant, chemotherapy, checkpoint inhibition, radical cystectomy, pelvic lymphadenectomy

Strengths and limitations of this study

- Completed predecessor study using the same therapeutic multimodality backbone
- Combination of local immunotherapy with chemotherapy, immune checkpoint blockade and radical cystectomy
- Open label, single arm phase II study
- Primary endpoint: pathological complete remission
- Population: MIBC cT2-T4a cN0-1 cM0

Introduction

Beside bladder sparing chemoradiation therapy, radical cystectomy is the accepted standard curative treatment modality for patients with muscle invasive bladder cancer (MIBC) without evidence of metastatic disease (cM0) (1). Despite the radical surgical approach, stage independent cure rates are however only around 50% at 5 years. Two phase III trials using cisplatin-based neoadjuvant chemotherapy demonstrated a significant improvement of overall survival of muscle-invasive bladder cancer of approximately 5% compared to radical cystectomy alone (2-3). These results were confirmed in a meta-analysis demonstrating that the addition of neoadjuvant cisplatin-based chemotherapy can improve overall survival (OS) by around 5% (4). Therefore, according to international guidelines, the use of cisplatin-based neoadjuvant chemotherapy is considered standard of care in all patients with localized MIBC with planned curative local treatment (1).

For a long time, there was no consensus which cisplatin-combination regimen (cisplatin/gemcitabine vs dose dense MVAC [ddMVAC, MVAC: methotrexate, vinblastine, adriamycin and cisplatin]) should be administered in the neoadjuvant setting. Recently, a phase III clinical trial (VESPER) suggested improved OS for the ddMVAC regimen compared to cisplatin/gemcitabine (5).

There remains a high unmet need to improve the cure rate for patients with localized MIBC. Moreover, establishment of a treatment with high local control omitting the need for either complete resection or irradiation of the bladder would substantially improve quality of life for those patients. Early results from clinical trials support the feasibility of bladder preserving approaches after immune-chemo-therapy (HCRN GU16-257) (6) In recent years, immunotherapy using PD-1 or PD-L1 targeting immune checkpoint inhibitors (ICI) proved to be beneficial for patients with metastatic bladder cancer and a significant improvement in OS was shown for pembrolizumab in the second-line setting (7). The first results have been presented and published using ICIs as neoadjuvant treatment for localized MIBC. Two monotherapy studies using either pembrolizumab (PURE-01) or atezolizumab (ABACUS) demonstrated pCR of 30-40% (8, 9).

Atezolizumab is a human monoclonal antibody (mAb) of the immunoglobulin G (IgG) 1 kappa subclass that inhibits binding of PD-L1. Atezolizumab was the first ICI to be tested in patients with urothelial carcinoma (UC). The published study program of atezolizumab in UC is broad, comprising phase I to phase IV trials in metastatic pretreated patients (10 - 13) and a phase II trial in metastatic treatment naïve cisplatin-ineligible patients (14). In the phase I trial, 95 pretreated metastatic UC patients received atezolizumab achieving a 40% response rate (10). The phase II trial included 310 platinum-pretreated patients and achieved a response rate of 15% including 5% complete remissions (CR) (11). 931 patients were randomized in the phase III trial comparing atezolizumab against chemotherapy of physician's choice (either docetaxel, paclitaxel or vinflunine). While the primary endpoint of improved OS for patients with high PD-L1 expression was not reached, the OS was numerically higher in the intention to treat population (12). Atezolizumab had a better safety profile than chemotherapy with 20% grade 3/4 toxicity as compared to 43% on chemotherapy. The efficacy and safety were confirmed in a large real-world population (N=1004) safety trial also including patients usually not eligible for immunotherapy trials such as patients with brain metastasis, autoimmune disease, renal insufficiency, HIV positivity as well as frail patients (13). Moreover, atezolizumab monotherapy demonstrated interesting efficacy in the first line treatment of cisplatin-ineligible patients with a response rate of 23% (9% CR) and an OS of 15.9 months (14).

The combination of cisplatin/gemcitabine chemotherapy with atezolizumab has been demonstrated to be effective and safe in a large phase III trial (15). The trial was

positive for the primary endpoint of progression free survival (PFS) without unexpected toxicity from the chemo-immunotherapy combination.

Intravesical instillation of Bacillus Calmette Guérin (BCG) is the recommended standard of care treatment for patients with intermediate/high risk for progression non-muscle invasive bladder cancer (NMIBC) after complete transurethral resection of the bladder tumor (TURB) (16). BCG was shown to cure carcinoma in situ (CIS) and prevent recurrence of high grade NMIBC and to prolong survival compared to TURB alone (16, 17). While the exact mechanism of BCG effect is not entirely understood, it is clear that intravesical BCG induces a local inflammation leading to induction of the innate immune system allowing for a tumor-specific immunity (adaptive immune response (18, 19). Several different BCG strains have been developed and used for intravesical therapy. It has been recognized that there might be differences in terms of immunogenicity and efficacy between strains (20). This has increased interest in developping novel BCG formulations.

A far developed and promising new BCG-derived vaccine is the recombinant Mycobacterium bovis (M. bovis) BCGΔureC::hly. rBCGΔureC::hly which was formulated as VPM1002BC for intravesical immunotherapy against NMIBC. This recombinant BCG (rBCG) VPM1002BC leads to translocation of proteins to the cytosol of infected host or cancer cells by perforation of the phagosome (21, 22). In preclinical models, these changes induce macrophage apoptosis, T cell priming, and proinflammatory cytokine expression, leading to CD4+ and CD8+ T cell responses that are superior compared to the parental BCG subtype Prague. These observations are potentially leading to an improved immune response. VPM1002BC has been used for intravesical therapy in patients with BCG refractory NMIBC in a clinical phase I/II trial (SAKK06/14). The phase I part demonstrated very good tolerance of the compound without need for dose modifications or grade 3 or 4 adverse events (23). The phase II part including 42 patients clearly met the primary endpoint resulting in a recurrence-free survival (RFS) rate in the bladder at 60 weeks in 49.3% of patients (24), while historical data from second-line treatment with conventional BCG results in a RFS rate of 12.5% (25). Only two patients (5%) did tolerate less than 5 instillations and this was not directly related to VPM1002BC. Over the whole course of therapy, treatment related grade 1, 2 and 3 adverse events (AEs) were observed in 14.3%, 54.8%, and 4.8% of the patients, respectively.

Methods/Design

The trial aims to implement a new immuno-immuno-chemotherapy induction therapy for MIBC combining rBCG intravesical installations and ICI followed by neo-adjuvant ICI in combination with chemotherapy followed by radical cystectomy and adjuvant ICI (figure 1).

The trial is a single arm phase 2 trial including patients with histologically proven urothelial cell carcinoma of the bladder (pT2 or cT2, cT3 or cT4a and ≤ cN1 (defined as a solitary lymph node ≤ 2 cm in the greatest dimension) and cM0 and be considered suitable for curative multimodality treatment including radical cystectomy by a multidisciplinary tumor board. Furthermore, location of tumor must allow placement of catheter without risk of bleeding. All histological subtypes are eligible with the exception of small cell neuroendocrine carcinoma. The renal function must be estimated to reach a glomerular filtration rate of (eGFR) > 50 mL/min/1.73m² to allow the use of cisplatin. Patients with prior intravesical BCG, with macrohematuria and those unable to retain BCG instillation for less than 1 hour are excluded.

The protocol includes additional research questions such as preoperative assessment of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and correlation with the pathological outcome, the tumor immunome before and after neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy endpoints, the effect of the gut microbiota on the response to immunotherapy, immune parameters in urine samples and their relation to efficacy endpoints.

Patients receive intravesical rBCG (VPM1002BC) by 3 weekly instillations of rBCG with single dose of VPM1002BC, live, 1-19.2 x 10^8 colony forming units (CFU) on day 1, 8 and 15 of the protocol. Atezolizumab 1200mg fixed dose is started with the first instillation of rBCG (1/-1 day) and continued in combination with the chemotherapy every 3 weeks (q3w) for 4 cycles. Chemotherapy consists of cisplatin and gemcitabine for 4 cycles and is started on day 22 after the first rBCG instillation. Cisplatin is used at a dose of 70mg/m^2 iv on d1 q3w and gemcitabine is used at a dose of 1000mg/m^2 iv on d1 and d8 q3w. Radical cystectomy with extensive lymph node dissection according to actual EAU guidelines is performed 4 to 8 weeks after completion of the last chemo-immunotherapy cycle. Adjuvant atezolizumab is given 1200mg fixed dose q3w for 13 cycles starting 4-16 weeks after date of surgery.

Endpoints

The primary endpoint of the trial is pCR after neoadjuvant treatment defined as ypT0 ypN0 and no evidence of non-muscle invasive bladder cancer (low grade, high grade or CIS). The primary analysis will be based on the results from central pathology review. This endpoint will only be calculated for patients in the resected patients set.

The secondary endpoints are the following:

Event-free survival (EFS)

EFS is defined as the time from treatment start until one of the following events, whichever comes first:

- Progression during neoadjuvant treatment leading to inoperability
- Recurrence or progression (in case of disease persistence) of locoregional disease after surgery
- Appearance of metastases at any localization
- Death

Patients without event at the time of analysis and patients starting a subsequent treatment in the absence of an event will be censored at the date of the last available assessment showing no event before the start of the subsequent treatment, if any. This endpoint will be calculated for patients in the full analysis set (FAS).

Recurrence free survival (RFS)

RFS after R0 resection is defined as the time from surgery until one of the following events, whichever comes first:

- Recurrence of locoregional disease
- Appearance of metastases at any localization
- Death

Patients without event at the time of analysis and patients starting a subsequent treatment in the absence of an event will be censored at the date of the last available assessment showing no event before the start of the subsequent treatment, if any.
This endpoint will only be calculated for patients in the R0 resection set.

Overall survival (OS)

OS is defined as the time from treatment start until death from any cause. Patients not experiencing an event will be censored at the last date they were known to be alive. This endpoint will be calculated for patients in the full analysis set (FAS).

Quality of resection

The quality of resection will be assessed in the following way:

- Complete resection (R0) defined as free resection margins proved microscopically
- Completeness of the lymphadenectomy and surgery using the photo documentation and histopathology
- Postoperative complications will be assessed using the Clavien-Dindo classification.

This endpoint will only be calculated for patients in the resected patients set.

Pathological response rate (PaR)

PaR is defined as pathological downstaging to <ypT2N0M0. The proportion of patients with PaR will be calculated for patients in the resected patients set. This endpoint will only be calculated for patients in the resected patients set.

Pattern of recurrence

Pattern of recurrence is defined as location of first tumor recurrence. Patterns can be locoregional or distant or any combination of these patterns.

Patients with secondary malignancies or patients with no recurrence will not be taken into consideration for this endpoint.

Feasibility

The following treatment feasibility criteria will be assessed:

- Completion of 3 instillations of intravesical VPM1002BC
- Completion of 4 cycles of neoadjuvant chemotherapy
- Completion of 4 cycles of neoadjuvant atezolizumab treatment
- Timely admission to and completion of planned surgery
- Timely initiation and completion of 13 cycles of adjuvant atezolizumab treatment

Adverse events (AE)

AEs will be assessed according to NCI CTCAE v5.0.
This endpoint will be calculated for patients in the safety set.

The protocol includes additional research questions such as preoperative assessment of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and correlation with the pathological outcome, the tumor immunome before and after neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy endpoints, the effect of the gut microbiota on the response to immunotherapy, immune parameters in urine samples and their relation to efficacy endpoints.

Statistics

The sample size is based on the primary endpoint pCR. The null hypothesis is a pCR rate ≤ 35% (based on reference 26) and the alternative hypothesis a pCR rate ≥ 55%. Using Simon's minimax two-stage design with a type I error of 5% and a power of 80%, 39 resected patients are needed. With an estimated drop-out rate of 15% (7 patients), we plan to recruit a total of 46 patients.

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.

After neoadjuvant therapy and resection of the first 21 patients an interim efficacy analysis will be performed. If the number of patients with pCR is 8 or less, the trial will be stopped for futility. If, however, the number of patients with pCR is 9 or more, the trial will continue to stage 2.

The primary analysis will take place after all patients have completed neoadjuvant therapy and had surgery, if applicable. The secondary analysis will be performed when all patients have reached a follow-up of at least 2 years.

For the primary endpoint, the point estimate of the pCR rate will be calculated using the uniformly minimum variance unbiased estimator (UMVUE) and the corresponding two-sided 90% confidence interval will be calculated using the "stage-wise ordering" based-method. If the lower bound of the confidence interval is above 35%, the null hypothesis can be rejected.

For all other binary endpoints the point estimate and exact 95% Clopper-Pearson confidence interval of the proportion will be calculated.

For the primary analysis of the primary endpoint the results from the central pathology review will be used. Supportive analyses are planned based on the following results:

- Local pathology
- MRI (local and central assessment) before surgery
- Cystoscopy and biopsy before surgery
- ctDNA

The following subgroup analyses are planned for the primary endpoint:

- high PD-L1 expression (assessed by standardized immunohistochemistry on tumor cells (TC) and tumor-associated immune cells (IC) using a ≥5% positivity on IC (i.e. IC2) as cutoff) versus no or low expression
- ypT0 vs rest
- ypN0 vs rest
- resection status of TUR-B (complete versus incomplete)

All time-to-event endpoints will have the median value estimated using the Kaplan-Meier method. The number and type of events of each endpoint will be presented descriptively by frequency and percentage.

Categorical variables will be summarized with frequency and percentage. The denominator for percentages will be the number of patients within the set of interest, unless otherwise specified. Continuous variables will be summarized using median and range.

Laboratory values will be expressed as the absolute values and as grading according to NCI CTCAE v5.0. AE grading will be presented by type, grade, and relation showing frequency and percentage of the within-patient worst grade. In addition, grade ≥ 3 AEs and AEs with relation to treatment ≥ 3 will be summarized separately.

Patient and Public Involvement

The protocol was developed within the SAKK network involving multiple stakeholders including physicians specialized in uro-onocology, nurses and the patient advisory board. The design of the trial is aimed to improve cure rates and to pave a scientific way to avoid radical cystectomy in the future, both clear aims to improve quality of live. Patients will be recruited within the SAKK network and the trial is accessible to the public via the SAKK webpage (https://www.sakk.ch/en/news/new-trial-patients-

bladder-cancer-sakk-0619). After closing and analysis of the trial results will be published in scientific journals. A lay abstract will be uploaded on the SAKK webpage.

Discussion

The herein presented clinical trial SAKK 06/19 is the further development of immuno-chemotherapies for MIBC within the SAKK network. SAKK has performed a predecessor single arm phase II trial using neoadjuvant chemo-immunotherapy with cisplatin/gemcitabine in combination with the PD-L1 inhibitor durvalumab (SAKK 06/17). In this trial a total of 61 patients were included in Switzerland and in one German center between 5/2018 and 9/2019. We presented the primary analysis at ASCO 2022 (26) as first trial in MIBC to report a primary endpoint of EFS (manuscript in preparation).

The rationale of the SAKK 06/17 trial was the addition of neo-adjuvant chemotherapy with cisplatin and gemcitabine to checkpoint inhibition to support the development of a therapeutic immune response by reducing the influence of the chronic inflammation caused by the immune suppressive innate cell network. Predominantly myeloid derived suppressor cells (MDSCs, including macrophages and neutrophils) are responsible for chronic inflammation hampering the immune response. Gemcitabine is known to reduce MDSCs and is therefore the ideal partner for an immuno-chemotherapy (27). As a consequence of immune activation, IFN-gamma is released resulting in TH1 T cell response. However, IFN-gamma also induces PD-1 expression on TH1 T cells leading to adaptive immune suppression aiming to stop the T-cell response (28). The use of ICIs is intended to block this negative feedback loop to allow a prolonged T-cell response. Furthermore, 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.

Several similar neo-adjuvant studies in MBIC using immunotherapy or the combination of immuno-chemotherapy have reported pCR rates in the same range of 30-40% and in addition, residual NMIBC can be found in approximately 15-20% (8, 9, 29, 30). Therefore there is hardly any improvement in the pCR rate compared to cisplatin-based chemotherapy, especially when compared to the more active regimen of ddMVAC (5).

In view of these rather modest results so far, strategies to further augment the immune response need to be evaluated. Beside concomitant application of radiotherapy and

immune checkpoint blockade, BCG appears to be a promising combination partner. BCG has been used for treatment of NMIBC for decades with very good success. It induces initial CR in 70-75% of patients with CIS and prevents recurrence in 55-65% of patients with high-risk papillary tumors (16, 17). However, 25-45% of patients don't respond initially and up to 40% experience relapse after initial response. BCG induces an intense local inflammatory response that mediates tumor immunity. Several steps are involved in mounting the inflammatory response including attachment to the urothelium with uptake by antigen presenting cells (APC) and putative internalization into urothelial cells followed by a boost of the innate immune response and induction of adaptive responses (18). Preclinical experiments demonstrated that intravesical BCG instillations induce a robust infiltration of T cells (CD4+ and CD8+) in the bladder wall (31). Moreover, a systemic immune response arises following intravesical BCG demonstrated by increased levels of different cytokines and chemokines including IFNγ, IL-1, IL-2, Il-8, TNF, CCL2, CCL5 (32).

Resistance mechanisms to BCG are not entirely understood but interestingly, granulomata found in patients not responding to BCG were found to be highly expressing PD-L1 (28) suggesting a T-cell exhaustion resulting from checkpoint activation. Patients with ARIDA1A mutation and CCNE1 amplification also appear to be at higher risk of relapse after BCG treatment (33). The immune response induced by intravesical BCG is, however, not solely restricted to the superficial urothelial layer but affects the whole bladder wall and also induces a systemic immune response (20). Therefore, the next logical step appears to use intravesical BCG also in patients with muscle-invasive bladder cancer as an adjuvant to prime and boost the immune response (both innate and adaptive) when using systemic immunotherapy with checkpoint inhibitors (figure 2). To avoid clinically relevant delay three installations of BCG were considered to be enough to prime and boost. This intended priming of the immune system might be better achieved by using the novel rBCG strain VPM1002BC which appears to have improved safety (21) immunogenicity (22). This is mediated by the exchange of the urease C gene with the lsteriolysin gene in rBCG VPM1002BC leading to a stronger adoptive and innate immune response. Furthermore, increased autophagy likely contributes to more rapid elimination of rBCG in the host and because listeriolysin is only active at acidic pH it is rapidly degraded in the cytosol of the host cell and it's effects are short-lived.

Our trial includes a broad translational research program evaluating different possible markers of treatment efficacy. We hope to help identify molecular predicitive biomarkers to tailor treatment more efficiently towards patients who are more likely to benefit and to spare the others unnecessary systemic treatment and proceed directly to radical local therapy.

In conclusion, this trial tests the hypothesis 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.

Ethics and dissemination: The study has received approval by ethical committee Zurich, Switzerland, BASEC-No. 2021-01872. Results will be made available by publication. Trial registration number: NCT04630730

Trial status

Recruitment started May 2022, estimated closure of accrual April 2025.

List of abbreviations

APC	Antigen presenting cells
BCG	Bacillus Calmette-Guerin
CIS	Carcinoma in situ
CR	Complete response
EFS	Event-free survival
eGFR	Estimated glomerular filtration rate
mAB	Monoclonal antibody
MDSC	Myeloid-derived suppressor cells)
MIBC	Muscle invasive bladder cancer
NCI CTCAE	NCI Common Terminology Criteria for Adverse Events
NMIBC	Non-Muscle invasive bladder cancer
OS	Overall survival
PaR	Pathological response
pCR	Pathological complete remission
PD-L1	Programmed cell death-ligand 1
PD1	Programmed cell death protein 1
PFS	Progression-free survival
RFS	Recurrence-free survival

SAKK Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung (Swiss Group for Clinical Cancer Research)

Ethics approval

Ethics approval 25.03.2022, ethical committee Zurich, Switzerland, BASEC-No. 2021-01872

Consent for publication

All authors have no objections for publication.

Availability of data and materials

Not applicable

Conflict of interest declaration

UP: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS, Merck, Pfizer, Roche, MSD, Janssen, Novartis

RC: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS, Merck, Pfizer, Roche, MSD, Ipsen, Janssen, Novartis; Honoraria (compensated, institutional) for Janssen, Astellas

MS: None

SH: None

CR: None

SR: Honoraria (compensated, institutional) from Roche, Astra Zeneca, BMS, Boehringer Ingelheim, MSD, Novartis, Amgen, Eli Lilly, Eisai, Merck Serono, Pfizer, Takeda, Bayer, Janssen, Otsuka, PharmaMar, Sanofi; Advisory role (institutional,compensated): Astea Zeneca, Boehringer Ingelheim, BMS, Pfizer, Eisai, Eli Lilly, Merck Serono, MSD, Roche, Novartis, Takeda, Amgen, Otsuka; Research Funding (institutional): Abbvie, BMS, Astra Zeneca, Boehringer Ingelheim, Merck Serono, Roche

MS: None

AO: Advisory role (compensated, institutional): Astra Zeneca, Astellas, Bayer, Janssen, Molecular Partners, MSD, Pfizer, Roche, Sanofi Aventis (compensated, institutional). Novartis, Janssen, Bayer, MSD, AstraZeneca, Merck, Astellas (compensated). Research support (institutional): TEVA, Janssen. Travel support

Astellas, Bayer, Janssen, Sanofi Aventis. Speakers Bureau (compensated, institutional): Astellas, Bayer, Janssen

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Provision of drugs

Atezolizumab by Roche

VPM1002BC by VPM/BBIO/SIIPL

Author contributions

Ulf Petrausch performed the study design and wrote the manuscript, Martin Spahn performed the study design, Martina Schneider submitted the protocol to authorities and ethical committee, Stefanie Hayoz performed the study design and did all statistical planning, Cyrill A. Rentsch performed the study design, Sacha I. Rothschild planned all translational research and will perform the analysis, Aurelius Omlin performed the study design and coordinated all centers for patient accrural, and Richard Cathomas performed the study design and wrote the manuscript.

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Figures

Figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

Figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFNγ 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.

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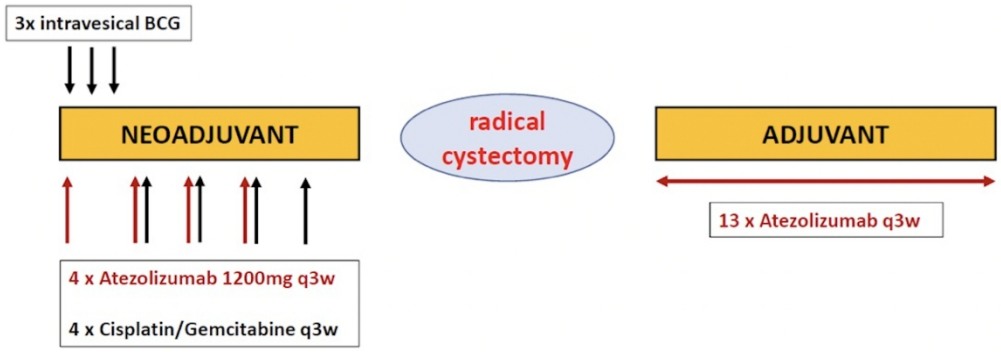

figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

480x167mm (144 x 144 DPI)

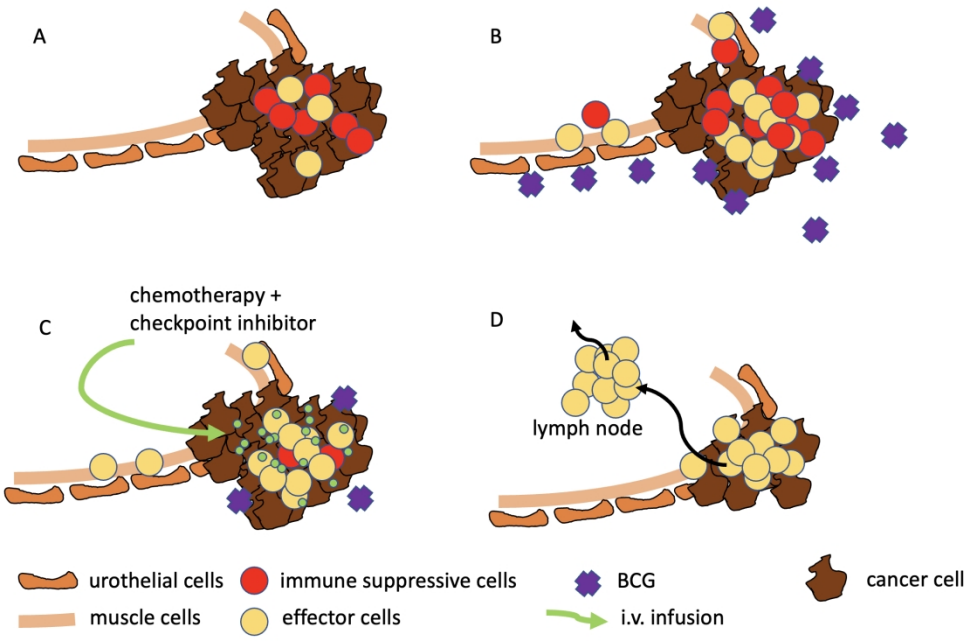

figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFN□ release resulting in increased number of immune suppressive immune cells (MDSC), and upregulation of PD-L1, C: chemotherapy diminishes MDSC, checkpoint inhibition 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.

416x267mm (144 x 144 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	11 -24 of 85
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	76 of 85
	2b	All items from the World Health Organization Trial Registration Data Set	11-24 of 85
Protocol version	3	Date and version identifier	1 of 85
Funding	4	Sources and types of financial, material, and other support	74 of 85
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	2 of 85
	5b	Name and contact information for the trial sponsor	1 of 85
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	74-85 of 85
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	74-85 of 85

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	25-27 of 85
	6b	Explanation for choice of comparators	63 of 85
Objectives	7	Specific objectives or hypotheses	28 of 85
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	63 of 85
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	76 of 85
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	30-31of 85
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	37–42 of 85
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	41 of 85
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	55-59 & 78 of 85
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	59 of 85
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	31 of 85
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	32 of 85

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	21 of 85
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	22 of 85
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n.a. phase II
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n.a. phase II
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n.a. phase II
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n.a. phase II
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n.a. phase II
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	55 – 59 of 85
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	75 of 85

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	62 of 85
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	63-64 of 85
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	63-64 of 85
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	64 of 85
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	74-75 of 85
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	63 of 85
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	43-54 of 85
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	74 of 85
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1 of 85
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	76 of 85

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	73-74 of 85
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	73-74 of 85
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	77 of 85
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	1 of 85
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	74 of 85
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	77 of 85
	31b	Authorship eligibility guidelines and any intended use of professional writers	77 of 85
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n.a.
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Separate file
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	67-72 of 85

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

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).

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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).

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Abstract

Introduction: The combination of checkpoint inhibition and cisplatin-based chemotherapy is investigated in muscle invasive bladder cancer (MIBC) and results from phase 2 trials have been presented. Intravesical Bacillus Calmette Guérin (BCG) has been used for non-muscle invasive bladder cancer (NMIBC) in patients with carcinoma in situ (CIS) and high-grade Ta/T1 tumors. BCG induces innate and adapted immune response and upregulation of PD-L1 in preclinical models. The proposed trial is intended to implement a new immuno-immuno-chemotherapy induction therapy for MIBC. The combination of BCG and checkpoint inhibition with chemotherapy aims higher intravesical responses and better local and systemic control of disease.

Methods and Analysis: SAKK 06/19 is an open label single arm phase II trial for patients with resectable MIBC T2-T4a cN0-1. Intravesical rBCG (VPM1002BC) is applied weekly for 3 instillations followed by 4 cycles of neoadjuvant cisplatin/gemcitabine every 3 weeks (q3w). Atezolizumab 1200mg q3w is started together with rBCG and given for 4 cycles. All patients then undergo restaging and radical cystectomy and pelvic lymphadenectomy. Atezolizumab is continued as maintenance therapy after surgery q3w for 13 cycles. Pathological complete remission is the primary endpoint. Secondary endpoints include pathological response rate (<ypT2N0), event-free survival, recurrence-free survival, overall survival, feasibility and toxicity. An interim safety analysis will be performed after the first 12 patients have completed neoadjuvant treatment specifically assessing toxicity possibly associated with intravesical rBCG application.

Keyword

muscle-invasive, resectable, urothelial cancer, Bacillus Calmette-Guérin, neo-adjuvant, adjuvant, chemotherapy, checkpoint inhibition, radical cystectomy, pelvic lymphadenectomy

Strengths and limitations of this study

- The completed predecessor study used the same therapeutic multimodality backbone.
- The study combines local immunotherapy with chemotherapy, immune checkpoint blockade and radical cystectomy.
- This is an open label, single arm phase II study.
- The primary endpoint is pathological complete remission.
- The population included consists of patients with MIBC cT2-T4a cN0-1 cM0.

Introduction

Beside bladder sparing chemoradiation therapy, radical cystectomy is the accepted standard curative treatment modality for patients with muscle invasive bladder cancer (MIBC) without evidence of metastatic disease (cM0) (1). Despite the radical surgical approach, stage independent cure rates are however only around 50% at 5 years. Two phase III trials using cisplatin-based neoadjuvant chemotherapy demonstrated a significant improvement of overall survival of muscle-invasive bladder cancer of approximately 5% compared to radical cystectomy alone (2-3). These results were confirmed in a meta-analysis demonstrating that the addition of neoadjuvant cisplatin-based chemotherapy can improve overall survival (OS) by around 5% (4). Therefore, according to international guidelines, the use of cisplatin-based neoadjuvant chemotherapy is considered standard of care in all patients with localized MIBC with planned curative local treatment (1).

For a long time, there was no consensus which cisplatin-combination regimen (cisplatin/gemcitabine vs dose dense MVAC [ddMVAC, MVAC: methotrexate, vinblastine, adriamycin and cisplatin]) should be administered in the neoadjuvant setting. Recently, a phase III clinical trial (VESPER) suggested improved OS for the ddMVAC regimen compared to cisplatin/gemcitabine (5).

There remains a high unmet need to improve the cure rate for patients with localized MIBC. Moreover, establishment of a treatment with high local control omitting the need for either complete resection or irradiation of the bladder would substantially improve quality of life for those patients. Early results from clinical trials support the feasibility of bladder preserving approaches after immune-chemo-therapy (HCRN GU16-257) (6) In recent years, immunotherapy using PD-1 or PD-L1 targeting immune checkpoint inhibitors (ICI) proved to be beneficial for patients with metastatic bladder cancer and a significant improvement in OS was shown for pembrolizumab in the second-line setting (7). The first results have been presented and published using ICIs as neoadjuvant treatment for localized MIBC. Two monotherapy studies using either pembrolizumab (PURE-01) or atezolizumab (ABACUS) demonstrated pCR of 30-40% (8, 9).

Atezolizumab is a human monoclonal antibody (mAb) of the immunoglobulin G (IgG) 1 kappa subclass that inhibits binding of PD-L1. Atezolizumab was the first ICI to be tested in patients with urothelial carcinoma (UC). The published study program of atezolizumab in UC is broad, comprising phase I to phase IV trials in metastatic pretreated patients (10 - 13) and a phase II trial in metastatic treatment naïve cisplatin-ineligible patients (14). In the phase I trial, 95 pretreated metastatic UC patients received atezolizumab achieving a 40% response rate (10). The phase II trial included 310 platinum-pretreated patients and achieved a response rate of 15% including 5% complete remissions (CR) (11). 931 patients were randomized in the phase III trial comparing atezolizumab against chemotherapy of physician's choice (either docetaxel, paclitaxel or vinflunine). While the primary endpoint of improved OS for patients with high PD-L1 expression was not reached, the OS was numerically higher in the intention to treat population (12). Atezolizumab had a better safety profile than chemotherapy with 20% grade 3/4 toxicity as compared to 43% on chemotherapy. The efficacy and safety were confirmed in a large real-world population (N=1004) safety trial also including patients usually not eligible for immunotherapy trials such as patients with brain metastasis, autoimmune disease, renal insufficiency, HIV positivity as well as frail patients (13). Moreover, atezolizumab monotherapy demonstrated interesting efficacy in the first line treatment of cisplatin-ineligible patients with a response rate of 23% (9% CR) and an OS of 15.9 months (14).

The combination of cisplatin/gemcitabine chemotherapy with atezolizumab has been demonstrated to be effective and safe in a large phase III trial (15). The trial was

positive for the primary endpoint of progression free survival (PFS) without unexpected toxicity from the chemo-immunotherapy combination.

Intravesical instillation of Bacillus Calmette Guérin (BCG) is the recommended standard of care treatment for patients with intermediate/high risk for progression non-muscle invasive bladder cancer (NMIBC) after complete transurethral resection of the bladder tumor (TURB) (16). BCG was shown to cure carcinoma in situ (CIS) and prevent recurrence of high grade NMIBC and to prolong survival compared to TURB alone (16, 17). While the exact mechanism of BCG effect is not entirely understood, it is clear that intravesical BCG induces a local inflammation leading to induction of the innate immune system allowing for a tumor-specific immunity (adaptive immune response (18, 19). Several different BCG strains have been developed and used for intravesical therapy. It has been recognized that there might be differences in terms of immunogenicity and efficacy between strains (20). This has increased interest in developping novel BCG formulations.

A far developed and promising new BCG-derived vaccine is the recombinant Mycobacterium bovis (M. bovis) BCGΔureC::hly. rBCGΔureC::hly which was formulated as VPM1002BC for intravesical immunotherapy against NMIBC. This recombinant BCG (rBCG) VPM1002BC leads to translocation of proteins to the cytosol of infected host or cancer cells by perforation of the phagosome (21, 22). In preclinical models, these changes induce macrophage apoptosis, T cell priming, and proinflammatory cytokine expression, leading to CD4+ and CD8+ T cell responses that are superior compared to the parental BCG subtype Prague. These observations are potentially leading to an improved immune response. VPM1002BC has been used for intravesical therapy in patients with BCG refractory NMIBC in a clinical phase I/II trial (SAKK06/14). The phase I part demonstrated very good tolerance of the compound without need for dose modifications or grade 3 or 4 adverse events (23). The phase II part including 42 patients clearly met the primary endpoint resulting in a recurrence-free survival (RFS) rate in the bladder at 60 weeks in 49.3% of patients (24), while historical data from second-line treatment with conventional BCG results in a RFS rate of 12.5% (25). Only two patients (5%) did tolerate less than 5 instillations and this was not directly related to VPM1002BC. Over the whole course of therapy, treatment related grade 1, 2 and 3 adverse events (AEs) were observed in 14.3%, 54.8%, and 4.8% of the patients, respectively.

Methods/Design

The trial aims to implement a new immuno-immuno-chemotherapy induction therapy for MIBC combining rBCG intravesical installations and ICI followed by neo-adjuvant ICI in combination with chemotherapy followed by radical cystectomy and adjuvant ICI (figure 1).

The trial is a single arm phase 2 trial including patients with histologically proven urothelial cell carcinoma of the bladder (pT2 or cT2, cT3 or cT4a and ≤ cN1 (defined as a solitary lymph node ≤ 2 cm in the greatest dimension) and cM0 and be considered suitable for curative multimodality treatment including radical cystectomy by a multidisciplinary tumor board. Furthermore, location of tumor must allow placement of catheter without risk of bleeding. All histological subtypes are eligible with the exception of small cell neuroendocrine carcinoma. The renal function must be estimated to reach a glomerular filtration rate of (eGFR) > 50 mL/min/1.73m² to allow the use of cisplatin. Patients with prior intravesical BCG, with macrohematuria and those unable to retain BCG instillation for less than 1 hour are excluded.

The protocol includes additional research questions such as preoperative assessment of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and correlation with the pathological outcome, the tumor immunome before and after neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy endpoints, the effect of the gut microbiota on the response to immunotherapy, immune parameters in urine samples and their relation to efficacy endpoints.

Patients receive intravesical rBCG (VPM1002BC) by 3 weekly instillations of rBCG with single dose of VPM1002BC, live, 1-19.2 x 10^8 colony forming units (CFU) on day 1, 8 and 15 of the protocol. Atezolizumab 1200mg fixed dose is started with the first instillation of rBCG (1/-1 day) and continued in combination with the chemotherapy every 3 weeks (q3w) for 4 cycles. Chemotherapy consists of cisplatin and gemcitabine for 4 cycles and is started on day 22 after the first rBCG instillation. Cisplatin is used at a dose of 70mg/m^2 iv on d1 q3w and gemcitabine is used at a dose of 1000mg/m^2 iv on d1 and d8 q3w. Radical cystectomy with extensive lymph node dissection according to actual EAU guidelines is performed 4 to 8 weeks after completion of the last chemo-immunotherapy cycle. Adjuvant atezolizumab is given 1200mg fixed dose q3w for 13 cycles starting 4-16 weeks after date of surgery.

Endpoints

The primary endpoint of the trial is pCR after neoadjuvant treatment defined as ypT0 ypN0 and no evidence of non-muscle invasive bladder cancer (low grade, high grade or CIS). The primary analysis will be based on the results from central pathology review. This endpoint will only be calculated for patients in the resected patients set.

The secondary endpoints are the following:

Event-free survival (EFS)

EFS is defined as the time from treatment start until one of the following events, whichever comes first:

- Progression during neoadjuvant treatment leading to inoperability
- Recurrence or progression (in case of disease persistence) of locoregional disease after surgery
- Appearance of metastases at any localization
- Death

Patients without event at the time of analysis and patients starting a subsequent treatment in the absence of an event will be censored at the date of the last available assessment showing no event before the start of the subsequent treatment, if any. This endpoint will be calculated for patients in the full analysis set (FAS).

Recurrence free survival (RFS)

RFS after R0 resection is defined as the time from surgery until one of the following events, whichever comes first:

- Recurrence of locoregional disease
- Appearance of metastases at any localization
- Death

Patients without event at the time of analysis and patients starting a subsequent treatment in the absence of an event will be censored at the date of the last available assessment showing no event before the start of the subsequent treatment, if any.
This endpoint will only be calculated for patients in the R0 resection set.

Overall survival (OS)

OS is defined as the time from treatment start until death from any cause. Patients not experiencing an event will be censored at the last date they were known to be alive. This endpoint will be calculated for patients in the full analysis set (FAS).

Quality of resection

The quality of resection will be assessed in the following way:

- Complete resection (R0) defined as free resection margins proved microscopically
- Completeness of the lymphadenectomy and surgery using the photo documentation and histopathology
- Postoperative complications will be assessed using the Clavien-Dindo classification.

This endpoint will only be calculated for patients in the resected patients set.

Pathological response rate (PaR)

PaR is defined as pathological downstaging to <ypT2N0M0. The proportion of patients with PaR will be calculated for patients in the resected patients set. This endpoint will only be calculated for patients in the resected patients set.

Pattern of recurrence

Pattern of recurrence is defined as location of first tumor recurrence. Patterns can be locoregional or distant or any combination of these patterns.

Patients with secondary malignancies or patients with no recurrence will not be taken into consideration for this endpoint.

Feasibility

The following treatment feasibility criteria will be assessed:

- Completion of 3 instillations of intravesical VPM1002BC
- Completion of 4 cycles of neoadjuvant chemotherapy
- Completion of 4 cycles of neoadjuvant atezolizumab treatment
- Timely admission to and completion of planned surgery
- Timely initiation and completion of 13 cycles of adjuvant atezolizumab treatment

Adverse events (AE)

AEs will be assessed according to NCI CTCAE v5.0.
This endpoint will be calculated for patients in the safety set.

The protocol includes additional research questions such as preoperative assessment of treatment response using MRI and circulating cell-free tumor DNA (ctDNA) and correlation with the pathological outcome, the tumor immunome before and after neoadjuvant chemo- and immunotherapy, tissue expression of PD-L1 and its relation to efficacy endpoints, biomarkers for anti-PD-L1 treatment and their relation to efficacy endpoints, the effect of the gut microbiota on the response to immunotherapy, immune parameters in urine samples and their relation to efficacy endpoints.

Statistics

The sample size is based on the primary endpoint pCR. The null hypothesis is a pCR rate ≤ 35% (based on reference 26) and the alternative hypothesis a pCR rate ≥ 55%. Using Simon's minimax two-stage design with a type I error of 5% and a power of 80%, 39 resected patients are needed. With an estimated drop-out rate of 15% (7 patients), we plan to recruit a total of 46 patients.

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.

After neoadjuvant therapy and resection of the first 21 patients an interim efficacy analysis will be performed. If the number of patients with pCR is 8 or less, the trial will be stopped for futility. If, however, the number of patients with pCR is 9 or more, the trial will continue to stage 2.

The primary analysis will take place after all patients have completed neoadjuvant therapy and had surgery, if applicable. The secondary analysis will be performed when all patients have reached a follow-up of at least 2 years.

For the primary endpoint, the point estimate of the pCR rate will be calculated using the uniformly minimum variance unbiased estimator (UMVUE) and the corresponding two-sided 90% confidence interval will be calculated using the "stage-wise ordering" based-method. If the lower bound of the confidence interval is above 35%, the null hypothesis can be rejected.

For all other binary endpoints the point estimate and exact 95% Clopper-Pearson confidence interval of the proportion will be calculated.

For the primary analysis of the primary endpoint the results from the central pathology review will be used. Supportive analyses are planned based on the following results:

- Local pathology
- MRI (local and central assessment) before surgery
- Cystoscopy and biopsy before surgery
- ctDNA

The following subgroup analyses are planned for the primary endpoint:

- high PD-L1 expression (assessed by standardized immunohistochemistry on tumor cells (TC) and tumor-associated immune cells (IC) using a ≥5% positivity on IC (i.e. IC2) as cutoff) versus no or low expression
- ypT0 vs rest
- ypN0 vs rest
- resection status of TUR-B (complete versus incomplete)

All time-to-event endpoints will have the median value estimated using the Kaplan-Meier method. The number and type of events of each endpoint will be presented descriptively by frequency and percentage.

Categorical variables will be summarized with frequency and percentage. The denominator for percentages will be the number of patients within the set of interest, unless otherwise specified. Continuous variables will be summarized using median and range.

Laboratory values will be expressed as the absolute values and as grading according to NCI CTCAE v5.0. AE grading will be presented by type, grade, and relation showing frequency and percentage of the within-patient worst grade. In addition, grade ≥ 3 AEs and AEs with relation to treatment ≥ 3 will be summarized separately.

Patient and Public Involvement

The protocol was developed within the SAKK network involving multiple stakeholders including physicians specialized in uro-onocology, nurses and the patient advisory board. The design of the trial is aimed to improve cure rates and to pave a scientific way to avoid radical cystectomy in the future, both clear aims to improve quality of live. Patients will be recruited within the SAKK network and the trial is accessible to the public via the SAKK webpage (https://www.sakk.ch/en/news/new-trial-patients-

bladder-cancer-sakk-0619). After closing and analysis of the trial results will be published in scientific journals. A lay abstract will be uploaded on the SAKK webpage.

Discussion

The herein presented clinical trial SAKK 06/19 is the further development of immuno-chemotherapies for MIBC within the SAKK network. SAKK has performed a predecessor single arm phase II trial using neoadjuvant chemo-immunotherapy with cisplatin/gemcitabine in combination with the PD-L1 inhibitor durvalumab (SAKK 06/17). In this trial a total of 61 patients were included in Switzerland and in one German center between 5/2018 and 9/2019. We presented the primary analysis at ASCO 2022 (26) as first trial in MIBC to report a primary endpoint of EFS (manuscript in preparation).

The rationale of the SAKK 06/17 trial was the addition of neo-adjuvant chemotherapy with cisplatin and gemcitabine to checkpoint inhibition to support the development of a therapeutic immune response by reducing the influence of the chronic inflammation caused by the immune suppressive innate cell network. Predominantly myeloid derived suppressor cells (MDSCs, including macrophages and neutrophils) are responsible for chronic inflammation hampering the immune response. Gemcitabine is known to reduce MDSCs and is therefore the ideal partner for an immuno-chemotherapy (27). As a consequence of immune activation, IFN-gamma is released resulting in TH1 T cell response. However, IFN-gamma also induces PD-1 expression on TH1 T cells leading to adaptive immune suppression aiming to stop the T-cell response (28). The use of ICIs is intended to block this negative feedback loop to allow a prolonged T-cell response. Furthermore, 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.

Several similar neo-adjuvant studies in MBIC using immunotherapy or the combination of immuno-chemotherapy have reported pCR rates in the same range of 30-40% and in addition, residual NMIBC can be found in approximately 15-20% (8, 9, 29, 30). Therefore there is hardly any improvement in the pCR rate compared to cisplatin-based chemotherapy, especially when compared to the more active regimen of ddMVAC (5).

In view of these rather modest results so far, strategies to further augment the immune response need to be evaluated. Beside concomitant application of radiotherapy and

immune checkpoint blockade, BCG appears to be a promising combination partner. BCG has been used for treatment of NMIBC for decades with very good success. It induces initial CR in 70-75% of patients with CIS and prevents recurrence in 55-65% of patients with high-risk papillary tumors (16, 17). However, 25-45% of patients don't respond initially and up to 40% experience relapse after initial response. BCG induces an intense local inflammatory response that mediates tumor immunity. Several steps are involved in mounting the inflammatory response including attachment to the urothelium with uptake by antigen presenting cells (APC) and putative internalization into urothelial cells followed by a boost of the innate immune response and induction of adaptive responses (18). Preclinical experiments demonstrated that intravesical BCG instillations induce a robust infiltration of T cells (CD4+ and CD8+) in the bladder wall (31). Moreover, a systemic immune response arises following intravesical BCG demonstrated by increased levels of different cytokines and chemokines including IFNγ, IL-1, IL-2, Il-8, TNF, CCL2, CCL5 (32).

Resistance mechanisms to BCG are not entirely understood but interestingly, granulomata found in patients not responding to BCG were found to be highly expressing PD-L1 (28) suggesting a T-cell exhaustion resulting from checkpoint activation. Patients with ARIDA1A mutation and CCNE1 amplification also appear to be at higher risk of relapse after BCG treatment (33). The immune response induced by intravesical BCG is, however, not solely restricted to the superficial urothelial layer but affects the whole bladder wall and also induces a systemic immune response (20). Therefore, the next logical step appears to use intravesical BCG also in patients with muscle-invasive bladder cancer as an adjuvant to prime and boost the immune response (both innate and adaptive) when using systemic immunotherapy with checkpoint inhibitors (figure 2). To avoid clinically relevant delay three installations of BCG were considered to be enough to prime and boost. This intended priming of the immune system might be better achieved by using the novel rBCG strain VPM1002BC which appears to have improved safety (21) immunogenicity (22). This is mediated by the exchange of the urease C gene with the lsteriolysin gene in rBCG VPM1002BC leading to a stronger adoptive and innate immune response. Furthermore, increased autophagy likely contributes to more rapid elimination of rBCG in the host and because listeriolysin is only active at acidic pH it is rapidly degraded in the cytosol of the host cell and it's effects are short-lived.

Our trial includes a broad translational research program evaluating different possible markers of treatment efficacy. We hope to help identify molecular predicitive biomarkers to tailor treatment more efficiently towards patients who are more likely to benefit and to spare the others unnecessary systemic treatment and proceed directly to radical local therapy.

In conclusion, this trial tests the hypothesis 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.

Ethics and dissemination: The study has received approval by ethical committee Zurich, Switzerland, BASEC-No. 2021-01872. Results will be made available by publication. Trial registration number: NCT04630730

Trial status

Recruitment started May 2022, estimated closure of accrual April 2025.

List of abbreviations

APC	Antigen presenting cells
BCG	Bacillus Calmette-Guerin
CIS	Carcinoma in situ
CR	Complete response
EFS	Event-free survival
eGFR	Estimated glomerular filtration rate
mAB	Monoclonal antibody
MDSC	Myeloid-derived suppressor cells)
MIBC	Muscle invasive bladder cancer
NCI CTCAE	NCI Common Terminology Criteria for Adverse Events
NMIBC	Non-Muscle invasive bladder cancer
OS	Overall survival
PaR	Pathological response
pCR	Pathological complete remission
PD-L1	Programmed cell death-ligand 1
PD1	Programmed cell death protein 1
PFS	Progression-free survival
RFS	Recurrence-free survival

SAKK Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung (Swiss Group for Clinical Cancer Research)

Ethics approval

Ethics approval 25.03.2022, ethical committee Zurich, Switzerland, BASEC-No. 2021-01872

Consent for publication

All authors have no objections for publication.

Availability of data and materials

Not applicable

Conflict of interest declaration

UP: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS, Merck, Pfizer, Roche, MSD, Janssen, Novartis

RC: Advisory board (compensated, institutional) for Astellas, Astra Zeneca, BMS, Merck, Pfizer, Roche, MSD, Ipsen, Janssen, Novartis; Honoraria (compensated, institutional) for Janssen, Astellas

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MS: None

AO: Advisory role (compensated, institutional): Astra Zeneca, Astellas, Bayer, Janssen, Molecular Partners, MSD, Pfizer, Roche, Sanofi Aventis (compensated, institutional). Novartis, Janssen, Bayer, MSD, AstraZeneca, Merck, Astellas (compensated). Research support (institutional): TEVA, Janssen. Travel support

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Provision of drugs

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VPM1002BC by VPM/BBIO/SIIPL

Author contributions

Ulf Petrausch performed the study design and wrote the manuscript, Martin Spahn performed the study design, Martina Schneider submitted the protocol to authorities and ethical committee, Stefanie Hayoz performed the study design and did all statistical planning, Cyrill A. Rentsch performed the study design, Sacha I. Rothschild planned all translational research and will perform the analysis, Aurelius Omlin performed the study design and coordinated all centers for patient accrural, and Richard Cathomas performed the study design and wrote the manuscript.

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Figures

Figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

Figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFNγ 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.

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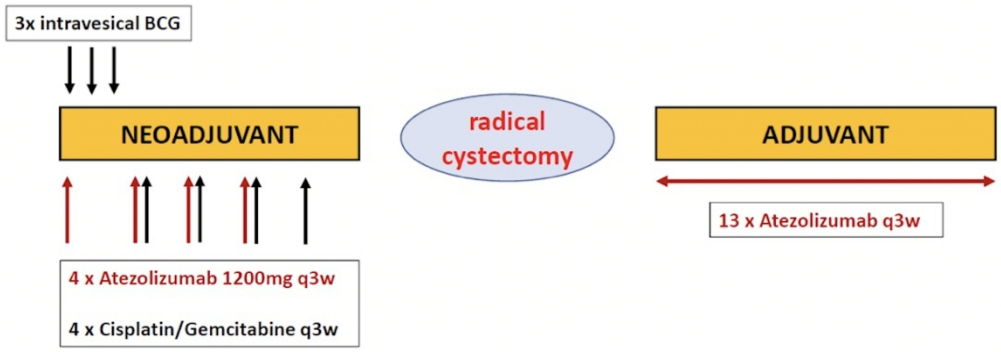

figure 1: Study schedule SAKK 06/19: Intravesical rBCG followed by perioperative chemo-immunotherapy for patients with muscle-invasive bladder cancer (MIBC). A multicenter, single arm phase 2 trial

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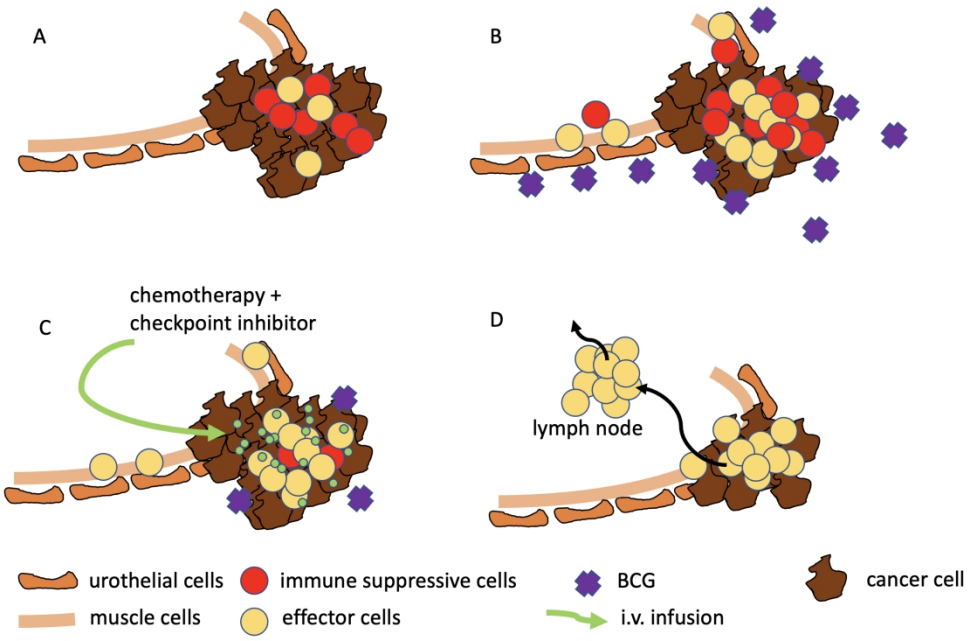

figure 2: A: the urothelial cancer (brown) is infiltrated by immune cells (yellow and red), B: BCG enhances the local inflammation by IFN□ release resulting in increased number of immune suppressive immune cells (MDSC), and upregulation of PD-L1, C: chemotherapy diminishes MDSC, checkpoint inhibition 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.

416x267mm (144 x 144 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	11 -24 of 85
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	76 of 85
	2b	All items from the World Health Organization Trial Registration Data Set	11-24 of 85
Protocol version	3	Date and version identifier	1 of 85
Funding	4	Sources and types of financial, material, and other support	74 of 85
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	2 of 85
	5b	Name and contact information for the trial sponsor	1 of 85
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	74-85 of 85
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	74-85 of 85

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	25-27 of 85
	6b	Explanation for choice of comparators	63 of 85
Objectives	7	Specific objectives or hypotheses	28 of 85
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	63 of 85
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	76 of 85
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	30-31of 85
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	37–42 of 85
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	41 of 85
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	55-59 & 78 of 85
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	59 of 85
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	31 of 85
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	32 of 85

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	21 of 85
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	22 of 85
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n.a. phase II
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n.a. phase II
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n.a. phase II
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n.a. phase II
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n.a. phase II
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	55 – 59 of 85
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	75 of 85

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	62 of 85
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	63-64 of 85
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	63-64 of 85
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	64 of 85
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	74-75 of 85
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	63 of 85
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	43-54 of 85
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	74 of 85
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	1 of 85
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	76 of 85

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	73-74 of 85
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	73-74 of 85
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	77 of 85
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	1 of 85
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	74 of 85
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	77 of 85
	31b	Authorship eligibility guidelines and any intended use of professional writers	77 of 85
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n.a.
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Separate file
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	67-72 of 85

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.